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Discriminative Stimulus Effects of Acute Morphine Followed by Naltrexone in the Squirrel Monkey: A Further Characterization

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Characterization of MOR→NTX Discriminative Cue in Monkeys

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LIST OF NON-STANDARD ABBREVIATIONS

Buprenorphine (BUP), Diprenorphine (DIP), Etorphine (ETOR), Fentanyl (FEN), Heroin (HER), Levorphanol (LEV), meperidine (MEP), Methodone (METH), Morphine (MOR), Saline (SAL), Nalbuphine (NBP), Nalorphine (NAL), Naltrexone (NTX), Morphine followed by naltrexone (MOR→NTX), Saline followed by naltrexone (SAL→NTX)

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ABSTRACT

The discriminative stimulus effects of acute morphine followed by naltrexone have been described previously in nonhuman primates. The purposes of this study were twofold: to extend the pharmacological characterization of the discrimination by testing mu-opioid agonists other than morphine and opioid-like compounds other than naltrexone; and to examine further the relationship between agonist pretreatment time and manifestation of the cue produced by morphine followed by naltrexone. Subjects were trained to discriminate 1.7 mg/kg morphine→ 0.1 mg/kg naltrexone (MOR→NTX) versus saline followed by 0.1 mg/kg naltrexone. When combined with 0.1 mg/kg naltrexone, all of the agonists tested, save buprenorphine, meperidine and nalbuphine, produced dose-dependent increases in MOR→NTX-appropriate responding, culminating in criterion levels of responding. Comparing agonist ED_{50}s revealed a rank order of potency of etorphine >> fentanyl >> levorphanol > heroin ≥ methadone ≥ nalbuphine ≥ morphine. ED_{50}s for buprenorphine and meperidine could not be calculated. MOR→NTX-appropriate responding following doses of agonist that produced criterion or near criterion levels of responding was also a function of naltrexone dose. After morphine pretreatment, diprenorphine and nalorphine, but not buprenorphine, dose-dependently substituted for naltrexone. The MOR→NTX discrimination was also dependent upon the interval between morphine and NTX administration. Finally, 1 h pretreatment with morphine and etorphine, but not buprenorphine, followed by naltrexone generalized to 4 h MOR→NTX. These results suggest a minimum efficacy requirement of acutely administered agonists together with the naltrexone training dose for stimulus control of behavior. However, in some cases this requirement can be overcome with higher doses of naltrexone.

The administration of an opioid antagonist after a single injection of a morphine-like drug elicits characteristic physiological and behavioral changes, in addition to subjective symptoms (e.g. negative mood states) (for review see Harris and Gewirtz, 2005)- much like the withdrawal syndrome following chronic morphine administration (Martin, 1983). These findings not only serve as evidence of acute opioid dependence they also suggest a common underlying mechanism with chronic opioid dependence. The phenomenon of acute dependence is predominantly mediated via mu-opioid receptors and is stereoselective (Harris and Gewirtz, 2005). The severity of withdrawal from acute opioid dependence is critically dependent upon the doses of agonist and antagonist employed as well as the interval between agonist and antagonist administration (Harris and Gewirtz, 2005).

Drug discrimination affords a useful animal model for studying interoceptive drug effects, including those associated with morphine withdrawal (Holtzman, 1990). Previously, we trained squirrel monkeys to discriminate 1.7 mg/kg morphine (4 h pretreatment) followed by 0.1 mg/kg naltrexone (15 min pretreatment) from pretreatment with saline followed by naltrexone (i.e. MOR→NTX versus SAL→NTX) (White and Holtzman, 2003). Stimulus control of behavior was an orderly function of both the dose of morphine and the dose of naltrexone. MOR→NTX-appropriate responding was also a function of morphine pretreatment time. Maximal responding occurred following 4 h pretreatment with morphine and had abated by 16 h. The discriminative stimulus appears to be mediated by opioid receptors as pretreatment with naltrexone 15 min prior to the training dose of morphine dose-dependently blocked MOR→NTX-appropriate responding. The stimulus is produced stereoselectively and is pharmacologically selective as well. Pretreatment with a congener of morphine, levorphanol (0.3 mg/kg), substituted fully for morphine when combined with naltrexone but its non-opioid stereoisomer dextrorphan and the kappa-selective agonist (5 alpha, 7 alpha, 8 beta)-(+)-N-

methyl-N-(7-(1-pyrrolidinyl)- 1-oxaspiro(4,5)dec-8-yl)benzeneacetamide (U69,593) did not. These data are consistent with previous findings with rats trained to discriminate 10 mg/kg morphine (4 h pretreatment) followed by 0.3 mg/kg naltrexone (15 min pretreatment) versus saline followed by naltrexone (Easterling and Holtzman, 1999;Holtzman, 2003). Taken together, these data suggest that the interoceptive stimuli associated with the MOR→NTX cue reflect antagonist-precipitated withdrawal from acute physical dependence upon morphine.

Our first objective was to extend the pharmacological characterization of the MOR→NTX discrimination by squirrel monkeys by testing mu-opioid agonists other than morphine and levorphanol and antagonists other than naltrexone. We initially determined the "optimal" doses to produce full MOR→NTX responding when followed by the training dose of naltrexone (0.1 mg/kg), for a variety of morphine-like agonists. These agonists represented a range of intrinsic efficacies (e.g., buprenorphine, nalbuphine, and meperidine: relatively low; morphine and heroin: intermediate; fentanyl, methadone, and etorphine: relatively high) (Schmidt et al., 1985;Emmerson et al., 1996;Selley et al., 1998). Intrinsic efficacy is a determinant of the magnitude of tolerance development to the antinociceptive effects of morphine-like drugs (Paronis and Holtzman, 1991). However, despite evidence that common mechanisms mediate tolerance and physical dependence (Way, 1993), until recently (Holtzman, 2003), there has been relatively little research on the role of intrinsic efficacy in the manifestation of acute opioid dependence or its associated interoceptive stimuli.

We then tested the "optimal" doses of the morphine-like agonists, which were estimated to be equi-effective with 1.7 mg/kg morphine (i.e., the training dose), and a range of naltrexone doses for stimulus generalization. In the case of buprenorphine, meperidine, and nalbuphine, criterion levels of responding could not be reached at any dose. Therefore, stimulus generalization was assessed following pretreatment with several doses of buprenorphine and

meperidine and 3.0 mg/kg nalbuphine followed by naltrexone. Naloxone substituted fully for naltrexone in squirrel monkeys and rats that were pretreated with a single dose of morphine (Easterling and Holtzman, 1999;White and Holtzman, 2003). We extended observations to two more opioid antagonists: diprenorphine and nalorphine.

The second objective of the current study was to examine further the functional relationship between agonist pretreatment interval and stimulus control of behavior. We extended our earlier observations (White and Holtzman, 2003) to include stimulus generalization curves for naltrexone following 1- and 2-h morphine pretreatment. Finally, we sought to determine if MOR→NTX-appropriate responding could be elicited at an earlier time point (1 h) following buprenorphine pretreatment. A Stimulus generalization curve for 1 h pretreatment with etorphine followed by NTX was also constructed for comparison. This last set of experiments was based on the finding that potentiation of naltrexone-induced suppression of water consumption in water-deprived rats was an inverse function of the interval between agonist and antagonist administration (White and Holtzman, 2001).

