# Changes in operant behavior as an index of a withdrawal state from morphine in rats*

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Rats previously trained to a fixed-ratio schedule (FR 50) were treated twice daily with saline or morphine sulfate (final dose, 40 mg/kg IP) for 20 days. On Days 21, 22, and 23, the morphine-treated animals received saline instead. Operant behavior was recorded during the first 14 days of treatment and again on Days 19-23. It was found that, during the first day of withdrawal, the mean leverpressing rate of the morphine group decreased significantly, indicating that an "abstinence syndrome" in rats can be detected by means of an operant behavior technique.

It is known that the morphine addict who is able to obtain an adequate supply of the drug can often behave in a way that cannot be discriminated from the behavior of nondrug users (Goodman & Gilman, 1970). However, when morphine is no longer available, the addict begins to experience various psychological and physiological disturbances and undergoes "a state characterized by behavioral disruption [Thompson & Pickens, 1969]." Following this observation, we have tried to detect the effects of morphine deprivation on behavior in rats by measuring the variations of an operant behavior maintained by a food-reinforced schedule.

Although several techniques have been proposed to quantify the withdrawal syndrome in rodents (Lorenzetti & Sancilio, 1970; Kaymakcalan & Woods, 1956), a need for more reliable and simple methods still exists. In fact, a fully developed abstinence syndrome can be elicited in rats only after a chronic treatment with very large doses of the alkaloid (Martin, Wikler, Eades, & Pescor, 1963). Therefore, a method by which a mild withdrawal state can be detected after a low-dosage schedule of morphine treatment may prove useful as a laboratory test in opiate addiction studies. The purpose of the present paper is to describe such a method.

## SUBJECTS

The Ss were 13 experimentally naive male rats of the Sprague-Dawley strain, weighing 250-280 g.

## APPARATUS

Six conventional Skinner boxes equipped with a lever and a dispenser for the delivery of 70-mg food pellets were used. Each box was enclosed in a separate sound-reducing compartment supplied with a ventilating fan. The sides of the boxes were made of Perspex; a houselight (15-W bulb) provided constant illumination during training and experimental session. A 65-V 25-microA dc current (which, in a preliminary test, was proved to be below the rats' threshold) was continuously delivered to the grid floor of the box so that each closure of the circuit made by Ss' feet could activate a digital counter through a solid-state amplifier. In this way, gross bodily movements of the animal were recorded. All stimulus events and contingencies were programmed automatically by electromagnetic switching and timing circuits, and responses were registered on Sodeco printing counters and pen recorders.

## PROCEDURE

The animals were gradually reduced to 85% of their original body weights by an adjusted feeding schedule. They were given 2 days of magazine training (60 reinforcements per session) and

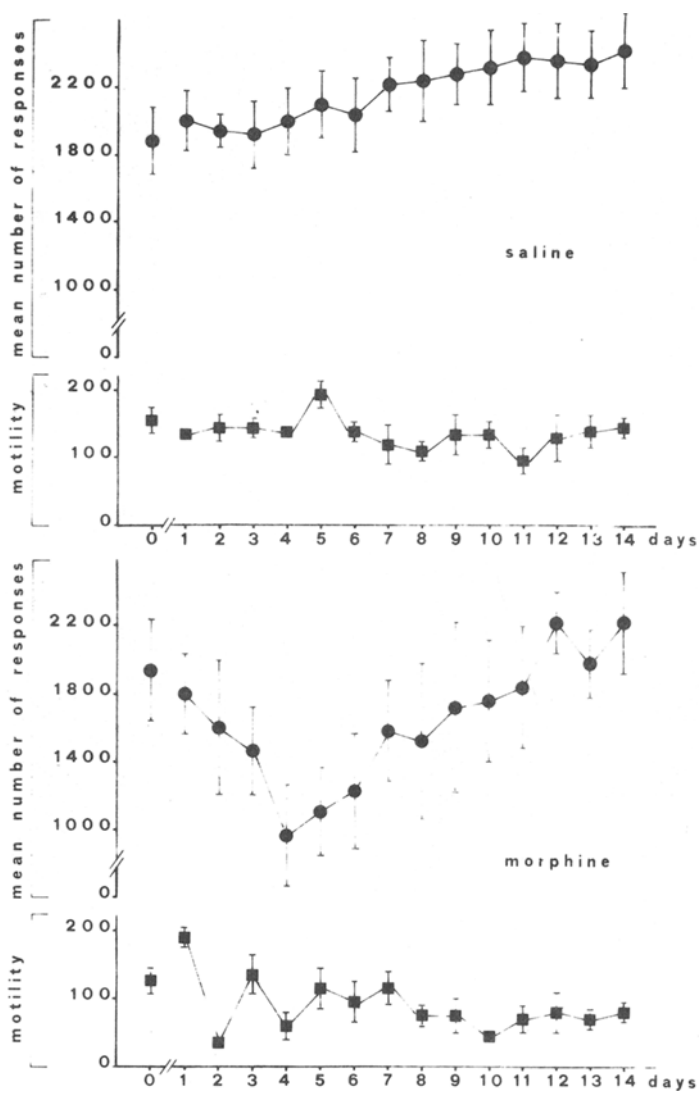


Fig. 1. Mean number of responses and mean motility values of saline- and morphine-treated rats during 14 days of treatment. Vertical bars are the SE.

