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# Sensitization and tolerance to the discriminative stimulus effects of mu-opioid agonists

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**Abstract.** The discriminative stimulus effects of several μ-opioid agonists were examined under conditions of opioid sensitization or tolerance, i.e., before and after 1-week SC infusions of naloxone or μ-opioid agonists. Rats were trained to discriminate 3.0 mg/kg morphine from saline using a two-lever, discrete trial, shock-avoidance/escape procedure. The rats generalized completely to morphine, fentanyl, meperidine, buprenorphine, and etorphine, and partially to pentazocine. A 7-day infusion of naloxone (0.3 mg/kg per h) potentiated the discriminative stimulus effects of all of these drugs. The magnitude of the increased potency varied indirectly with the efficacy of the μ-opioid agonists; potency ratios (pre-infusion $ED_{50}$/post-infusion $ED_{50}$) ranged from 1.58 (etorphine) to 3.58 (pentazocine). Stimulus generalization to morphine, fentanyl, and meperidine also was examined following infusions of equieffective doses of each of these three drugs. Differences among drugs were generally small, and failed to reach statistical significance. Nonetheless, the induction of μ-opioid tolerance did seem to vary with the efficacy of the three μ-opioid agonists. Thus, meperidine (6.25 mg/kg per h), which has the lowest efficacy of the drugs infused, produced the greatest shift to the right of the stimulus-generalization curves of these three drugs; the post-meperidine PR ranged between 0.40 and 0.61. Fentanyl (0.1 mg/kg per h), a drug with a higher efficacy at μ-opioid receptors, did not produce tolerance to the discriminative stimulus effects of morphine, fentanyl, or meperidine; potency ratios ranged from 0.50 to 0.75. Potency ratios for buprenorphine, etorphine, fentanyl, meperidine, and morphine after 7-day morphine infusions (0.75 mg/kg per h) ranged from 0.38 (buprenorphine) to 0.80 (etorphine). Morphine induced significant tolerance only to the discriminative stimulus effects of fentanyl. Our results suggest that different cellular mechanisms underlie the development of tolerance and sensitization to the discriminative stimulus effects of μ-opioid agonists.

**Key words:** Drug discrimination – Drug tolerance – Sensitization – μ-Opioid receptors – Morphine – Naloxone – Meperidine – Fentanyl

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Continuous exposure to opioid antagonists such as naloxone or naltrexone, increases the number of opioid binding sites in the central nervous system. The increase in μ-opioid receptors is accompanied by an increase in the potency of morphine to produce electrophysiological and neurochemical effects (Bardo et al. 1983; Ahtee et al. 1990), as well as analgesic effects (Tang and Collins 1978; Yoburn and Inturrisi 1988; Paronis and Holtzman 1991). However, studies on the potency of μ-opioid agonists as discriminative stimuli following naltrexone infusions have produced mixed results. A 7-day infusion of 6 mg/kg per day naltrexone did not change the discriminative stimulus effects of fentanyl in rats (Ayesta et al. 1992). However, the potency of morphine increased by two-fold for both stimulus control of behavior and suppression of food-reinforced responding following 8-day infusions of 18 mg/kg per day naltrexone (Young et al. 1991a). To date, the effects of continuous naloxone infusions on the discriminative stimulus effects of other μ-opioid agonists have not been assessed.

In contrast, numerous studies have focused on the development of tolerance to the discriminative stimulus effects of μ-opioid agonists. Early reports suggested that tolerance did not develop (Colpaert et al. 1976, 1978). Since then, however, it has become apparent that tolerance to the discriminative stimulus effects of μ-opioid agonists will develop if the discrimination training is interrupted for the duration of the supplemental administration of the tolerance-inducing drug (Miksic and Lal 1977; Young et al. 1991b).

In the current experiments, we examined whether the morphine-like discriminative stimulus effects of μ-opioid

agonists demonstrate different degrees of sensitization and tolerance following continuous infusions of either naloxone or μ-opioid agonists. Furthermore, we investigated the abilities of three drugs with varying degrees of efficacy at the μ-opioid receptor to induce tolerance to morphine-like discriminative stimulus effects. Morphine was used as the prototypical μ-opioid agonist, fentanyl as a μ-opioid agonist with a higher efficacy than morphine, and meperidine as a μ-opioid agonist with a lower efficacy. The discriminative stimulus effects of morphine, meperidine, and fentanyl were examined before and after 1-week infusions of either naloxone or one of the three agonist drugs. Thus, the stimulus generalization of morphine, meperidine, and fentanyl were examined under four different treatment conditions, so that relative sensitization could be compared to relative tolerance, as a function of agonist efficacy. The role of efficacy in the ability of a drug to produce morphine-like discriminative stimulus effects following continuous drug treatment was also examined. The discriminative stimulus effects of the mixed agonist/antagonists pentazocine and buprenorphine, and the potent, full μ-opioid agonist etorphine were determined in rats that had received naloxone, and the stimulus generalization of buprenorphine and etorphine was also examined following morphine infusions.

## Materials and methods

*Subjects.* Male Sprague-Dawley rats (Charles River, Inc., Raleigh, N.C.) weighing 150–300 g were used. Subjects were experimentally and drug naive at the start of the experiment. Rats were initially housed in groups of three, in standard laboratory cages. Occasionally, cagemates would attack each other; this was independent of drug treatment. When this behavior persisted, subjects were separated and housed individually for the remainder of the study. All animals were kept in a temperature-controlled colony room with a 12-h light/dark cycle (lights on at 7:00 a.m.). Food (Purina Rodent Chow; Purina Mills, St. Louis, Mo.) and water were available ad libitum.

