Morphine Lowering of Self-Stimulation Thresholds: Lack of Tolerance with Long-Term Administration

Abstract. *Rats were given increasing amounts of morphine over a period of weeks in order to achieve tolerance. Doses of the drug which initially reduced the threshold for self-stimulation behavior continued to do so after long-term administration. These results demonstrate a persistent central effect of morphine which may be related to the opiate "high."*

The reports of human patients receiving electrical stimulation to positively reinforcing portions of the brain (*1*) have striking similarities to the reports of morphine users describing the opiate "high," suggesting a possible relationship between these two phenomena. This relationship has also been pointed out by Kumar *et al.* (*2*) who noted that both morphine and hypothalamic stimulation can affect eating and drinking behaviors and that both can function as rewards for maintaining behavior in animals. The possible relationship between morphine-induced euphoria and hypothalamic-reward mechanisms has also been suggested by Kerr and Pozuelo (*3*) who further proposed that physical dependence may represent a functional disorganization of the hypothalamic centers concerned with basic consummatory behaviors.

Recent work in our laboratory has demonstrated that a single dose of morphine can affect both the neural activity of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (*4*) and the threshold for self-stimulation behavior at this neuroanatomical site (*5*). The amplitude of the electroencephalogram recorded from this site in rats decreases after a single dose of morphine (*4*). Such decreases generally reflect increased functional activity in the neuronal system and, in accordance with this, it was demonstrated (*5*) that single doses of morphine (4 to 8 mg/kg) lowered the threshold for intracranial self-stimulation to the same brain area. Higher doses (10 to 16 mg/kg) resulted in increases in the threshold. The "double staircase" psychophysical method was used in the last study (*5*) to determine self-stimulation threshold. This method avoided the theoretical and practical difficulties which plagued previous attempts to determine the reward strength of self-stimulation [for a review, see (*6*)] but proved to be impractical in our initial attempts at long-term studies. For the study described herein, a modification of the psychophysical method of limits was used to ascertain the effects of long-term morphine administration on self-stimulation behavior in rats.

Male Charles River rats (CDF strain), each weighing approximately 300 g, were stereotactically implanted with bipolar stainless steel electrodes, 0.005 inch (0.0002 cm) in diameter. The electrodes were insulated, except at the tips, and were aimed at the MFB at the level of the lateral hypothalamus (*7*).

The animals were trained on a threshold procedure in a Plexiglas chamber (20 by 20 cm). Mounted in an opening in one wall of the chamber was a wheel manipulandum which was 15 cm long and 7.5 cm in diameter. Four equally spaced cams were positioned on one of the end plates such that they operated a microswitch when the wheel was rotated. Reinforcement was obtained only after two closures of the microswitch within 1 second. This requirement ensured that only discrete "goal directed" responses would be reinforced. A constant current stimulator (Nuclear-Chicago) was used to deliver the stimuli which consisted of a half-second train of biphasic symmetrical pulses. Each train occurred at a frequency of 100 hertz, with a pulse width of 0.2 msec, and a delay of 0.2 msec between the positive and negative pulses. Pulse amplitude was varied according to the procedural requirements for threshold determination.

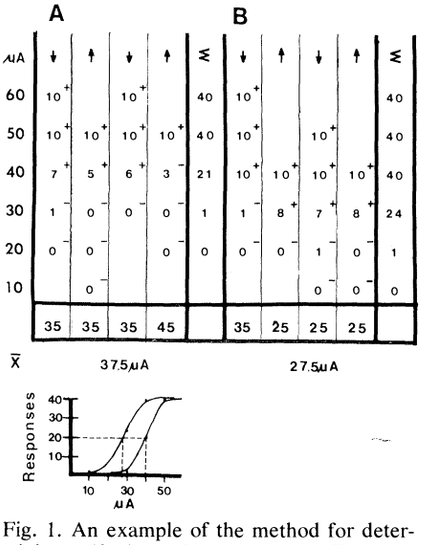

Fig. 1. An example of the method for determining self-stimulation thresholds. The data are for rat 211 on day 5 of drug administration (morphine sulfate, 6 mg/kg). Ascending and descending series for sessions 1 (A) and 2 (B) are indicated by arrows, with individual series thresholds at the bottom of each column. The numbers within the columns represent the number of contingent responses at each intensity. The total number of responses at each intensity is indicated in the right-hand column after each session. Responses as a function of intensity can also be represented as in the graph at the bottom left.

