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Schedule-Controlled Behavior in the Morphine-Dependent Rat[1]

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FORD, R. D. AND R. L. BALSTER. *Schedule-controlled behavior in the morphine-dependent rat.* PHARMAC. BIOCHEM. BEHAV. 4(5) 569–573, 1976. – The behavioral effects of acute and chronic administration of morphine and its withdrawal were studied using schedule-controlled responsing in the rat under a differential reinforcement of low rates of responding (DRL) schedule of food presentation. Acute morphine administration had a biphasic effect on response rate. Low doses (1.8–5.6 mg/kg) tended to produce a small increase and higher doses (10–30 mg/kg) decreased responding. Physical dependence was produced by twice daily injections, with an initial dose of 40 mg/kg/day which was increased by 80 mg/kg/day until reaching 600 mg/kg/day which was continued for 14 days. Throughout chronic administration the pattern of responding remained disrupted resulting in a 27 47 percent decrease in presentations of the reinforcer, while response rate was more variable and generally decreased. The effects of morphine withdrawal lasted 5 days and produced an initial marked decrease in reinforcements per hour and a biphasic change in response rate. A marked decrease in responding early in withdrawal (22.5 hr) was followed by a marked and more prolonged (70.5 - 118.5 hr) response rate increase.

Morphine Physical dependence Operant behavior Differential reinforcement of low rate schedule
Withdrawal Tolerance

CHRONIC administration of morphine to laboratory animals and humans often results in the development of physical dependence which is manifested by a number of characteristic autonomic and behavioral signs when drug administration is discontinued. Although the physiological correlates of chronic morphine administration and withdrawal in rats have been carefully studied, there is relatively little information on the behavioral correlates and this data is often in disagreement. For example, some investigators have characterized the behavioral effects of morphine withdrawal in the rat as "sedation" [11] and decreased motor activity [14], while others describe "extreme irritability" [2] and "increased excitability and increased activity" [12]. Certainly these discrepancies are due in no small part to differences in pharmacological variables such as dose, length and frequency of administration, tolerance development, whether the withdrawal syndrome was produced by cessation of morphine administration or by the administration of a narcotic antagonist and when the behavior was measured. However, an important contributing factor as noted by Martin *et al.* [13] is the behavior was characterized "on the basis of unquantified observations under environmental conditions that were not rigorously defined."

Schedule-controlled behavior has been used successfully as a stable and sensitive baseline upon which to study the development of behavioral tolerance to the effects of a wide range of drugs, in several animal species, using various schedules of reinforcement [5, 6, 7, 16, 20]. However, rarely have studies sought to use these behavioral baselines to detect, on cessation of administration, a withdrawal syndrome indicative of physical dependence. Such procedures might be useful for qualitatively and quantitatively assessing the behavioral effects associated with a withdrawal syndrome.

Profound withdrawal syndromes occur on the cessation of chronic administration of the narcotic analgesic and sedative-hypnotic classes of drugs [4]. Recently marked alterations in schedule-controlled behavior, indicative of withdrawal syndrome, were demonstrated to occur on cessation of chronic morphine administration to rhesus monkeys [10] and following abstinence from prolonged ethanol drinking in rats [1]. We report here on the effects of chronic morphine administration and withdrawal on the schedule-controlled behavior of rats. The behavior was engendered by the same differential reinforcement of low rate (DRL) schedule as that used by Ahlenius and Engel [1] to detect an ethanol withdrawal syndrome. The

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regimen of chronic morphine administration used here was similar to those previously demonstrated to produce physical dependence on morphine in rats [3,13].

METHOD

Animals

Six male Sprague-Dawley (Holtzman) rats with no previous experimental history and weighing between 314 and 364 g when given free access to food and water were used. They were deprived of food until they reached 85% of their free-feeding weight, and subsequently maintained at this weight by adjusted feedings after each experimental session. Except for an hour each day consumed by the experimental session, rats were housed individually with free access to water.

