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Dose and Physical Dependence as Factors in the Self-Administration of Morphine by Rats*

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Abstract. Groups of naive rats were offered morphine sulfate for self-administration in doses of 0.0032 – 10 mg/kg for 6 days. On day 7 saline was substituted for morphine. Loss of weight was taken as physiological evidence of dependence. Rats that did not lose weight formed a single population whose mean injection rate did not differ from control rats receiving only saline injections. Injection rates for rats losing weight were log-normally distributed, and the mean of the logarithms of the injection rates was linearly related to the logarithm of the dose. Mean daily injection rates averaged 12 for controls, 23 at 10 mg/kg, and 411 at 0.01 mg/kg. A transient increase in morphine intake after an injection of nalorphine was taken as behavioral evidence of dependence. Nalorphine increased morphine intake when rats were self-injecting 0.32 and 1.0 mg/kg of morphine, but not 0.032 or 0.1 mg/kg. The reinforcing property of morphine may occur without behavioral evidence of dependence.

Key words: Morphine – Rat – Self-administration – Physical dependence – Addiction – Reinforcement – Behavior.

Many studies have demonstrated that rats and monkeys self-administer morphine and other opiates IV on a sustained basis (Schuster and Thompson, 1969). If, as a consequence of drug intake, physical dependence is induced, self-administration could be due to relief of an impending withdrawal reaction, that is, "escape training" (Nichols et al., 1956). However, both monkeys (Deneau et al., 1969) and rats (Smith et al., 1976) will initiate self-administration of morphine without prior drug treatment indicating that relief of physical dependence is not needed initially for morphine to serve as a reinforcing agent. In monkeys, Woods et al. (1968) showed that the reinforcing property of morphine was a function of unit dose and that 10 μg/kg supported self-administration without concomitant development of physical dependence. Hoffmeister and Schlichting (1972) noted that no physical dependence was associated with monkeys self-administering codeine in 3-h sessions several days a week. Crowder et al. (1972) noted that morphine injections could elicit conditioned responses without gross evidence of physical dependence. However, the relapse to morphine self-administration by previously physically dependent ("post-addict") rats seemed most likely to be a consequence of a conditioned withdrawal reaction (Weeks and Collins, 1968). The contribution of physical dependence to maintenance of sustained morphine self-administration is still not clear.

Loss of body weight after discontinuing morphine injections is a commonly used and sensitive measure of the withdrawal syndrome in rats (Akera and Brody, 1968; Nurimoto, 1973; Nozaki et al., 1975). Injections of an antagonist, such as nalorphine, to an opiate-dependent animal will precipitate withdrawal. If a rat or monkey is maintaining dependence by morphine self-administration, a series of injections will follow the injection of the antagonist, presumably in an attempt to overcome the induced withdrawal (Weeks, 1962; Goldberg et al., 1971).

In this study we analyzed the contribution of dose and physical dependence to the initiation and maintenance of morphine self-administration in rats. In the first experiment, groups of naive rats were offered morphine at different doses for 6 days, followed by substitution of

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saline for 1 day. A significant loss of weight after discontinuation of morphine was described as physiological evidence of dependence. To determine the appropriate statistical treatment, the distribution of the injection rates among individual rats was analyzed. A second experiment showed that motor-stimulant properties of chronic morphine administration (Rosloff and Freeman, 1971) are not likely to account for the observed results. The third experiment evaluated the contribution of dose to an increase in self-injection rate following a nalorphine challenge. Such an increased rate was described as behavioral evidence of dependence. This experiment demonstrated that rats may have physiological dependence without behavioral dependence. All rats that initiated morphine self-administration showed physiological dependence, but at low individual morphine doses, morphine intake was well-maintained without behavioral dependence.

