

# Discriminative Stimulus Properties of Fentanyl and Morphine: Tolerance and Dependence

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COLPAERT, F. C., J. J. M. D. KUYPS, C. J. E. NIEMEGEREERS AND P. A. J. JANSSEN. *Discriminative stimulus properties of fentanyl and morphine: tolerance and dependence*. PHARMAC. BIOCHEM. BEHAV. 5(4) 401–408, 1976. — Using a food-reinforced two-lever operant procedure, rats were trained to discriminate 0.04 mg/kg fentanyl from saline. At different time intervals after the establishment of discriminative responding, stimulus generalization experiments were performed with equivalent dose ranges of fentanyl (0.0025 to 0.02 mg/kg) and morphine (2.5 to 20 mg/kg). It was found that the  $ED_{50}$  values of both compounds for generalization with the narcotic discriminative stimulus complex, did not change over a 4-month period. The subjects used in this study demonstrated marked tolerance to narcotic analgesia, but none of them showed signs reminiscent of narcotic dependence. It is concluded that the discriminative stimulus properties of narcotic analgesics are not subject to tolerance.

Fentanyl	Morphine	Narcotic	Drug discrimination	State-dependent	Discriminative stimulus
Tolerance	Dependence				

PHARMACOLOGICAL knowledge of drugs is almost entirely based upon experimental studies which aim to determine the organisms' unconditioned responses to drug administration, and thus is limited to the unconditioned stimulus properties of drugs. This approach disregards the ability of stimuli to subserve a number of different functions, including eliciting, reinforcing, discriminative and emotional [31]. Hence, recent research (e.g. [2,28]) has shown that drugs may control operant responding in laboratory animals, thus confirming that drugs can possess stimulus properties other than merely unconditioned ones. The two major types of learning phenomena occurring in animals being exposed to drugs have been denoted as drug discrimination and state-dependent learning. The distinction between the two types pertains basically to the training drug subserving different stimulus functions in the experimental conditions which give rise to these learning phenomena [10]. State-dependent learning is evidenced by the lack of transfer of a given response to the no-drug condition, and indicates that the drug constitutes a critical part of the set of stimuli upon which the occurrence of the response is conditional (dependent). Drug discrimination learning consists of the acquisition of (at least) two responses under the discriminative control of two physically distinct injections; the drug and saline injections (or, alternatively, two different drug injections) then constitute a pair of discriminative stimuli upon which, however, neither response is conditional.

Extensive evidence has become available which indicates that fentanyl [5, 6, 7, 8], morphine [18, 21, 22, 29, 35] and numerous other narcotic analgesic drugs [6, 7, 8, 9] are able to control operant responding in rats. However, a major inconsistency has arisen with respect to the problem of whether operant response control by narcotic analgesics is subject to tolerance. Hirschhorn and Rosecrans [22] have shown that the ability of morphine to control operant responding in rats decreases with repeated administration of the drug. In contrast, using fentanyl as the training drug, no tolerance seemed evident in another study [5] which nevertheless covered a period of over two months. Among the various conceivable explanations for this inconsistency, one intriguing possibility is that the narcotic analgesics subserved different stimulus functions in the two studies. Whereas the fentanyl study [5] employed a procedure which specifically assesses the discriminative stimulus properties of drugs [10], the nature of the stimulus function involved in the morphine study [22] seems less clear-cut (see Discussion in [10]). Therefore, the inconsistency may reflect a possible difference between the drug actions underlying drug discrimination and state-dependent learning using a narcotic analgesic as the training drug.

The present study therefore sought to determine whether the discriminative stimulus properties of narcotic analgesics are subject to tolerance. Some further aspects of the above mentioned inconsistency were also investigated.

Fentanyl was used as the training drug because this compound, as compared to morphine and other morphine-like analgesics, is relatively atoxic [23].

### EXPERIMENT 1

The first experiment was designed to investigate whether the discriminative stimulus properties of narcotic analgesic drugs are subject to tolerance. To this end, rats were trained to discriminate 0.04 mg/kg fentanyl from saline; in animals so trained, the possible occurrence of tolerance to fentanyl and of cross-tolerance to morphine was studied by repeatedly determining the  $ED_{50}$  values of both drugs for stimulus generalization with the standard fentanyl treatment. The study pertains to a period of 4 months not including training.

### METHOD

#### *Animals*

The experimental animals were twelve male Wistar rats weighing  $210 \pm 10$  g (age: 7–8 weeks) at the beginning of the experiments; they were housed in individual living cages, stored in a continuously illuminated and air conditioned room ( $21 \pm 1^\circ\text{C}$ ; Relative humidity:  $65 \pm 5\%$ ). Tap water was freely available; access to dry powdered standard laboratory food was limited to 2 hr a day, as specified below.