METHODS

Subjects

Six male squirrel monkeys (Saimiri sciureus), weighing between 700-1100 g at the beginning of experimentation, were pair-housed with unlimited access to food and water. Monkeys were provided with fresh fruit, peanuts, or a vitamin supplement mixture each day in the home cage. All of the monkeys were opioid drug experienced and had previously been trained to discriminate 1.7 mg/kg morphine followed by 0.1 mg/kg naltrexone versus saline followed by naltrexone (White and Holtzman, 2003). Animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" (National Academy of Sciences, 1996), and

all procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

Apparatus

During experimental sessions, monkeys were seated in small primate chairs housed in ventilated and sound-attenuated chambers (BRS/LVE Inc., Laurel MD). The chairs were equipped with a small stock and two brass electrodes through which electric current was delivered to a shaved portion of the monkey's tail. Two response levers were mounted 9.5 cm apart on the front panel and 3 cm from the sidewalls. A Plexiglas partition extended from the ceiling to the waist-plate of the chair, creating a wall 6 cm out from the front panel. Two slots approximately 10 cm apart, measuring 4 X 5 cm each were cut out of this partition just in front of each lever with approximately 10 cm between the slots to prevent the monkey from reaching and pressing both levers simultaneously. A red stimulus light was mounted at eye level and centered between the two response levers on the front panel. A white house light was positioned above the red stimulus light or on the rear wall of the chamber, depending on the chamber used. The chambers were equipped with white noise to mask extraneous sounds.

Drug Discrimination Procedure

This procedure has been described previously (White and Holtzman, 2003). In short, experimental sessions were conducted three to five days a week, Monday through Friday and consisted of 25 trials. Subjects did not receive drug on non-experimental days. Monkeys were trained to press the response levers under a fixed-ratio 1 (FR1) schedule of stimulus termination/avoidance. At the beginning of each trial, the house light was illuminated, and the monkey had 5 s to press the lever appropriate for the injection combination received before the

session in order to avoid a 2-4 mA electrical stimulus to the tail. If the monkey failed to press the correct lever within 5 s, the electrical stimulus was delivered in 1-s pulses every 2 s until the monkey responded on the correct lever or until 15 stimuli were delivered. At the end of the trial the house light was turned off and the red stimulus light was turned on for a 30 s time-out period. Each response on the incorrect lever resulted in an electrical stimulus and a 3-s change-over delay, during which responses on the correct lever did not end the trial. Each response during the 30-s time-out period also resulted in the delivery of an electrical stimulus to the tail in order to discourage responding between trials.

For training sessions, monkeys received an i.m. injection of either saline or 1.7 mg/kg morphine 4 h before each daily session, followed by 0.1 mg/kg naltrexone 3.75 h later (SAL→NTX and MOR→NTX, respectively). Lever assignments for each training-drug combination were balanced across subjects. Drug discrimination training continued for each monkey until it achieved a criterion of emitting the first response on the injection-appropriate lever in ≥ 88% (i.e. ≥ 22) of the trials in a session for four consecutive daily sessions. Monkeys then underwent two test sessions, one for each condition. Test sessions were similar to training sessions, except there was not a "correct" lever, so that a trial was terminated by a response on either lever. However, subjects were still required to respond within 5 s avoid an electrical stimulus. If monkeys responded on the injection-appropriate lever in ≥ 88% of the trials on both test days, they were considered to have met criteria for acquisition of the discrimination. Following acquisition of the discrimination, novel doses and/or drugs were tested one or two times per week with at least 3 days between successive tests. Between test sessions, monkeys were required to perform at criterion (≥ 88% injection-appropriate responding) in at least two consecutive training sessions.

The first experiment involved varying the dose of the opioid agonists buprenorphine (0.01-0.1 mg/kg), etorphine (0.56-1.78 µg/kg), fentanyl (0.01-0.03 mg/kg), heroin (0.1-0.56 mg/kg), levorphanol (0.03-0.3 mg/kg), meperidine (3.0-17.8 mg/kg), methadone (0.1-1.78 mg/kg), morphine(0.56-1.7 mg/kg), and nalbuphine (0.1-17.8 mg/kg), while holding the pretreatment interval (3.75 h) and naltrexone dose constant (0.1 mg/kg, 0.25 h). This experiment was performed to determine the "optimal" dose of agonist required to elicit criterion levels of MOR→NTX-appropriate responding when followed by the training dose of naltrexone. Drugs were tested up to doses that either substituted completely for MOR→NTX or produced observable side effects (e.g., muscle tremors, sedation, and/or respiratory depression) that suggested that higher doses could be a threat to the well-being of the monkeys. For the next experiment, the "optimal" dose and pretreatment time of the agonists were held constant and the dose of naltrexone was varied to determine whether the interoceptive effects of withdrawal from acute opioid dependence upon the different agonist are a function of the naltrexone dose. The only exceptions were following buprenorphine, meperidine and nalbuphine, where criterion levels of MOR→NTX-appropriate responding were not achieved. For buprenorphine and meperidine, naltrexone dose-response curves were constructed following all the doses of agonist tested in the initial experiment to determine if criterion levels of responding could be reached. For nalbuphine, the dose at which maximal (80%) responding occurred (i.e. 3.0 mg/kg) was used prior to naltrexone to determine if criterion levels of responding could be reached. Following this experiment, other drugs, including buprenorphine (0.1, 1.0 mg/kg), diprenorphine (0.001-1.0 mg/kg) and nalorphine (0.03-10 mg/kg), were then tested in place of naltrexone following morphine pretreatment. We also examined the effects of varying agonist pretreatment time on MOR→NTX-appropriate responding by first constructing a time-course for 1.7 mg/kg morphine given 1-24 h prior to testing and with the naltrexone dose and pretreatment time held constant.

We then constructed naltrexone dose-response curves following morphine pretreatment at 1, 2, and 4 h prior to testing. In the last set of experiments, we wanted to determine if we could elicit greater MOR→NTX-appropriate responding following a shorter buprenorphine pretreatment time. Therefore, a naltrexone dose-response curve was constructed following 1 h pretreatment with a maximal dose of buprenorphine (0.1 mg/kg). Naltrexone dose-response curves following 1 h pretreatment with the determined "optimal" doses of morphine and etorphine at 4 h were also constructed for comparative purposes. Naltrexone was administered 0.25 h before testing. All experiments were performed on 2-6 subjects.

Drugs

The drugs used and their source were: buprenorphine hydrochloride, etorphine hydrochloride, fentanyl hydrochloride, heroin hydrochloride, meperidine hydrochloride (National Institute on Drug Abuse, Bethesda, MD); morphine sulfate (Penick, Newark, NJ); naltrexone hydrochloride, nalbuphine hydrochloride (Sigma-Aldrich, St Louis, Mo., USA); levorphanol tartrate (Roche Laboratories, Nutley, NJ); methadone hydrochloride (Mallinkrodt, St. Louis, MO); and nalorphine hydrochloride (Merck Research Labs, West Point, PA). Buprenorphine was dissolved in distilled water and all of the other drugs were all dissolved in normal (0.9%) saline. All drugs except nalbuphine were injected i.m. in a volume of 0.25 mL/kg body weight to minimize irritation. Doses of nalbuphine up to 3.0 mg/kg were prepared in a volume of 0.25 mL/kg body weight higher doses were administered in a volume of 0.5-1.0 ml/kg due to the limited solubility of the drug. Drug doses are expressed as the free base.

Data analysis

Stimulus-generalization data are expressed as the mean (n=2-6) number of trials completed on the response lever appropriate for the MOR→NTX condition; the remaining trials of the session were completed on the lever appropriate for SAL→NTX. Complete substitution (generalization) for MOR→NTX was defined as completion of ≥ 88% of the trials in the test session on the MOR→NTX-appropriate lever. Agonist and antagonist ED_{50} values (i.e., the dose at which 50% of the trials are completed on the MOR→NTX-appropriate lever) were estimated for each monkey by log-linear interpolation of ascending or descending portions of the dose-response curve; group means and SEM were calculated from those data. ED_{50} data were analyzed with either a one-factor ANOVA and Newman-Keuls test post hoc or with Student's t-test, as appropriate. The time until responding on the MOR→NTX-appropriate lever reached and returned to one-half its maximal value ($T_{½}$) was calculated by linear regression of the ascending or descending part of the time-course curve. These served as estimates of the onset and offset rates of acute morphine dependence.

