Effects of opioids in morphine-treated pigeons trained to discriminate among morphine, the low-efficacy agonist nalbuphine, and saline


Department of Psychology, University of North Carolina at Chapel Hill, NC USA
Running title: Discrimination in morphine-treated pigeons

Ellen A. Walker, Ph.D.
Department of Pharmaceutical Sciences
Temple University School of Pharmacy
3307 North Broad Street
Philadelphia, PA 19140

Tel: 215-707-6770
Fax: 215-707-3678
Email: ellen.walker@temple.edu

Text pages: 18
Tables: 2
Figures: 4
References: 39
Abstract: 224
Introduction: 631
Discussion: 1,591

Abbreviations: CTAP [D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH₂]; DAMGO, [(D-Ala², N-Me-Phe⁴, Gly⁵-ol)-enkephalin)]; FR, fixed ratio; 95% C.L., 95% confidence limits.

Section Assignment: Behavioral Pharmacology
Abstract

In opioid-dependent subjects, the low-efficacy \( \mu \) agonist nalbuphine generally precipitates withdrawal or withdrawal-like stimulus effects. To provide a more complete characterization of the discriminative stimulus effects of nalbuphine in opioid-treated subjects, seven White Carneux pigeons were treated daily with 10 mg/kg morphine, i.m. and trained 6 h later to discriminate among 10 mg/kg morphine, 1.0 mg/kg nalbuphine and saline by responding on one of three different keys. When tested, morphine produced morphine-key responding and nalbuphine produced nalbuphine-key responding. Replacing the daily morphine injection with saline produced nalbuphine-key responding and this effect was reversed by the administration of morphine. In substitution tests with other compounds, the antagonists naltrexone (i.m.) and CTAP [D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH\(_2\)] (i.c.v.) produced nalbuphine-key responding. High-efficacy agonists fentanyl and etorphine produced morphine-key responding. The intermediate-efficacy agonists buprenorphine, dezocine, and butorphanol produced a pattern of morphine-, saline-, and/or nalbuphine-key responding that differed across individual pigeons. The lower efficacy agonists nalorphine and levallorphan produced predominantly nalbuphine-key responding. Kappa agonists spiradoline and U50,488, the \( \delta \) agonist SNC80, the nonopioid \( d \)-amphetamine, and saline produced predominantly saline-key responding. Naltrexone and nalbuphine dose-dependently reversed the morphine-key responding produced by the training dose of morphine. Taken together, these data suggest that the discriminative-stimulus effects of the low efficacy \( \mu \) agonist nalbuphine in morphine-treated pigeons are similar to those of other low efficacy agonists, naltrexone, and the termination of daily morphine treatment.
Drug discrimination assays offer investigators unique perspectives on the relative quantitative and qualitative aspects of the stimulus effects of drugs. In these assays, subjects are trained to make one response after administration of a specific dose of drug and to make a different response in the absence of that drug. For example, rats and pigeons can be readily trained to respond on one of two keys or levers depending on whether they received morphine (morphine-appropriate responding) or saline (saline-appropriate responding). When other high efficacy µ opioid agonists such as fentanyl, methadone, and etonitazene (Emmerson et al., 1996; Selley et al., 1998; Walker et al., 1998) are substituted for morphine in animals that have been trained in this manner, they respond on the morphine-appropriate key or lever (Shannon and Holtzman, 1977; Morgan and Picker, 1998). Similar effects have been reported in rats trained to discriminate fentanyl from saline (Colpaert et al., 1980; Zhang et al., 2000).

The discriminative stimulus effects of compounds possessing intrinsic efficacy lower than fentanyl or morphine are particularly interesting. For example, compounds such as cyclazocine, nalbuphine, or nalorphine (Traynor and Nahorski, 1995; Alt et al., 2001), only produce morphine or fentanyl-appropriate responding when lower training doses of fentanyl or morphine are used to establish the discriminations (Shannon and Holtzman, 1979; Colpaert et al., 1980; Young et al., 1992; Zhang et al., 2000). Interestingly, the same dose of nalbuphine that substitutes for a low dose of fentanyl or morphine can antagonize the discriminative stimulus effects of high training doses of fentanyl or morphine, demonstrating that nalbuphine produces both agonist and antagonist effects through similar, presumably µ opioid receptors (Young et al., 1992; Zhang et al., 2000).
The pattern of substitution is less complicated when low efficacy agonists such as nalbuphine and butorphanol are trained as discriminative stimuli. Under these conditions, both high and low efficacy agonists produce full substitution for nalbuphine or butorphanol, generally independent of the training dose (Walker and Young, 1993; Gerak and France, 1996; Picker et al., 1996). Moreover, the discriminative stimulus effects of nalbuphine (Walker and Young, 1993; Gerak and France, 1996), butorphanol (Picker et al., 1996), and dezocine (Picker, 1997) are blocked by μ, but not κ or δ opioid receptor antagonists, indicating that the effects are mediated through μ opioid receptors.