#### *Apparatus*

Standard small animal test cages (Model E 10–10; Coulbourn Instr., Inc.) were used as experimental chambers; they were programmed by solid state logic modules and fitted with a house light and two levers. Between the two levers, a food pellet receptacle was mounted 2 cm above the cage floor.

#### *Training Procedure*

Daily discrimination training started after habituation of the experimental conditions and initial shaping. Thirty min before it was placed in the test cage, the rat was injected subcutaneously with either 0.04 mg/kg fentanyl or saline. Depending on whether the animal was injected with fentanyl or saline, it could obtain reinforcement by pressing either the drug lever (DL) or the saline lever (SL) respectively. That is, after every tenth press (fixed-ratio 10) on the correct lever a pellet (45 mg P. J. Noyes Precision Food Pellets) was delivered through a pellet dispenser. Responses on the incorrect lever (i.e. the SL after fentanyl and the DL after saline) produced no programmed consequences. The lever assignments were DL: left, SL: right in one half of the animals, and DL: right, SL: left in the other half. The number of responses made on either lever before the first food pellet was obtained (and, thus, before 10 responses were made on the correct lever) was recorded (symbol: FRF). The FRF reflects the accuracy of the animals' lever selection; the number by which the FRF exceeds 10, equals the number of incorrect responses emitted before the first reinforcement was obtained. Fifteen min after the rat was put into the test cage, the session was stopped and all correct and incorrect responses made in the course of the 15-min period were recorded (symbol: TR). After the session, the animal was removed to its living cage; one hr later it was allowed to feed freely for

2 hr. On weekend days (when no training was given), free access to food was allowed between 10 and 12 a.m.

Every week, each rat was run once a day on 5 consecutive days. Daily fentanyl or saline injections were given according to two weekly alternating sequences. The first sequence was: fentanyl-saline-saline-fentanyl-fentanyl; the second sequence was: saline-fentanyl-fentanyl-saline-saline. The learning criterion was reached when the animals' FRF did not exceed 12 on at least 15 consecutive training sessions. When this was accomplished, the rat was submitted to the experimental program described below.

#### *Experimental Program*

The experimental program covered a period of 17 consecutive weeks. During Weeks 1, 4, 5, 7, 8, 11, 12, 14 and 15 the above described training conditions were continued in order to provide appropriate base-line data on the reliability of lever selection and on response output; fentanyl (0.04 mg/kg) or saline injections were given according to the alternating sequences indicated above. During Weeks 2, 9 and 16 test sessions were carried out on which four doses of morphine (i.e. 2.5, 5, 10 and 20 mg/kg) were submitted for test; during Weeks 3, 6, 10, 13, and 17 four doses of fentanyl (i.e., 0.0025, 0.005, 0.01 and 0.02 mg/kg) were likewise tested. The morphine- and fentanyl test weeks consisted of four consecutive days (from Monday to Thursday) on which daily sessions were preceded by the injection of different doses of the test drug; the sequence in which those different doses were given was randomized for each rat individually and for every test week separately. If an animal were to generalize all four test treatments of either drug with the standard 0.04 mg/kg fentanyl injection, then that animal was submitted to a fifth test session which took place on Friday of the same week; the latter session was preceded by injection with the subsequent lower dose of the drug, i.e., 1.25 mg/kg morphine or 0.00125 mg/kg fentanyl. Otherwise, on Fridays, Saturdays and Sundays, no experiments were done, and the animals were allowed to feed freely between 10 and 12 a.m. For test sessions, the animal was subcutaneously injected and, 30 min later, was placed in the test cage. It was then determined on which of the two levers the animal totaled 10 responses first; this lever will be further referred to as the selected lever. Once this choice had been established, the rat obtained a first food pellet and subsequent reinforcement was made contingent upon pressing (fixed-ratio 10) the selected lever. Pressing the alternative lever again produced no programmed consequences. The following data were recorded: selected lever (DL or SL), FRF value, and TR. Body weight was measured daily, 5 days a week. Following those 15-min test sessions, the rat was transferred to its living cage; one hr later, it was allowed to feed freely for 2 hr.

#### *Drugs and Doses*

Fentanyl citrate and morphine hydrochloride were freshly prepared as aqueous solutions. All injections, including those of saline, were given subcutaneously at a volume of 1 ml/100 g body weight. The dosage levels were selected on the basis of earlier studies [5, 9, 23] indicating that the dose range 0.0025–0.02 mg/kg fentanyl is equivalent to 2.5–20 mg/kg morphine.