The latency to emit the first response was recorded and averaged over the 25 trial session. Mean group (saline or drug pretreatment) response latencies were calculated and analyzed using one-factor repeated measures ANOVA and Dunnett's test post hoc. For control sessions in which an injection of either a drug or saline preceded an injection of saline, mean response latencies were compared with either a one-factor ANOVA followed by Newman-Keuls test or by Student's t-test, as appropriate. P values equal to or less than 0.05 were accepted as statistically significant.

RESULTS

All of the monkeys responded within 5 s of the start of a trial, thereby avoiding the electrical stimulus to the tail. This performance was not altered within the dose ranges tested; therefore avoidance data are not presented.

Determination of "Optimal" Agonist Doses

Six of the nine of drugs tested dose-dependently and completely substituted for morphine when administered 4 h before testing and 3.75 h before the 0.1 mg/kg training dose of naltrexone (Fig 1.). Buprenorphine, meperidine, and nalbuphine were the only exceptions. Following meperidine pretreatment, MOR→NTX-appropriate responding dose-dependently increased, but only to a maximum average of 6.5 trials (26%) at a dose of 10 mg/kg. However, this effect was bi-phasic: pretreatment with 17.8 mg/kg of meperidine occasioned responding exclusively on the SAL→NTX-appropriate lever (Fig. 1), and produced seizures in one monkey (data excluded from group average). Regardless of the pretreatment dose, buprenorphine occasioned virtually no MOR→NTX-appropriate responding. Because 0.1 mg/kg buprenorphine clearly produced observable side effects (e.g. behavioral and respiratory depression), higher doses were not tested. Pretreatment with nalbuphine dose-dependently increased MOR→NTX-appropriate responding to a maximum average of 19.5 trials (78%) at a dose of 3.0 mg/kg; at higher doses (10 and 17.8 mg/kg), slightly fewer trials were completed on the MOR→NTX-appropriate lever (18.5 and 18.3 trials, respectively). Comparison of agonist ED_{50}s revealed a significant difference among them (Table 1; $F[6,30]=87.8$), with a resultant rank order of potency of etorphine >> fentanyl >> levorphanol > heroin ≥ methadone ≥ nalbuphine ≥ morphine. The average latency to respond following various doses of each agonist and 0.1 mg/kg naltrexone ranged between 0.51 ± 0.07 and 1.64± 0.25 s and did not adversely affect responding.

Stimulus-generalization Curves Following Different Agonist or Antagonist Pretreatment

Following pretreatment with "optimal" doses of the agonists etorphine, fentanyl, heroin, levorphanol, methadone, and morphine, increasing doses of naltrexone produced dose-dependent increases in MOR→NTX-appropriate responding (Fig. 2A). The curve for 0.3 mg/kg levorphanol naltrexone was biphasic: full MOR→NTX-appropriate responding occurred following 0.1 mg/kg naltrexone but 40% fewer trials were completed on this lever following 1.0 mg/kg naltrexone. A similar result was seen following pretreatment with 0.03 mg/kg fentanyl, albeit to a lesser degree, as fentanyl pretreatment followed by 1.0 mg/kg naltrexone resulted in an average of 21.2 responses on the MOR→NTX lever, just below criterion. The ED_{50}s for naltrexone following 3.75 h pretreatment with an agonist (including nalbuphine-see below) spanned only a 6.4-fold range, which nevertheless was statistically reliable ($F[6,29]=3.72$; Table 2). Presumably, this variance would have been even less with a finer titration of the agonist doses (i.e. finer than ¼ log unit) in the "optimal" agonist doses study (see above). Regardless, only the naltrexone ED_{50} following fentanyl pretreatment differed significantly from the naltrexone ED_{50} following morphine pretreatment (Table 2).

To determine how much MOR→NTX-appropriate responding could be attained with buprenorphine, meperidine, and nalbuphine, naltrexone dose-response curves were constructed following 0.01, 0.03, and 0.1 mg/kg buprenorphine, all the doses of meperidine used in the "optimal" agonist dose study and 3.0 mg/kg nalbuphine- the dose at which the greatest MOR→NTX-appropriate responding occurred. Meperidine substituted for morphine dose-dependently but partially, a maximum average of 11.75 trials to the MOR→NTX-appropriate lever after 3.0 mg/kg meperidine and 10 mg/kg naltrexone (Fig. 2B). Similar to the findings with meperidine, buprenorphine in combination with naltrexone failed to substitute fully for

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MOR→NTX (Fig 2B). When naltrexone was preceded by 0.1 mg/kg buprenorphine, there was a dose-dependent increase in responding, reaching a maximum average of 6 responses at 10 mg/kg. However, pretreatment with lower doses of buprenorphine, resulted in virtually no responding on the MOR→NTX lever regardless of naltrexone dose. Unlike the findings with meperidine and buprenorphine, pretreatment with 3.0 mg/kg nalbuphine in combination with naltrexone completely substituted for MOR→NTX (Fig. 2B). Responding was naltrexone dose-dependent, reaching criterion at a dose of 1.0 mg/kg. Four-hour agonist pretreatment followed by 15-min saline pretreatment did not occasion significant MOR→NTX responding (Figs. 2A and 2B).

After 4-h agonist and 15-min saline pretreatment, the average response latency ranged between 1.21 ± 0.27 and 1.93 ± 0.66 s, and did not differ among agonists (data not shown). There was a significant effect of naltrexone following pretreatment with 0.01 and 0.03 buprenorphine ($F[3,23]=3.95$; $F[3,23]=6.59$, respectively), etorphine ($F[5,35]=14.10$), fentanyl ($F[5,35]=12.36$), and 5.6, 10, and 17.8 mg/kg meperidine ($F[3,23]=4.04$; $F[3,23]=28.55$; and $F[3,23]=130.0$, respectively). Latencies were usually lower, often significantly, after agonist followed by naltrexone pretreatment versus agonist followed by saline pretreatment. For example, the average latency to respond after etorphine followed by naltrexone (0.1 and 1.0 mg/kg) was 50-60% shorter compared to etorphine followed by saline (saline: 1.93± 0.66 s versus 0.03 mg/kg: 0.67± 0.01 s and 0.1 mg/kg: 0.99± 0.33 s).

Morphine followed by diprenorphine or nalorphine substituted for MOR→NTX fully and dose-dependently, but morphine followed by buprenorphine did not (Fig. 3). The ED_{50} of diprenorphine was comparable to that of naltrexone whereas the ED_{50} of nalorphine was almost 50-fold higher ($t[1,9]=-15.50$; Table 2).

The combination of saline followed by diprenorphine or buprenorphine occasioned little or no MOR→NTX-appropriate responding (Fig. 3), even, in the case of diprenorphine, at doses up to 80-fold higher than the ED_{50} after morphine pretreatment (Table 2). Similarly, no MOR→NTX appropriate responding occurred after pretreatment with saline followed 3.75 h later by 1.0 mg/kg nalorphine (Fig. 3), a dose equivalent to the ED_{50} of nalorphine following morphine pretreatment (Table 2). However, a 10-fold higher dose of nalorphine elicited an average of 11.25 trials to the MOR→NTX-appropriate lever. ED_{50}s could not be calculated for diprenorphine, nalorphine or buprenorphine after saline pretreatment (Table 2).

Following morphine pretreatment, diprenorphine decreased the overall average latency to respond ($F[4,29]=4.58$; 1.03± 0.21 s following 0.001 diprenorphine versus 0.79± 0.16, 0.59± 0.11, 0.70± 0.23, and 0.63± 0.15 s following 0.003, 0.01, 0.1 and 1.0 mg/kg diprenorphine, respectively), but nalorphine ($F[3,23]=1.25$) and buprenorphine ($F[1,5]=0.45$) did not (data not shown). Neither diprenorphine ($F[1,11]=0.17$) or nalorphine ($F[1,7]=0.001$) altered average response latency following saline pretreatment (data not shown). The average latency to respond after saline and 0.1 mg/kg buprenorphine was 1.53 s.