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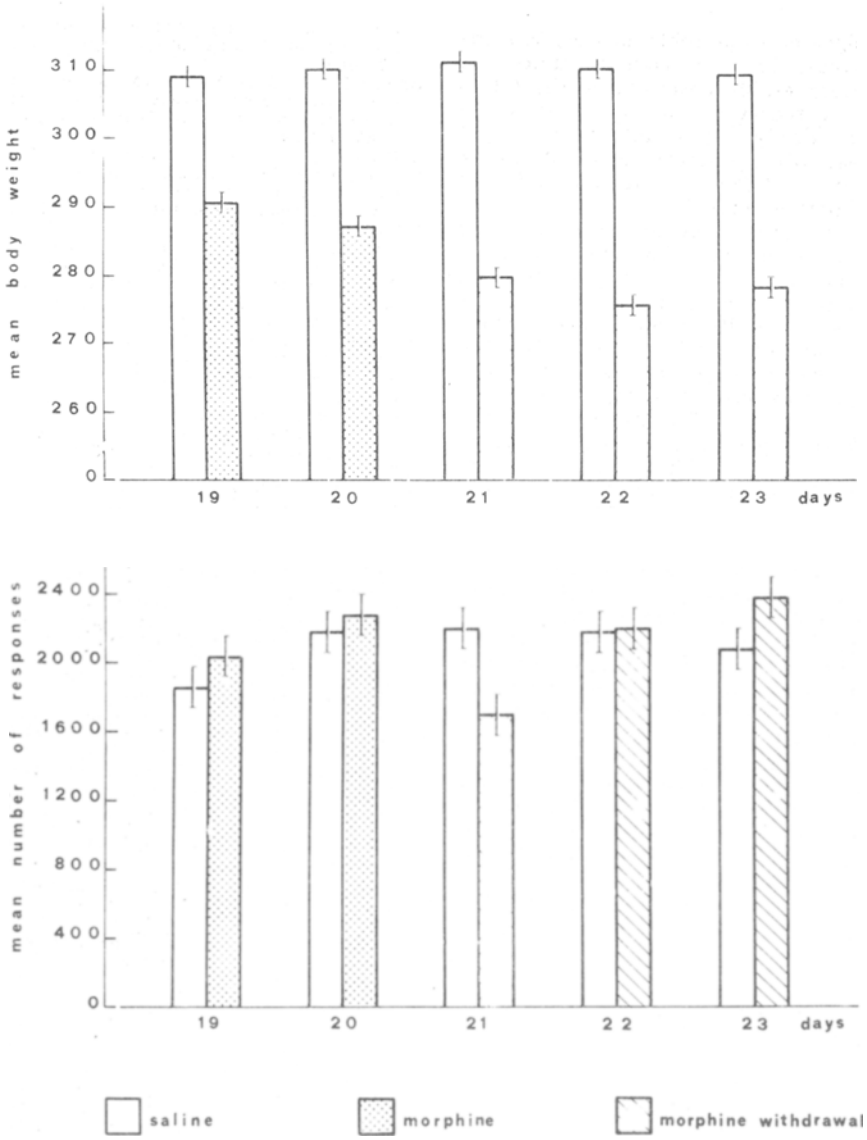


**Fig. 2. Mean number of responses and mean body weight of saline- and morphine-treated rats during Days 19-23. Vertical bars are the SE calculated from ANOVA.**

several days of leverpress training on a fixed-ratio schedule (FR 50) until all Ss reached stable performance.

The animals were then divided into two groups, the first (six Ss) being treated chronically with saline and the second (seven Ss) with morphine sulfate IP. The rats were injected twice daily at 0830 and 2030 h. The dose per injection on the first day was 10 mg/kg, on the second 20 mg/kg, and on the third (and thereafter) 40 mg/kg. The Ss were tested every day during the first 14 days of the chronic treatment period. The test session was run at 1530 h. The daily food intake of both groups was kept constant (20 g), giving an additional number of pellets to the Ss in their home cages after the trial in order not to confound any morphine effect on body weight. In each test session, S was placed in a Skinner box, and 5 min later, its operant behavior (number of leverpresses) and motility (number of closures of the circuit by rat's feet) were recorded for ½ h.

