*Pharmacology Biochemistry & Behavior*, Vol. 8, pp. 119–123. Printed in the U.S.A.

# Morphine and Self-Stimulation: Evidence for Action on a Common Neural Substrate[1]

ALLAN N. MAROLI,[2,3] WAH-KWAN TSANG AND ROBERT M. STUTZ

*Department of Psychology, University of Cincinnati, Cincinnati, OH 45221*

(Received 23 May 1977)

MAROLI, A. N., W. K. TSANG AND R. M. STUTZ. *Morphine and self-stimulation: evidence for action on a common neural substrate.* PHARMAC. BIOCHEM. BEHAV. 8(2) 119–123, 1978. – Recent studies have demonstrated that the self-stimulation phenomenon may provide a useful technique for investigating the rewarding properties of potentially addictive drugs such as morphine. The present study attempted to examine the nature of morphine's effects on self-stimulation by observing changes in rate-intensity functions following morphine administration. The results indicate that morphine markedly enhanced bar pressing for low intensity stimulation when the intensities were presented in an ascending sequence but morphine produced only slight changes in self-stimulation rates when a descending series was used. The failure of morphine to facilitate responding in the descending series suggests that adaptation of the self-stimulation system can block morphine's effects on this system. These findings appear to support the hypothesis that morphine affects the excitability of the neural system which mediates self-stimulation.

Morphine Self-stimulation Rate-intensity functions

IN the past several years a number of investigators have reported a facilitation of self-stimulation (SS) behavior in the rat following morphine administration [1, 3, 11]. These observations have been accompanied by findings that morphine lowers positive reinforcement thresholds for electrical stimulation of the medial forebrain bundle (MFB) [8,12]. The latter results are consistent with earlier findings which showed that morphine decreases the amplitude of EEG waves recorded from sites in the MFB, indicating that morphine increases the excitation of neural elements in this structure [13]. These results lead to speculations that the mechanism through which morphine enhances SS may be related to the neural processes which mediate the positively reinforcing characteristics of this drug (i.e., euphoria). That is, it may be that morphine exerts its reinforcing effects by increasing the excitability of central reward structures to endogenous stimulation, thus enhancing the reward value of tonic levels of neural activity [12].

This hypothesis is supported by a number of pharmacological and behavioral studies which indicate that catecholamine-containing neurons may have a critical role, perhaps providing a final common pathway, in mediating the reinforcing effects of morphine and SS. For example, it has been shown that both morphine [18,19] and SS [2, 17, 21] produce an increase in the turnover of catecholamines in many of the brain structures which are anatomically related to the MFB. Furthermore, administration of alpha-methyl-para-tyrosine (AMPT), a tyrosine hydroxylase inhibitor which depletes brain catecholamines, has been found to suppress SS [9,16] and to block the self-administration and the secondary reinforcing properties of intravenous injections of morphine [5,6]. Pretreatment with AMPT has also been found to attenuate morphine's facilitation of SS [14].

These findings suggest that the SS phenomenon may provide an avenue for investigating the neurochemical mechanisms which mediate the reward induced by potentially addictive agents such as morphine and other narcotics. Therefore, the present study was designed to further examine the nature of morphine's facilitory effect on SS by observing the changes in response rates induced by morphine over a range of intensities which are capable of maintaining SS behavior. Previous studies on SS rate-intensity functions have typically found that with electrodes in the lateral hypothalamic region of the MFB, SS accelerates with increasing intensity up to some optimal level, beyond which further increases produce either no facilitation or even a decrement in response rates [15,24]. It is hypothesized that if the facilitory effect of morphine on SS is due to a potentiation of the activity of the MFB, as suggested by its ability to lower positive reinforcement thresholds, then morphine should produce a shift to the left of these rate-intensity functions. This effect has been demonstrated previously with amphetamine [22] which is also reported to facilitate SS behavior in rats [20] and to produce the subjective experiences of euphoria in humans [4]. It is not known, however, whether morphine and amphetamine induce these effects through a common neural mechanism.