### RESULTS

Of the 12 rats that took part in the drug discrimination

study, 3 animals died in the course of the experimental program. The results reported here therefore pertain to the 9 surviving animals.

### Training

The 9 rats participating in the drug discrimination study required a median number of 40 training sessions (95% confidence limits (C. L.): 35–45; the numbers include the 15 criterion sessions) in order to meet the learning criterion. During the training period, mean TR ( $\pm$  SEM) increased significantly (one-tailed  $p < 0.01$ ; Wilcoxon test [30]) from 668 ( $\pm$  53.8) to 926 ( $\pm$  59.4) on saline sessions, and from 373 ( $\pm$  96.9) to 732 ( $\pm$  76.4) on 0.04 mg/kg fentanyl sessions. The comparisons are based on the first 2 and the last 2 weeks of the training period. The mean response level on 0.04 mg/kg fentanyl sessions (i.e., TR on fentanyl sessions expressed as a percentage of TR on saline sessions) also increased significantly ( $p < 0.05$ ) from 50.9% ( $\pm$  12.2) to 78.4% ( $\pm$  5.5). In contrast to the above mentioned increases which were clearly progressive, the increase of body weight (226 g  $\pm$  6.7 to 357 g  $\pm$  9.9), although significant ( $p < 0.01$ ), was not progressive and showed a high variability as a function of time.

### Experimental Program

Base-line data were obtained on Weeks 1, 4, 5, 7, 8, 11, 12, 14 and 15. The data reveal that all 9 rats reliably selected the appropriate lever after both standard treatments, i.e. the SL following saline and the DL following 0.04 mg/kg fentanyl. Lever selection was always correct in all animals, except for one inappropriate (DL-) selection by Rat 4 following saline, and for one error (SL selection) by Rat 9 following 0.04 mg/kg fentanyl. Otherwise, no errors occurred and the accuracy of lever selection, as evidenced by the FRF values, was virtually perfect; in none of the animals did the median (and 95% C. L.) FRF exceed 10 under either standard treatment (FIG. 1). As a result, each individual rat reached a significant level (one-tailed  $p < 0.001$ ; binomial test [30]) of correct lever selection. Figure 1 shows that the group mean TR on saline sessions varied between 990 (Week 12) and 1200 (Week 11). In none of the rats did the individual mean TR bear a systematic relation to time (Spearman Rank correlation coefficient:  $r_s < 0.55$ ;  $p > 0.05$  [30]), and neither did the group mean TR ( $r_s = 0.217$ ;  $p > 0.05$ ). The slight attenuation during Week 12 was due to a faulty lever which affected performance in two rats. The group mean TR on 0.04 mg/kg fentanyl sessions varied between 730 (Week 1) and 920 (Week 12). In only one rat (No. 3) was there a significant positive relationship between individual mean TR and time ( $r_s = 0.993$ ;  $p < 0.01$ ); the group mean TR did not show a significant correlation ( $r_s = 0.50$ ;  $p > 0.05$ ). The group mean response level on fentanyl sessions varied between 69% (Weeks 1 and 14) and 78% (Week 11); apart from that in Rat 3, neither individual nor group mean response levels showed a significant correlation with time ( $p > 0.05$ ). The accuracy of responding, as demonstrated by the percentage of TR on the correct lever, was constantly perfect (Fig. 1).

Test data were obtained on Weeks 3, 6, 10, 13 and 17 for fentanyl, and on Weeks 2, 9 and 16 for morphine. It follows that DL selection was always dose-related (Fig. 2). In two instances (Weeks 3 and 13), the lower fentanyl dose (i.e. 0.00125 mg/kg) had to be tested in one animal; the SL