When pigeons are trained to discriminate among morphine, nalbuphine, and saline in a three-choice discrimination procedure, low efficacy compounds such as butorphanol, nalorphine, and levallorphan produce predominantly nalbuphine-appropriate responding (Walker et al., 2001). Low doses of morphine, fentanyl, etorphine, buprenorphine, and dezocine also produce nalbuphine-appropriate responding whereas high doses of these compounds produce morphine-appropriate responding.

A different pattern is observed in opioid-dependent subjects. Under these conditions, low efficacy agonists such as nalbuphine generally precipitate withdrawal or produce withdrawal-like stimulus effects. For example, nalbuphine precipitates withdrawal signs in methadone-dependent human subjects (Preston et al., 1989; Preston et al., 1990) and morphine-dependent rhesus monkeys (Villarreal and Karbowski, 1974). Similarly, in opioid-dependent rats (Young et al., 1991), pigeons (France and Woods, 1990), and rhesus monkeys (France and Woods, 1989), nalbuphine produces saline or naltrexone-appropriate responding in drug discrimination assays. These data suggest that
under some conditions of opioid dependence, nalbuphine produces effects similar to opioid antagonists such as naltrexone.

The present study uses a three choice discrimination procedure to examine further the discriminative stimulus effects of the low efficacy agonist nalbuphine in morphine-treated pigeons. The following questions are addressed: First, can pigeons be trained to discriminate among a low efficacy agonist, such as nalbuphine, a high efficacy agonist, such as morphine and saline under conditions of daily morphine treatment? Second, what patterns of substitution will a range of high and low efficacy agonists and opioid antagonists produce under these conditions? Finally, how are the discriminative stimulus effects of nalbuphine and morphine altered during morphine withdrawal?
Methods

Subjects: Seven, White Carneux pigeons maintained at approximately 85% of their free feeding weights (401-482 g) were used. Pigeons were fed mixed grain and Purina Pigeon Chow during and after each experimental session. Each pigeon was housed individually in a colony maintained on a 12 h light-dark cycle and had free access to grit and water. Pigeons were injected with 10 mg/kg morphine, i.m., seven days a week in the morning.

Apparatus: Seven operant conditioning chambers were used. Each chamber contained three response keys that were 2.5 cm in diameter and located 23 cm from the bottom of the intelligence panel and centered approximately 12 cm apart. An opening located on the intelligence panel centered 8 cm above the floor of the chamber allowed access to a hopper filled with mixed grain when the hopper was raised. A 7-w white bulb illuminated the opening when the hopper was raised. A houselight mounted 33 cm above the chamber floor provided ambient illumination. Each chamber was equipped with an exhaust fan and white noise. A microcomputer with software and interfacing (MED Associates, Georgia, VT) was used for scheduling of experimental events and data collection.

Discrimination training and testing. Six hours after the morning morphine injections, pigeons were injected with 10 mg/kg morphine, saline, or 1.0 mg/kg nalbuphine and placed in the darkened operant chambers. Fifteen minutes later, the three response lights were illuminated and the pigeons were trained to respond on either the right, center, or left key on a continuous reinforcement schedule for food delivery (3-sec access to mixed grain) for 15 min. Responses on the injection-inappropriate keys were
counted but had no programmed consequences. The number of responses required for food delivery was increased to 30 (fixed ratio FR30) over several experimental sessions. Morphine, saline, and nalbuphine training sessions were conducted randomly with the restriction that a given training drug was not administered for more than two consecutive sessions. Training conditions continued until an individual pigeon met the following conditions: 1) the first 30 responses completed were made on the correct training key; 2) less than 60 responses were made before the first reinforcer; 3) the percentage of responses emitted on the appropriate key during the entire session was $\geq 80\%$; and 4) the above conditions were met for nine out of 12 consecutive days. In addition, the pigeon needed to meet criteria 1-3 for three saline training days, three nalbuphine training days, and three morphine training days. After initial testing criteria were met, tests were performed whenever pigeons met the above criteria for each of the three training conditions for three consecutive days.

Test sessions were conducted using a multiple-trial, cumulative-dosing procedure (Walker et al., 2001). Each trial consisted of a 15-min pretreatment period and a testing component that ended after ten reinforcers or 5 min, whichever came first. During the testing component, completion of an FR30 on any key produced food. For cumulative dosing, the dose of drug administered before each trial increased the total dose by 0.25 to 1.0 log$_{10}$ unit. Each test consisted of five to eight trials. For repeated saline tests, an injection of saline was administered at the beginning of every trial for five trials. In the naltrexone and nalbuphine reversal tests, the training dose of morphine was administered to the pigeons and was followed by cumulative doses of naltrexone or nalbuphine. In the morphine withdrawal study, the daily 10 mg/kg morphine injection was replaced by
saline and the pigeons were tested six hours later with saline. Following the saline injection, cumulative doses of morphine were tested. The following day, the morning morphine injections were reinstated.