[1] Supported, in part, by a grant to Robert M. Stutz, from the University of Cincinnati Research Council.
[2] Send reprint requests to A. N. Maroli, Department of Psychology, University of Cincinnati, Cincinnati, OH 45221.
[3] Supported, in part, by a Summer Research Fellowship Award from the University of Cincinnati Research Council.



0091-3057/78/0802-0119$00.75

## METHOD

### *Animals*

The animals were six male Sprague-Dawley albino rats from the University of Cincinnati animal colony. At the time of surgery, the animals were approximately 115 days old and weighed between 335–435 g. All animals were given food and water ad lib and housed in individual cages throughout the experiment.

### *Surgery*

Each animal was implanted with a single bipolar stainless steel electrode (Plastic Products Company, MS–303–.018–.312–.010), insulated except at the cross section of the tip. Surgery was performed using sodium pentobarbital (Nembutal) anesthesia (42 mg/kg, IP). Atropine sulfate (0.2 mg, IP) was administered 20 min prior to surgery in order to reduce respiratory congestion. Electrodes were implanted in the MFB using flat head coordinates: 4.5 mm posterior to bregma, 1.5 mm lateral to the midline, and 8.5 mm below the surface of the skull.

### *Apparatus*

All animals were tested individually in an operant chamber (23 cm x 20 cm x 24 cm) with a Plexiglas front and top. The metal lever (2.5 cm x 5.0 cm), which activated the stimulator, was positioned 5 cm above the floor at one end of the chamber. A Grass Model–S8 stimulator delivered biphasic rectangular pulses through a constant current circuit. Pulse pairs were delivered at a frequency of 100 pairs per second with a pulse width of 2 msec and a delay of 2 msec between the positive and negative phases. Train duration was held constant at 250 msec. The intensity of the stimulation was varied in accordance with the experimental procedure and was continuously monitored on an oscilloscope (Hewlett-Packard, Model 120AR) by measuring the voltage drop across a 10 kΩ resistor.

### *Procedure*

After a minimum of 14 days following surgery, the animals were shaped to self-stimulate in the experimental chamber. Preliminary training consisted of daily 30 min sessions on four consecutive days. On each of the next four days each animal was presented with an ascending and descending sequence of stimulation intensities in order to determine the approximate range of current levels that would support SS on a continuous reinforcement schedule. Each animal's range was selected according to the criterion that the lowest intensity would not consistently support SS and the highest intensity would support SS without producing gross motor disturbances or convulsions. Overall, the current levels selected ranged from 76 μA to 400 μA.

Seven current levels were selected within each animal's range for the collection of rate-intensity data. The increments in stimulation intensity were made in equal log units for individual animals, but varied across animals due to differences in their ranges. Bar-pressing rates were stabilized by allowing the animals to SS for seven min at each intensity. Ascending and descending sequences were presented on alternate days until the animals showed consistent responding.

The experimental regimen consisted of eight daily sessions, given at the same time each day, in which the animals were tested at each of the seven intensities in succession. Testing at each intensity lasted for 7 min, after which the current was immediately adjusted to the next level. SS rates were recorded only during the last five min of each trial in order to allow the animals time (two min) to adapt to the new current level. If an animal failed to bar press during the first minute of any trial, priming was given for one min to encourage the animal to respond.

On a given day each animal was exposed to either an ascending or descending series of stimulation intensities, with each sequence being presented on alternate days. The order of the two sequences was balanced across animals.

During the first four days of the experiment, baseline rate-intensity data were collected for each animal. Two hr prior to testing, the animals were injected with the control substance, physiological saline (1 ml/kg, IP). Drug testing was carried out in the same manner on Days 5 through 8 of the experiment, with injections of morphine (10 mg/kg, IP) preceding test sessions by two hr. The drug was prepared by dissolving morphine sulfate in physiological saline and adjusting the volume of the solution such that each animal received one milliliter of the vehicle per kilogram of body weight.