*Drug discrimination training.* Rats were trained to discriminate between 3.0 mg/kg morphine and saline in a two-choice discrete-trial avoidance/escape paradigm. Morphine or saline was injected SC 30 min before the start of a training session. Sessions were conducted in an operant chamber equipped with three levers, an "observing" lever mounted on one wall, and two "choice" levers, separated by a Plexiglas partition, mounted on the opposite wall. The beginning of a trial was signalled by the illumination of the house light and onset of white noise. Beginning 5.0 s after the onset of the trial, a constant current shock generator delivered a brief electrical stimulus (1.0–1.5 mA) to the grid floor of the chamber every 3.0 s in 0.5-s pulses. The rat was required to press the observing lever, then turn and press one of the two choice levers. The observing response terminated the white noise and the appropriate choice response extinguished the light, terminated the electrical stimulus, and ended the trial. Completion of the two-response chain within the first 5.0 s of the trial avoided delivery of the electrical stimulus. Pressing the incorrect choice lever reactivated the white noise and required that the observing lever be pressed again before a correct choice could be made. For half of the animals the right lever was designated as the saline lever, and the left lever was the morphine lever; lever assignments were reversed for the remaining rats. Each trial lasted a maximum of 65 s, and was followed by a 50-s time-out, during which the chamber was dark. Experimental sessions consisted of 21 trials or 30 min, whichever occurred first. Generally each rat completed one session per day except on test days, and training sessions were conducted 5–7 days/week. A microcomputer controlled schedule contingencies and also collected session data. The first trial of each session was considered a "warm up", and data from these trials were not collected.

*Drug discrimination testing.* Stimulus generalization testing began after the rat reliably completed 90% of the trials on the appropriate choice lever for four consecutive training sessions. Criteria for maintaining discrimination was 90% accuracy over the four consecutive sessions immediately preceding a test day. During test sessions both levers were activated so that a response on either lever terminated the trial. Animals received saline before the first session of each test day, and then a cumulative dosing procedures was used to determine drug effects. After the first session the subject was injected with a low dose of the test drug; 15 or 20 min later the animal was retested, and immediately after the session was injected with the next dose of drug. The cumulative dose was increased by 0.25 or 0.5 log units. For example, if the animal initially received 0.1 mg/kg, the second injection would be at a dose of 0.20 mg/kg, for a cumulative dose of 0.30 mg/kg. This procedure continued until complete stimulus generalization occurred, or for a maximum of five sessions. Some session parameters were altered on test days: the time-out period was shortened to 30 s and the maximum session length and pretreatment time were both decreased to 20 min. On days when fentanyl or etorphine were tested, the pretreatment time was shortened further to 15 min. A minimum of 6 days intervened between initial generalization tests, and a recovery period of at least 3 weeks, during which training sessions were conducted, followed each 7-day drug infusion. If an animal failed to maintain criterion performance at any point in the study, testing of that animal was temporarily suspended and training continued until criterion performance resumed.

*Experimental design.* Rats that attained criterion performance, 90% condition-appropriate responding over four consecutive (daily) sessions, were tested with four "cumulative" saline injections, using the test contingencies described above, followed by a test session with the training dose of morphine, to check for any nonspecific effects of completing five sessions in 1 day. Subsequently, stimulus-generalization curves for morphine, fentanyl, and meperidine were completed in each rat that successfully discriminated the morphine training dose after the four cumulative saline sessions. The cumulative dosing procedure allowed individual dose-response curves to be completed for each animal in a single day. Phencyclidine (PCP), a non-opioid psychoactive drug, was tested as a control for the specificity of stimulus generalization.

The tests of morphine, fentanyl, and meperidine were conducted in a randomized fashion across subjects. Following these initial generalization tests, the subjects were retested with morphine. Twenty-four hours later the rats were implanted for 1 week with sham osmotic pumps, i.e., empty pumps that had been used previously. Training sessions were suspended during these 7 days. Twenty-four hours after removal of the pumps, the morphine stimulus-generalization curve was redetermined in each rat. Subjects that showed an effect of the sham pump, that is, the dose-response curve for stimulus generalization after removal of the pump was shifted more than 1/4 log unit from the previous determination, were excluded from further study.

The general testing procedure, following the initial tests described above, was as follows: the morphine-like discriminative stimulus effects of a test drug were determined using the cumulative dosing procedure. An osmotic pump was implanted the next day, releasing naloxone (0.3 mg/kg per h), morphine (0.75 mg/kg per h), fentanyl (0.01 mg/kg per h), or meperidine (6.25 mg/kg per h). The infused dose of naloxone was chosen based on the results of an earlier study (Paronis and Holtzman 1991), which showed that a 1-week infusion of 0.3 mg/kg per h naloxone increased the analgesic potency of several μ-opioid agonists. The infused doses of agonists also were used in an earlier study (Paronis and Holtzman 1992). These doses of morphine, fentanyl, and meperidine were equated on

the basis of their acute analgesic potency, i.e., for each drug, the dose delivered hourly represents one quarter of the acute analgesic $ED_{50}$ of the drug. At these doses, each of the three infused drugs produced some degree of cross-tolerance to the analgesic effects of μ-opioid agonists. After 7 days, the osmotic pump was removed and the stimulus-generalization curve of the test drug was redetermined 24 h later. The rat was then retrained for 3 weeks and the testing procedure was repeated for another drug. Challenge drugs and infused drugs were assigned in a random order; each rat was tested with a specific combination of test drug/infused drug only once. At least five rats were tested with each combination of test drug/infused drug.

*Surgery.* Osmotic pumps (2ML1; Alza Corp., Palo Alto, Calif.) were filled with naloxone, morphine, fentanyl, or meperidine solutions at concentrations of 17.5–42.1 mg/ml, 44.6–57.3 mg/ml, 0.58–0.69 mg/ml, or 357.4–421.3 mg/ml, respectively, depending on the weight of the animals, and the infusion rate of the pump. The final doses of the infusions were: naloxone, 0.3 mg/kg per h; morphine, 0.75 mg/kg per h; fentanyl, 0.01 mg/kg per h; meperidine, 6.25 mg/kg per h. Pumps were implanted SC through an incision in the mid-scapular region of the back while the rat was under light halothane anesthesia (Halocarbon Laboratories, North Augusta, S.C.). The incision was closed with surgical wound clips. One week later the pumps were removed through this incision, using the same procedure.

*Data analysis.* Data were analyzed according to the number of drug lever responses. Individual $ED_{50}$s (the log dose needed to produce ten drug lever responses) were calculated by linear regression of the stimulus-generalization curves. Subjects for which an $ED_{50}$ could not be determined, i.e., animals that emitted fewer than ten responses on the drug lever, had an $ED_{50}$ set at the dose of the drug 0.25–0.5 log unit higher than the last dose tested, depending upon the progression of doses used. Potency ratios (PR) were determined by dividing the pre-infusion $ED_{50}$ of a rat by its post-infusion $ED_{50}$. The individual $ED_{50}$ and PR based upon the $\text{logarithm}_{10}$ of the dose, were averaged to determine group means and 95% confidence limits (CL). Potency ratios greater than 1.0 indicated increased potency, PR; less than 1.0 reflected decreased potency. Paired *t*-tests of the $ED_{50}$s were used to determine levels of statistical significance. Individual PR, following naloxone infusion, were compared using a one-way analysis of variance; a two-way analysis of variance (infused drug versus test drug) was used to compare PR following agonist infusions.