Experiment I: Relationship of Morphine Dosage to the Incidence of Physiological Evidence of Dependence

Materials and Methods

Female rats of Sprague-Dawley origin (305–434 g) were used. The method of drug delivery was essentially as previously described (Weeks, 1972; Weeks and Collins, 1976). Injection volumes were 25, 50, or 400 µl/kg, given as infusion at a rate of 10.8 µl/s. The two smaller volumes were used for morphine doses 0.0032–0.1 mg/kg and for some rats at 0.32 mg/kg. The lever switch was BRS/LVE 121-03 (5301 Holland Drive, Beltsville, MD 20705), modified by a shield such that the rat's tail could not trip the lever (Weeks, 1977). Rats had 24-h access to the lever. At 8 a.m. rats were observed, experimental conditions changed, and any challenging procedures carried out. Morphine sulfate was dissolved in isotonic saline. Doses are of the salt.

Statistical Interpretations. Means and SD of daily injection rates were calculated from the logarithms of the individual values. For clarity, antilogarithms of the means are also given in the text. If zero values were present in any of the groups being compared, each value x was analyzed after the transformation log $(x + 1)$, and the mean expressed as the antilog-1. Significance of differences between groups was determined by Student t-test. The test for log-normality of distributions was by the Fisher G tests on the log-transformed values (Fisher, 1973).

Protocol. Eight groups of naive rats, at least ten per group, were offered morphine doses of 0.0032–10 mg/kg, spaced at 0.5 log intervals, for 6 days. On day 7 saline was substituted for morphine and the change in weight recorded. A control group of 28 rats received only saline for 7 days.

Results

The change in weight (mean ± SD) of the control group on day 7 was 0.0 ± 6.41 g. A loss of weight greater than 11 g for any one rat would be significant at the 95% level (one-tailed), and was interpreted as physiological evidence of physical dependence.

The number of rats showing physiological dependence at each dose level is given in Table 1. No rats were dependent at 0.0032 mg/kg. The incidence of dependence increased in a dose-related manner until virtually all rats were dependent at 1.0 mg/kg and above. Among rats showing such dependence, mean daily intake and loss of weight were directly related to the dose.

Distribution of Self-Injection Rates in Relation to Dose and Physiological Evidence of Dependence. The distribution of injection rates on the day 6 is plotted for all rats in Fig. 1. The distribution of the raw data was not only skewed to the right, but also it seemed that those rats which did not lose weight formed a separate population. We analyzed these distributions by posing the following hypotheses: The injection rates are log-normally distributed for (1) the saline control group (saline operant rate); (2) those rats with physiological dependence (closed circles) within each dose group; and (3) those rats without physiological dependence (open circles) taken as a separate population regardless of dose.

Table 1. Morphine self-administration on day 6 of access to graded doses of morphine in naive rats

Dose (mg/kg)	Number of rats tested	Rats with physiological evidence of dependence			
		Dependent (%)	Injections per day ± SE[a]	Morphine intake (mg/kg/day)	Weight loss (%)
0.0032	11	0	– –	–	–
0.010	10	30	411 ± 3.12	4.1	3.7
0.032	17	65	320 ± 1.25	10.2	7.6
0.10	30	57	121 ± 1.20	12.1	8.1
0.32	17	65	125 ± 1.17	40.0	13.2
1.0	11	91	44.9 ± 1.27	44.9	12.7
3.2	11	100	33.5 ± 1.17	107	13.8
10	10	100	23.3 ± 1.17	233	16.3

[a] Antilogarithms of mean ± SE of log values. For arithmetic SE limits, multiply and divide mean by the antilogarithm