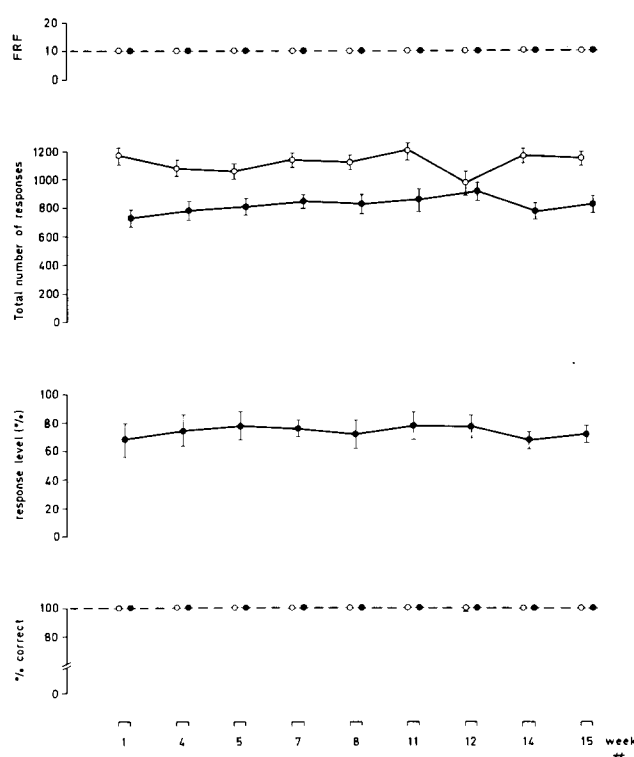


FIG. 1. Baseline data obtained in rats ( $n=9$ ) trained to discriminate 0.04 mg/kg fentanyl ( $\bullet$ ) from saline ( $\circ$ ). FRF represents the total number of responses made before the first reinforcement was obtained. The response level represents the number of responses made on drug sessions expressed as a percentage of the number of responses made on saline sessions. FRF and % on correct lever are expressed as the median and 95% confidence limits; other values are expressed as the mean  $\pm$  SEM.

was selected in each case. The baseline data obtained on 0.04 mg/kg fentanyl (100% DL selection) were incorporated into the computation of  $ED_{50}$  values [25] for stimulus generalization. These  $ED_{50}$  values appear to be remarkably constant for fentanyl as well as for morphine (Fig. 2); thus, the  $ED_{50}$  value of fentanyl on week No.17 (0.012 mg/kg) did not differ significantly (two-tailed  $p > 0.05$  [25]) from that on Week 3 (0.011 mg/kg). Likewise, no difference could be demonstrated ( $p > 0.05$ ) between the  $ED_{50}$  values of morphine on Weeks 16 (6.2 mg/kg) and 2 (6.5 mg/kg). The FRF values show that the accuracy of lever selection on test sessions was quite high; in no case was there a significant deterioration of these values, as compared to individual median values derived from baseline data (one-tailed  $p > 0.05$ ; Wilcoxon test).

For the computation of response levels, the individual TR on test sessions was expressed as a percentage of the overall (all baseline weeks combined) individual mean TR on saline sessions. Fentanyl exerted response rate increasing effects (two-tailed  $p < 0.05$ ; Wilcoxon test) in some instances, and a rate decreasing effect was found only once (0.02 mg/kg fentanyl on Week 6); no systematic change of these rate altering effects of fentanyl with time could be detected. At the dose of 2.5 mg/kg, morphine did not affect responding in any case; however, higher doses (5–20 mg/kg) consistently decreased response rate ( $p < 0.05$ ) in a dose-related way. Again, no systematic alteration on these

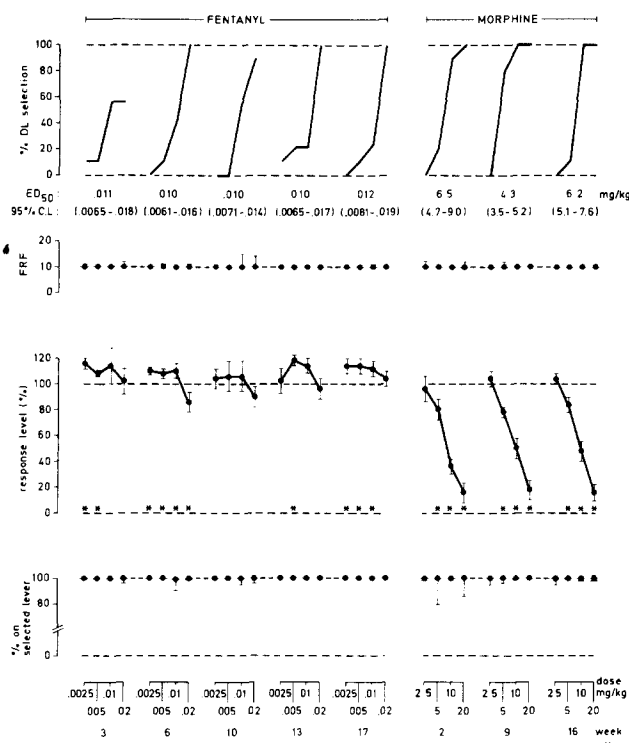


FIG. 2. Data from generalization experiments with fentanyl (left) and morphine (right). The upper graph represents the percentage of animals that select the DL upon injection with different doses of fentanyl or morphine. The asterisk denotes two-tailed  $p < 0.05$  (Wilcoxon). Other specifications as in Fig. 1.

effects with time was evident. As a rule, the percentage of TR on the selected lever amounted to 100%; although in some instances this value was slightly lower than the corresponding baseline data, no significant deterioration was ever found.