*Drugs.* The following drugs were dissolved in normal (0.9%) saline: naloxone hydrochloride, (Sigma, St. Louis, Mo.), morphine sulfate and meperidine hydrochloride (Penick Corp., Newark, N.J.), fentanyl citrate (McNeil Laboratories, Fort Washington, Pa.), etorphine hydrochloride, buprenorphine hydrochloride, and phencyclidine hydrochloride (National Institute on Drug Abuse, Rockville, Md.). Pentazocine hydrochloride (Sigma, St. Louis, Mo.) was dissolved in distilled water. All doses are expressed as the free base, and were injected SC in a volume of 1.0 ml/kg.

## Results

### *Stimulus generalization of μ-opioid agonists in rats trained to discriminate morphine*

Twenty-one rats met the criteria for discriminating 3.0 mg/kg morphine from saline; three of these rats were subsequently excluded from the study, as discussed below, and their data are not included in any grouped data. The mean number of sessions to criterion was 67 ± 3 (range: 50–80 sessions). Cumulative injections of saline did not affect the ability of the rats to discriminate the training dose (3.0 mg/kg) of morphine. All of the rats used in this study demonstrated a dose-dependent generalization to morphine, fentanyl, and meperidine from the morphine cue. The mean $ED_{50}$s of the μ-opioid agonists for morphine-like discriminative effects are shown in Fig. 1(A–C). Phencyclidine (PCP), which was tested as a non-opioid control, was not generalized from the morphine cue when tested up to a dose of 1.78 mg/kg (Fig. 1D). At this dose of PCP five rats did not complete

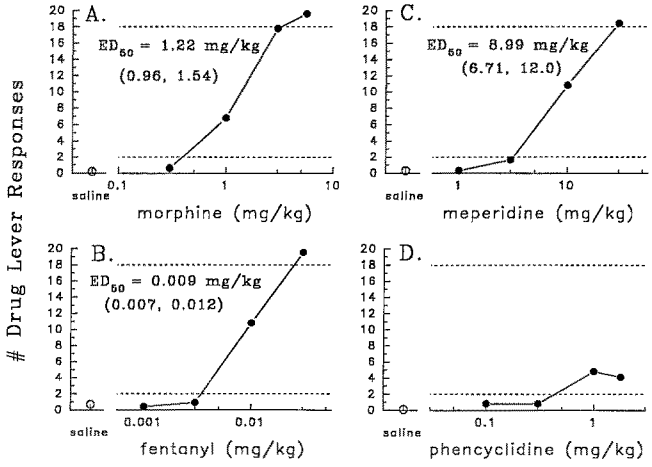


**Fig. 1.** Cumulative doses of morphine (**A**), fentanyl (**B**) and meperidine (**C**) all engendered morphine-appropriate responding in rats trained to discriminate 3.0 mg/kg morphine from saline. The two highest doses of meperidine decreased responding in two animals, i.e., these animals completed fewer than the maximum 20 trials of the session. Cumulative doses of phencyclidine (*PCP*) did not produce morphine-appropriate responding (**D**). The highest dose of PCP (1.78 mg/kg) reduced responding in five animals. A saline session always preceded the lowest dose of the drug tested; these data are presented in each panel to the left of the graph. *Numbers* indicate the mean $ED_{50}$ values (with 95% CL) for stimulus generalization. *Abscissa*: cumulative drug dose in milligrams per kilogram. *Ordinates*: mean number of trials terminated by a response on the morphine-appropriate choice lever. Upper and lower *dashed lines* indicate the criterion levels of responding for morphine and saline, respectively. Each point represents the mean number of trials completed on the morphine lever, based on a single observation in 18 animals

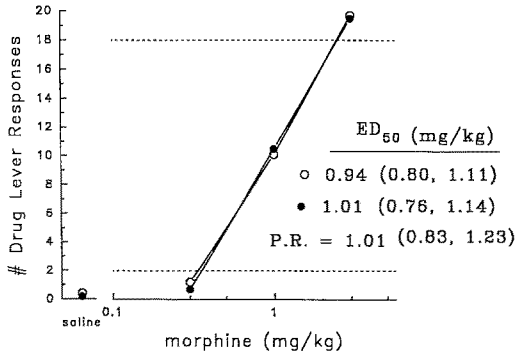


**Fig. 2.** In 18 of 21 animals, the dose-response curve of morphine was unaffected by 7-day implantation of a sham pump, and consequent interruption of training. Dose-response curves were determined twice in each animal, 24 h before implanting the sham pumps (*open circles*) and 24 h after removing the pumps (*closed circles*) *Abscissa*: cumulative morphine dose in mg/kg. *Ordinates*: mean number of trials terminated by a response on the morphine-appropriate choice lever. Each point represents the mean number of trials completed on the morphine lever, based on observations in 18 animals. Other details as in Fig. 1

all of the trials of the session, hence higher doses of PCP were not tested.

Three rats had an enhanced response to the discriminative stimulus effects of morphine following the implantation and subsequent removal of a sham pump; these rats were excluded from further study. Interruption of training for 1 week, and implantation of a sham pump did not affect the ability of the remaining 18 rats to discriminate morphine (Fig. 2).

*Effects of continuous naloxone infusion on the discriminative stimulus effects of μ-opioid agonists*

In the groups of animals tested with morphine ($n = 5$) and fentanyl ($n = 6$), the $ED_{50}$s for stimulus generalization before the naloxone infusion, 1.05 and 0.013 mg/kg, respectively, were similar to the baseline $ED_{50}$s for the stimulus generalization of these drugs calculated for all 18 rats (see Fig. 1). The mean prenaloxone $ED_{50}$ for stimulus generalization in the rats tested with meperidine ($n = 6$) was outside the upper 95% CL of the baseline $ED_{50}$ of meperidine for the whole group of 18 rats; however, this was due primarily to one rat that had a prenaloxone $ED_{50}$ of 43.5 mg/kg.