Apparatus

Rats were trained to bar press for food reinforcement in standard operant chambers, each equipped with one response bar. A bar press with a force of approximately 15 g constituted a response. Centered in the wall containing the response bar was an opening through which 45 mg Noyes food pellets could be made accessible by means of an electrically operated dispenser. Each chamber was illuminated by a 6 W bulb and placed in a ventilated enclosure for light and sound attenuation. Solid state programming equipment located in an adjacent room was used to control the experimental contingencies and record data.

Procedure

On the initial day in the experimental chamber, rats were trained to bar press and each response resulted in the presentation of the food reinforcer. On subsequent days, the session time was set at 1 hr and the rats were placed on a 15-sec differential reinforcement of low rates of responding (DRL 15) schedule. On this schedule interresponse times (IRTs) of 15 sec or longer were reinforced by the presentation of a food pellet. An IRT distribution was obtained for 3 of the animals by recording successive 3-sec IRTs in nine successive intervals, and all IRTs of 27 sec or more were recorded in the tenth interval.

Drug Procedure

Morphine sulfate was dissolved in normal saline and doses are in terms of the salt. After responding on the DRL 15 schedule stabilized (about 20 sessions) an acute dose-response curve for morphine was obtained. All injections were IP 15 min before the initiation of a session. The volume injected was 1 ml/kg. Control injections consisted of only the saline vehicle. Doses were administered weekly in an unsystematic order.

After an acute morphine dose-response curve was obtained, chronic doses of morphine were administered in a regimen similar to that described by Buckett [3] and Martin *et al.* [13] as producing physical dependence to morphine in rats. All rats were injected twice each day. Four rats were injected at 0900 and 1630 hr, and the remaining 2 rats were injected at 1015 and 1745 hr. Experimental sessions were initiated at 1500 and 1615 hours, 6 hr after the morning injection. The initial dose of 20 mg/kg was administered twice on Day 1. Subsequently, the dose was increased by 40 mg/kg each day until on Day 8 the dose reached 300 mg/kg. The 300 mg/kg dose continued to be administered twice daily for 14 days. After Day 21, morphine administration was stopped.

During the period of chronic administration, doses of morphine of 100 mg/kg or less were injected in a volume of 1 ml/kg. A 100 mg/ml solution was used to administer larger doses, so that the volume administered varied with dose and was 3 ml/kg for the largest dose of 300 mg/kg. Injections during the initial period of chronic administration were IP; however, on the last 5 days of administration, injections were given s.c. due to signs of ulceration of the peritoneum.

RESULTS

The DRL 15 schedule of food reinforcement engendered in each rat the characteristic steady pattern of low rate responding which has been previously described [15,18]. The control data for the acute morphine dose-response curve was obtained from saline control sessions on the day preceding each drug session. The control data as mean response rate and mean reinforcements per hr is shown in Fig. 1, while the mean IRT distribution is shown in the upper left panel of Fig. 2. During control sessions the mean response rate was 0.068 responses per sec, and this produced on the average 151 reinforcements during an hour session. The IRT distribution for three rats shows that most responses occurred with IRTs of 15–18 and 18–21 sec and that shorter and longer IRTs were relatively infrequent.

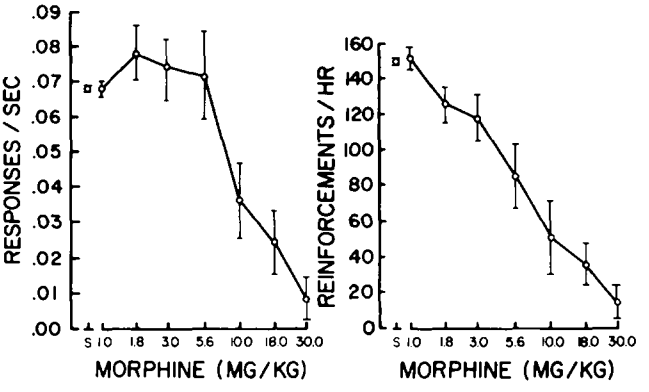

FIG. 1. Effects of acute doses of morphine on rate of responding and reinforcements per session under DRL 15 schedule of reinforcement. Abscissas: dose, log scale. Ordinates: response rate and number of presentations of the food reinforcer during an hour session. The vertical lines show one standard error above and below the mean. The points at S are the means of saline injection sessions for 6 rats on the day before each drug session (42 sessions). Other points represent the mean of one sessions for six rats at each dose of morphine.