#### DISCUSSION

The baseline data obtained in the present experiments indicate that all 9 rats had learned to use the injections of 0.04 mg/kg fentanyl and saline as discriminative stimuli; each animal reached a highly significant level ( $p < 0.001$ ) of correct lever selection, and the accuracy of lever selection (FRF) and of further responding (% of TR on correct lever) was virtually perfect. The TR data (Fig. 1) show that there was a minor week-to-week variation, but no systematic relation to time was evident. In all animals except one (No. 3), the progressive tolerance to the response rate decreasing effect of 0.04 mg/kg fentanyl, which was evident during training, apparently had ceased in the course of the experimental program. The stimulus generalization experiments (Fig. 2) show that the  $ED_{50}$  value of fentanyl to produce the narcotic cue did not appreciably shift over a period of 4 months; this lack of tolerance to the discriminative stimulus properties is the more striking as, during this period, the animals were repeatedly given a fentanyl dose (0.04 mg/kg) 4 times higher than the  $ED_{50}$  value (0.01 mg/kg). The morphine experiments likewise failed to provide any evidence for the occurrence of cross-tolerance.

Therefore, it is concluded that the discriminative stimulus properties of narcotic analgesic drugs are not subject to tolerance when assessed under the conditions described here.

#### EXPERIMENT 2

It has been shown recently [9] that there exists a virtually perfect correlation between the discriminative stimulus properties of narcotic analgesics on the one hand, and the analgesic effects of these drugs on the other. In this study [9], the stimulus properties were investigated in a group of rats trained as described here (Experiment 1), whereas analgesic effects were studied in drug-naïve animals. However, although it is well-known that rats and other animal species become readily tolerant to the analgesic effects of narcotic analgesics, Experiment 1 indicated that rats do not to any appreciable degree become tolerant to the discriminative stimulus properties of these drugs. This strongly suggests that the above mentioned correlation merely reflects drug potency and, more important, that the discriminative stimulus properties and analgesic effects of narcotic analgesics are based upon distinct pharmacological actions.

As a test for the latter hypothesis, Experiment 2 attempted to verify whether tolerance to the analgesic effect of a single fentanyl dose would be evident in the rats that had failed (Experiment 1) to develop any tolerance to the discriminative stimulus properties of the same drug.

#### METHOD

##### Animals

The experimental group consisted of the 9 rats that had completed the entire experimental program of Experiment 1. The control group was composed of 9 experimentally naïve male Wistar rats (age:  $24 \pm 3$  weeks) the body weight of which was matched ( $\pm 10$  g) with that of the experimental animals. These 9 controls were selected from a group of rats whose food access had been restricted between the ages of 2 and 6 months in order to match the body weight of the experimental group as closely as possible.

##### Procedure

The procedure used to assess analgesic drug effects measures the inhibitory effects of drugs on the withdrawal reflex induced by application of warm water to the tail, and has been extensively described previously [23]. The animals were placed individually in standard rat holders with the tail hanging free outside the holder. The pre-injection reading in both groups consisted of dipping the tail into a warm ( $55 \pm 1^\circ\text{C}$ ) water bath and determining the reaction time for tail withdrawal. Cut-off time was 10 sec. Immediately after the preinjection reading, all rats were given 0.02 mg/kg fentanyl citrate (volume: 0.2 ml/100 g body weight), injected into a tail vein. Thereafter, readings were done at 1/2, 1, 5, 10, 15, 20, 30, 40, 60 and 90 min after fentanyl injection. Experiment 2 took place at 2 p.m. on Friday of Week 17.

#### RESULTS AND DISCUSSION

Large-scale experiments with the tail withdrawal procedure in rats [23] have shown that 0% of the saline

treated (control) rats ( $n = 580$ ) show a reaction time  $\geq 10$  sec under the conditions applied here. Therefore, the data obtained in the present experiments are represented (Fig. 3) as the number of rats exhibiting a reaction time  $\geq 10$  sec; any such rat is considered to be positively affected by the analgesic effect of the drug. The preinjection reading ( $t_0$ ) showed that the median reaction time (and 95% C. L.) of the experimental group (2.0 sec (1.2–3.0)) did not differ (two-tailed  $p > 0.05$ ; Mann-Whitney U-test [30]) from that of the drug-naive control group (2.0 sec (1.8–2.8)). The subsequent intravenous injection of 0.02 mg/kg fentanyl induced analgesia within one min in all control rats, as well as in all experimental animals (Fig. 3). However, the duration of analgesia markedly differed ( $p < 0.001$ ) between the two groups; the median value was 40 min (20–60) in the control group, and only 10 min (5–10) in the experimental group.