The stimulus-generalization curves for μ-opioid agonists before and after the naloxone infusions are shown in Fig. 3; the $ED_{50}$ values and potency ratios are listed in Table 1. A 1-week infusion of 0.3 mg/kg per h naloxone shifted to the left the dose-response curves for all of the drugs tested. Following continuous administration of naloxone, the mean potency ratios of both morphine and fentanyl were close to 2.0. The potency of morphine as a discriminative stimulus increased in each of the five rats tested with morphine, potency ratios ranging from 1.12 to 3.07. Five of the six rats tested with fentanyl showed an increased sensitivity to the discriminative stimulus effects of fentanyl. Potency ratios for fentanyl in these five rats ranged from 1.60 to 3.12. One rat tested with fentanyl before and after a naloxone infusion showed a decreased response to fentanyl (PR = 0.80). Meperidine, a μ-opioid agonist with a lower efficacy than morphine, had a mean potency ratio of 2.7 after the continuous naloxone infusion; the potency of meperidine increased in each of the six rats tested.

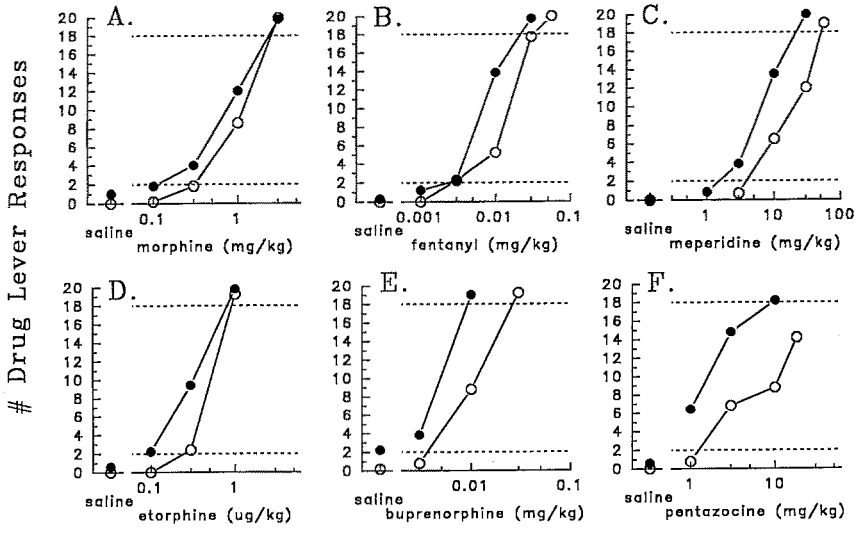


**Fig. 3.** Stimulus generalization of morphine ($n = 5$), fentanyl ($n = 6$), meperidine ($n = 6$), etorphine ($n = 5$), buprenorphine ($n = 5$), and pentazocine ($n = 5$) in rats continuously treated with naloxone. Dose-response curves were generated twice in each animal, before (*open circles*) and after (*closed circles*) a 7-day infusion of 0.3 mg/kg per h naloxone. *Abscissa*: cumulative drug dose in mg/kg. *Ordinates*: mean number of trials terminated by a response on the morphine-appropriate choice lever. Each point represents the mean of five to six animals. Other details as in Fig. 1

**Table 1.** Mean $ED_{50}$ (mg/kg) with 95% confidence limits ($n = 5$–6) for the discriminative stimulus effects of μ-opioid agonists before and after a 7-day infusion of 0.3 mg/kg per h naloxone

| Test drug | Prenaloxone $ED_{50}$ | Postnaloxone $ED_{50}$[a] | Potency ratio[b] | *P* value[c] |
|---|---|---|---|---|
| Buprenorphine | 0.010 (0.006, 0.017) | 0.0004 (0.002, 0.004) | 2.34 (1.13, 5.06) | 0.032 |
| Etorphine | 0.0005 (0.0003, 0.00007) | 0.0003 (0.0002, 0.0004) | 1.58 (1.02, 2.47) | 0.044 |
| Fentanyl | 0.013 (0.006, 0.026) | 0.007 (0.005, 0.010) | 1.82 (1.09, 3.04) | 0.032 |
| Meperidine | 16.7 (7.2, 38.7) | 6.1 (3.3, 11.1) | 2.74 (1.55, 4.86) | 0.006 |
| Morphine | 1.05 (0.44, 2.54) | 0.56 (0.18, 1.73) | 1.88 (1.15, 3.06) | 0.027 |
| Pentazocine | 5.92[d] (1.79, 19.6) | 1.65 (0.59, 4.62) | 3.58 (2.69, 4.76) | 0.0002 |

[a] Determined 24 h after removal of pump
[b] Calculated by dividing prenaloxone $ED_{50}$ by postnaloxone $ED_{50}$ for each subject, then determining the group mean
[c] For pre- versus postnaloxone $ED_{50}$
[d] Includes value estimated for one rat that completed fewer than 10 trials on the drug lever (see Methods)

The potency of etorphine as a discriminative stimulus increased after the naloxone infusion in four of the five rats tested and was unchanged in one. The same occurred with buprenorphine. Effects of the naloxone infusion on the discriminative stimulus effects of pentazocine were particularly marked. The potency of pentazocine as a discriminative stimulus was increased in all rats tested: potency ratios in individual rats were all greater than 2.5, ranging from 2.69 to 4.68. Prior to the naloxone infusion, one rat completed only four trials on the morphine lever after receiving 17.8 mg/kg pentazocine, yet an $ED_{50}$ of 6.92 mg/kg was determined in this rat after the naloxone infusion.

A one-way analysis of variance on the potency ratios across the different agonists approached statistical significance ($F_{15,26} = 2.27$; $P = 0.077$). The lower efficacy agonists tended to have greater increases in potency after naloxone infusion than did the higher efficacy drugs. Post hoc tests indicated that the potency ratios for pentazocine were significantly higher than those for morphine, fentanyl, and etorphine ($P < 0.05$).

*Effects of continuous morphine infusion on the discriminative stimulus effects of μ-opioid agonists*

The mean premorphine $ED_{50}$s for stimulus generalization to morphine, fentanyl, and meperidine, in the groups of five to six animals tested with these drugs, were 1.33, 0.011, and 7.29 mg/kg, respectively. These values are similar to the mean baseline $ED_{50}$s of these three drugs for the whole group of 18 rats (see Fig. 1).