Temporal Dependency of MOR→NTX-appropriate responding

To determine the temporal dependency of morphine pretreatment on MOR→NTX-appropriate responding, pretreatment with morphine (1.7 mg/kg) was systematically varied from 0.5–24 h, while pretreatment with naltrexone (0.1 mg/g) remained constant at 0.25 h (Fig. 4). At 1 h after the morphine injection, an average of 5.7 (approximately 23% of maximum) responses occurred on the MOR→NTX lever. However, MOR→NTX-appropriate responding reached criterion levels when pretreatment was lengthened to 2 h and remained there as the pretreatment intervals was lengthened further to 4, 6 or 8 h (Fig. 4). Responding occurred exclusively on the

SAL→NTX-appropriate lever when the morphine pretreatment was 24 h. The onset and offset of MOR→NTX-appropriate responding occurred with $t_{½}$s of 1.68 ± 0.07 and 12.74 ± 1.47 h, respectively. A number of studies have reported signs of spontaneous withdrawal following acutely administered morphine (for review see Harris and Gewirtz, 2005), including subjective signs in humans (Kirby and Stitzer, 1993). Morphine followed 16 h later by saline resulted only in responding on the SAL→NTX-appropriate lever (Fig. 4), eliminating the possibility that interoceptive cues associated with spontaneous withdrawal following morphine administration at longer pretreatment intervals contributed to MOR→NTX-appropriate responding.

Given that the interoceptive effects of withdrawal from acute dependence are a function of morphine dose and duration of morphine exposure, a shorter pretreatment interval should produce less dependence and smaller interoceptive effects. As a result, a larger antagonist dose would be required to produce discriminative stimulus effects equivalent to those occurring during training. Therefore, we assessed MOR→NTX-appropriate responding at 1, 2 and 4 h after morphine pretreatment (Fig. 5A). Compared to pretreatment with 1.7 mg/kg morphine at 4 h, shorter pretreatment times of 2 and 1 h resulted in rightward shifts of the morphine-naltrexone stimulus-generalization curve. There was a significant effect of morphine pretreatment time on the ED_{50}s of naltrexone (Table 2; $F[2,13]=19.83$), with approximately two- and seven-fold higher ED_{50}s following 2 and 1 h pretreatment, respectively.

Four-hour pretreatment with buprenorphine resulted in very little MOR→NTX-appropriate responding. To determine if greater responding could be achieved at an earlier time point, we assessed the effects of 1 h buprenorphine (0.1 mg/kg) pretreatment on MOR→NTX-appropriate responding. For comparative purposes we included a naltrexone dose-response curve following 1 h pretreatment with the "optimal" dose etorphine (0.00178 mg/kg; Fig. 5B).

One-hour etorphine pretreatment followed by naltrexone dose-dependently produced MOR→NTX-appropriate responding, which reached maximum at a dose of 0.3 mg/kg naltrexone (Fig. 5B). Consistent with the outcome following 1 and 4 h morphine pretreatment (Fig. 5A), the etorphine→naltrexone curve 1 h after etorphine pretreatment was shifted to the right of the 4 h curve, and naltrexone had a 3.5-fold higher ED_{50} (Table 2). The dose-response curves for 0.1 mg/kg buprenorphine at 1 and 4 h pretreatment followed by naltrexone were very similar to each other (Fig. 5B): there was little MOR→NTX-appropriate responding, even at doses as high as 3.0 and 10 mg/kg naltrexone.

DISCUSSION

This study extends previous findings characterizing the discriminative effects of acutely administered morphine followed by naltrexone in squirrel monkeys by, among other things, including opioid agonists other than morphine and opioid antagonists other than naltrexone. Six of the nine morphine-like drugs studied when combined with 0.1 mg/kg naltrexone elicited criterion levels of MOR→NTX-appropriate responding. Dose-dependent stimulus generalization also occurred when "optimal" doses of the six agonists were followed by varying doses of naltrexone. The three exceptions were buprenorphine, meperidine, and nalbuphine. Various dose combinations of meperidine followed by naltrexone occasioned peak MOR→NTX-appropriate lever selection of less than 50%, and combinations of various buprenorphine and naltrexone doses resulted in almost exclusive selection of the SAL→NTX-appropriate lever. Nalbuphine dose-dependently increased MOR→NTX lever selection but failed to elicit criterion levels of responding in combination with the training dose of naltrexone. However, when nalbuphine (3.0 mg/kg) was used in combination with a higher dose of naltrexone (1.0 mg/kg), criterion levels of MOR→NTX-appropriate responding was achieved.

Our data suggest there is a minimum agonist efficacy requirement for MOR→NTX-like stimulus control of behavior when the training dose of naltrexone is administered. buprenorphine and meperidine paired with naltrexone occasioned little or no responding on the MOR→NTX-appropriate lever, even at doses that were behaviorally active and were well within the range of doses shown to be effective in other behavioral models using squirrel monkeys (Schaefer and Holtzman, 1977;Dykstra, 1983;DeRossett and Holtzman, 1984;Dykstra, 1985;Negus et al., 1991;Dykstra et al., 2002;Allen et al., 2003). Likewise, pretreatment with the partial mu-opioid receptor agonist nalbuphine in combination with 0.1 mg/kg naltrexone failed to elicit criterion levels of responding, even at a dose ten times higher than that of the training dose of morphine. These results might be a consequence of the training doses used. Perhaps greater responding would have occurred if the monkeys had been trained with a lower dose of morphine and/or naltrexone, resulting in a lower degree of dependence and less intense discriminative stimuli with less stringent efficacy requirements. The degree of opioid dependence can alter efficacy requirements and subsequently impact the qualitative effects of morphine-like drugs. For example, in opioid dependent rhesus monkeys trained to discriminate 0.0178 mg/kg naltrexone from saline, buprenorphine exhibited agonistic or antagonistic properties, depending on the drug (morphine or L-α-acetylmethadol) upon which the monkeys were dependent (Sell et al., 2003). There are also examples of lower-efficacy agonists substituting completely for higher efficacy agonists when the training dose of the latter was low and substituting only partially or not at all when the training dose was high (Shannon and Holtzman, 1979;Picker et al., 1993;Holtzman, 1997). When we used nalbuphine in combination with a higher dose of naltrexone, criterion MOR→NT responding was attained, demonstrating agonist efficacy requirements can be overcome with higher doses of naltrexone.

Buprenorphine, which binds the mu-opioid receptor with very high affinity and has slow pharmacokinetics (for a recent review see Tzschentke, 2002), produces long lasting behavioral effects (Dykstra, 1983;DeRossett and Holtzman, 1984). This could account for the fact that there was not an increase in MOR→NTX responding even at the highest dose of naltrexone. Our inability to precipitate withdrawal from buprenorphine is consistent with the clinical literature (Heel et al., 1979;Lewis, 1985;Lange et al., 1990;Bickel and Amass, 1995;Ling et al., 1998). However, the opioid antagonist naloxone administered almost 2 h after buprenorphine completely reversed the effect of buprenorphine on squirrel monkeys responding in a shock-titration procedure (Dykstra, 1985).

High agonist affinity for the receptor and slow dissociation from it can not account for the failure of meperidine followed by naltrexone to substitute completely for MOR→NTX. It is well documented that one of the metabolites of meperidine, normeperidine, is a convulsant (Dykstra and Leander, 1978;Leander and Carter, 1982)- effectively limiting the dose range at which meperidine can be tested. However, in the dose range tested this study, meperidine was dose-dependently and fully generalized to morphine by squirrel monkeys trained to discriminate morphine (3.0 mg/kg) from saline (Schaefer and Holtzman, 1977). Meperidine also elicits morphine-like analgesia and suppresses scheduled-controlled responding (Leander, 1980;Witkin et al., 1983). The latter effect is antagonized by naloxone and exhibits cross-tolerance with morphine (Witkin et al., 1983), indicating that morphine and meperidine share a common site of action.