Determination of the thresholds involved a discrete trial procedure identical in part to that used previously (*5*). A trial began with the delivery of a noncontingent 0.5-second pulse train. A response within 7.5 seconds of this stimulus resulted in immediate delivery of a contingent stimulus, identical in all parameters to the noncontingent stimulus, and terminated the trial. Failure to respond had no scheduled consequences, and the trial terminated after 7.5 seconds. Intervals between trials varied with an average of 15 seconds. Responses during the intertrial interval resulted in a 15-second delay before the start of the next trial. The initial noncontingent stimulation thus served both as a discriminative stimulus indicating availability of response-contingent stimulation, and as a comparative stimulus in the sense that it was a predictor of the parameters of the contingent stimulus.

Stimulus intensities for the threshold determinations were varied according to the classical method of limits with slight modification. Stimuli were presented in alternating descending and ascending series with a step size of 10 μa. Ten trials were given in succession at each step size or interval. A descending series was initiated at a previously determined intensity which invariably yielded a contingent response in at least nine out of ten trials, and then ten more successive trials were conducted at the next lowest interval and so on. Five or more responses at a particular intensity were arbitrarily scored as a plus for the interval, while less than five responses were scored as a minus for the interval. Descending series were conducted until minus scores were achieved in two successive intervals. An ascending series was started at one step size below the lowest intensity in the descending series, and continued until a level was reached in which there were at least nine responses out of ten trials, whereupon a descending series would be initiated at least one interval above the last intensity used in the ascending series. Threshold was determined by calculating the arithmetic mean ($\bar{x}$) in microamperes of the midpoints between intervals in which the animal made greater than five responses (a plus score) and less than five responses (a minus score).

Each day the animals were given four test series (session 1) before and four

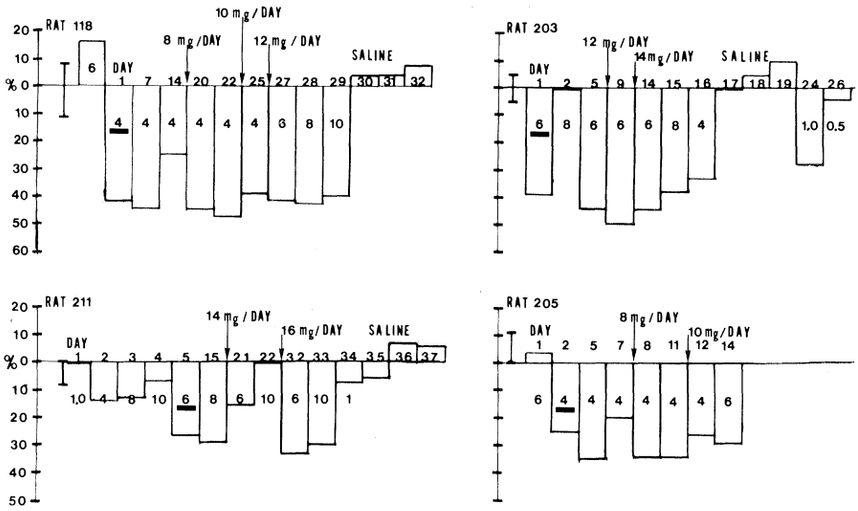

Fig. 2. The ordinate of each graph indicates the percentage change (session 2 threshold minus session 1 threshold × 100 divided by the session 1 threshold) in threshold from session 1 to session 2. The range obtained on the days that saline was injected is indicated at the left side of the graph for each animal. The bars represent the percentage change in scores for selected days on which morphine was administered, followed by bars for scores obtained on days on which saline was administered after cessation of the drug treatment. A bar above the zero line indicates a raising of the threshold, and a bar below the zero line indicates a lowering of the threshold. The days on which the administration of a second dose of morphine was initiated are indicated by the arrows. Thus, for example, on day 14, rat 118 was given a second injection of 4 mg/kg after completion of session 2 (total daily dose, 8 mg/kg), and this second dose was given daily until day 22 when the second dose level was raised to 6 mg/kg, making a total daily dose of 10 mg/kg, and so on.