The stimulus-generalization curves for μ-opioid agonists before and after the morphine infusions are shown in Fig. 4; $ED_{50}$ values and potency ratios are listed in Table 2. A 7-day SC infusion of 0.75 mg/kg per h morphine did not produce significant tolerance to the discriminative stimulus effects of morphine. Although the potency of morphine was decreased in five of the six rats tested, it was increased in one rat; the mean potency ratio of morphine for the group of six rats was 0.70 (Table 2). Continous exposure to morphine appeared to have no affect on the discriminative stimulus effects of etorphine. The mean potency ratio of etorphine following the SC

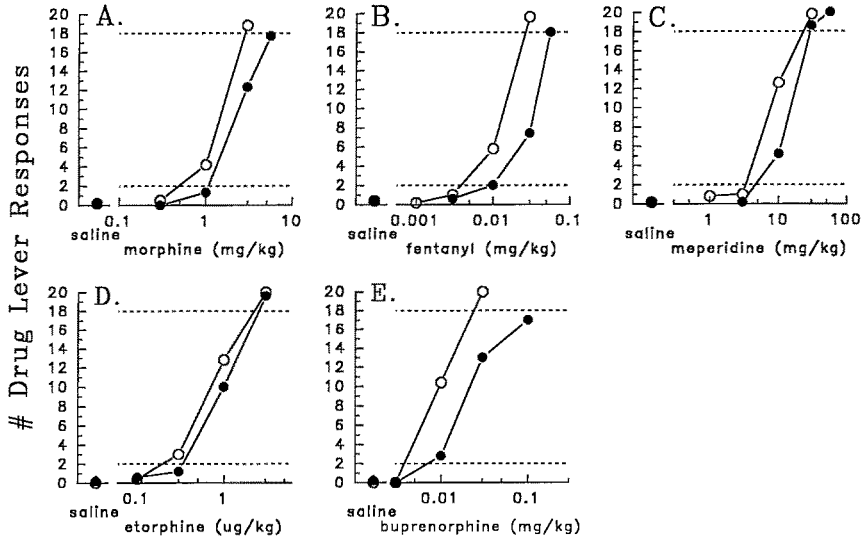


**Fig. 4.** Stimulus generalization of morphine ($n = 6$), fentanyl ($n = 5$), meperidine ($n = 5$), etorphine ($n = 5$), and buprenorphine ($n = 5$) in rats continuously treated with morphine. Dose-response curves were generated twice in each animal, before (*open circles*) and after (*closed circles*) a 7-day infusion of 0.75 mg/kg per h morphine. The highest dose of fentanyl decreased responding in one animal after the morphine infusion. *Abscissa*: cumulative drug dose in mg/kg. *Ordinates*: mean number of trials terminated by a response on the morphine-appropriate choice lever. Each point represents the mean of five or six animals. Other details as in Fig. 1

**Table 2.** Mean $ED_{50}$ (mg/kg) with 95% confidence limits ($n = 5$–6) for the discriminative stimulus effects of μ-opioid agonists before and after a 7-day infusion of 0.75 mg/kg per h morphine

| Test drug | Premorphine $ED_{50}$ | Postmorphine $ED_{50}$[a] | Potency ratio[b] | $P$ value[c] |
|---|---|---|---|---|
| Buprenorphine | 0.010 (0.007, 0.014) | 0.027[d] (0.005, 0.152) | 0.38 (0.09, 1.64) | 0.141 |
| Etorphine | 0.0007 (0.0003, 0.0016) | 0.0009 (0.0003, 0.0023) | 0.80 (0.23, 2.86) | 0.656 |
| Fentanyl | 0.011 (0.006, 0.020) | 0.028 (0.018, 0.044) | 0.40 (0.26, 0.062) | 0.005 |
| Meperidine | 7.29 (4.19, 12.7) | 12.7 (6.93, 23.4) | 0.57 (0.29, 1.12) | 0.082 |
| Morphine | 1.33 (1.06, 1.68) | 2.37 (1.30, 4.34) | 0.56 (0.25, 1.26) | 0.127 |

[a] Determined 24 h after removal of pump
[b] Calculated by dividing premorphine $ED_{50}$ by postmorphine $ED_{50}$ for each subject, then determining the group mean
[c] For pre- versus postmorphine $ED_{50}$
[d] Includes value estimated for one rat that completed fewer than 10 trails on the drug lever (see Methods)

infusion of morphine was 0.80 (Table 2); however, there was a large degree of individual variability in the responses to etorphine. The continuous administration of morphine did not affect the ability of one rat to discriminate etorphine (PR = 1.0); however, two of the rats exhibited a profound tolerance (PR < 0.3), to the discriminative stimulus effects of etorphine, whereas the remaining two rats exhibited a sensitization (PR > 2.0)

The stimulus-generalization curves for fentanyl, meperidine, and buprenorphine, were all shifted to the right after the morphine infusions, indicating that cross-tolerance to the interoceptive cues of these drugs did develop. The largest decrease in potency was seen in the responses to fentanyl. Following the morphine infusion all five of the rats tested with fentanyl showed a decreased sensitivitiy to of fentanyl and, similarly, all five of the rats tested with meperidine showed a decreased sensitivity to meperidine, although this latter difference only approached statistical significance (Table 2). Although the differences between the pre-and postmorphine $ED_{50}$s for buprenorphine also failed to reach significance, four of the five rats tested had decreased sensitivity to buprenorphine. In fact, one rat responded on the morphine lever only 6 times in the postmorphine condition. This inability to surmount the morphine-induced tolerance is reflected in the large confidence limits of the postmorphine $ED_{50}$ for the stimulus generalization to buprenorphine (Table 2).