At the end of the experiment, the morning morphine injection was replaced with saline for twenty-three consecutive days. Six hours later, on days 1, 3, 8, 15, 18 and 23, the pigeons were injected with saline again and placed into the experimental chambers for a single trial test. Fifteen minutes later, the three stimulus lights were illuminated for fifteen min and completion of an FR30 on any key produced food. Under similar testing conditions, the training doses of 10 mg/kg morphine and 1.0 mg/kg nalbuphine were tested on days 16 and 17 and 3.2 mg/kg morphine and 10 mg/kg nalbuphine were examined on days 19 and 22, respectively. On those days on which the pigeons were not tested, they received saline injections in the morning and 6 h later they were fed in their home cage.

Surgery. To deliver drugs centrally, a permanent in-dwelling cannula (Plastic One, Inc., Roanoke, VA) was placed into the lateral ventricle of each pigeon with a stereotaxic instrument (Stoelting Co., Wood Dale, Illinois) and a Rezvin adapter (Karten and Hodos, 1967) using methods previously described (France et al., 1985; Jewett et al., 1996). Briefly, each pigeon was anesthetized with 8 mg/kg of ketamine and 2.5-2.75 ml/kg of Chloropent (chloral hydrate and pentobarbital: Fort Dodge Laboratories, Fort Dodge, Iowa). After surgery, a removable 28 g dummy cannula was inserted into the guide cannula. Patency of each cannula was tested by injecting 17.2 µg of 2-amino-5-phosphonovalerate (Sigma, St. Louis, MO) and observing catalepsy (Koek et al., 1986).
These tests occurred once before, during, and after the i.c.v. experiments to insure cannula patency over the five-month i.c.v. testing period.

**Data analyses:** Drug discrimination data are presented as the mean percentage of morphine- or nalbuphine-appropriate responding to total responding of all pigeons during the test session. Rate of responding was calculated as a percentage of the rate of responding during the saline training session conducted prior to the test session. Data were determined for all pigeons tested at a given dose and plotted as mean values. S.E.M. is used to express variance. Data from pigeons responding less than 30 times during a trial were included in the response rate figures but not the discrimination figures. The dose required to produce 50% drug-appropriate responding (ED$_{50}$ value), reduce drug-appropriate responding to 50% (ID$_{50}$ value), and reduce response rates to 50% (ED$_{50}$ value) and 95% C.L. were calculated by nonlinear regression analysis using unconstrained maximum and minimum values for the logistic equations (GraphPad Prism Version 3.0; San Diego, CA).

**Drugs:** The following compounds were used: buprenorphine hydrochloride (HCl), etorphine HCl, morphine sulfate, CTAP [D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH$_2$], DAMGO [(D-Ala$^2$, N-Me-Phe$^4$, Gly$^5$-ol)-enkephalin]], U50,488 [trans-3,4-dichloro-N-methyl-N(2-[1-pyrrolidinyl]cyclohexyl)benzeneacetamide methanesulfonate] (generously supplied by National Institute on Drug Abuse, Rockville, MD), butorphanol tartrate, sodium pentobarbital (Sigma Chemical, Inc., St. Louis, MO), nalbuphine HCl, nalorphine HCl, naltrexone HCl, spiradoline mesylate (Research Biochemicals International, Natick, MA), levallorphan tartrate (Hoffman-LaRoche, Inc., Nutley, NJ), fentanyl citrate (Janssen Pharmaceuticals, Inc., Beerse, Belguim), SNC80 (Tocris,
Ballwin, MO), and dezocine HCl (Dalgen: Astra Pharmaceutical, Westborough, MA). Nalbuphine, morphine, etorphine, U50,488, butorphanol, buprenorphine, nalorphine, spiradoline, levallorphan, fentanyl, SNC80 and naltrexone were dissolved in distilled water. Dezocine was purchased as a solution and diluted with distilled water. Solutions were generally prepared to deliver each injection in a volume of 0.1 to 0.7 ml per pigeon into the breast muscle. Doses are expressed as the forms listed above. Saline was injected in a volume of 1 ml/kg of b.wt. As observed in previous studies (Walker et al., 2001), 10 mg/kg buprenorphine disrupted training performance for one to four weeks. Therefore, higher doses of buprenorphine were not examined.

Nalbuphine and the peptides CTAP and DAMGO were administered i.c.v. using a cumulative-dosing procedure (Jewett et al., 1996). For cumulative dosing, the drugs were dissolved in filtered sterile water and were prepared to deliver each injection in a volume of 3-5 µl. The dose of drug administered before each trial increased the total i.c.v. dose by 0.25 to 1.0 log\textsubscript{10} units. Using a handheld 50 µl Hamilton syringe outfitted with PE50 tubing and a 28 g injector (Plastic One, Inc., Roanoke, VA), 3-5 µl of solution was infused slowly for 30 sec into the lateral ventricle. The injector remained in place for an additional 30 sec to allow the solution to absorb into the tissue. A maximum total volume of 30 µl (5 to 6 trials) was injected during an experiment.
Results

Substitution experiments with the training drugs. The morphine, nalbuphine, and saline discrimination was acquired by all seven pigeons in an average of 42 days with a range of 10 to 120 days. After the pigeons met testing criteria, cumulative doses of nalbuphine and morphine were administered. Low doses of nalbuphine produced saline-key responding and higher doses of nalbuphine produced >80% nalbuphine-key responding in all pigeons (figure 1, left panels; Table 1). Low doses of morphine produced saline-key responding and higher doses of morphine (10 mg/kg morphine or more) produced morphine-key responding in all pigeons (figure 1, right panels; Table 1). Five trials of repeated saline injections produced predominantly >80% saline-key responding (Table 1). Doses of 100 mg/kg nalbuphine and morphine decreased response rates to 25% and 0% of saline control values, respectively. Doses of 32 and 100 mg/kg nalbuphine produced vomiting in three pigeons. ED50 values and 95% C.L. are reported in Table 1.