As indicated in Fig. 1, a 1 mg/kg dose of morphine did not alter the mean response rate nor the number of presentations of the reinforcer. Acute morphine doses of 1.8, 3.0 and 5.6 mg/kg only tended to increase the mean response rate. Although the mean response rates at these doses do not differ significantly from control rates as determined by a paired t comparison, one of more of these doses produced a substantial response rate increase in each

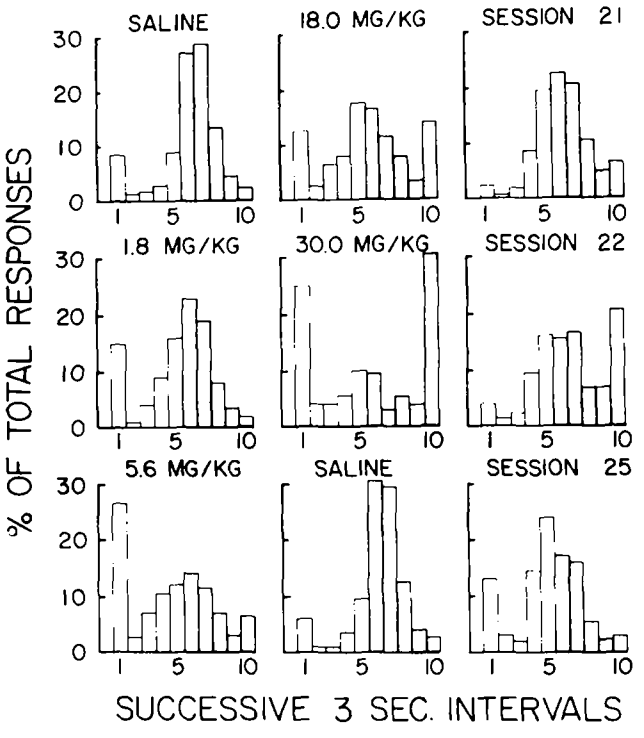

FIG. 2. The mean inter-response time (IRT) distribution for 3 rats during acute and chronic administration of morphine and its withdrawal. IRTs are distributed into nine successive 3-sec class intervals and all IRTs longer than 27 sec are in the tenth class interval. The shaded area indicates the percentage of IRTs which were longer than 15 sec and reinforced. The unshaded area indicates the percentage of IRTs which were shorter than 15 sec and not reinforced. The saline distribution (top left) is the mean of saline injection sessions for 3 rats on the day before each acute drug session (21 sessions). Each IRT distribution for acute morphine doses (1.8, 5.6, 18 and 20 mg/kg) is the mean of 1 session for 3 rats at each dose of morphine. The saline distribution (center at bottom) is the mean of daily saline injection sessions for 3 rats on the 6 days preceeding chronic administration (18 sessions). The IRT distributions for Sessions 21, 22 and 25 are each the mean of 1 session for 3 rats.