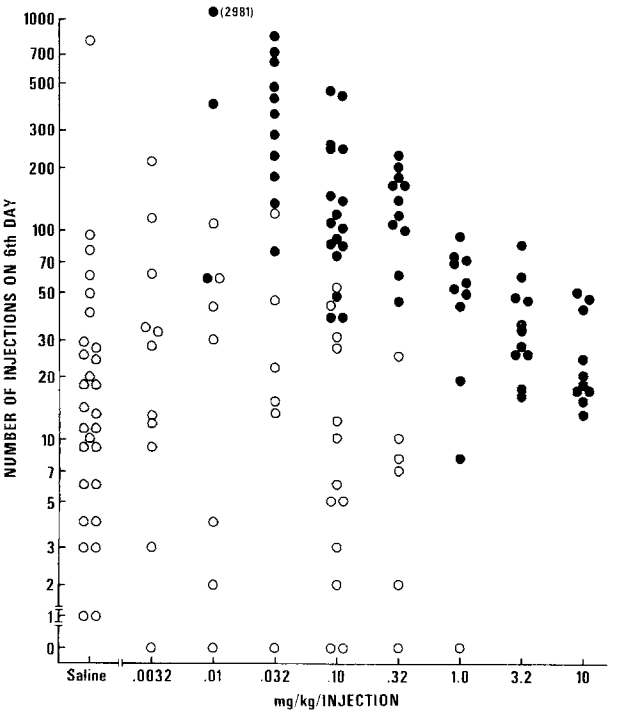

Fig. 1. Distribution of self-injection rates of morphine by rats after 6 days: Closed circles = rats with a significant weight loss following withdrawal of morphine; open circles = control rats and rats without weight loss

The saline control group includes one rat that took 793 injections while the next highest value was only 94. A later study included similarly determined saline operant rates for a group of 30 rats over a 9-day period (Collins and Weeks, 1976). The largest number of injections on any one day was 97, and on the day 6 the mean ($\pm$ SD) was 13.8 (antilog 1.140 $\pm$ 0.426) injections. Accordingly, the rat with 793 injections was considered as an extreme and omitted from calculations. For the remaining 27 rats the mean ($\pm$ SD) was 12.3 (antilog 1.089 $\pm$ 0.516) injections per day, and the upper 95% confidence limit was 93 injections per day. The Fisher G test supports a log-normal distribution.

For the rats with physiological dependence, the individual dose groups contained so few observations that the likelihood of detecting a significant deviation from normality would be low. Accordingly, analysis was applied to the combined doses 10 – 0.032 mg/kg. Each log-transformed observation was first standardized by substracting the mean of the corresponding dose group and then dividing by the SD of that dose group. The assumption was that all of the standardized deviates were normally distributed, versus the alternative that the deviates in any one of the dose groups were otherwise distributed. Since the analysis showed $P < 0.25$, the hypothesis of log-normal distribution was supported.

For the 33 morphine-treated rats without physiological dependence, the log-transformed mean ($\pm$ SD) was 9.6 (antilog 0.981 $\pm$ 0.636) injections per day. The Fisher G test supported the hypothesis that the distribution was log-normal independent of dose. Further, this injection rate did not differ significantly from the saline operant rate.

Since saline injections are presumably nonreinforcing and the injection rate of rats not losing weight did not differ significantly from the saline operant rate, weight loss was assumed to be an independent measure of rats reinforced by morphine. Therefore, daily injection rates and morphine-intake calculations were based only on those rats that had lost weight. Results for day 6 are summarized in Table 1. The mean injection rate for each dose differed significantly from the saline operant rate ($P < 0.05$). The log of the injection rate was linearly related to the log of the dose, the regression equation being (log injections per day) = -0.436 (log dose) + 1.75. The correlation was -0.78, the lack of fit was not significant ($F = 1.20$), and the overall SD was 0.32. The regression equation means that for each ten-fold increase in dose (log 1.0) the injection rate will be 0.37 (antilog -0.436) times as great, or conversely, a ten-fold decrease in the dose results in only 2.7-times as many injections. The injection rate does not fully compensate for a decrease in dose; as the morphine dose decreased so did the daily morphine intake. These relationships are summarized in Table 1.

Temporal Pattern of Self-Injections at High and Low Doses. At 10 mg/kg morphine, rats seldom took more than one injection at a time (Weeks, 1962). When the dose was reduced to 3.2 mg/kg, double and occasional triple injections were seen. We noted this same pattern in these experiments. With the much smaller doses, rats usually took morphine in a series of closely spaced injections, and then a pause with only sporadic injections until the next series. Examples of this pattern are shown in Fig. 2. The speed on our event recorder was too slow to resolve the number of injections in each series. Sometimes rats operated the lever continuously at a rapid rate so the motor on the syringe driver did not stop between injections. On several occasions we observed that the series consisted of 20 – 50 injections.

