

# Biphasic Effects of Morphine on Locomotor Activity in Hamsters

PAUL SCHNUR, FRANCINE BRAVO, MARK TRUJILLO AND STEVAN ROCHA

Center for Psychology and Mental Health  
University of Southern Colorado, Pueblo, CO 81001

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SCHNUR, P, F BRAVO, M TRUJILLO AND S ROCHA *Biphasic effects of morphine on locomotor activity in hamsters* PHARMACOL BIOCHEM BEHAV 18(3) 357-361, 1983 —Two experiments investigated the dose and time related effects of morphine sulfate on the running wheel activity of golden Syrian hamsters. Compared with saline controls, a low dose (5 mg/kg) increased locomotor activity, whereas high doses (10, 20 and 40 mg/kg) produced an initial dose-related depression in activity, a gradual dose-related recovery and finally a period of hyperactivity. The results are discussed in terms of similar biphasic effects of morphine observed in rats.

Hamsters    Morphine    Opiates    Locomotor activity

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THE behavioral effects of morphine appear to be dose and species related. Stimulant or depressant actions depend upon the dose administered and the species tested. For example, in humans and monkeys, morphine produces sedation or behavioral depression [9], whereas in cats and mice, behavioral stimulation is the rule [5, 10, 12]. In rats, both stimulant and depressant effects have been observed [1, 2, 6, 7], depending upon the dose and the time since drug administration. At low doses of morphine sulfate (5 mg/kg or less), rats become hyperactive, but at higher doses (10 mg/kg or more), an initial period of behavioral depression precedes the hyperactivity [1, 2, 6, 7]. As noted by Domino *et al* [7], the biphasic pattern of effects observed in rats suggests the possibility that "morphine may have both depressant and excitatory effects in all species and the reason depression or stimulation is not observed may be due to variations in dose and time after administration" (p 362). The plausibility of this suggestion would be strengthened by demonstrations of biphasic morphine effects in species other than rats. The present work was designed to investigate the dose and time related effects of morphine in the golden Syrian hamster.

According to a number of sources [4, 8, 15], the hamster is uniquely unresponsive to the sedative effects of morphine. For example, in an authoritative summary of information about the hamster's response to chemicals, Wills [15] concluded that compared with other species, the hamster is resistant to the sedative/hypnotic effects of morphine. Similarly, Houchin [8] reported that morphine had no noticeable effects even at doses of 150 mg/kg and that at 300 mg/kg it produced "some incoordination in the extremities, increased activity" (p 339). According to Houchin, this lack of responsiveness was evident regardless of route of administration, sex, body weight, or age. In a recent study, Tseng *et al* [13] compared the behavioral effects of opioid peptides with morphine sulfate in rats and hamsters. The principal difference between the peptides and morphine was

found among hamsters: beta-endorphin and enkephalin both induced loss of the righting reflex, whereas morphine sulfate, even in high doses, did not.

In marked contrast to these findings, Ostrowski *et al* [11] reported that hamsters are not insensitive to the effects of morphine. Ostrowski *et al* [11] tested the effects of four doses of morphine sulfate (10, 20, 40 or 80 mg/kg) on exploratory activity (distance travelled in a C-shaped alley) and on general motor activity (number of shuttles in a shuttle box). Compared with saline controls, dose related impairments of behavior were found on both measures, though the impairments produced by the two lowest doses were small and unreliable. Moreover, there was no evidence in this study of the dose and time related biphasic pattern of effects observed in rats. Perhaps this is not surprising since morphine effects were studied for only two minutes and doses less than 10 mg/kg were not included. In rats, stimulant effects are evident at doses less than 10 mg/kg and the time related stimulant/depressant pattern obtains over the course of several hours. In light of these considerations, the present study included a 5 mg/kg dose and monitored the behavioral effects of morphine for several hours.

## EXPERIMENT 1

Monitoring the behavioral effects of any drug for several hours requires a stable baseline. Pilot observations in our laboratory indicated that running wheel activity would provide a reasonably stable baseline against which to assess the dose and time related effects of morphine in the hamster. Further, our pilot work indicated that the baseline could be maintained without the use of deprivation schedules or nociceptive stimuli, an important consideration when testing the effects of a potent analgesic and anorectic drug such as morphine. The first experiment investigated the effects of four doses of morphine on hamster running wheel activity for two hours.