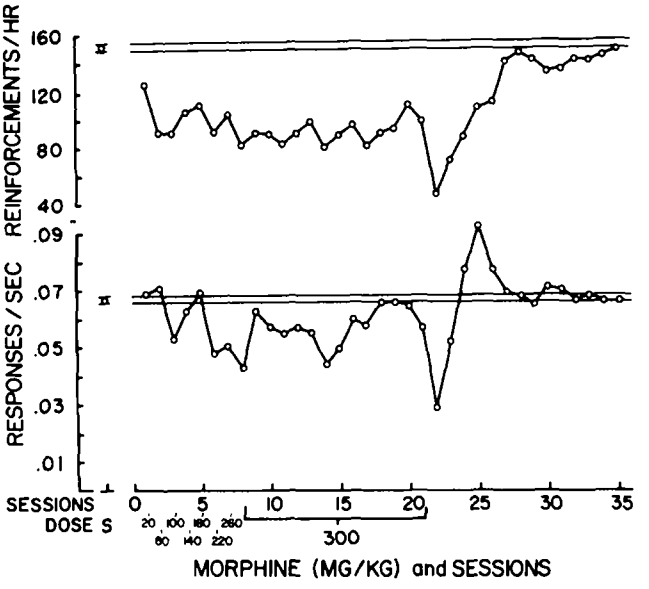

FIG. 3. Effects of chronic morphine administration and its withdrawal on rate of responding and reinforcements per session under DRL 15 schedule of reinforcement. Abscissa: daily sessions and dose of morphine administered twice each day. Ordinates: response rate and number of presentation of the food reinforcer during an hour daily session. The points at S are the means of daily saline injection sessions for 6 rats on the 6 days preceeding chronic administration (36 sessions). The horizontal lines show 2 standard errors above and below the mean values of these 6 days. Other points represent the mean of 1 daily session for 6 rats.

rat. Higher doses (10–30 mg/kg) of morphine resulted in a dose-related decrease in responding ($p \leq 0.05$). The number of presentations of the reinforcer was progressively decreased as a function of acute morphine doses over the entire dose range. The effects on the IRT distribution produced by acute morphine doses are illustrated in Fig. 2. Doses of 1.8 and 5.6 mg/kg produced an increase in the percentage of short IRTs, while the highest doses of 18 and 30 mg/kg resulted in increases in the relative frequency of both short and long IRTs. Of the few responses that did occur following 30 mg/kg, nearly all responses had IRTs of less than 3 sec or more than 27 sec.

The control baseline performance for chronic morphine administration is provided in terms of mean response rate and reinforcements per hour by the two points at the left in Fig. 3. These values were obtained from the 6 days immediately preceding chronic administration. A low response rate was engendered (0.067 responses/sec) which produced a mean of 152 reinforcements per hr. The control IRT distribution for 3 rats, shown in the lower center panel of Fig. 2, was almost identical to the control distribution during the determination of the acute dose-response curve and indicates that most responses had IRTs of 15 to 21 sec and were reinforced.

The effects of 21 days chronic morphine administration and subsequent withdrawal on mean reinforcements per hour and response rate are shown in Fig. 3. During the first daily session, 6 hrs after 20 mg/kg, the mean rate of reinforcement was decreased to 82% of baseline. Subsequently, the mean reinforcements per hr remained decreased, from 53 to 73% of baseline, throughout the period of chronic administration. The mean response rate was more variable but was generally decreased during chronic morphine administration. However, some tolerance to this effect is suggested during the last 5 days, as the mean response rate was near or within the control baseline on three of these days.

Since, toward the end of chronic morphine administration, the mean response rate was within or near the control baseline, while at the same time the number of reinforcements per hr was markedly decreased, this indicates an alteration in the pattern of responding. It is shown in Fig. 2 that the IRT distribution for Session 21, the last day of morphine administration, is shifted to the left from the control distribution. In comparison with the control distribution, there is a substantial increase in the percentage of responses with IRTs of 12–15 sec, while the percentage of IRTs of 15 21 sec is decreased. In addition and unlike the effects produced by acute doses of morphine, the percentage of very short (<3 sec) and very long (>27 sec) IRTs is not much altered from the control distribution. This change in the IRT distribution produced

by chronic doses of morphine suggests an effect that is distinct from those obtained with acute morphine doses.

Morphine administration was stopped following the second injection on Day 21. The sessions on Day 22 were initiated 22.5 hr after the last injection and, as shown in Fig. 3, both the mean response rate and number of reinforcements per hour were markedly decreased. On subsequent days, the mean response rate progressively increased until a maximum on Day 25, which was markedly increased from the control baseline. By Day 28 both the mean response rate and number of reinforcements per hour approximated control values.

Figure 2 shows the mean IRT distribution for session 22, when the mean response rate was markedly decreased, and for session, 25, when the mean response rate was markedly increased. Similar to the effects produced by high acute morphine doses, the mean IRT distribution for session 22 was shifted to the right with an increase in the percentage of long IRTs. However, there was no increase in very short IRTs as seen with acute morphine administration. On the other hand, much like the effects produced by low acute doses of morphine, the mean IRT distribution for session 25 was shifted to the left by an increase in the percentage of short IRTs.