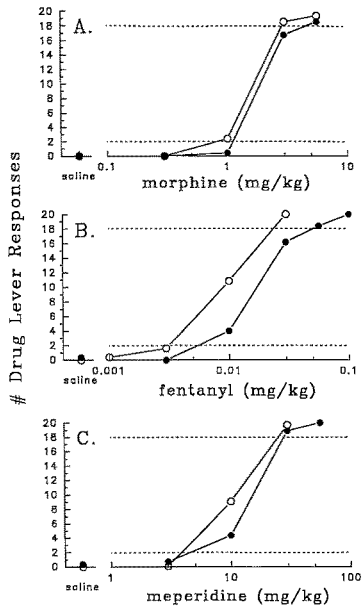


**Fig. 5.** Stimulus generalization of morphine ($n$ = 5), fentanyl ($n$ = 5), and meperidine ($n$ = 7) in rats continuously treated with fentanyl. Dose-response curves were generated twice in each animal, before (*open circles*) and after (*closed circles*) a 7-day infusion of 0.01 mg/kg per h fentanyl. *Abscissa*: cumulative morphine (**A**), fentanyl (**B**), and meperidine (**C**) dose in mg/kg. *Ordinates*: mean number of trials terminated by a response on the morphine-appropriate choice lever. Each point represents the mean of five to seven animals. Other details as in Fig. 1

*Effects of continuous fentanyl infusion on the discriminative stimulus effects of μ-opioid agonists*

The mean prefentanyl $ED_{50}$s for stimulus generalization to morphine ($n$ = 5) fentanyl ($n$ = 5), and meperidine ($n$ = 7), for the groups of animals tested with these drugs, were 1.55, 0.009, and 9.77 mg/kg, respectively. These values are in accordance with the mean baseline $ED_{50}$s of these three drugs for the whole group of 18 rats (see Fig. 1).

Following infusions of 0.01 mg/kg per h fentanyl, the generalization curves for morphine, fentanyl, and meperidine were shifted slightly to the right (Fig. 5); however, in each case the differences between the pre- and post-fentanyl $ED_{50}$s failed to reach statistical significance (Table 3). The dose-response curve for the group of rats tested with fentanyl shifted to the right the most, as the mean $ED_{50}$ for stimulus generalization to fentanyl almost doubled, from 0.009 mg/kg to 0.017 mg/kg, following the continuous exposure to fentanyl. Two of the five animals in this group, however, showed no change (PR = 1.0) in their responses to fentanyl. Similar individual differences were apparent in the groups tested with morphine or meperidine.

**Table 3.** Mean $ED_{50}$ (mg/kg) with 95% confidence limits ($n$ = 5–7) for the discriminative stimulus effects of μ-opioid agonists before and after a 7-day infusion of 0.01 mg/kg per h fentanyl

| Test drug | Prefentanyl $ED_{50}$ | Postfentanyl $ED_{50}$[a] | Potency Ratio[b] | $P$ value[c] |
|---|---|---|---|---|
| Fentanyl | 0.009 (0.005, 0.016) | 0.017 (0.007, 0.044) | 0.50 (0.22, 1.16) | 0.085 |
| Meperidine | 9.77 (6.57, 14.5) | 14.1 (10.2, 19.5) | 0.69 (0.42, 1.14) | 0.123 |
| Morphine | 1.55 (1.17, 2.05) | 2.07 (1.25, 3.42) | 0.75 (0.50, 1.12) | 0.114 |

[a] Determined 24 h after removal of pump
[b] Calculated by dividing prefentanyl $ED_{50}$ by postfentanyl $ED_{50}$ for each subject, then determining the group mean
[c] For pre- versus postfentanyl $ED_{50}$

*Effects of continuous meperidine infusion on the discriminative stimulus effects of μ-opioid agonists*

The mean pre-meperidine $ED_{50}$s for stimulus generalization to morphine, fentanyl, and meperidine, in the groups of five animals tested with these drugs, were 1.42, 0.013, and 12.3 respectively. These values are similar to the mean baseline $ED_{50}$s of these three drugs for the whole group of 18 rats (see Fig. 1).

A 7-day infusion of 6.25 mg/kg per h meperidine produced tolerance and cross-tolerance to the discriminative stimulus effects of meperidine, morphine, and fentanyl (Fig. 6; Table 4). As was the case with infusion of morphine and fentanyl, the infusion of meperidine induced the most cross-tolerance to fentanyl, although the potency of fentanyl was unchanged in one of five rats. The meperidine infusion also decreased the potency of morphine as a discriminative stimulus in four of five rats, giving a mean potency ratio for the group of 0.61. The

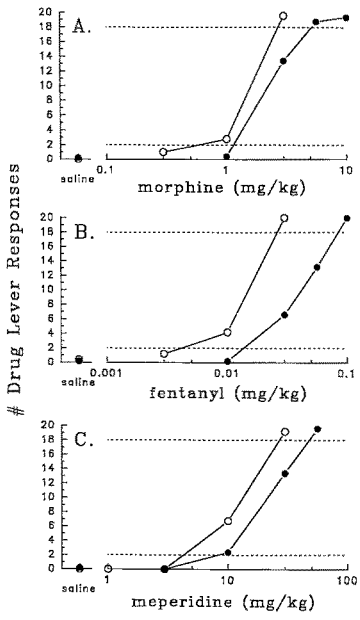


**Fig. 6.** Stimulus generalization of morphine, fentanyl, and meperidine in rats continuously treated with meperidine. Dose-response curves were generated twice in each animal, before (*open circles*) and after (*closed circles*) a 7-day infusion of 6.25 mg/kg per h meperidine. *Abscissa*: cumulative morphine (**A**), fentanyl (**B**), and meperidine (**C**) dose in mg/kg. *Ordinates*: mean number of trials terminated by a response on the morphine-appropriate choice lever. Each point represents the mean of five animals. Other details as in Fig. 1

**Table 4.** Mean $ED_{50}$ (mg/kg) with 95% confidence limits ($n=5$) for the discriminative stimulus effects of μ-opioid agonists before and after a 7-day infusion of 6.25 mg/kg per h meperidine

| Test drug | Pre-meperidine $ED_{50}$ | Post-meperidine $ED_{50}$[a] | Potency ratio[b] | $P$ value[c] |
|---|---|---|---|---|
| Fentanyl | 0.013 (0.006, 0.025) | 0.033 (0.019, 0.058) | 0.40 (0.14, 1.00) | 0.052 |
| Meperidine | 12.3 (6.12, 24.8) | 20.3 (12.1, 34.0) | 0.61 (0.28, 0.98) | 0.046 |
| Morphine | 1.42 (1.01, 2.01) | 2.35 (1.58, 3.49) | 0.60 (0.34, 1.09) | 0.076 |

[a] Determined 24 h after removal of pump
[b] Calculated by dividing premeperidine $ED_{50}$ by postmeperidine $ED_{50}$ for each subject, then determining the group mean
[c] For pre- versus postmeperidine $ED_{50}$

mean potency ratio for meperidine was similar to that for morphine, 0.60. Although the potency of meperidine as a discriminative stimulus decreased in all five rats, with individual potency ratios ranging from 0.24 to 0.87, variability among subjects prevented the group data from reaching statistical significance.