Substitution experiments with other compounds. Etorphine and fentanyl produced a pattern of substitution similar to morphine in that low and high doses of etorphine and fentanyl produced saline- and morphine-key responding, respectively. Etorphine (figure 2, left panels) and fentanyl (figure 2, left middle panels) produced >80% morphine-key responding in most pigeons (Table 1). Buprenorphine, dezocine (figure 2, right middle and right panels) and butorphanol (figure 3, left panels) produced a combination of saline-, nalbuphine-, and morphine-key responding in the morphine-treated pigeons (Table 1). The lower efficacy agonists nalorphine and levallorphan (figure 3, left middle, right middle panels) as well as the opioid antagonists naltrexone
(figure 3, right panels) and CTAP (i.c.v.) produced predominantly nalbuphine-key responding in a majority of pigeons (Table 1). The κ agonists U50,488 and spiradoline, the delta agonist SNC80 and the nonopioid d-amphetamine produced predominantly saline-key responding (Table 1). Nalbuphine (i.c.v.) and DAMGO (i.c.v.) produced 50-100% nalbuphine- or morphine-key responding, respectively, in four of the five pigeons tested.

All compounds with the exception of buprenorphine were examined at doses up to those that decreased rates of responding to less than 25% of saline control values (figures 1-3, bottom panels; Table 1). Since a dose of 10 mg/kg buprenorphine disrupted training performance for one to four weeks, higher doses were not examined. A dose of 10 mg/kg naltrexone reduced rates to approximately 20% of control levels and produced vomiting in two pigeons. Rate of responding was reduced by 10-32 µg CTAP, 10-320 µg nalbuphine, and 10-560 µg DAMGO. The large degree of intersubject variability observed for the i.c.v. dose of nalbuphine and DAMGO to suppress response rates limited the testing of higher doses.

**Reversal experiments.** In the reversal experiments, a single injection of the training dose of 10 mg/kg morphine produced approximately 80% morphine-key responding. Doses of both nalbuphine and naltrexone reversed the stimulus effects of the training dose of morphine in a dose-dependent manner (figure 4, left and left middle panels, respectively). As morphine-key responding decreased, there was a concomitant increase in nalbuphine-key responding as the dose of nalbuphine or naltrexone was increased. In these experiments, the ID₅₀ values for nalbuphine and naltrexone to block morphine-key responding were 0.25 mg/kg and 0.0017 mg/kg, respectively. In the
presence of the training dose of morphine, the ED$_{50}$ value for nalbuphine increased from 0.96 mg/kg to 5.4 mg/kg (figure 4, left panel) and the ED$_{50}$ value for naltrexone increased from 0.0034 mg/kg to 0.025 mg/kg (figure 4, middle left panel). Therefore, nalbuphine and naltrexone are approximately 5- and 7-fold less potent in producing nalbuphine-key responding in the presence of the training dose of morphine. Similarly, nalbuphine and naltrexone reduced rates of responding with ED$_{50}$ values of 11 mg/kg and 0.082 mg/kg, respectively under these conditions, indicating that nalbuphine and naltrexone are approximately 3- and 4-fold more potent in producing rate-decreasing effects in the presence of the training dose of morphine. Time course studies revealed that an injection of 10 mg/kg morphine followed by six trials of saline injections produced 60-100% morphine-key responding for 140 min. In general, response rates were not altered in saline control tests (figure 4, right middle panel).

**Single and daily morphine withdrawal experiments.** When saline was substituted for the morning 10 mg/kg morphine injection, a saline injection six hours later produced approximately 80% nalbuphine-key responding. This nalbuphine-key responding was reversed by morphine (figure 4, right panels; Table 1). However, the potency of morphine was not altered in the absence of the morning morphine injection (Table 1).

After the completion of the above experiments, the morning morphine injection was replaced with saline for twenty-three days and the pigeons were tested 6 h later on various days with saline, morphine, or nalbuphine (Table 2). On days 1, 3, 8, 15, and 23 morphine-withdrawn pigeons responded predominantly on the nalbuphine-key after a 15-min pretreatment of saline in a single trial test. Although response rates were very low,
the training dose of 10 mg/kg morphine produced morphine-key responding on day 16. The training dose of 1.0 mg/kg nalbuphine produced 71% nalbuphine-key responding on day 17. On day 19, a lower dose of 3.2 mg/kg morphine evoked 64% morphine-key responding. A higher dose of 10 mg/kg nalbuphine was injected on day 22 and produced 29% nalbuphine-key responding and 71% saline-key responding. On the final day of the experiment, saline injection prior to the test session produced 70% nalbuphine key-responding.
Discussion

The data collected in this study indicate that the low efficacy $\mu$ agonist nalbuphine produces a unique pattern of stimulus effects in morphine-treated pigeons. Under these conditions, nalbuphine’s discriminative effects are similar to those of the opioid antagonist, naltrexone, other low efficacy $\mu$ agonists, and the termination of daily morphine treatment.