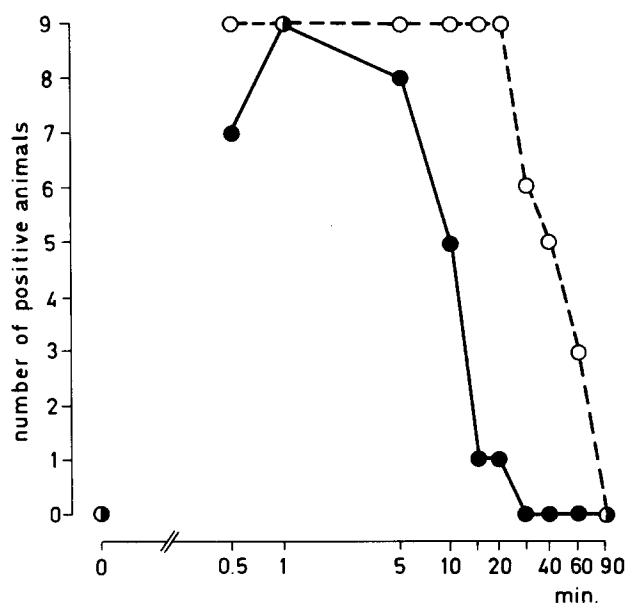


FIG. 3. Analgesic effect of the intravenous injection of 0.02 mg/kg fentanyl in the 9 rats that participated in the drug discrimination experiments (●), as well as in a group of 9 drug-naive animals (○). The data pertain to a reaction time experiment for tail withdrawal from a warm water bath. An animal is considered positive if its reaction time for tail withdrawal is equal to or exceeds 10 sec.

The present data in drug-naive animals essentially duplicate the earlier finding [23] that the intravenous injection of 0.02 mg/kg fentanyl produces immediate analgesia in 100% of the rats tested, the duration of the effect being  $\pm 40$  min. In the group of animals that failed to show any tolerance to the discriminative stimulus properties of fentanyl (Experiment 1), the duration of analgesia produced by the same treatment was reduced to 1/4 of the control value. The difference between both groups was highly significant ( $p < 0.001$ ), and indicates that tolerance to the analgesic effect of fentanyl had developed in the experimental group. In addition it was found that, within the experimental group, there was no significant correlation ( $r_s = -0.03$ ;  $p > 0.05$ ) between the duration of analgesia on the one hand, and the fentanyl threshold dose for stimulus generalization (DL selection) during Week 17 on the other.

These results strongly evidence that the discriminative stimulus properties and analgesic effects of narcotic analgesics are based upon pharmacological actions which, to a major extent, are distinct. They further imply that the correlation which exists between both phenomena [9] does not indicate similarity of action and, hence, merely reflects drug potency.

### EXPERIMENT 3

In their study on the ability of morphine to control operant behavior in rats, Hirschhorn and Rosecrans [22] showed that this ability is subject to tolerance. However, the latter conclusion was based on data obtained from animals which appeared to be dependent on narcotic drugs. That is, the intraperitoneal injection of the narcotic antagonist [4] naloxone (1 mg/kg) in these rats immediately elicited diarrhea, salivation and some loss of body weight [22]. It is conceivable, therefore, that the morphine produced stimulus controlling operant responding in that study, was constituted by the drug action underlying narcotic produced relief from (dysphoric) narcotic withdrawal symptoms. This would fit the well known fact that the dose of narcotic drug required to prevent the narcotic withdrawal syndrome in narcotic dependent organisms increases with increasing degree of dependence. Were this interpretation valid, then the inconsistency which seemingly exists between the present study (experiment 1) and that by Hirschhorn and Rosecrans [22] would be explainable on the basis of the relative degree of narcotic addiction in the rats which participated in these studies. Experiment 3 therefore sought to determine whether there was any evidence for narcotic dependence in the rats that participated in Experiment 1. The dependence study comprised an acute (naloxone-precipitated abstinence) as well as a chronic (narcotic withdrawal) phase.

### METHOD

#### Precipitated Abstinence

Precipitated abstinence was looked for upon intravenous (tail vein) injection of 10 mg/kg naloxone hydrochloride (volume: 0.2 ml/100 g body weight); this injection took place at 10 a.m. on Monday of Week 18, and was followed by a 2 hr observation period during which a number of narcotic abstinence signs were assessed. The signs were: diarrhea, salivation, lacrimation, teeth chattering, myoclonic twitch and repetitive shaking movements (wet dog shakes), irritability to handling, and palpebral opening upon handling (ptosis). In addition, body weight was measured at the end of the 2 hr observation period.