Extinction Response Pattern in Relation to Dose. To evaluate the rate of extinction, injections following substitution of saline (day 7) were recorded at 2-h intervals for 10 h. Analysis was limited to rats with physiological evidence of dependence. Since there were only three such rats in the 0.01 mg/kg group this dose was omitted. Results (Fig. 3) are plotted as percent of the average 2-h injection rate for day 6. Although there was much variation, a consistent pattern seems present.

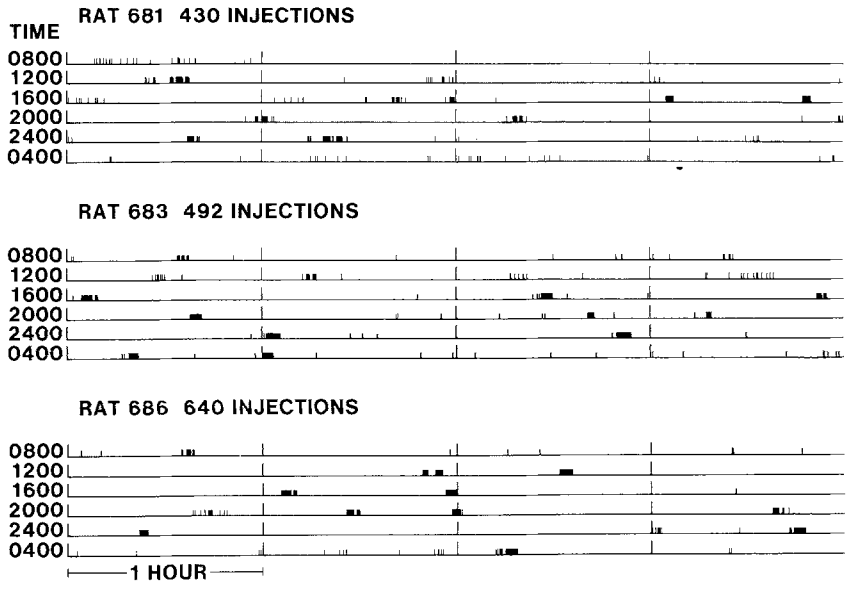

Fig. 2. Temporal distribution of 0.032 mg/kg morphine injections. Rats illustrated are three of those shown in Fig. 1

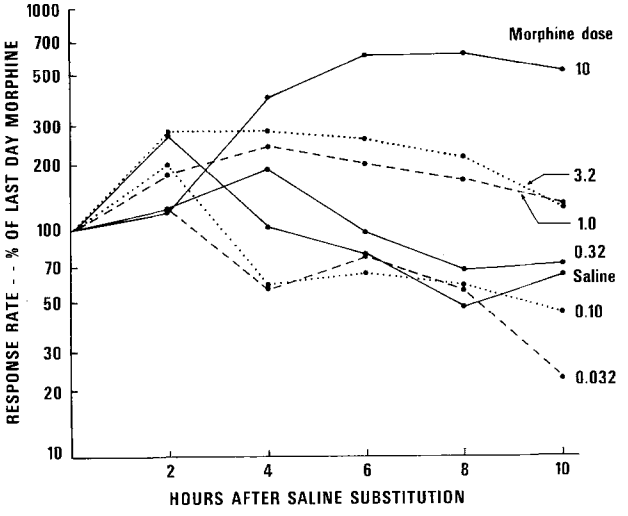

Fig. 3. Extinction of self-injection following substitution of saline for morphine in rats showing physiological evidence of physical dependence. Morphine injection rates on day 6 are given in column 4, Table 1

During the first 2 h there was an increase in response rate which may have been secondary to stimulation from handling when rats were removed from cages for weighing. For the saline controls, following the initial increase, rates remained only slightly below the previous day's average. After the three highest doses of morphine, injection rates were two- to 6-fold above the previous day for at least 8 h, and then started to decline. Presumably, this sustained increase was a heightened opiate-directed state due to morphine withdrawal. After the 0.32 mg/kg dose the injection rate was increased only during the 2–4 h period and then declined. After 0.032 and 0.10 mg/kg doses extinction was already under way during the 2–4-h period. The pattern of injections after the 0.0032 mg/kg dose (not illustrated) in which no rats showed physiological dependence was virtually identical to saline controls.