## METHOD

## Subjects

Forty adult golden Syrian hamsters (19 males, 21 females), with a mean weight of 101 g, were used. Thirty-three animals were obtained from SASCO, Inc (Omaha, NE), seven were offspring of SASCO hamsters born in our lab. Animals were housed individually in stainless steel cages, maintained on a 12-hour light-dark cycle, and given free access to food and water throughout the experiment.

## Apparatus and Materials

The apparatus consisted of eight identical activity wheels (Wahmann Co., Model LC-34) which were housed in a room dimly illuminated by two 15 watt bulbs. An ambient noise level of 79 dB (re 0.0002 dynes/cm<sup>2</sup>, A scale) was maintained.

Morphine injections consisted of 5, 10, 20 or 40 mg/kg doses of morphine sulfate, expressed as the salt, dissolved in 1 ml of physiological saline. Morphine as well as saline injections were administered subcutaneously in the dorsal surface of the neck in 1 ml/kg volumes.

## Procedure

Experimental procedures were conducted over the course of 11 days. On the first three days, each animal was weighed, injected with saline and placed in the running wheel for a two hour baseline session. The number of wheel revolutions was recorded every 20 minutes for each animal. The baseline sessions served to accustom the animals to the running wheel and to the handling/injection procedures. In addition, baseline data were used to assign hamsters to two equal sized "blocks." Those with running rates above the median baseline for all animals (high responders) and those with running rates below the median baseline for all animals (low responders). Animals within each block were randomly assigned to experimental groups so that the mean baseline running rates were approximately equal across drug treatment conditions. Five treatment groups (n=8) were included: Group Sal (saline controls), Group MS-5 (5 mg/kg morphine sulfate), Group MS-10 (10 mg/kg morphine sulfate), Group MS-20 (20 mg/kg morphine sulfate) and Group MS-40 (40 mg/kg morphine sulfate).

Following a two day respite during which animals were left undisturbed in their home cages, eight consecutive days of testing were carried out. On test days, each animal was weighed, injected with saline or the appropriate dose of morphine, and placed in the running wheel for a two hour session. The number of wheel revolutions was recorded every 20 minutes for each animal.

## RESULTS AND DISCUSSION

Figure 1 shows mean activity (number of wheel revolutions) across days for all groups. A biphasic dose-effect relationship is evident. Compared with saline controls, Group MS-5 was hyperactive and Group MS-40 was hypoactive. A similar though less consistent pattern is seen at intermediate doses with Group MS-10 hyperactive and Group MS-20 hypoactive compared with Group Sal. Moreover, the differences among groups were relatively stable throughout the experiment. That is, though all groups showed an increase in activity across days, stimulant and depressant effects were manifested on the first day and then maintained for the dura-

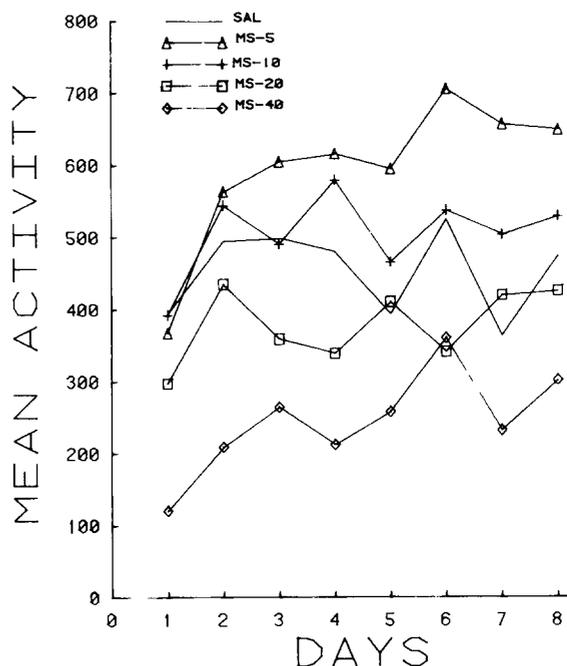


FIG 1 Mean activity (number of wheel revolutions) as a function of days for all groups in Experiment 1

tion of the experiment. Furthermore, under the schedules of administration used here, little tolerance developed to the effects of morphine. Although Group MS-40 did show an increase in activity as a function of days, it was relatively small compared with a similar increase in Group Sal. And, Group MS-5 showed increased hyperactivity over days, a pattern akin to sensitization. These conclusions are corroborated by a 5 (Dose)  $\times$  8 (Days) randomized blocks mixed factorial analysis of variance (ANOVA) which indicated that the effect of dose was significant,  $F(4,34)=6.91$ ,  $p<0.005$ , that the increase across days was significant,  $F(7,245)=5.53$ ,  $p<0.005$ , but that the Dose  $\times$  Days interaction was not significant,  $F(28,245)=1.09$ .