Among the three drugs that were both infused and tested for morphine-like discriminative effects, morphine, fentanyl, and meperidine, meperidine appeared to induceä the most tolerance and cross-tolerance when it was infused and fentanyl appeared to undergo the biggest decrease in potency when it was tested. However, a two-way analysis of variance failed to reveal a significant difference among drugs infused ($F_{2,39} = 0.71$; $P > 0.1$), among drugs tested for discriminative effects ($F_{2,39} = 2.15$; $P > 0.1$), or a significant interaction between infused and tested drugs ($F_{4,39} = 0.02$).

## Discussion

We examined the discriminative stimulus effects of μ-opioid agonists before and after 7-day infusions of either naloxone or one of three μ-opioid agonists, morphine, fentanyl, or meperidine. In initial tests of stimulus generalization, each of the three μ-opioid agonists, but not PCP, generalized from the morphine cue at some dose. This is consistent with results from other studies examining the discriminative stimulus effects of opioid drugs in rats trained to discriminate between 3.0 mg/kg morphine and saline (Shannon and Holtzman 1976; Young et al. 1992).

Stimulus control of behavior by morphine was stable; neither spontaneous sensitization nor tolerance to the discriminative stimulus effects of morphine occurred over the approximately 1.5-year period following the initial training. This despite week-long infusions of naloxone and μ-opioid agonists, and associated periods without daily training sessions. The mean $ED_{50}$s for the discriminative stimulus effects of morphine, determined for individual groups of five or six rats over the course of the experiments, did not vary more than 0.2 mg/kg from the mean $ED_{50}$ (1.22 mg/kg) calculated for the entire group of 18 rats at the beginning of the experiments. The mean $ED_{50}$s for generalization to fentanyl and meperidine from the morphine cue were similarly consistent across time. The stability of the learned behavior in drug discrimination paradigms is well documented. Behavioral instability can occur in some animals over the course of an experiment; however, the behavior of most animals can be maintained under the stimulus control of a drug for several months, provided that training sessions are regularly completed (Schechter et al. 1989; Pugh et al. 1992).

It has been reported that a suspension of drug discrimination training for up to 14 days does not affect the stimulus generalization curve for morphine (Sannerud and Young 1987). In the present study, a 1-week interruption of training and implantation of a sham pump also had no effect on the discriminative stimulus effects of morphine. On the other hand, a 1-week interruption of training accompanied by an infusion of naloxone produced 1.5- to 4-fold shifts to the left in the dose-response curves of the μ-opioid agonists.

Continuous exposure to naloxone will produce up to 2-fold increases in the number of μ-opioid binding sites in the brain (Morris et al. 1988), as well as two-fold increases in the potency of morphine in assays of analgesic and locomotor activity (Bardo and Neisewander 1987; Millan et al. 1988). The effects of continuous exposure to μ-opioid antagonists on other μ-opioid agonist effects are not as well understood. There is one report of an enhancement of the reinforcing effects of morphine as measured in a conditioned place preference paradigm (Bardo

and Neisewander 1987). In another study, the discriminative stimulus effects of fentanyl were examined following a 7-day infusion of 6.0 mg/kg per day naltrexone (Ayesta et al. 1992). No change in the potency of fentanyl as a discriminative stimulus was noted in the fentanyl-trained rats. However, in another study that examined the discriminative stimulus properties of morphine following continuous exposure to naltrexone there was a 2-fold increase in the potency of morphine as a discriminative stimulus (Young et al. 1991a). In the latter study, rats had received 18 mg/kg per day naltrexone for 8 days. In our experiments, the potencies of both morphine and fentanyl as discriminative stimuli were almost doubled following a 1-week infusion of naloxone, in agreement with the results of Young et al. (1991a).

The present study represents the first time that the discriminative stimulus effects of μ-opioid agonists other than morphine or fentanyl were examined following a period of antagonist administration. Etorphine, meperidine, buprenorphine, and pentazocine all exhibited some degree of increased potency as discriminative stimuli following the continuous administration of naloxone. This is in contrast to the results of a previous study of the analgesic potency of μ-opioid agonists following an infusion of naloxone (Paronis and Holtzman 1991). For example, in the current study the stimulus-generalization curve for pentazocine was shifted in a parallel fashion almost 4-fold to the left following naloxone, whereas an equivalent naloxone infusion enhanced the analgesic response only to the highest dose of pentazocine. Similarly, although there was no change in the analgesic potency of either etorphine or buprenorphine subsequent to naloxone infusions (Paronis and Holtzman 1991), both of these drugs exhibited enhanced discriminative stimulus effects after the naloxone infusions. Thus, the discriminative stimulus effects of μ-opioid agonists appear to be more readily enhanced by a continuous exposure to naloxone than are the analgesic effects of these drugs.

There were differences among drugs in the magnitude of the changes in potency induced by naloxone, as the potency ratios spanned a 2-fold range, from 1.58 to 3.58 (Table 1). Although these differences among drugs only approached statistical significance, the potency ratios nonetheless appeared to vary with the efficacy of the test drugs. That is, drugs with a higher efficacy, for instance etorphine, were more resistant to naloxone-induced increases in potency than were drugs of lower efficacy, such as buprenorphine and pentazocine. The large degree of individual variability inherent in measuring the discriminative stimulus effects of drugs (Locke and Holtzman 1986) might be a factor in the differences that were seen. However, the individual variability between rats cannot be the sole explanation for the differences seen among drugs in these experiments. After the naloxone infusions, the potency ratio of pentazocine was greater than 2.6 in each one of the rats tested, but only one of the rats tested with etorphine had an etorphine potency ratio greater than 2.0.

In contrast to the effects of the naloxone infusions, the continuous administration of the μ-opioid agonists generally produced rightward shifts in the stimulus-generalization curves of the drugs tested. In accordance with results from other investigators (Emmett-Oglesby et al. 1988; Young et al. 1991b), rats that received 7-day infusions of morphine were tolerant to morphine, fentanyl, meperidine, and buprenorphine. However, in contrast to the results of Young and colleagues, we saw no tolerance to the discriminative stimulus effects of etorphine.