Discussion

Relationship Between Dose and Self-Injection Rate. Smith et al. (1976) allowed naive rats to self-administer morphine IV 10 h daily for 5 days in doses of 0.03–10 mg/kg. They noted the same relationship between dose, number of injections, and total morphine intake, but the hourly injection rate at each dose was about 4-fold greater. Injections were given in a smaller volume (0.023 ml) and over a much shorter time (0.25 s). In another study (Weeks and Collins, 1978), we found that rats self-administered 3.2 mg/kg (rapid bolus injections) at a stable rate of 120 injections per day compared to 33.5 injections per day in this study. Thus, speed of injection, as well as intermittent access and a buzzer superimposed upon the injection, may explain the differences in otherwise similar experiments. Harrigan and Downs (1978) allowed monkeys access to morphine for 15 min every 4 h and observed the same relationships over the doses of 0.01–0.25 mg/kg. At 0.002 and 0.005mg/kg, however, injection rates decreased. It is possible that these low doses of morphine were not adequate reinforcement to sustain a high response rate.

Log-Normal Distribution of Injection Rates Between Rats. Many statistical procedures, such as the commonly used SD, SE, and Student *t*-test, are based upon the assumption that the data are drawn from a normally distributed population. If the population is

not normally distributed before such procedures are used the data must be transformed to at least an approximately normal distribution. The demonstration here that both morphine injection rates and saline operant rates are log-normally distributed dictates that the data should use log-transformations. Indeed, several years ago we noted that log-transformed self-injection rates gave the smallest error variance in parallel line assays comparing several opiates (Collins and Weeks, 1965). It may be that data from other behavioral studies are also log-normally distributed, but few such studies use sufficient numbers of animals to test this hypothesis.

Saline Operant Rate. The very large variation between rats in saline operant rate complicates establishing a statistically significant increase in rate for drug-reinforced injections. However, in any individual rat, the operant rate may be only a minor factor in determining rate of administration of pharmacologically active substances. Satiation, incapacitating pharmacological effects, or aversive toxic effects may limit drug intake. For example, monkeys administer pentobarbital until anesthetized (Yanagita and Takahashi, 1970).

Temporal Pattern of Injection of Low Doses. The clustered pattern of injections with relatively long pauses raises some questions concerning the nature of the morphine reinforcement and satiation. The intermittent pattern suggests that the drive to consume morphine is abruptly triggered, and injections will be continued until satiation is achieved.

Experiment II: Nonspecific Effect of Morphine on Operant Rate

Materials and Methods

Two groups (12 rats each) were prepared; one group given a continuous IV infusion of morphine at 20 mg/kg/day in a volume of 2.1 ml/day, the other only a saline infusion. The apparatus was the same as in Experiment I except that pressing the lever only measured operant rate. Infusions were continued for 6 days, and on the morning of day 7 rats were weighed and saline infused to all rats for 1 more day.

Results

Results are summarized in Fig. 4. For the saline-infused rats, the operant rate was high on day 1 and then remained constant at about the same rate as the control rats receiving saline in the first experiment. The greater rate on day 1 may have been the result of exploratory behavior in an unfamiliar environment. Operant rate during morphine infusion was initially much greater than saline, then declined; by days 5 and 6 they did not differ significantly from saline. The day 1 mean rate was 58 injections per day, less than half the stabilized rate predicted for self-injection of 20 mg/kg of morphine. Thus, nonspecific behavioral effects are unlikely to contribute to maintenance of morphine self-injection at low doses. The increased operant rate when saline was substituted for morphine was probably a consequence of increased motor activity during withdrawal. Physiological dependence was also present. Morphine-treated rats lost 9.2% of their body weight compared to a 0.6% loss by the controls. This loss is comparable to that shown by rats self-injecting comparable doses of morphine (see Table 1).