The same procedure was used on Days 19 and 20, while on Days 21, 22, and 23, the Ss of the morphine group received saline instead of morphine. Operant behavior and activity were registered as usual. A three-way analysis of variance with repeated measures on one factor (Winer, 1962) was used to analyze the data throughout. As the sizes of the two groups were unequal, an unweighted means solution was employed (Winer, 1962).

## RESULTS

As shown in Fig. 1, morphine caused a decrease in the level of operant behavior; this reached a maximum on the 4th day of treatment. However, on succeeding days, the leverpressing behavior was gradually resumed, attaining the predrug values on the 12th day of treatment. Motility was slightly above the control level on Day 1, heavily depressed on Day 2, and remained below the predrug level during all the period of chronic morphine treatment.

The mean number of responses and the mean body weight of morphine and saline groups on Days 19, 20, 21, 22, and 23 are shown in Fig. 2. Analysis of variance of the mean number of responses yielded, as expected, significant differences among Ss within treatments ($F = 36.55$, $df = 11/44$, $p < .01$), but also a significant Day by Treatment interaction, which indicated that the difference between morphine and saline groups varied from day to day. Duncan's range test showed that the difference was reliably greater on Day 21 than on any other day ($p < .05$); that is, on the first day of withdrawal, the number of leverpresses of morphine-treated animals was significantly decreased. An inspection of the data of each animal showed that this decrease was mainly due to longer postreinforcement pauses. Two Ss showed also a complete suppression of the leverpressing behavior towards the end of the session.

In regard to body weight, an analysis of variance gave a significant Day by Treatment interaction, and Duncan's range test indicated that the differences between morphine and saline groups on Days 19 and 20 were significantly lower than those on the 3 days of withdrawal. Analysis of variance of motility scores on Days 19-23 yielded a significant difference between treatments ($F = 5{,}12$, $df = 1/11$, $p < .05$), the means of the saline group being always higher than those of the morphine group, while neither days nor the Day by Treatment interaction were significant.

## DISCUSSION

These results indicate that it is possible to detect a withdrawal state from morphine in rats by means of an operant behavior technique. The fact that the dosage schedule in our experiment was quite low compared to those commonly employed to induce physical dependence in rats suggests that the method may be more sensitive than those already described by others (Buckett, 1964). The present findings are also in agreement with results obtained by others in different

organisms. Thompson & Schuster (1964) found that withdrawal from self-administered morphine in monkeys progressively reduced the tendency to work for food on a FR-35 schedule. Goldberg (1971) described an immediate suppression of food-reinforced responding in morphine-dependent monkeys when nalorphine was given intravenously.

The main point that remains to be explained is whether or not the reduction in leverpressing behavior that we found on the first day of morphine withdrawal can be considered a true symptom of an abstinence syndrome. An alternative explanation is that morphine has caused a dissociate learning. In a review on state-dependent learning, Overton (1967) pointed out that an alternative explanation for this kind of learning can be drug-withdrawal symptoms; the converse might be true. In fact, in a recent paper, Hill et al (1971) have stated that all highly addicting drugs produce strong state-dependent learning. However, as our animals were initially trained in the undrugged condition, a return to this condition would not be expected to produce a retrieval deficit, because the Ss are well trained in this condition. To support a state-dependent learning hypothesis, it would be necessary to assume that the withdrawal state was somewhat dissociated from the no-drug state, and no evidence supporting this argument currently exists in the literature. It would therefore be more reasonable to argue that withdrawal induces anorexia, diffuse malaise, or that both of these effects produce the reduced barpressing. The increased postreinforcement pauses we found is a further indication that anorexia might be responsible, at least to some extent, for the reduced number of responses. Moreover, during the 3 days of withdrawal, the rats showed a statistically significant body weight loss, which, according to Akera & Brody (1968), "is the best index of addiction in rats."

Of course, any conclusions of the present paper are limited by the use of only one dosage schedule. Further studies are necessary to explain the present results; these would include the use of morphine antagonists, the single-dose suppression technique with morphine-like drugs, the use of other dose or reinforcement schedules. These experiments are in progress in our laboratory. The results of motility records are not in agreement with our previous finding (Babbini & Davis, 1967) that, during chronic morphine treatment, the depressive effect of the alkaloid gradually is changed into an excitatory one. However, the present motility data are not free from the influence of leverpressing behavior, and the schedule of treatment and dosage are different from those used in that case.

A final point is worth mentioning. The decrease in leverpressing behavior is evident only on the first day of withdrawal. This could suggest either that the abstinence syndrome is mild or that the kind of behavioral disturbance changes over days. In fact, some preliminary data seem to indicate that the leverpressing rate increases (at least in some Ss) on the later days of withdrawal to above the prewithdrawal values.

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