Interestingly, substitution tests with high efficacy opioid agonists in the current studies revealed that the nalbuphine discriminative stimulus no longer reflects agonist actions in morphine-treated pigeons whereas the nalbuphine discriminative stimulus clearly reflects agonist actions in non-treated pigeons. For example, when non-treated pigeons are trained to discriminate a low dose of morphine from a high dose, high efficacy agonists produce responding on the key appropriate to a low-dose of morphine followed by responding on the key appropriate to a high-dose of morphine (Vanecek and Young, 1995). Similarly, in non-treated pigeons trained to discriminate among morphine, nalbuphine, and saline, high efficacy agonists produce nalbuphine-key responding at low doses and morphine-key responding at higher doses (Walker et al., 2001). In contrast, data collected in the current study indicate that the high efficacy agonists, etorphine, morphine and fentanyl, do not produce nalbuphine-key responding at any dose in morphine-treated pigeons (figure 2). Instead, etorphine, fentanyl, and morphine produce a greater proportion of saline-key responding in morphine-treated pigeons as compared to non-treated pigeons (Walker et al., 2001). However, in both studies, etorphine, fentanyl and morphine produced morphine-key responding.
In the current study, substitution tests with intermediate efficacy agonists buprenorphine, dezocine, and butorphanol revealed a variable pattern of responding across the morphine, saline, and nalbuphine keys in morphine-treated pigeons. Generally, in morphine-treated pigeons, these intermediate efficacy agonists produced a greater proportion of saline-key responding than either morphine- or nalbuphine-key responding in comparison to previous results from non-treated pigeons (Walker et al., 2001). For example, buprenorphine produced morphine-key responding in greater than 75% of the non-treated pigeons (Walker et al., 2001), whereas buprenorphine only produced morphine-key responding in 25% of the morphine-treated pigeons (figure 2). Similarly, butorphanol produced nalbuphine-key responding in greater than 75% of the non-treated pigeons, whereas butorphanol only produced nalbuphine-key responding in 25% of the morphine-treated pigeons (figure 3).

Interestingly, both nalorphine and levallorphan produced high levels of nalbuphine-key responding in both non-treated (Walker et al., 2001) and morphine-treated pigeons (present study) trained to discriminate among morphine, nalbuphine, and saline. In studies using two choice discriminations, nalorphine, levallorphan, and nalbuphine produce morphine- or fentanyl-appropriate responding as long as the training doses of morphine or fentanyl are low (Holtzman, 1985; Young et al., 1992; Zhang et al., 2000). Similarly, nalorphine and levallorphan produce nalbuphine-appropriate (Walker and Young, 1993), butorphanol-appropriate (Picker et al., 1996) or dezocine-appropriate (Picker, 1996) responding when the discriminative stimuli are lower efficacy agonists (e.g., nalbuphine, butorphanol or dezocine). In morphine-treated pigeons, as in previous studies in non-treated pigeons and rats (Walker et al., 2001; Walker and Young, 1993)
nalorphine and levallorphan retained the capacity to produce nalbuphine-key responding supporting the notion that these compounds all possess very low efficacy at µ opioid receptors.

The discriminative stimulus effects of the κ and δ agonists observed in morphine-treated pigeons in the present study were different from those observed previously in non-treated pigeons. The κ agonists, U50,488 and spiradoline, have been shown to produce some nalbuphine-appropriate responding in non-treated pigeons (Walker et al., 2001). However, in the current study, U50,488 and spiradoline did not produce nalbuphine-appropriate responding in pigeons treated daily with 10 mg/kg morphine and trained to discriminate a lower dose of nalbuphine. Similarly, the δ agonists failed to produce morphine- or nalbuphine-key responding under these conditions. Therefore, these observations provide further confirmation that neither the κ nor the δ opioid receptor play a prominent role in the morphine, nalbuphine, and saline discrimination in morphine-treated pigeons (Picker, 1994; Picker and Cook, 1997; 1998; France and Woods, 1993).