At the completion of the experiment the animals were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with physiological saline followed by 10% Formalin. After removal, the brains were embedded in celloidin, sectioned, and stained with cresyl-fast violet. Brain sections were then mounted and microscopically examined for localization of the electrode tips.

## RESULTS

For each animal separate rate-intensity functions were generated for the ascending and descending sequences of trials in the pre- and postdrug conditions. The response rates were determined by calculating the means of the two trials at each intensity for a given order of presentation. Since the rate-intensity functions varied from animal to animal, response rates were converted to percentages, as in a previous investigation [10], in order to allow treatment effects to be assessed for the group. This transformation was made by considering each animal's highest baseline response rate as a score of 100% and expressing all of its other response rates as percentages of that maximum. The maximum response rates ranged from 114 to 445 bar presses per five min for the various animals. One of the animals lost its electrode assembly on Day 4 of the experiment and had to be discontinued. Therefore, all results reported are based on the five animals which completed the entire experiment.

The mean percentages of maximum bar-pressing rates are plotted as a function of intensity level for the morphine and saline conditions in Fig. 1A and Fig. 1B. These figures illustrate the effects of morphine on SS rates for ascending and descending sequences of stimulus intensity presentation, respectively.

A comparison of the saline curves in Fig. 1A and Fig. 1B demonstrates the effect of order of stimulus intensity presentation on the rate-intensity functions. This effect may be described as a shift to the left or right in the rate-intensity functions which depends on whether the intensity changes were made in an ascending or descending sequence. In the ascending sequence (Fig. 1A) a shift to the

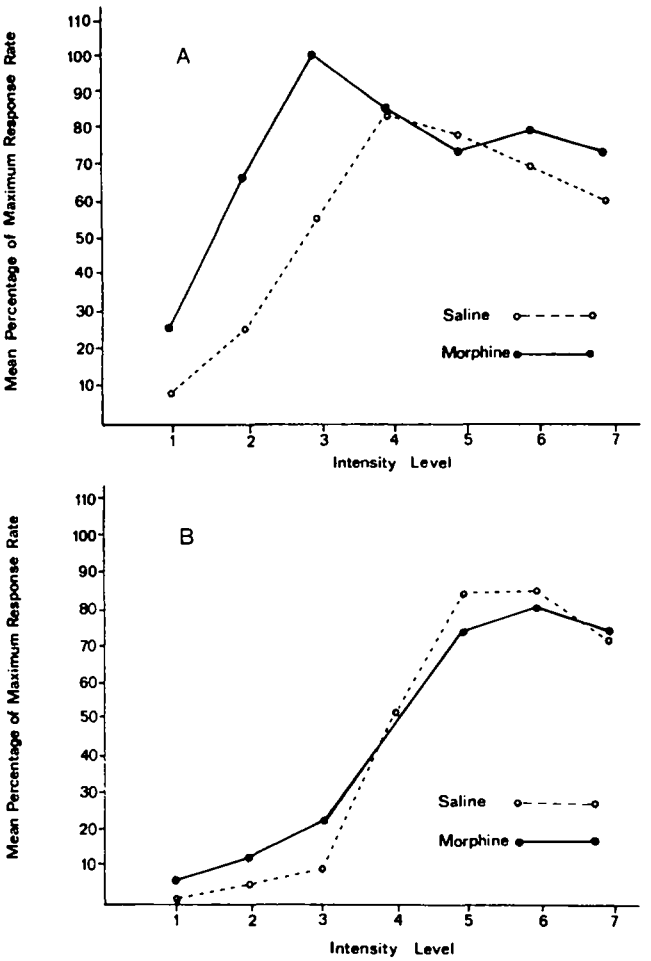


FIG. 1. Mean percentages of maximum bar-pressing rates following saline and morphine injections for ascending (A) and descending (B) sequences of stimulus intensity presentation.