The initial pharmacological characterizations indicated that the discriminative effects associated with MOR→NTX are similar in rats and squirrel monkeys (Easterling and Holtzman, 1999;White and Holtzman, 2003). However, broadening the pharmacological characterization of the phenomenon has revealed differences between the two species. In rats trained to

discriminate 4-h pretreatment with 10 mg/kg morphine and 15-min pretreatment with 0.3 mg/kg naltrexone from pretreatment with saline and naltrexone, only heroin and levorphanol substituted completely for morphine when administered acutely in combination with naltrexone (Holtzman, 2003). Other agonists substituted for morphine only partially, regardless of whether their intrinsic efficacy was higher (e.g. etorphine, fentanyl, and methadone) or lower (e.g. buprenorphine and meperidine) than that of morphine. In the present study, only lower intrinsic efficacy drugs failed to substitute for morphine when the training dose of naltrexone was administered, suggesting a minimum efficacy requirement. It appears that once this efficacy requirement has been satisfied, or overcome with a higher dose of naltrexone, as in the case with nalbuphine, mu-opioid agonists combined with naltrexone more uniformly produce the MOR→NTX associated cues in the squirrel monkey than they do in the rat. The results of our study are consistent with the clinical literature on acute opioid dependence (Wright et al., 1991;Greenwald et al., 1996), and provide one more example of inter-species differences in opioid pharmacology.

The pure antagonist diprenorphine, but not the partial mu-opioid agonist nalorphine, fully substituted for naltrexone in rats (Holtzman, 2003). This discrepancy was attributed to the fact that diprenorphine, like naltrexone, is devoid of intrinsic efficacy whereas nalorphine is not (Holtzman, 2003). In our study, full dose-dependent stimulus generalization occurred following both diprenorphine and nalorphine in monkeys pretreated with morphine. Together with data from our previous report (White and Holtzman, 2003) the relative antagonist potency of naltrexone = diprenorphine ≥ naloxone >nalorphine reflects the affinity of those drugs for the mu-opioid receptor. Thus, in this case it was the action of an antagonist that differed between rats and squirrel monkeys.

Our results revealed a significant effect of morphine pretreatment time on MOR→NTX responding. Monkeys responded at criterion levels of MOR→NTX-appropriate responding as early as 2 h after morphine, and significant MOR→NTX responding persisted as long as 16 h after morphine administration. This is in contrast to our previous report, where criterion MOR→NTX responding only occurred at 4 h after morphine administration (White and Holtzman, 2003). It is possible that classical conditioning occurred to the unconditioned stimulus effects of naltrexone after morphine or perhaps to naltrexone alone. However, reassessment of the naltrexone dose-response curve following morphine pretreatment revealed that the stimulus-generalization curve was unchanged from one study to the next: the naltrexone ED50s were virtually identical between the two studies. Additionally, in our previous study (White and Holtzman, 2003), sensitization to the stimulus effects of naltrexone did not occur following repeated training or testing; the combination of saline pretreatment and as much as1.0 mg/kg NTX exclusively elicited selection of the SAL→NTX-appropriate lever. The reason(s) why the time-course of discriminative effects changed between studies, while the morphine→naltrexone stimulus-generalization curve did not is obscure.

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FOOTNOTES

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LEGENDS FOR FIGURES

Fig 1. MOR→NTX appropriate responding is a function of agonist dose. The discriminative stimulus effects of the opioid agonists buprenorphine (BUP; 0.01-0.1 mg/kg), etorphine (ETOR; 0.56-1.78 ug/kg), fentanyl (FEN; 0.01-0.03 mg/kg), heroin (HER; 0.1-0.56 mg/kg), levorphanol (LEV; 0.03-0.3 mg/kg), meperidine (MEP; 3.0-17.8 mg/kg), methadone (METH; 0.1-1.78 mg/kg), morphine (MOR; 0.56-1.7 mg/kg) (all given s.c.; 4 h pretreatment), and *nalbuphine (NBP; 0.1-17.8 mg/kg)* followed 0.1 mg/kg naltrexone (NTX; s.c.; 0.25 h pretreatment) were determined in monkeys trained to discriminate 1.7 mg/kg morphine→0.1 mg/kg naltrexone (MOR→NTX) from saline→naltrexone (SAL→NTX). Each point represents a mean of one observation in each of three to five monkeys, except 17.8 mg/kg meperidine (n=2). The dashed lines at the top and bottom of the figure represent criteria for full MOR→NTX and SAL→NTX appropriate responding, respectively. The "optimal" doses of agonist needed to produce full MOR→NTX appropriate responding were determined and used in subsequent studies. All of the agonists except buprenorphine and meperidine dose-dependently produced criterion levels of responding. The partial agonist nalbuphine dose-dependently increased responding but did not reach criterion.

Fig 2. Agonist→NTX discrimination is a function naltrexone dose. (**A**) The discriminative stimulus effects of the opioid agonists etorphine (ETOR), fentanyl (FEN), heroin (HER), levorphanol (LEV), methodone (METH), and morphine (MOR) (s.c.; 4 h pretreatment) followed by saline (SAL) or naltrexone (NTX; 0.001-10 mg/kg, s.c.; 0.25 h pretreatment) were determined. "Optimal" doses of agonist (i.e. those eliciting criterion MOR→NTX responding when followed by 0.1 mg/kg NTX) were used (See Fig 1.). Each point represents a mean of one

observation in each of two to five monkeys. (**B**) The discriminative stimulus effects of doses of buprenorphine (BUP; 0.01-0.1 mg/kg), meperidine (MEP; 3.0-17.8 mg/kg), and nalbuphine (NBP; 3.0 mg/kg) followed 3.75 h later by saline or naltrexone (0.001-10 mg/kg, s.c.; 0.25 h pretreatment) were also assessed to determine if greater MOR→NTX appropriate responding could be achieved with higher doses of naltrexone. Each point represents a mean of one observation in each of two to four monkeys. Other details are as in Fig. 1.

Fig 3. Diprenorphine and nalorphine but not buprenorphine substitute for naltrexone following 4 h pretreatment with morphine. The discriminative stimulus effects of 1.7 mg/kg morphine (MOR) or saline followed by buprenorphine (BUP; 0.01, 0.1 mg/kg, s.c.; 0.25 h pretreatment), diprenorphine (DIP; 0.001-1.0 mg/kg, s.c.; 0.25 h pretreatment), or nalorphine (NAL; 0.03-10 mg/kg, s.c.; 0.25 h pretreatment) were determined. MOR→NTX curve is reproduced from Fig. 2. Each point represents a mean of three to six monkeys. Other details are as in Figs. 1 and 2.

Fig 4. MOR→NTX discrimination is a function of morphine pretreatment time. The discriminative stimulus effects of naltrexone (NTX; 0.1 mg/kg, s.c.; 0.25 h pretreatment) following morphine (MOR; 1.7 mg/kg, s.c) given 1.0 to 24 h prior to testing were assessed. Each point represents a mean of four monkeys. Full MOR→NTX responding occurred with 2 h of morphine administration but was gone by 24 h following morphine. Other details are as in Figs. 1 and 2.

Fig 5. MOR→NTX discrimination is a function of morphine and etorphine but not buprenorphine pretreatment time and naltrexone dose. (**A**) The discriminative stimulus

effects of naltrexone (NTX; 0.001-1.0 mg/kg, s.c; 0.25 h pretreatment) following morphine (MOR; 1.7 mg/kg, s.c) given 1.0 or 2.0 h prior to testing were assessed. (**B**) The discriminative stimulus effects of naltrexone following 1 h pretreatment with 0.1 mg/kg buprenorphine (BUP) or 1.78 µg/kg etorphine (ETOR). The curves represent means of three to six monkeys. The 4 h pretreatment curves for morphine, etorphine, and buprenorphine are reproduced from Fig. 2. Other details are as in Figs. 1 and 2.