test series (session 2) after they were injected. After session 1, the animals were injected subcutaneously with either saline or the drug, and then allowed 10 minutes to rest in the chamber before session 2 was begun. The time needed to complete session 1 or session 2 varied from 60 to 90 minutes. The critical dependent measure was the percentage change in threshold from session 1 to session 2. (The percentage change was calculated as the session 2 threshold minus the session 1 threshold × 100 divided by the session 1 threshold) (see Fig. 1).

Animals were run for at least 4 days to determine the extent of the changes that occurred between sessions 1 and 2 when the animals were injected with saline. They were then injected daily with various single doses of morphine sulfate delivered in a 0.9 percent saline vehicle, in order to determine the optimal dose in terms of greatest reduction of threshold. This dose was then used as the daily test dose during the long-term administration of the drug. In order to achieve tolerance, the animals were given gradually increasing amounts of morphine each day with second injections given 15 minutes after the completion of the daily threshold procedures.

The results for selected days in this study are shown in Fig. 2. Animal 118, for example, first received a 6 mg/kg dose of morphine which resulted in a raising of the threshold. He was then given a 4 mg/kg dose which caused a marked drop in the threshold. When given the same dose daily for 14 consecutive days this animal showed little evidence of tolerance. On day 14 the total daily dose was raised to 8 mg/kg but the test dose remained at 4 mg/kg. There was still no evidence of tolerance to this dosage on day 25, at which time the total daily dose was raised to 12 mg/kg. On day 27 the animal was retested with 6 mg/kg; this dose, which previously raised the threshold, now lowered the threshold. Furthermore, when injected with saline only on days 30 to 32 the thresholds did not differ from those obtained with saline before the drug injections were begun. It is significant that despite some variation in the session 1 thresholds (approximately 3 to 15 μa), there was no trend in any of the animals tested for these session 1 "baseline" scores to go either up or down. This was true even during the morphine withdrawal period, which is of particular interest since the animals did lose weight (20 to 45 g) during the withdrawal period and showed irritability, diarrhea, and some "wet-dog" shakes. Tolerance did not develop to the threshold-lowering effect of the original test dose. It is also important to note that the relatively higher doses that did not initially lower the threshhold, or did so only slightly, significantly lowered the threshold after the animals were on daily administration of the drug for a number of weeks.

Animal 211 was tested on day 34 with a dose of 1.0 mg/kg (a dose which on day 1 of long-term administration had resulted in a percentage change of zero) for a second time in order to determine if daily testing plus the daily administration of the drug had resulted in some sensitization of the animal to morphine. As shown in Fig. 2, the effect of the second dose of 1.0 mg/kg was within the range of change for saline alone. Animal 203 showed results similar to the other animals previously described, in that a dose of 6 mg/kg which produced significant reductions in threshold on day 1, still did so on day 14 even though the total daily dose had been increased to 14 mg/kg. As with the other animals, a dose which did not initially reduce the threshold did so after long-term administration of the drug. On day 24 (after 3 days of saline alone) this animal showed a substantial threshold-lowering effect to a dose of morphine as low as 1.0 mg/kg. Animal 205, was found to have a loosening skull platform on day 14. However, up to that time, the data obtained from this animal were similar to those obtained from the other animals. An initial 6 mg/kg dose did not change the threshold but on day 14 the threshold was significantly lowered by a dose of 6 mg/kg. Histological examination of the animals indicated all the electrodes were located in the MFB at the level of the lateral hypothalamus (*8*).