The opioid antagonist naltrexone and the µ-selective peptide antagonist, CTAP, both produced nalbuphine-key responding in morphine-treated pigeons. Previous studies have shown that both nalbuphine and naltrexone can reverse the stimulus effects of morphine with potencies indicative of µ receptor blockade in morphine-treated subjects (France et al., 1990; Walker et al., 1996). In the present study, pigeons respond on the nalbuphine key when saline injections were substituted for the morning morphine injections and increasing doses of morphine reversed responding on the nalbuphine key. Both of these observations are in keeping with findings from other studies with
naltrexone (France and Woods, 1987; France and Woods, 1989; France and Woods, 1990). In previous studies, this kind of evidence was used to support the notion that the stimulus effects of naltrexone represent a state of opioid withdrawal in rats, pigeons, and rhesus monkeys (Gellert and Holtzman, 1979; Holtzman, 1985; France and Woods, 1989; France and Woods, 1990). ED$_{50}$ values for naltrexone to produce either naltrexone- or nalbuphine-key responding in pigeons treated daily with 10 mg/kg were approximately 0.0056 mg/kg (France and Woods, 1990) and 0.0034 mg/kg (present study), respectively. This observation demonstrates the similar degrees of morphine withdrawal between the two studies despite the fact that naltrexone or nalbuphine were trained as the discriminative stimuli in morphine-treated pigeons. Taken together, these data suggest that the discriminative stimulus effects of nalbuphine in morphine-treated pigeons parallel the discriminative stimulus effects of morphine withdrawal. Indeed, high doses of nalbuphine and naltrexone produced vomiting in some pigeons, a sign indicative of physical withdrawal.

Despite these similarities between naltrexone and nalbuphine, it should be noted that nalbuphine and naltrexone do not always produce similar stimulus effects. For example, naltrexone fails to substitute for nalbuphine and actually blocks the stimulus effects of nalbuphine in non-treated rats, pigeons, and rhesus monkeys trained to discriminate nalbuphine from saline (Gerak and France, 1996; Walker and Young, 1993; Walker et al., 2001). In morphine-treated pigeons trained to discriminate naltrexone from saline, nalbuphine produces approximately equal saline and naltrexone responding (France and Woods, 1990). Yet, in morphine-treated pigeons trained to discriminate between nalbuphine and saline, naltrexone substitutes fully for nalbuphine. This
asymmetrical substitution suggests that under some morphine treatment conditions, nalbuphine is able to reverse the discriminative effects produced by naltrexone; however, under other morphine treatment conditions, nalbuphine’s discriminative stimulus effects are similar to those of naltrexone. Taken together, these observations suggest that nalbuphine may represent a withdrawal stimulus similar to a lower training dose of naltrexone.

It is also possible that nalbuphine produces a discriminative stimulus that represents the absence of morphine in pigeons that have been treated with morphine. For instance, pigeons treated with morphine may be discriminating among high dose morphine (10 mg/kg morphine in the a.m. plus 10 mg/kg morphine in the p.m.), low dose morphine (10 mg/kg morphine in the a.m. plus saline in the p.m.), and absence or reversal of morphine (10 mg/kg morphine in the a.m. and 1.0 mg/kg nalbuphine in the p.m.). Substituting saline for the morning morphine injection could produce nalbuphine-key responding by evoking a morphine withdrawal state or by just representing the absence of morphine. In either case, these effects would be reversed by increasing doses of morphine (figure 4).

The results from the daily morphine withdrawal study may support the notion that nalbuphine-key responding in morphine-treated pigeons represents the absence of morphine. Saline produced predominantly nalbuphine-key responding for as long as twenty-three days in morphine-withdrawn pigeons. Indeed, a low dose of nalbuphine (1.0 mg/kg) in morphine-withdrawn pigeons produced predominantly nalbuphine-key responding, whereas a higher dose of 10 mg/kg nalbuphine produced 70% saline-key responding and 29% nalbuphine-key responding. These data are in agreement with the
suggestion that saline key responding may represent a low dose of morphine under conditions of daily morphine treatment. Morphine withdrawal did not appear to alter the discriminative stimulus effects of morphine with the exception that the pigeons became more sensitive to the rate-decreasing effects of 10 mg/kg morphine. When the morphine test dose was lowered to 3.2 mg/kg, the pigeons distributed their responding relatively evenly between the morphine (proposed high dose) and saline (proposed low dose morphine) keys.

One feature of the nalbuphine discrimination in the present experiment, however, does not agree with the hypothesis that the nalbuphine stimulus represents the absence of morphine, whereas the saline stimulus represents a low dose of morphine. Studies in non-treated subjects have firmly established that high doses of nalbuphine evoke generalization with low dose morphine training stimuli (Morgan and Picker, 1998; Walker et al., 1996; Vanecek and Young, 1995; Young et al., 1992; Holtzman, 1985). Therefore, if the above hypothesis is true, as the nalbuphine dose is increased, responding should move from the nalbuphine key (proposed absence of drug stimuli) to the saline key (proposed low dose morphine stimulus). This did not occur, however. In contrast, increasing the dose of nalbuphine above the training dose produced only nalbuphine-key responding and occasional vomiting, suggesting the presence of a mild state of withdrawal. This outcome lends strong support to the alternate hypothesis that the nalbuphine stimulus comprised a withdrawal cue. It is possible that the discriminative stimulus effects of nalbuphine observed in the present study resemble the stimuli produced in models of acute dependence, in which animals are treated with morphine, followed 4 h later by naltrexone (Easterling and Holtzman, 1999; Holtzman, 2003; White
and Holtzman, 2003). This acute dependence model produces a pattern of discrimination that is unique from that observed when naltrexone is administered without morphine pretreatment. In the present study, 6 h pretreatment of morphine followed by nalbuphine may also produce a different set of interoceptive stimuli. Moreover, the data collected here suggest that these interoceptive stimuli are shared with opioid antagonists, low efficacy µ agonists, and single or daily morphine withdrawal.
Acknowledgements