Table 1. ED_{50s} of opioid agonists given 4 hr prior to testing followed by 0.1 mg/kg naltrexone administered 15 min prior to testing

Agonist	Doses (mg/kg)	Agonist ED_{50} and 95% C.I. (mg/kg)	Potency Relative to Morphine	*n*
Etorphine	0.00056-0.00178	0.001 (0.00033,0.0024)*	1060	4
Fentanyl	0.01-0.03	0.02 (0.017,0.023)*	48	5
Levorphanol	0.03-0.3	0.10 (0.04, 0.29)*	9.2	4
Heroin	0.1-0.56	0.31 (0.22, 0.44)*	3.0	4
Methadone	0.1-1.78	0.57 (0.37, 0.89)	1.6	5
Nalbuphine	*0.1-17.8*	*0.82 (0.14, 4.68)*	*1.1*	*4*
Morphine	0.56-1.7	0.94 (0.60, 1.47)	1.0	4
Buprenorphine	0.01-0.1	ineffective	---	5
Meperidine	3.0-17.8	ineffective	---	3[†]

*Significantly different from ED_{50} of morphine; one-factor ANOVA, Dunnett's t-test *post hoc*, $p<0.05$

[†]N=2 at 17.8 mg/kg

Table 2. The effects of opioid agonists 1-4 hr prior to testing on the ED_{50} of opioid antagonists administered 15 min prior to testing

Agonist	Dose (mg/kg)	Pretreatment Time	Antagonist	Antagonist ED_{50} and 95% C.I. (mg/kg)	Potency relative to 4 h MOR→NTX[a]	*n*
Buprenorphine	0.1	1 h	Naltrexone	--	--	4
	0.01	4 h		--	--	4
	0.03	4 h		--	--	4
	0.1	4 h		--	--	6
Etorphine	0.00178	1 h	Naltrexone	0.042 (0.006, 0.28)	0.54	4
	0.00178	4 h		0.012 (0.002, 0.061)	1.88	4
Fentanyl	0.03	4 h	Naltrexone	0.077 (0.030, 0.19)*	0.30	5
Heroin	0.56	4 h	Naltrexone	0.012 (0.007, 0.022)	1.85	4
Levorphanol	0.3	4 h	Naltrexone	0.036 (0.012, 0.11)	0.63	4
Meperidine	3.0	4 h	Naltrexone	--	--	4
	5.6	4 h		--	--	4
	10	4 h		--	--	3
	17.8	4 h		--	--	2
Methadone	1.0	4 h	Naltrexone	0.024 (0.003, 0.22)	0.97	5
Morphine	1.7	1 h	Naltrexone	0.17 (0.094, 0.303)*	0.14	5
	1.7	2 h		0.044 (0.018, 0.104)	0.52	5
	1.7	4 h		0.023 (0.001, 0.042)	1.0	4

	1.7	4 h	Diprenorphine	0.012 (0.005, 0.027)	1.88	4
	1.7	4 h	Nalorphine	1.09 (0.25, 4.65) †	0.02	6
	1.7	4 h	Buprenorphine	--	--	4
Nalbuphine	3.0	4 h	Naltrexone	0.06 (0.019, 0.22)	0.36	4
Saline	0	4 h	Diprenorphine	--	--	4
	0	4 h	Nalorphine	--	--	4
	0	4h	Buprenorphine	--	--	4

*Significantly different from ED_{50} of 4 hr morphine pretreatment group; one-factor ANOVA, Dunnett's t-test *post hoc*, $p<0.05$

†Significantly different from ED_{50} of 4 hr morphine pretreatment group followed by naltrexone; Student's t-Test, $p<0.05$

[a]Fold change was calculated as (NTX ED_{50} following 4 hr MOR/ED_{50} from experimental group)