Figure 2 shows the time effect relation for all groups. Mean activity (collapsed across days) is shown for the two hours following drug administration. It is clear that morphine's time effect curve varies as a function of dose. Compared with Group Sal, Group MS-5 showed increased activity for the entire two hour daily session. Groups MS-10, MS-20 and MS-40 were all initially depressed compared with saline controls and the magnitude of that depression was a direct function of dose. Furthermore, Groups MS-10, MS-20 and MS-40 showed increased activity over the course of the two hour session and the rate of that increase was inversely related to dose. Indeed, compared with Group Sal, Groups MS-10 and MS-20 were hyperactive for some portion of the session. Group MS-40 remained depressed for the duration of the daily session. These conclusions are corroborated by a 5 (Dose)  $\times$  6 (Time) randomized blocks mixed factorial ANOVA which indicated that the effect of dose was significant,  $F(4,34)=6.91$ ,  $p<0.005$ , that the increase during the session was significant,  $F(5,175)=30.94$ ,  $p<0.005$ , and that the interaction between dose and session time was also significant,  $F(20,175)=3.97$ ,  $p<0.005$ .

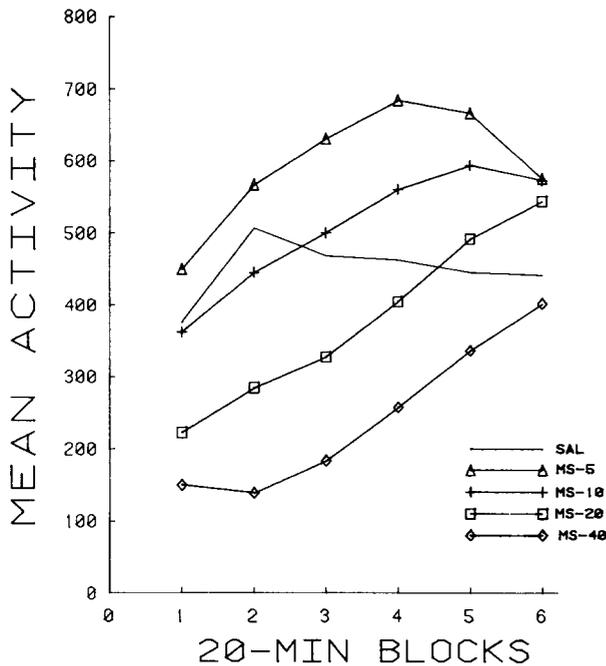


FIG 2 Mean activity (number of wheel revolutions) as a function of 20-minute blocks of time for all groups in Experiment 1

The results of this experiment indicate that morphine has biphasic effects on running wheel activity in the hamster, a pattern similar to that found in rats [1, 6, 7]. As in the rat, low doses are excitatory and high doses are inhibitory, at least over the course of a two hour session. At intermediate doses (e.g., 10 and 20 mg/kg), an initial period of behavioral depression gives way to subsequent hyperactivity. Moreover, the depth and duration of the initial depression are dose-related. Although a period of hyperactivity following a dose of 40 mg/kg was not evident in the present study, the increase in activity shown by Group MS-40 during the two hour session suggests that hyperactivity might be evident if session duration were lengthened.

#### EXPERIMENT 2

The purpose of Experiment 2 was to investigate the time effect relation for three doses of morphine found to have initially depressant effects in the first experiment. In particular, Experiment 2 was designed to determine whether a dose of 40 mg/kg would produce hyperactivity in hamsters following an initial period of depressed activity. In rats, Babbini and Davis [1] monitored activity for eight hours post-injection and found a biphasic pattern using 40 mg/kg doses of morphine. Similarly, Domino *et al* [6,7] monitored activity in rats for seven hours and observed a biphasic pattern using a 32 mg/kg dose. The present experiment monitored hamsters' running wheel activity for six hours following the injection of morphine sulfate at doses of 10, 20 and 40 mg/kg.