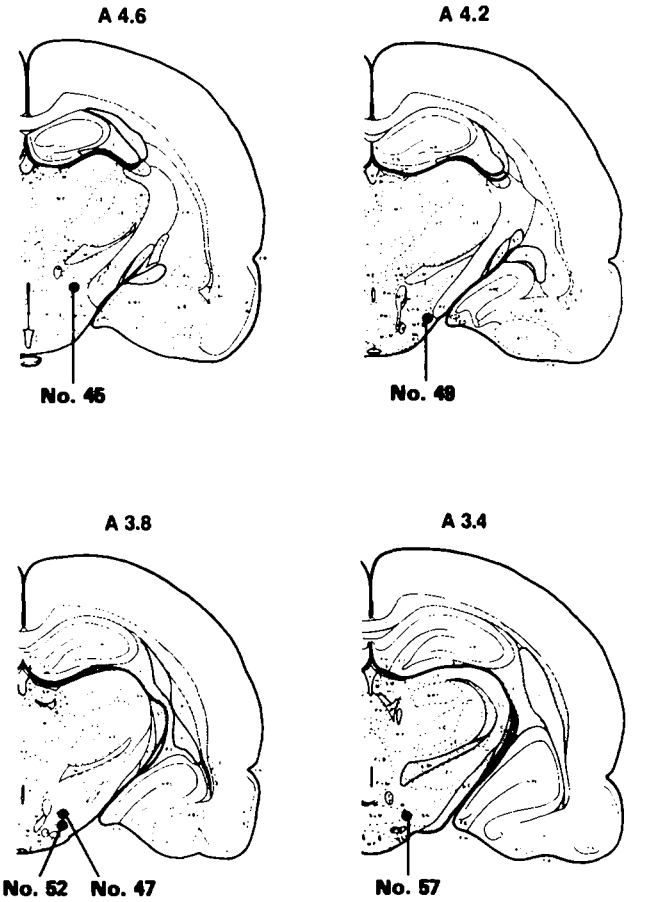


FIG. 2. Drawings of coronal sections of the rat brain showing the location of the electrode tip for each of the five animals. Sections are taken from the deGroot [7] atlas.

left is apparent in the rapid increase in responding at the low and intermediate intensities and the gradual decline in responding at the higher intensities. On the other hand, in the descending sequence (Fig. 1B) response rates reached their maximum at the higher intensities and then dropped off sharply at the intermediate and low intensities producing a shift to the right of the rate-intensity function.

Examining Fig. 1A, it is apparent that in the ascending sequence of stimulus presentations morphine enhanced bar-pressing rates for low intensity stimulation. However, morphine produced little change in responding at the intermediate intensities where the animals showed their highest rates during the saline control condition. On the other hand, Fig. 1B reveals that morphine produced only slight changes in SS rates at any of the intensities tested in the descending sequence of stimulus presentations.

The data were analyzed by a three-factor analysis of variance with repeated measures on each factor (i.e., intensity, order of stimulus presentation, and drug treatment). This analysis yielded a significant effect for intensity levels, $F(6,24) = 23.9$, $p<0.05$, and significant interactions for drug x order, $F(1,4) = 8.7$, $p<0.05$, and intensity x order, $F(6,24) = 8.2$, $p<0.05$. None of the other main effects or interactions were statistically significant.

In Fig. 2, drawings of coronal sections of the rat brain taken from the deGroot [7] atlas are presented to illustrate the positions of the electrode tips. As shown, the electrodes in four of the five animals were localized in the MFB at about the level of the posterior hypothalamus. As a result of accidental damage to the ventral aspect of the brain of animal No. 45 it was impossible to obtain an exact verification of this animal's electrode placement. An examination of the undamaged sections from this animal indicated that the electrode descended at least as far as the zona incerta – approximately 1.5 mm dorsal to its intended placement. However, since this animal's behavior did not deviate from that of the other animals, and in view of the fact that all of the electrodes were precut to the correct length prior to surgery, it seems reasonable to assume that this electrode was also in or adjacent to the MFB.