Fig. 1

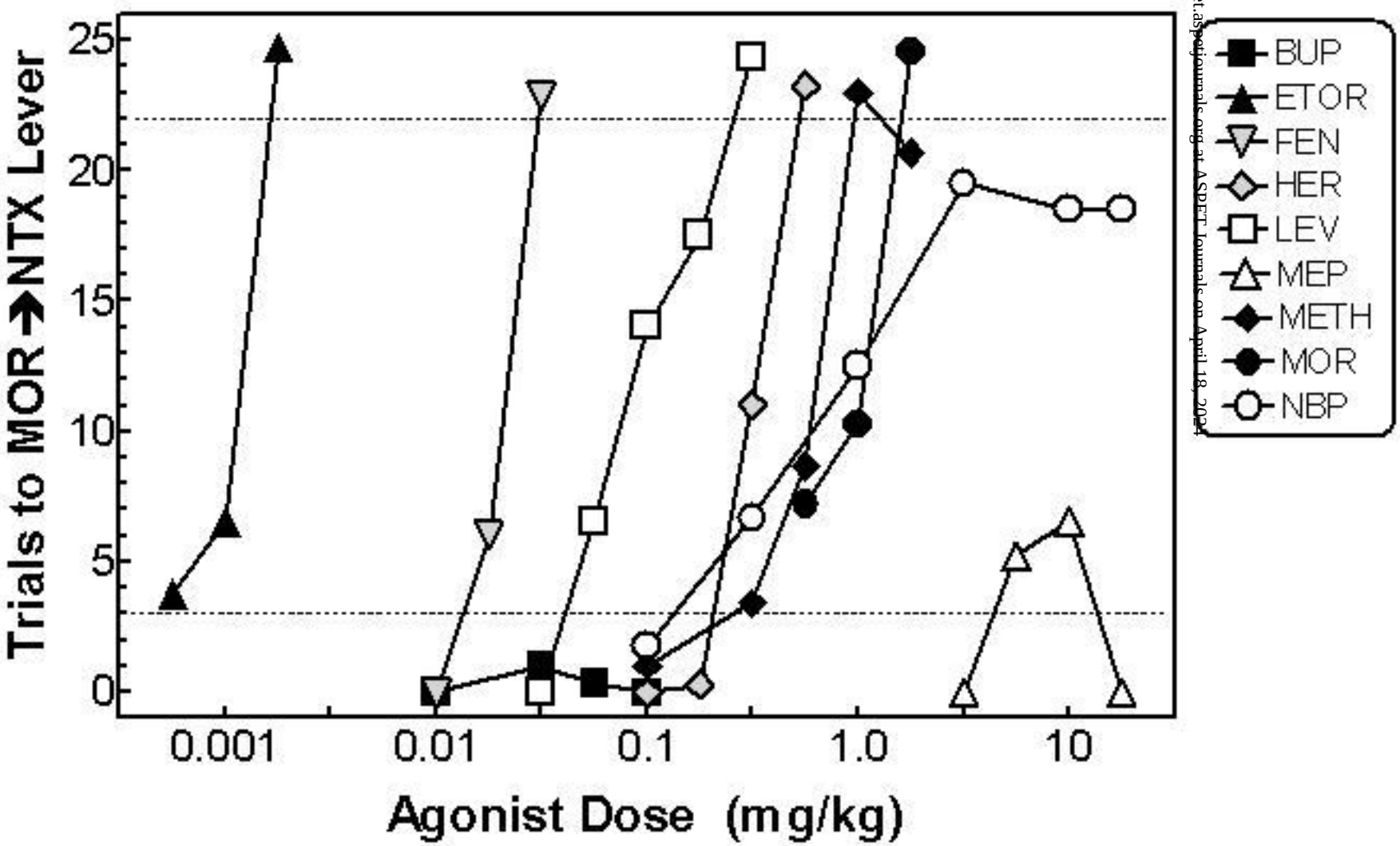


Fig. 2

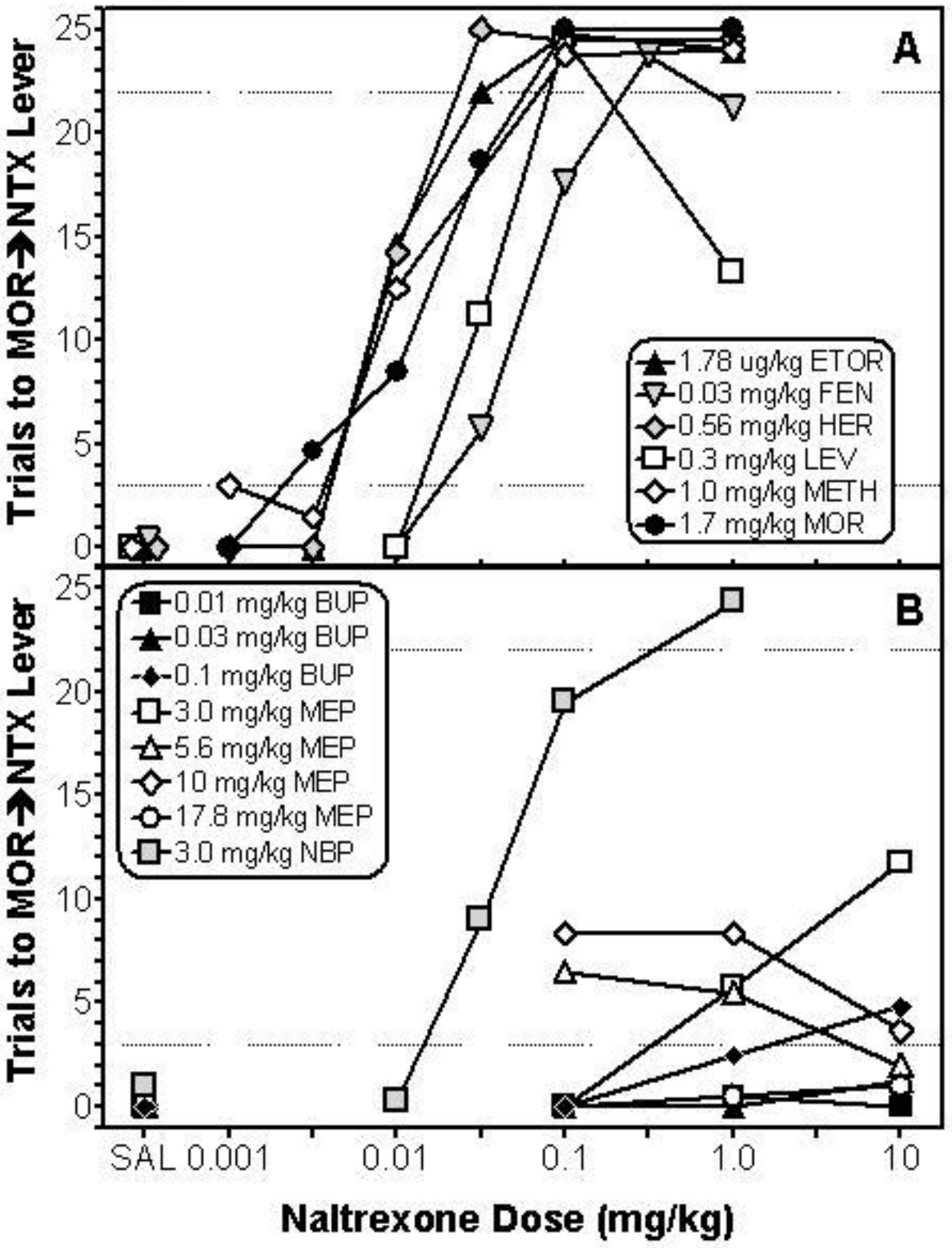

A
B
Trials to MOR→NTX Lever
Trials to MOR→NTX Lever
25
20
15
10
5
0
1.78 ug/kg ETOR
0.03 mg/kg FEN
0.56 mg/kg HER
0.3 mg/kg LEV
1.0 mg/kg METH
1.7 mg/kg MOR
0.01 mg/kg BUP
0.03 mg/kg BUP
0.1 mg/kg BUP
3.0 mg/kg MEP
5.6 mg/kg MEP
10 mg/kg MEP
17.8 mg/kg MEP
3.0 mg/kg NBP
SAL 0.001
0.01
0.1
1.0
10
Naltrexone Dose (mg/kg)