DISCUSSION

Acute morphine administration resulted in a biphasic effect on mean response rate. Low doses (1.8–5.6 mg/kg) tended to produce a small increase in responding and higher doses (10–30 mg/kg) produced a decreased response rate. This biphasic effect on low baseline response rates has previously been demonstrated in the rat [8,9] and pigeon [7,17]. The effects of morphine on the pattern of responding was, at low doses, an increase in the frequency of very short IRTs and at higher doses an increase in the frequency of both very short and very long IRTs. This disruption in the pattern of responding, along with the effects on mean response rate, resulted in a dose-dependent decrease in reinforcements per hour over the entire range of active doses.

Throughout the period of chronic morphine administration response patterning remained disrupted resulting in a consistently lower frequency of reinforced responses. Overall response rate was more variable and mostly decreased from the control baseline. There was some evidence that mean response rate was returning to baseline levels toward the end of chronic administration after daily increases in dose were terminated and the route of administration was changed from IP to SC.

Tolerance to the effects of morphine on schedule-controlled behavior have been reported to occur in the pigeon [7], cat [5] and rhesus monkey [10]. In all these studies a single dose was administered daily until tolerance development was observed. In the present study the purpose was to attain high dose levels as quickly as possible so that tolerance development was not allowed to occur before dose increases. It is probable that this masked most of the evidence for tolerance. However, the general lack of effect on overall response rate, the decrease in very short IRTs relative to acute morphine effects, and only a 35% decrease in reinforcement frequency for Session 21, six hours after 300 mg/kg was administered (600 mg/kg over the preceding 24 hr) provide some evidence that behavioral tolerance occurred with this dose regimen.

A marked alteration of behavior from both the control baseline and the more variable baseline during chronic administration occurred following the cessation of morphine administration. That this alteration in behavior is indicative of a morphine withdrawal syndrome is suggested by its onset and duration, as well as the regimen of prior morphine administration. There is general agreement in the literature that the chronic administration of high doses of morphine (100 mg/kg/day or more) to rats results on cessation in a withdrawal syndrome with an onset of from 8–16 hr which largely subsides in less than 168 hr [2, 13, 14, 19]. In the present case, the schedule-controlled behavior of rats was assessed during one hour daily sessions. The onset of the effects produced by stoppage of morphine administration was less than 22.5 hr, as indicated by the marked decrease in mean response rate and frequency of reinforcement during the sessions on the first day off of chronic drug. Six days (142.5 hr) after the last dose of morphine, responding of rats approximated control baseline levels. This duration of a morphine withdrawal syndrome in rats is consistent with those of 5 to 6 days as determined by increased incidence of "wet dog" shakes [13], and of 120 hours for the "fighting of rats during post-morphine withdrawal" [19].

Morphine withdrawal produced a biphasic effect on mean response rate. A marked decrease in responding early in withdrawal (22.5 hr), was followed by a marked and more prolonged (70.5 to 118.5 hr) response rate increase. During the first phase of decreased response rates, characterized by long IRTs and few response bursts, the animals demonstrated overt signs of morphine withdrawal. Diarrhea and weight loss were the most notable. The period of high response rates may correspond to the "increased excitability and increased activity" observed by others [2,12]. Holtzman and Villarreal [10] observed an increase in low rates of punished responding during morphine withdrawal in rhesus monkeys. They suggested that low rate responding may in general be increased by morphine withdrawal. Our results would support and extend this finding.

In conclusion, we have shown that the behavioral sequelae to chronic morphine administration and withdrawal can be studied under conditions suitable for careful quantification. It has been suggested [10] that schedule-controlled behavior may provide a useful means of evaluating drugs for physical dependence liability since a highly stable and specific baseline is used. Drugs which produce morphine-like physical dependence should readily substitute for morphine and reverse the behavioral effects during withdrawal. Since such a drug would be expected to prevent both the response rate decreasing and increasing effects of withdrawal, a great deal of specificity would be required.

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