## DISCUSSION

The rate-intensity shifts found in the saline conditions (compare saline results in Fig. 1A and Fig. 1B) are consistent with the findings of Koob [10] who observed similar changes in SS rates as a function of the order of presentation of the stimulus intensities. Koob explained these findings solely in terms of incentive shifts without making any inferences about the underlying neurophysiological mechanisms which may have contributed to these effects. This is understandable, however, since it appears

that Koob's primary concern was with methodological issues, viz., that presentation of ordered sequences of stimulus intensities can distort the reinforcement value of any given intensity.

An explanation which takes into account possible neurophysiological processes would seem to be of greater heuristic value for the present study. It is suggested, therefore, that the dramatic differences observed between the ascending and descending rate-intensity functions are the result of a change in the excitatory state of the neuron population being stimulated. That is to say, neural adaptation can provide a plausible explanation for the rapid decline in response rates seen in the descending sequence.

This hypothesis is supported by an earlier study in which Thalmann [23] found that prestimulation of the SS system with long duration, low voltage, high frequency stimulation could depress subsequent responding. Since this effect was limited to ipsilateral stimulation and was found to diminish with time following prestimulation, Thalmann concluded that the depression in responding was due to adaptation (i.e., decreased excitability) in the neural system underlying SS.

Furthermore, the present study shows that the ability of morphine to influence SS at a given intensity is a function of the previous levels of stimulation to which the organism has been exposed. This is evident in the striking contrast between morphine's effects on ascending as opposed to descending sequences of stimulus intensity presentation. For the ascending sequence, the morphine-induced changes observed at the lower intensities are consistent with the outcomes which would be expected to follow from a decrease in threshold, viz., increased responding at low intensities and a shift toward the left of the point at which maximum bar-pressing occurs [22]. However, in the case where intensity presentations were made in a descending sequence, morphine had little effect. This suggests that the facilitory effects of morphine on SS can be blocked by decreasing the excitability (i.e., producing adaptation) of the underlying neural substrate. Thus, it appears that the increased excitation normally produced in this neural system by morphine (with the dosage employed) was overriden by the decreased excitation induced by the initially high current levels.

The present findings provide behavioral evidence indicating that morphine's facilitation of SS is due to a potentiation of the activity of this system. These results are consistent with those of Nelsen and Kornetsky [13] who reported that administration of morphine produced an increase in the neural activity recorded from the MFB. This suggests that morphine and rewarding brain stimulation act upon this common neural substrate. Taken together with the results of previous experiments (e.g. [1, 3, 4, 8, 11, 12]) these findings reaffirm the usefulness of SS as a model for investigating the psychotropic (i.e., euphorigenic) effects of morphine and related compounds.

ACKNOWLEDGEMENTS

The authors acknowledge the advice and assistance of Drs. Marvin Schwartz and Lloyd Hastings in various phases of the experiment.