The discrepancies between our results and those of other investigators may be due to the different durations of the morphine treatment. There is evidence that the magnitude of morphine tolerance is related to the duration of the chronic administration of morphine (Young et al. 1991c). The rats used in the studies of Young et al. (1991c) received 15 mg/kg per day morphine for 2 weeks, whereas the rats in the current experiments were exposed to a roughly equivalent dose of morphine for only 1 week. Therefore, it is not surprising that less μ-opioid tolerance was apparent in the present studies.

Although infusions of fentanyl also lessened the discriminative stimulus effects of fentanyl, morphine, and meperidine, in each case this effect failed to reach levels of statistical significance. These results are in accordance with those of other studies of the stimulus generalization of fentanyl and morphine to a fentanyl cue after a 1-week exposure to either morphine or fentanyl. Tolerance to the discriminative stimulus effects of both morphine and fentanyl were seen after 7 days of twice daily injections of 8 mg/kg morphine, yet no tolerance was evident following twice daily injections of 0.08 mg/kg fentanyl, a cumulative dose equal to four times the training dose of fentanyl (Emmett-Oglesby et al. 1988). Tolerance to the discriminative stimulus effects of morphine and fentanyl were seen only when the daily dose of fentanyl was tripled, to four injections of 0.12 mg/kg fentanyl.

When a higher dose of fentanyl, 0.48 mg/kg per day, twice the dose used in the present experiments, was infused via osmotic pumps (Emmett-Oglesby et al. 1989), no tolerance to the discriminative stimulus effects of fentanyl was seen 24 h after terminating the infusion. However, 50% of the animals did demonstrate tolerance when tested 12 h after the fentanyl treatment was halted. Fentanyl has a shorter duration of action than morphine and meperidine, although its biological half-life, 1–2 h, is similar to that of meperidine (Dahlström et al. 1979; Hug and Murphy 1981). It is possible that in the present experiment the animals that received infusions of fentanyl were transiently tolerant to the discriminative stimulus effects of μ-opioid agonists, but had recovered from this tolerance sooner than 24 h following the removal of the osmotic pumps. However, if the development of μ-opioid tolerance involves an alteration at the level of the receptor, than the duration of action of the drug used to induce the tolerance should be relatively inconsequential, provided the drug is continuously administered. Indeed, duration of opioid tolerance will vary with the length of the continuous opioid treatment, and recovery from this tolerance is gradual, lasting from days (Young et al. 1990, 1991b) to weeks (Craft et al. 1989). A more likely explanation for the lack of tolerance following infusions of fentanyl is that too few receptors were down-regulated by the 0.01 mg/kg per h dose of fentanyl. Hence, although a

relatively large dose of fentanyl was infused (7/8 $ED_{50}$ for generalization per h), apparently this was insufficient to occupy and consequently inactivate enough receptors to produce tolerance in all animals to the subsequent discriminative stimulus effects of morphine, meperidine, or fentanyl. Among the three μ-opioid agonists, infusions of fentanyl also induced the least tolerance and cross-tolerance to analgesic effects (Paronis and Holtzman 1992).

The tolerance and cross-tolerance to meperidine, morphine, and fentanyl seen following 7-day infusions of meperidine can then be explained by the relatively lower efficacy of meperidine, and the consequently higher fractional receptor occupation by meperidine. The dose of meperidine that was infused, 6.25 mg/kg per h, was relatively lower than the dose of fentanyl. That is, meperidine was infused at a rate of 1/2 $ED_{50}$/h, whereas fentanyl was delivered at a rate of 7/8 $ED_{50}$/h. Therefore, the meperidine dose should have activated fewer receptors than did fentanyl, and yet the meperidine infusions shifted the dose-effect curves of the test drugs further to the right than did the fentanyl infusions. As a drug with a lower intrinsic activity than fentanyl (Adams et al. 1990), meperidine must occupy more receptors to produce the same effect as fentanyl. Whether a dose of 1/2 the $ED_{50}$ of meperidine occupied more receptors than did a dose of 7/8 of the ED50 of fentanyl is not known. Nonetheless, that meperidine induced tolerance whereas fentanyl did not, suggests that more receptors were occupied by meperidine, and that receptor occupation, not merely activation, is important in the degree of tolerance produced by μ-opioid agonists.

Repeatedly inducing tolerance/sensitization to μ-opioid agonists in the same animals may have produced a confound that makes interpretation of our results difficult. These repeated exposures to the continuous drug treatments may have exceeded the plasticity of the opioid systems in these animals. In particular, this might explain the large degree of individual variability seen following similar drug treatments. However, when the data from the individual animals are examined, there does not appear to be an effect of time on the magnitude of the changes in response following the drug infusions. Likewise, there were not any systematic alterations over time in the baseline stimulus generalization curves of morphine, meperidine, or fentanyl. Furthermore, the repeated induction of tolerance in rats trained to discriminate drugs is not unprecedented (Miksic and Lal 1977; Young et al. 1991c; Pugh et al. 1992). Therefore, although repeated induction of sensitization and tolerance to μ-opioid agonists may indeed have had some effect on the magnitude of the changes following the continuous drug treatments, there were no indications that the results of the experiments were unduly affected by the various treatments. Perhaps exposing the animals to the drug infusions in a random sequence helped prevent time-dependent changes in sensitivity to the various drugs that were tested.

In summary, all of the μ-opioid agonists tested demonstrated enhanced discriminative stimulus effects subsequent to infusions of the opioid antagonist naloxone. This consistent increase in potency is in contrast to the differential effects of μ-opioid agonist infusions on the stimulus generalization to morphine, fentanyl, and meperidine. The large amount of variability in the responses of the animals to the continuous drug treatments precludes forming broad based conclusions; nonetheless, it appears that meperidine infusions produced the most tolerance, and fentanyl the least tolerance, to μ-opioid agonists. The consistent increases in agonist potency following the naloxone infusion and the lack of such consistency following any of the μ-opioid agonist infusions suggests that antagonist-induced increases and agonist-induced decreases in the potency of μ-opioid agonists are two distinct phenomena rather than the reciprocal outcomes of a single mechanism of action.

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