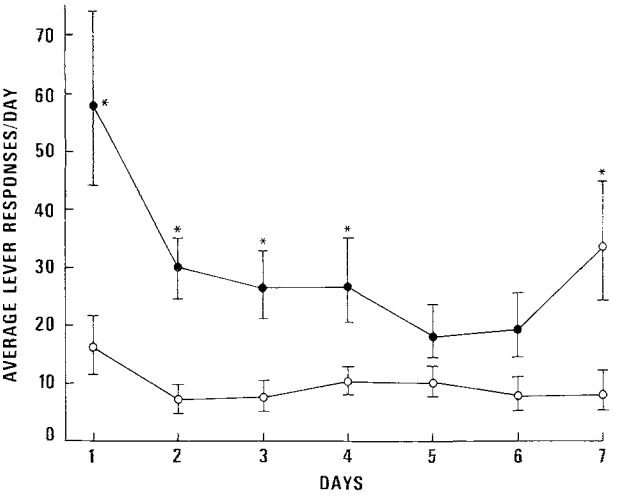

Fig. 4. Effect of morphine infusion on the operant rate of lever pressing activity: Closed circles = morphine, 20 mg/kg/day ($N = 12$); open circles = saline, 2.1 ml/day ($N = 12$). Points are mean ± SE of log-transformed values; * indicates difference from saline ($P < 0.05$)

Experiment III: Behavioral Evidence as Related to Dose

This experiment measured behavioral dependence as an increase in morphine injection rate during the hour immediately following an injection of nalorphine.

Materials and Methods

Morphine injections were delivered as in Experiment I. Bolus injections of nalorphine were administered without handling the rats or opening the cage. These injections were given through a second polyethylene tube (size PE 10) which was threaded through the coiled spring leash to the saddle on the rat. A small hypodermic tubing T-piece (TC-20-3, Small Parts, 6901 NE 3rd Ave., Miami, FL 33138) joined the rat cannula, morphine syringe, and this extra tube. Injections were followed by a 0.15 ml saline flush. Nalorphine hydrochloride was dissolved in isotonic saline (dose as the salt).

Protocol. An increase in response rate might follow a mere discontinuation of morphine as well as being brought about as a pharmacological consequence of nalorphine blockade of morphine.

Accordingly, the nalorphine effect was evaluated for each rat as the ratio of the responses following nalorphine to the responses when the tubing from the syringe was disconnected for 1h. Behavioral evidence of physical dependence would be present if the ratio were significantly greater than 1. The nalorphine challenge was a 2.5 mg/kg bolus injection and the control challenge was an equal volume of saline. The challenges, on successive days, were saline, disconnect, and nalorphine.

The entire protocol required 31 days. Morphine was offered at 0.032 mg/kg for the first 7 days; the challenges were given on days 5, 6, and 7. The daily morphine intake and level of physiological dependence were increased by raising the dose to 1 mg/kg for 4 days, followed by the 3-day challenge series. The dose was then progressively decreased; to 0.32 mg/kg on day 16, followed by the challenges; to 0.1 mg/kg on day 20, followed by the challenges; and finally to the original 0.032 mg/kg on day 24 for 4 days, and a final challenge series. All rats did not complete the entire protocol.

If it is to be demonstrated that behavioral dependence is not always associated with physiological dependence, it is essential that challenges be given only to rats virtually certain to show physiological evidence of dependence. From the data in Fig. 1, all rats given 0.032 mg/kg with injection rates of 134 or more lost weight after withdrawal of morphine. Based upon the log-transformed means of the total daily injections following the first three challenges, rats were clearly separated into two groups, one ($N = 9$) with a mean of 25.7 injections per day (range 12.9–71.2) and the other ($N = 11$) with a mean of 284 injections per day (range 164–546). One rat whose injection rate varied erratically was discarded. Accordingly, the 11 rats in the latter goup, which were selected for subsequent nalorphine challenges, almost certainly would have shown physiological dependence.