Although morphine administration has been found to facilitate lever pressing in rats for intracranial reinforcement (*9*), rate as a dependent variable has been criticized on both empirical and logical grounds (*10*). In rats given a choice between two levers, each activating a different electrode, the rate of responding did not correlate significantly with lever preference in a choice situation (*11*) nor with measures of resistance to competition from other reinforcers such as food and the avoidance of foot shock (*12*). Thus, the threshold measure used in the present experiments is probably a more valid method of determining the reinforcement value of the stimulation.

One possible interpretation of the threshold-lowering effects of morphine is that nonspecific motor-stimulating effects become manifest after repeated drug administration. Since there was no evidence of an increase in the rate of intertrial responding with long-term morphine administration, we consider this interpretation as invalid.

These results have a parallel in the

findings of Clouet and Ratner (*13*) who found little evidence of tolerance to increased synthesis of catecholamines during long-term morphine administration. Also, our failure to demonstrate tolerance to the threshold-lowering effect of morphine parallels the report of Roffman *et al.* (*14*) who found tolerance to morphine induced changes in the concentration of MHPG (*15*) in a variety of brain areas with the exception of the hypothalamus.

We believe that the threshold-lowering effect of morphine may be related to the euphoria-producing and the reinforcing properties of the opiates in man. In this context a study of Mirin *et al.* (*16*) is of interest. In an effort to understand the continued working for, and self-administration of heroin in human subjects who had acquired tolerance to the drug and showed marked clinical and social deterioration, these workers measured changes in mood during the period of peak drug effect, 30 minutes after each injection. Using the Osgood semantic differential scale they found significant alterations in mood following the intravenous administration of heroin. Their subjects reported feeling more carefree, relaxed, calm, clear, and elated. These effects were sustained over the entire course of the addiction cycle with no significant decrement in the drug's ability to produce such mood alterations. To the extent that our threshold-lowering effect may be related to these mood alterations, we think that we have demonstrated a mechanism of primary importance to the sustained reinforcing and addictive properties of the opiates.

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References and Notes

1. C. W. Sem-Jacobsen and A. Torkildsen, in *Electrical Studies on the Unanesthetized Brain*, E. R. Ramey and D. S. O'Doherty, Eds. (Hoeber, New York, 1960), p. 275.
2. R. Kumar, E. Mitchell, I. P. Stolerman, *Br. J. Pharmacol.* **42**, 473 (1971).
3. F. W. L. Kerr and J. Pozuelo, *Mayo Clin. Proc.* **46**, 653 (1971).
4. J. M. Nelsen and C. Kornetsky, *Fifth Int. Congr. Pharmacol.* (1972), p. 166.
5. R. Marcus and C. Kornetsky, *Psychopharmacologia* **38**, 1 (1974).
6. E. S. Valenstein, *Psychol. Rev.* **71** 415 (1964).
7. Coordinates from bregma were −4.0 mm, anterior-posterior; ± 1.4 mm, lateral; and −8.5 mm, dorsal-ventral.
8. Animal 205 evidenced loosening of the skull platform after the final day of testing. Histological verification of the placement of the tip of the electrode was made by locating the electrode tract and superimposing the length of the actual electrode from the removed platform, while accounting for the thickness of the skull.
9. J. Olds and R. P. Travis, *J. Pharmacol. Exp. Ther.* **128**, 397 (1960); W. J. Adams, S. A. Lorens, C. L. Mitchell, *Proc. Soc. Exp. Biol. Med.* **140**, 770 (1972); S. A. Lorens and C. L. Mitchell, *Psychopharmacologia* **32**, 271 (1973); H. D. Bush, M. F. Bush, A. Miller, L. D. Reid, *Physiol. Psychol.* **4**, 79 (1976).
10. E. S. Valenstein, *Psychol Rev.* **71**, 415 (1964).
11. W. Hodos and E. S. Valenstein, *J. Comp. Physiol. Psychol.* **55**, 80 (1962).
12. E. S. Valenstein and B. Beer, *Science* **137**, 1052 (1962).
13. D. H. Clouet and M. Ratner, *ibid.* **168**, 854 (1970).
14. M. Roffman, G. Cassens, J. Schildkraut, in preparation.
15. The compound MHPG (3-methoxy-4-hydroxyphenylglycol) is a major central metabolite of norepinephrine.
16. S. M. Mirin, R. E. Meyer, H. B. McNamee, *Arch. Gen. Psychiatry*, in press.
17. Supported in part by NIDA grant DA 00377, NIMH grant MH 12568, and NIMH research scientist award MH 1759 to C.K.