The authors would like to thank William Prizer and Michael Tiano for their technical assistance as well as members of the behavioral pharmacology laboratory for their assistance with daily injections.
References


Footnotes

This research was supported by National Institute on Drug Abuse Grants DA10776 (E.A.W.), DA10277 (M.J.P.) and DA02749 (L.A.D.). These experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

A portion of these data were presented and published as a part of the Fifth International Meeting on Drug Discrimination, Beerse, Belgium, August 1998 (Pharmacology Biochemistry and Behavior 64:445-448, 1999).
Figure 1. The effects of training stimuli nalbuphine (left panels) and morphine (right panels) on the % of morphine and nalbuphine-key responding and response rates (N=7). Ordinate (top panels): Percentage of total responses made on the nalbuphine key (triangles) or morphine key (circles). Percentages of responses made on the saline key are not shown but are the difference of 100% and the sum of the total responses made on the morphine and nalbuphine key. Ordinate (bottom panels): Rate of responding on all three keys is shown as a percentage of the rate of responding on the last saline training session conducted prior to the test session. Baseline saline rates for the group were 2.42 ± 0.53 and 2.49 ± 0.36 responses per second for nalbuphine and morphine, respectively. Data from pigeons making less than 30 responses were included in the rate figures but not the discrimination figures. Abscissa: cumulative doses of drug, in mg/kg. Vertical bars represent ± S.E.M.

Figure 2. The effects of high efficacy agonists, etorphine (left panels) and fentanyl (left middle panels) and intermediate efficacy agonists, buprenorphine (right middle panels) and dezocine (left panels) on the % of morphine- and nalbuphine-key responding and response rates. Baseline saline rates for the group were 2.29 ± 0.52, 2.13 ± 0.25, 2.36 ± 0.29, and 2.11 ± 0.38 responses per second for etorphine (N=6), fentanyl (N=6), buprenorphine (N=7), and dezocine (N=7), respectively. Other details as in fig. 1.

Figure 3. The effects of lower efficacy agonists, butorphanol (left panels), nalorphine (left middle panels), levallorphan (right middle panels) and opioid antagonist naltrexone (right panels) on the % of morphine- and nalbuphine-key responding and response rates.
Baseline saline rates for the group were 2.56 ± 0.35, 2.42 ± 0.37, 2.70 ± 0.57, and 2.52 ± 0.44 responses per second for butorphanol (N=7), nalorphine (N=6), levallorphan (N=6) and naltrexone (N=7), respectively. Other details as in fig. 1.

Figure 4. Reversal of the stimulus effects of the morphine-training dose (10 mg/kg) by nalbuphine (left panels), naltrexone (left middle panels), and saline (right middle panels) and reversal of the stimulus effects of morphine withdrawal (right panels). Baseline saline rates were 2.51 ± 0.43, 2.52 ± 0.44, 2.08 ± 0.18, and 2.77 ± 0.46 responses per second for nalbuphine reversal (N=6), naltrexone reversal (N=5), multiple saline injections (N=6), and morphine withdrawal (N=5), respectively. Points above M represent the effects of an injection of 10 mg/kg morphine alone. Points above S represent the effects of a saline injection. Dotted lines represent the range of effects (± S.E.M) of nalbuphine (left panels), naltrexone (left middle panels), and morphine (right panels) under control conditions, i.e., after the morning morphine injection (see figures 1 and 3). Nalbuphine, naltrexone or multiple injections of saline followed the injection of the training dose of morphine (left, left middle, and right middle panels). Saline injected in 30-hr morphine-withdrawn pigeons produced nalbuphine-key responding that was reversed by morphine (right panels). Other details as in fig. 1.
Table 1

Discriminative stimulus and rate-decreasing effects for drugs tested in pigeons treated with 10 mg/kg morphine and trained to discriminate 1.0 mg/kg nalbuphine, 10 mg/kg morphine, and saline six hours later.