Results

Results are summarized in Table 2. The daily injection rate is that following the saline challenge. During the initial 0.032 mg/kg dose, the number of injections following all challenges did not differ significantly from each other and the 95% confidence limits of the nalorphine/disconnect ratio included unity. Thus, there was no behavioral evidence of dependence, even though these rats certainly would have manifested physiological dependence. When the dose was increased to 1.0 mg/kg the daily injection rate decreased and the daily morphine intake increased. In the 2 h following the challenges the number of injections was very low following saline (mean 2.2 injections), but was significantly greater after disconnect (mean 6.8 injections) and still greater after nalorphine (mean 18.5 injections). The nalorphine/disconnect ratio likewise was significantly greater than unity. Thus, these rats displayed the expected behavioral evidence of dependence. When the morphine dose was reduced to 0.32 mg/kg, the daily injection rate increased as did the number of injections following the saline challenge. Behavioral dependence was still present. However, when the dose was reduced to 0.1 and 0.032 mg/kg, the challenges no longer differed significantly and the nalorphine/disconnect ratios did not differ significantly from unity.

Discussion

The nalorphine challenging dose for eliciting behavioral evidence of dependence must be adequate to block morphine action completely. In rats self-injecting morphine at 10 mg/kg on a fixed-ratio 10 schedule, the average daily morphine intake was 95 mg/kg/day. An infusion of 0.5–1 mg/kg/h or nalorphine was maximally effective in increasing morphine injection rate (Weeks and Collins, 1964). In this experiment morphine intake ranged from about 10–68 mg/kg/day, depending upon the dose. The 2.5 mg/kg bolus of nalorphine used here should be a fully blocking dose.

The nalorphine challenges show that when morphine is being self-administered to the point that physiological dependence (withdrawal weight loss) is present, behavioral evidence will not be evident when the morphine dose is relatively small. The design of the

Table 2. Behavioral evidence of physical dependence in relation to the dose of morphine

	Morphine dose (mg/kg)				
	0.032	1.0	0.32	0.1	0.032
Number of rats	11	10	10	9	7
Injections/day after saline challenge[a]	265	68.4	207	348	344
Daily morphine intake (mg/kg)	8.5	68.4	66.2	34.8	11.0
Injections 1 h after challenge[a]					
Saline	12.7	2.2[b]	7.8	19.7	13.3
Disconnect	21.0	6.8	6.3	23.8	26.9
Nalorphine (2.5 mg/kg)	10.3	18.5[b]	22.4[b]	28.3	23.6
Ratio nalorphine/disconnect[c]	0.48	2.80	3.79	1.20	0.83
Upper 95% confidence limit	1.0	4.87	8.82	2.60	2.11
Lower 95% confidence limit	0.007	1.61	1.62	0.55	0.32

[a] Antilog of mean of log values
[b] Different from disconnect challenge $P < 0.05$
[c] Antilog of mean of logarithm of individual ratios. Zero values calculated as unity (two cases)

experiment clearly shows that this phenomenon is dose-related since both were present when the dose was increased to 1.0 mg/kg, and then as the dose was progressively decreased the behavioral effect of the nalorphine again was lost.

The extinction of lever pressing following substitution of saline is consistent with the concept that at low doses withdrawal from morphine does not increase the drive toward self-injection. Following morphine doses of 0.032 and 0.1 mg/kg (behavioral dependence not evident), saline substitution did not increase the injection rate, although it did after greater doses.

One cannot conclude from these experiments that pharmacological and behavioral evidence of dependence are different phenomena, since at higher individual doses the daily morphine intake and presumably also physical dependence are greater. Weight loss may only be a more sensitive indicator of dependence than the behavioral changes following nalorphine. Nevertheless, the data imply that at low doses the reinforcing property of morphine can occur in the absence of behavioral dependence insofar as it can be revealed by a morphine antagonist.

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