#### METHOD

##### Subjects

Twenty-four adult golden Syrian hamsters (9 males, 15

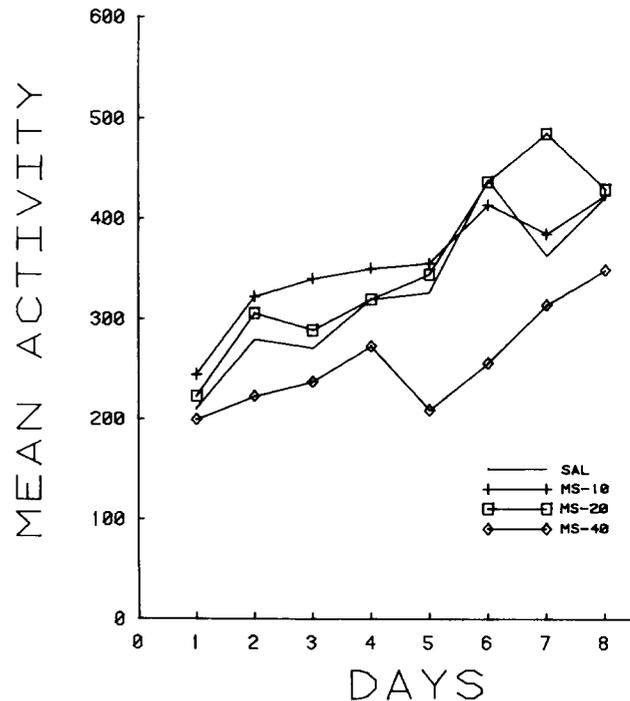


FIG 3 Mean activity (number of wheel revolutions) as a function of days for all groups in Experiment 2

females), with a mean weight of 102 g, were used. Thirteen animals were obtained from SASCO, Inc (Omaha, NE), eleven were offspring of SASCO hamsters born in our lab. Animals were housed individually in stainless steel cages, maintained on a 12-hour light-dark cycle, and given free access to food and water throughout the experiment.

##### Apparatus and Materials

The apparatus was the same as that used in Experiment 1. Morphine injections consisted of 10, 20 or 40 mg/kg doses of morphine sulfate, expressed as the salt, dissolved in 1 ml of physiological saline. Morphine as well as saline injections were administered subcutaneously in the dorsal surface of the neck in 1 ml/kg volumes.

##### Procedure

Experimental procedures were identical to those of Experiment 1 except that daily sessions (baseline and test) were six hours in duration and only four treatment groups ( $n=6$ ) were included: Group Sal (saline controls), Group MS-10 (10 mg/kg morphine sulfate), Group MS-20 (20 mg/kg morphine sulfate) and Group MS-40 (40 mg/kg morphine sulfate).

#### RESULTS AND DISCUSSION

Figure 3 shows mean activity (number of wheel revolutions) across days for all groups. It is evident that all groups showed an increase in activity as a function of days and that differences among groups in daily running were relatively small. It should be noted that daily sessions lasted six hours in this experiment and, as will become apparent below, total

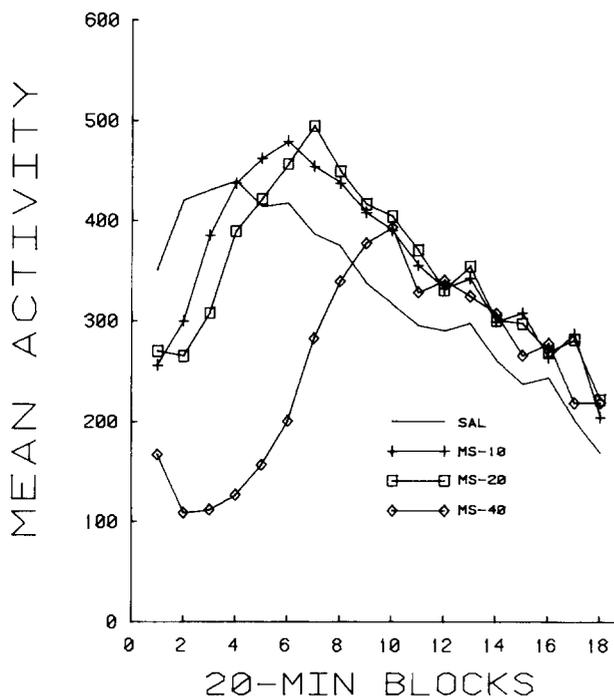


FIG 4 Mean activity (number of wheel revolutions) as a function of 20-minute blocks of time for all groups in Experiment 2

daily activity provides an incomplete picture of morphine's effects over time. As in Experiment 1, little tolerance developed to the effects of morphine, the increase in activity among groups given morphine being no different from the increase in Group Sal. These conclusions are substantiated by a 4 (Dose)  $\times$  8 (Days) randomized blocks mixed factorial ANOVA which indicated that the activity increase over days was significant,  $F(7,140)=14.12$ ,  $p<0.005$ , but that neither the effect of dose,  $F(3,19)=0.38$ , nor the interaction between dose and days,  $F(21,140)=0.80$ , was significant.