#### Narcotic Withdrawal

On the subsequent days of Week 18 (i.e. from Tuesday to Friday) further 1-hr observations were done to assess the possible occurrence of the withdrawal signs listed above. Thus, since the fentanyl (0.02 mg/kg, intravenous injection on Friday of Week 17 (Experiment 2) the animals had not been given any further injection, and apart from the above indicated naloxone treatment, neither drugs nor saline were administered during Week 18. In addition to those daily observation periods, the animals were allowed to bar press for food on the lever they selected at the onset of each 15-min session in the test cages. Maintenance conditions were similar to those described under Experiment 1.

## RESULTS AND DISCUSSION

The intravenous injection of 10 mg/kg naloxone in all 9 rats employed in Experiment 1 failed to induce any of the behavioral signs reminiscent of narcotic dependence. None of those signs was observed in any animal either during the 2-hr observation period immediately following the naloxone challenge, or during the 1-hr observation sessions held on the subsequent days of Week 18. Also, neither the mean body weight two hrs after naloxone ( $360 \text{ g} \pm 20.9$ ), nor the overall mean value for Week 18 ( $367 \text{ g} \pm 17.9$ ) differed significantly (one-tailed  $p > 0.05$ ; Wilcoxon test) from the overall mean value for Week 17 ( $365 \text{ g} \pm 17.7$ ). From Tuesday to Friday of Week 18, the animals were allowed to bar press for food during daily 15-min sessions in the test cage; all rats always selected the SL with a FRF value of 10. Individual mean TR values for those 4 sessions were computed (group mean:  $1147 \pm 99.7$ ), and were compared to the individual overall (all weeks combined) mean TR values derived from the baseline data (group mean:  $1120 \pm 68.4$ ); there was no significant difference (two-tailed  $p > 0.05$ ; Wilcoxon test) between the two samples.

There can be little doubt that the intravenous injection of a naloxone dose as high as 10 mg/kg effectively precipitates the abstinence syndrome in narcotic dependent rats [15, 32, 33], and the relevance of the abstinence signs which were assessed in the present study is likewise well-documented [e.g. 1, 3, 11, 14, 20, 27]. Nevertheless, neither the naloxone challenge, nor the withdrawal from narcotic injection for a period of 7 days induced any of those signs in any of the 9 rats tested. In addition, it was found that neither naloxone nor narcotic withdrawal decreased operant responding for food, even though this responding is very sensitive to those events in narcotic dependent rats [16, 17, 34].

This warrants the conclusion that the rats which participated in the drug discrimination study (Experiment 1) were not at all dependent on narcotic drugs.

## GENERAL DISCUSSION

The major outcome of the present study (Experiment 1) is that 9 rats, following a training period of 2 months, failed to demonstrate even the slightest tolerance to the discriminative stimulus effects of the narcotic analgesics fentanyl and morphine. During the course of the 4-month test period, neither the level selection nor the percentage of responding on the DL upon 0.04 mg/kg fentanyl injection was attenuated (Fig. 1). Also, there was no detectable change of the  $ED_{50}$  values of either fentanyl ( $\pm 0.01 \text{ mg/kg}$ ) or morphine ( $\pm 6 \text{ mg/kg}$ ) for generalization with the standard fentanyl treatment (Fig. 2). This result sharply contrasts with the conclusions reached by Hirschhorn and Rosecrans [22] who found that, in 5 rats that were given a training of the same duration, the ability of morphine to control operant responding significantly decreased over a period of 2 months. The authors interpreted their findings in terms of the observation [19] that tolerance to the various actions of morphine develops unequally, and speculated that tolerance to the stimulus effects of morphine would develop more slowly than that to the analgesic or lethal effects of the drug.

Four alternatives emerge to explain these seemingly contradictory findings.

One is that the training drug used here, fentanyl, may produce relatively less tolerance than morphine, or that the amount of narcotic drugs injected during the course of the experiments was too low to produce any tolerance at all.

This interpretation is invalidated by the fact that, in the present study, tolerance did occur to the response rate decreasing (Experiment 1) as well as to the analgesic effects (Experiment 2) of fentanyl, and comparative data on fentanyl and morphine [23] likewise fail to suggest any significant qualitative difference between the two drugs. It is interesting to note that the tolerance to the response rate depressant effects which occurred during training, was only partial (response level from 50.9 to 78.4%), and did not develop further during the subsequent 4 months of the experiments (Fig. 1). Nevertheless, it is unlikely that a similar phenomenon would account for the lack of tolerance to the discriminative stimulus properties of fentanyl because there is no relationship between those properties and the response rate modulating effects of narcotic analgesics [5, 6, 7, 8], and because Hirschhorn and Rosecrans [22] observed tolerance long after a 2-month training period during which comparable amounts of narcotic drugs were injected.