<table>
<thead>
<tr>
<th>Drug or Condition</th>
<th># Pigeons Selecting NLBa</th>
<th>% NLB ED50 value (mg/kg) (95% C.L.)</th>
<th># Pigeons Selecting MSa</th>
<th>% MS ED50 value (mg/kg) (95% C.L.)</th>
<th>Rate % saline control ED50 value (mg/kg) (95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalbuphine</td>
<td>7/7</td>
<td>0.96 (0.21-4.3)</td>
<td>1/7</td>
<td>---b</td>
<td>35 (28-42)</td>
</tr>
<tr>
<td>Morphine</td>
<td>1/7</td>
<td>---b</td>
<td>7/7</td>
<td>3.6 (1.2-11)</td>
<td>21 (13-33)</td>
</tr>
<tr>
<td>Saline</td>
<td>1/7</td>
<td>---b</td>
<td>1/7</td>
<td>---b</td>
<td>---b</td>
</tr>
<tr>
<td>Etorphine</td>
<td>0/6</td>
<td>---b</td>
<td>5/6</td>
<td>0.0025 (0.0012-0.0054)</td>
<td>0.013 (0.0082-0.020)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>1/6</td>
<td>---b</td>
<td>5/6</td>
<td>0.048 (0.021-0.10)</td>
<td>0.16 (0.13-0.21)</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>2/7</td>
<td>---b</td>
<td>2/7</td>
<td>---b</td>
<td>---c</td>
</tr>
<tr>
<td>Dezocine</td>
<td>4/7</td>
<td>---b</td>
<td>4/7</td>
<td>---b</td>
<td>14 (5.7-35)</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>2/7</td>
<td>2.3 (2.1-2.5)</td>
<td>1/7</td>
<td>---b</td>
<td>15 (8.2-27)</td>
</tr>
<tr>
<td>Nalorphine</td>
<td>6/6</td>
<td>0.042 (0.00052-34)</td>
<td>0/6</td>
<td>---b</td>
<td>7.8 (7.7-7.9)</td>
</tr>
<tr>
<td>Levallophan</td>
<td>5/6</td>
<td>0.12 (0.0021-7.3)</td>
<td>1/6</td>
<td>---b</td>
<td>8.1 (3.1-21)</td>
</tr>
</tbody>
</table>

---b: No significant effect.
<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>ED50 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naltrexone</td>
<td>7/7</td>
<td>0.0034 (0.00086-0.013)</td>
</tr>
<tr>
<td>Spiradoline</td>
<td>1/6</td>
<td>---&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>U50,488</td>
<td>0/5</td>
<td>---&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SNC80</td>
<td>1/5</td>
<td>---&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>d-amphetamine</td>
<td>1/5</td>
<td>---&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CTAP, i.c.v.</td>
<td>4/5</td>
<td>4.6 (0.41-52)</td>
</tr>
<tr>
<td>Nalbuphine, i.c.v.</td>
<td>2/5</td>
<td>---&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DAMGO, i.c.v.</td>
<td>0/5</td>
<td>---&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>30 hr withdrawal&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5/5</td>
<td>4.5 (0.92-22)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of pigeons producing ≥80% nalbuphine (NLB) or morphine (MS) appropriate responding at some dose out of the number of pigeons tested.

<sup>b</sup>An ED<sub>50</sub> value could not be calculated.

<sup>c</sup>Rate of responding was >50%.

<sup>d</sup>Morning morphine injection was replaced with saline and the pigeons were tested with saline 6 h later. After the saline test, cumulative doses of morphine were studied.
Table 2. Effects of daily morphine withdrawal on the patterns of responding in previously morphine-treated pigeons (N=6).

<table>
<thead>
<tr>
<th>Day</th>
<th>Afternoon Injection</th>
<th>% Nalbuphine-key Responding (+ S.E.M.)</th>
<th>% Morphine-key Responding (+ S.E.M.)</th>
<th>Rate % saline control (+ S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>99 (0.33)</td>
<td>0.83 (0.83)</td>
<td>86 (10)</td>
</tr>
<tr>
<td>3</td>
<td>Saline</td>
<td>84 (16)</td>
<td>0</td>
<td>90 (13)</td>
</tr>
<tr>
<td>8</td>
<td>Saline</td>
<td>71 (17)</td>
<td>0.16 (0.20)</td>
<td>106 (7.1)</td>
</tr>
<tr>
<td>15</td>
<td>Saline</td>
<td>80 (16)</td>
<td>0</td>
<td>108 (9.2)</td>
</tr>
<tr>
<td>16</td>
<td>10 mg/kg morphine</td>
<td>0</td>
<td>94 (5.4)</td>
<td>23 (14)</td>
</tr>
<tr>
<td>17</td>
<td>1.0 mg/kg nalbuphine</td>
<td>71 (15)</td>
<td>2.5 (2.5)</td>
<td>103 (11)</td>
</tr>
<tr>
<td>18</td>
<td>Saline</td>
<td>54 (19)</td>
<td>0.33 (0.33)</td>
<td>103 (5.0)</td>
</tr>
<tr>
<td>19</td>
<td>3.2 mg/kg morphine</td>
<td>7.2 (7.2)</td>
<td>64 (16.2)</td>
<td>62 (13)</td>
</tr>
<tr>
<td>22</td>
<td>10 mg/kg nalbuphine</td>
<td>29 (17)</td>
<td>2.2 (2.2)</td>
<td>65 (14)</td>
</tr>
<tr>
<td>23</td>
<td>Saline</td>
<td>71 (17)</td>
<td>0</td>
<td>108 (10)</td>
</tr>
</tbody>
</table>

\(^a\) Consecutive days of saline injections replacing morning morphine injections.
Rate of responding on all three keys as a percentage of the rate of responding on the last saline training session prior to morphine withdrawal
Figure 1
Figure 2
Figure 3