Figure 4 shows the time effect relation for all groups. Mean activity (collapsed across days) is shown for the six hours following drug administration. As in Experiment 1, it is clear that morphine's time effect curve varies as a function of dose. Furthermore, a biphasic pattern is evident at all doses. Compared with Group Sal, Groups MS-10, MS-20 and MS-40 showed an initial dose-related decrease in activity. This finding replicates the results of Experiment 1 for the groups receiving the same doses of morphine. In fact, if one examines morphine's effects for only the first two hours of Experiment 2, the findings of the first experiment are replicated very closely in Groups MS-10, MS-20 and MS-40. When morphine's effects for six hours are considered, the hyperactive portion of the biphasic pattern is observed at all doses tested. That is, compared with saline controls, all morphine groups became hyperactive for some portion of the session. As in Experiment 1, the duration of the initial depression was directly related to morphine dose. After approximately three hours, Group MS-40 was hyperactive compared with saline controls.

A 4 (Dose)  $\times$  18 (Time) randomized blocks mixed factorial ANOVA indicated that the effect of dose was significant

$F(3,19)=3.19$ ,  $p<0.05$ , as was the change in activity over the six hour session,  $F(17,340)=5.36$ ,  $p<0.005$ . However, the Dose  $\times$  Time interaction was not significant,  $F(51,340)=1.09$ . This nonsignificant interaction is partly attributable to the stability of drug effects for the last half of the six hour experimental session (see Fig. 4). If one analyzes mean activity for the first four hours post-injection, a significant Dose  $\times$  Time interaction is revealed,  $F(33,220)=2.95$ ,  $p<0.005$ .

The results of this experiment confirm and extend the findings of Experiment 1. As in Experiment 1, morphine at doses of 10 and 20 mg/kg first decreased then increased hamster running compared with saline controls, a biphasic pattern common at least in rats [1, 6, 7]. And, as suggested by the first experiment, a 40 mg/kg dose of morphine was shown in Experiment 2 to produce biphasic effects on activity: the hyperactivity occurring three hours post-injection and following a dramatic period of behavioral depression.

### GENERAL DISCUSSION

The present study leaves little doubt that the hamster's behavioral response to morphine is strikingly similar to the biphasic pattern documented in rats. At low doses (e.g., 5 mg/kg or less), sustained hyperactivity follows the injection; at higher doses (e.g., 10 mg/kg or more), an immediate dose-related decrease in activity is followed by a gradual, dose-related recovery and subsequent hyperactivity. The failure of other investigators to observe these effects in hamsters is presumably due to the use of insensitive behavioral measures and/or to a paucity of time effect data. The presence of biphasic morphine effects in a species other than rats lends credence to the hypothesis that morphine's effects may be characterized by excitatory and inhibitory phases in many species. For example, in an apparent exception to the typical finding of morphine-induced activation in mice, Carroll and Sharp [3] noted a brief (10–15 minutes) decrease in spontaneous activity followed by prolonged activation. The fact that baseline rates of spontaneous activity are typically low in tests of mice may contribute to the difficulty in observing decreases in activity.

At the present time, there is no adequate explanation for the mixed depressant and stimulant actions of morphine, though a number of hypotheses have been advanced. One hypothesis [1] suggests that different drug-receptor interactions underlie the biphasic effects: one producing behavioral excitation, the other behavioral inhibition. Another hypothesis [6,7] relates both effects to acetylcholine (ACh) levels in the brain. According to Domino *et al.* [7], morphine's depressant effects are related to a decrease in ACh utilization by the brain, whereas subsequent stimulant actions are related to an increase in ACh utilization. Still another hypothesis suggests that only one mechanism is involved, producing stimulant effects at low doses and depressant effects at high doses. According to this hypothesis [14], the late stimulant actions observed with higher doses of morphine could be due to a decrease in the brain level of drug to a concentration equal to that achieved with a low dose initially'' (p. 856). The present data provide no basis for choosing among these alternatives, but we believe that additional research using locomotor activity in the hamster will contribute to an understanding of morphine's biphasic effects.

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