Fig. 3

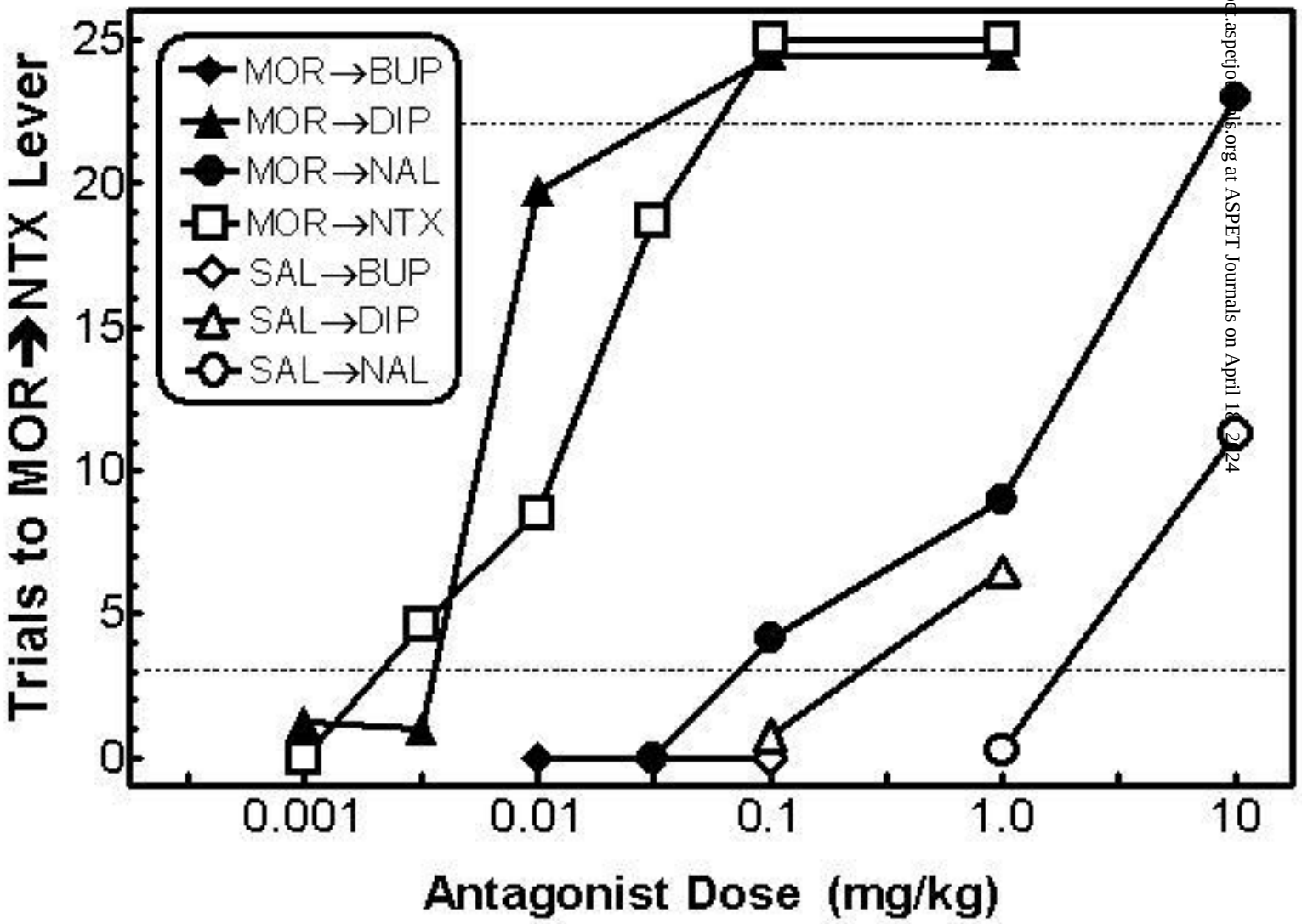


Fig. 4

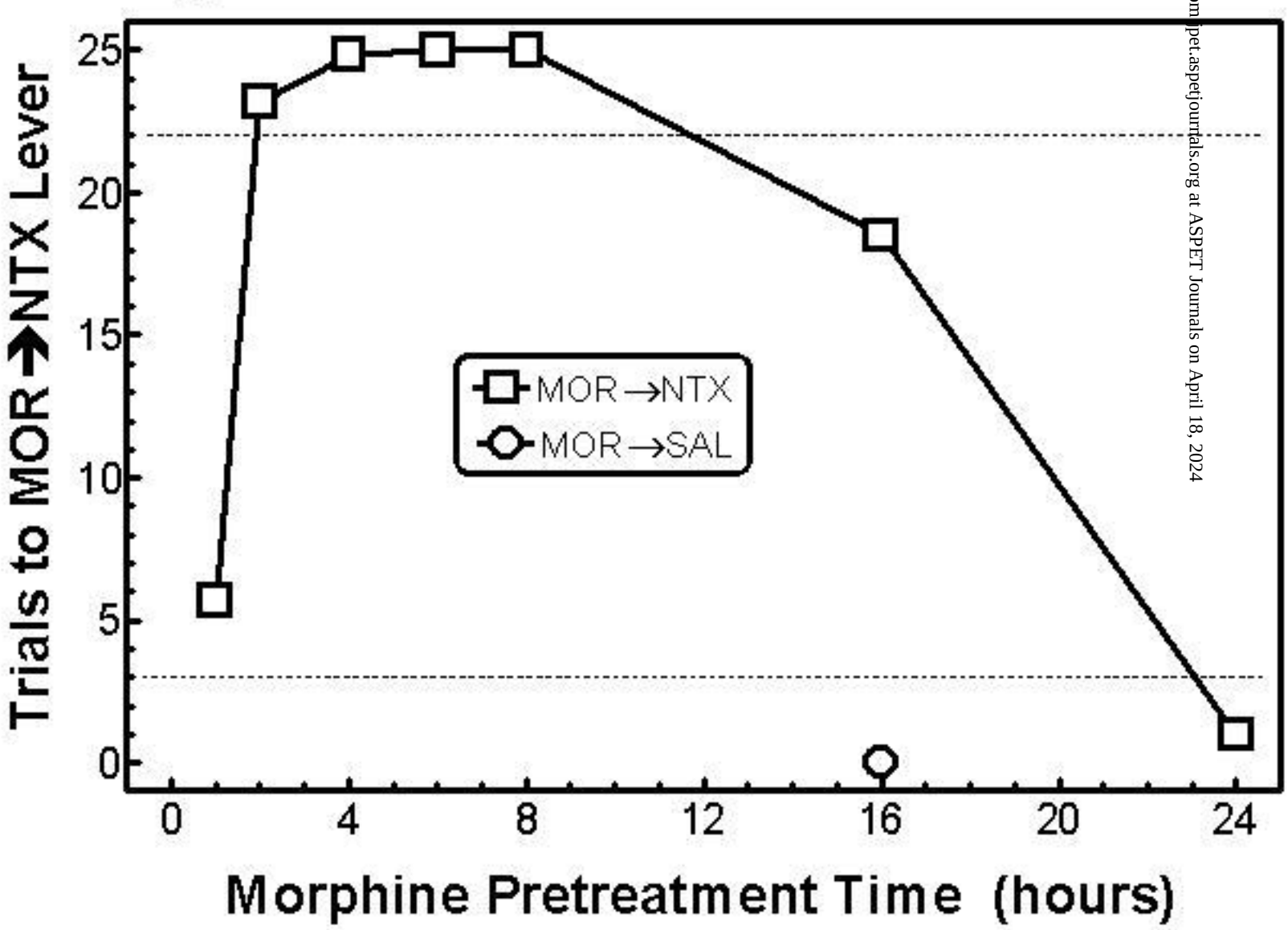
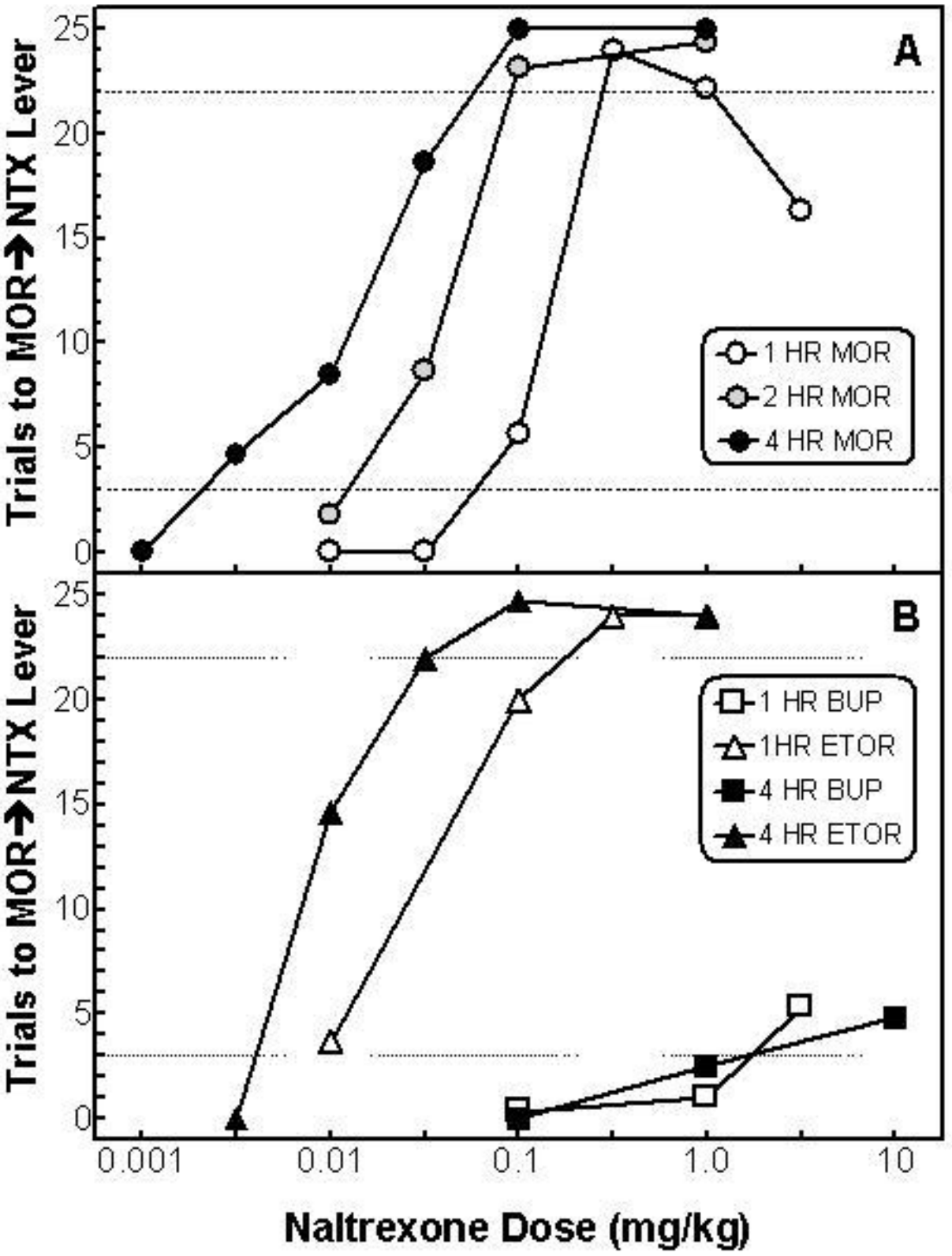


Fig. 5


A
Trials to MOR→NTX Lever
25
20
15
10
5
0
1 HR MOR
2 HR MOR
4 HR MOR
B
Trials to MOR→NTX Lever
1 HR BUP
1HR ETOR
4 HR BUP
4 HR ETOR
0.001
0.01
0.1
1.0
10
Naltrexone Dose (mg/kg)