## REFERENCES

1. Adams, W. J., S. A. Lorens and C. I. Mitchell. Morphine enhances lateral hypothalamic self-stimulation in the rat. *Proc. Soc. exp. Biol. Med.* 140: 770–771, 1972.
2. Arbuthnott, G. W., K. Fuxe and U. Ungerstedt. Central catecholamine turnover and self-stimulation behavior. *Brain Res.* 27: 406–413, 1971.
3. Bush, H. D., M. F. Bush, M. A. Miller and L. D. Reid. Addictive agents and intracranial stimulation: Daily morphine and lateral hypothalamic self-stimulation. *Physiol. Psychol.* 4: 79–85, 1976.
4. Collier, H. O. J. The experimental analysis of drug-dependence. *Endeavour* 31: 123–129, 1972.
5. Davis, W. M. and S. G. Smith. Alpha-methyltyrosine to prevent self-administration of morphine and amphetamine. *Curr. Ther. Res.* 14: 814–819, 1972.
6. Davis, W. M. and S. G. Smith. Blocking of morphine based reinforcement by alpha-methyltyrosine. *Life Sci.* 12: 185–191, 1973.
7. deGroot, J. The rat forebrain in stereotaxic coordinates. *Verh K. ned. Akad. Wet.* 52: 1–40, 1959.
8. Esposito, R. and C. Kornetsky. Morphine lowering of self-stimulation thresholds: Lack of tolerance with long-term administration. *Science* 195: 189–191, 1977.
9. Gibson, S., E. G. McGeer and P. L. McGeer. Effect of selective inhibitors of tyrosine and tryptophan hydroxylases on self-stimulation in the rat. *Expl Neurol.* 27: 283–290, 1970.
10. Koob, G. F. Incentive shifts in intracranial self-stimulation produced by different series of stimulus intensity presentations. *Physiol. Behav.* 18: 131–135, 1977.
11. Lorens, S. A. and C. L. Mitchell. Influence of morphine on lateral hypothalamic self-stimulation in the rat. *Psychopharmacologia* 32: 271–277, 1973.
12. Marcus, R. and C. Kornetsky. Negative and positive intracranial reinforcement thresholds: Effects of morphine. *Psychopharmacologia* 38: 1-13, 1974.
13. Nelsen, J. M. and C. Kornetsky. Morphine-induced EEG changes in central motivational systems: Evidence for single dose tolerance. *Fifth. Int. Congr. Pharmacol., Abstr. Contrib. Papers*, 1972, p. 166.
14. Pert, A. and R. Hulsebus. Effect of morphine on intracranial self-stimulation behavior following brain amine depletion. *Life Sci.* 17: 19–20, 1975.
15. Reynolds, R. W. The relationship between stimulation voltage and rate of hypothalamic self-stimulation in the rat. *J. comp. physiol. Psychol.* 51: 193–198, 1958.
16. Saint-Laurent, J., R. Leclerc, J. Beaugrand, M. Mitchell and M. Paradis. Alpha-methyl-para-tyrosine and alpha-methyl-para-tyrosine methyl ester and self-stimulation in rats. *Rev. Can. Biol.* 32: 137–141, 1973.
17. Saint-Laurent, J., M. F. Roizen, E. Miliaressis and D. M. Jacobwitz. The effects of self-stimulation on the catecholamine concentration of discrete areas of the rat brain. *Brain Res.* 99: 194–200, 1975.
18. Smith, C. B., M. I. Sheldon, J. H. Bednarczyk and J. E. Villarreal. Morphine-induced increases in the incorporation of $^{14}$C–dopamine and $^{14}$C–norepinephrine in the mouse brain: Antagonism by naloxone and tolerance. *J. Pharm. exp. Ther.* 180: 547–557, 1972.
19. Smith, C. B., J. E. Villarreal, J. H. Bednarczyk and M. I. Sheldon. Tolerance to morphine-induced increases in $^{14}$C–catecholamine synthesis in mouse brain. *Science* 170: 1106–1107, 1970.
20. Stein, L. Self-stimulation of the brain and the central stimulant action of amphetamine. *Fedn Proc.* 23: 836–850, 1964.

21. Stein, L. and C. D. Wise. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. *J. comp. physiol. Psychol.* 67: 189–198, 1969.
22. Steiner, S. S. and S. N. Stokely. Methamphetamine lowers self-stimulation thresholds. *Physiol. Psychol.* 1: 161–164, 1973.
23. Thalmann, R. H. *The Effect of Intracranial Stimulation on Subsequent Self-Stimulation.* Doctoral dissertation, University of Michigan, Ann Arbor, University Microfilms, 1967, No. 68–07743.
24. Wauquier, A., C. J. Niemegeers and H. A. Geivers. Intracranial self-stimulation in rats as a function of various stimulus parameters. I. An empirical study with monopolar electrodes in the medial forebrain bundle. *Psychopharmacologia* 23: 238–260, 1972.