The second possibility is that the tolerance to morphine's stimulus properties observed by these authors [22] is related to the induction of dependence; the lack of any such tolerance observed in the present study would then be consistent with there being no evidence whatsoever for dependence in the subjects used here (Experiment 3). Thus, the morphine produced stimulus controlling operant responding in narcotic dependent rats would be constituted by the drug action underlying narcotic-produced relief from withdrawal symptoms, and it is readily conceivable that such drug action may differ from the one underlying the intrinsic discriminative stimulus properties of narcotic drugs in nondependent subjects. It is also conceivable that narcotic dependence may have exerted an unspecific deterioration of differential responding. However, any interpretation of the possible significance of dependence to the occurrence of tolerance in the study by Hirschhorn and Rosecrans [22] remains inconclusive, as it is unknown whether the subjects used in that study were only dependent after, or also during the stimulus-experiments.

Thirdly, the possible occurrence of tolerance to the cuing action of fentanyl and morphine may have been compensated for by an adaptive learning process. This is to say that the control sessions which were held between test weeks may have provided the opportunity to learn an increasingly difficult discrimination task. The latter interpretation would imply that progressively fewer animals detect fentanyl doses lower than 0.04 mg/kg, and that the stimulus generalization gradient steepens progressively. This is because the learning effect — if present — pertains to the 0.04 mg/kg fentanyl dose, and there is no reason to assume that it might be transferred to the lower dosages as well. In the present study, the slopes of the dose-response curves for fentanyl (Weeks 3 and 17) and morphine (Weeks 2 and 16) were parallel, and this result seems to rule out the third alternative. Also, possible learning effects can have occurred in a small portion only of the rats used, since but two of them only once selected the lever inappropriate to the control injection given.

The fourth alternative is that the stimulus functions subserved by morphine [22] and fentanyl (Experiment 1) were different in the two studies. Whereas the method

employed here is designed to specifically investigate the discriminative stimulus properties of drugs [10], the stimulus function involved in the morphine study [22] is less clear-cut. There seem to be two major differences between the methods used. One is that the level of response control achieved in the morphine study amounted to approximately 80%, whereas that in the present study persisted at 100% over a period as long as 4 months (Figs. 1 and 2). This is intriguing, as the duration of training was virtually the same in the two studies. The second difference refers to the measurement of discriminative responding itself; in the present study, the animals were required to select one of two levers on the basis of the perceived difference between the injection of a narcotic and that of saline, and lever selection was immediately followed by food reward. In the morphine study [22], the measurement consists of the percentage of responding on the morphine-appropriate lever, assessed during an extinction trail. The latter procedure, in addition to providing a valid indication of differential responding, may be confounded [10] by state-dependent learning effects, as well as by response probing due to absence of reinforcement. It is obvious that response probing may account for any approach of the measurement to the 50% level; however, state-dependent learning effects may affect this percentage only during the drug condition, but not during the saline condition. The significant decrease of the percentage of drug-appropriate responses during the drug condition in the morphine study [22] thus may reflect tolerance to state-dependent learning effects. As a result, the inconsistency mentioned above would be based upon the training drug serving a different stimulus function in the two studies, and hence would reflect that state-dependent, but not drug

discrimination learning with narcotic analgesics is indeed subject to tolerance.

The lack of tolerance to the discriminative stimulus properties of narcotic analgesics, as established here, is consistent with clinical observations on subjectively experienced effects of these drugs in humans. These observations on subjectively experienced effects of these drugs in humans. These observations [12, 13, 24, 26] indeed indicate that the ability of humans to discern the injection of a narcotic analgesic from that of either placebo or a nonnarcotic drug, is not subject to tolerance. In fact, addicts or post-addicts appear to be even more sensitive and reliable in this respect than naive animals [12,26]. The similarities between the clinical technique applied in those studies [12,13] and the method used here (Experiment 1) are striking, and support the suggestion [5,6] that the discriminative stimulus properties of drugs, as assessed by this animal method [10], may be relevant to subjectively experienced drug effects in humans.

In conclusion, the present studies indicate that, in rats, the discriminative stimulus properties of narcotic analgesics are not subject to any detectable tolerance. This result was obtained in rats which, though not being dependent, demonstrate clear-cut tolerance to the analgesic effects of fentanyl. Dependence and state-dependent learning effects may be involved in seemingly conflicting data [22] on the stimulus properties of narcotic analgesics. The present results also evidence that the discriminative stimulus properties and analgesic effects of narcotic analgesics are based upon distinct pharmacological actions. The previously reported [9] correlation between both phenomena probably reflects mere drug potency.

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