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Experimental Separation of Sensory and Motor Functions in Pea Tendrils

Abstract. *When illuminated pea tendrils from light-grown plants are rubbed on their abaxial side, they rapidly coil in a spiral fashion. If similar tendrils are held in the dark for 3 days and then rubbed, however, they will not coil until they are subsequently illuminated. They can remain uncoiled in the dark for as long as 2 hours after stimulation, and will still coil immediately when they are illuminated. Tendrils that are rubbed and held at 25°C will coil, but those treated at 5° or 10°C will not. However, tendrils rubbed at 25°C and kept from coiling for an hour at 5°C, will immediately coil when restored to the higher temperature. These observations are interpreted to imply separation of sensory and motor functions.*

Tendrils are thin, hairlike organs by means of which some weak-stemmed plants anchor themselves upright to supporting structures. They do so by slowly rotating through space by the process of circumnutation (*1*) until they touch a potential support, at which time they cease to circumnutate and begin to coil around the support (*2*). This latter movement, called contact coiling (*3*), is quite rapid, and the tendril can throw more than one complete coil around its support in less than 1 hour (*3*). A conceptual model has been proposed (*4*) which links the sensory function (absorption of stimulus energy) with the motor function (motile response) by one or more transduction steps. Although many correlations of contact coiling with both physiological and biochemical events have been reported (*4*), no one has been able to discern if they were part of the sensory or the motor function. For example, the utilization of adenosine triphosphate has been shown to be necessary for contact coiling (*5*), but it is not known whether this occurs during absorption of the mechanical stimulus, or afterward, during the actual coiling itself.

Therefore, a technique was sought which would permit experimental separation of the motor function from the sensory function. The first experiment shows that tendrils can store sensory information and retrieve it and respond at

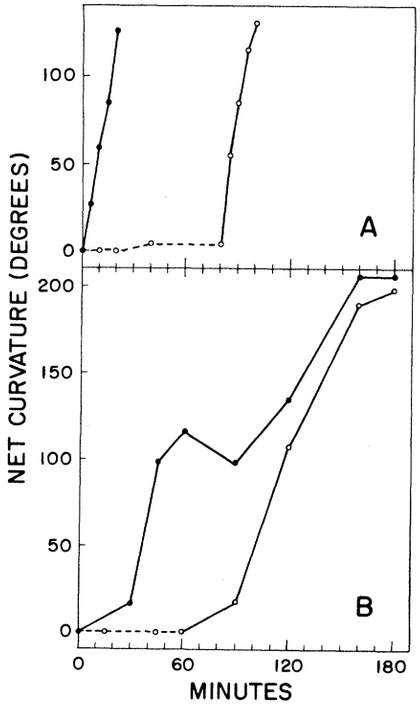

Fig. 1. The effect of low temperature (A) and prolonged darkness (B) on both the sensory and motor functions of contact-stimulated pea tendrils. For the temperature experiment (A), tendrils were excised at the base into petri dishes containing 0.01 percent Tween-20 in 0.05*M* phosphates buffer, *p*H 6.4. After floating for 1 hour to recover from excision, they were stimulated and either held at 25°C (•——•) or at 5°C (o-----o) for 80 minutes and then at 25°C (o——o). For the light experiment (B), preparations were made by excising material at the base of the petiole, and standing the petiole in a jar of wet vermiculite for 3 days in the dark at 26°C. The tendrils were then stimulated and immediately brought out into the light (•——•), or held in the dark for 60 minutes more (o-----o), and then brought out into the light (o——o).