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Locomotor Activity and Opiate Effects in Male and Female Hamsters

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SCHNUR, P. AND P. BARELA. *Locomotor activity and opiate effects in male and female hamsters.* PHARMACOL BIOCHEM BEHAV 21(3) 369–374, 1984.—Locomotor activity of golden Syrian hamsters was investigated in three experiments. In Experiment 1, running wheel activity of male and female hamsters was compared under the following conditions: no injection, saline injection, morphine injection (15 mg/kg) and naltrexone injection (1 mg/kg). During two hour test sessions, females maintained high levels of activity, whereas males slowed down considerably during the second hour. The difference between males and females was evident under all conditions except following morphine which produced a biphasic time-effect pattern in both sexes. Naltrexone, however, had no detectable effects on either males or females. Experiment 2 investigated the effects of four doses of naltrexone (0.3, 1, 3, 10 mg/kg) on hamster locomotion. Results indicated that none of the doses tested had an effect. Experiment 3 demonstrated that a 1 mg/kg dose of naltrexone antagonizes two of morphine's (15 mg/kg) effects on activity. First, naltrexone partially blocked morphine elicited sedation. Second, naltrexone blocked the increase in activity that characterizes recovery from morphine.

Hamsters Sex differences Locomotor activity Opiates Morphine Naltrexone

HAMSTERS, given access to wheels that spin or to surfaces that rotate, run at stable rates and in characteristic patterns [3]. As a result, the hamster has been the animal of choice for studies of circadian rhythm [6, 8, 9, 19]. Such studies have demonstrated that internal (e.g., gonadal hormones) and external (e.g., photoperiod) factors interact to control locomotor activity. In our laboratory, running wheel activity has been used to provide a reliable and sensitive behavioral assay for the effects of morphine in the hamster. We have found that, over a wide range of doses (0.5–40 mg/kg), morphine produces a biphasic time-effect pattern on locomotor activity: An initial dose-related decrease in activity is followed by a dose-related rate of recovery, and then by a period of hyperactivity [16,17]. The present work continues our study of opiate effects on locomotor activity in hamsters.

EXPERIMENT 1

Experiment 1 was designed to examine sex differences in hamster locomotor activity. Although previous investigations of locomotor activity in hamsters have documented sex differences in the hormonal and photoperiodic control of circadian rhythmicity [6,19], procedural differences between long-term circadian rhythm studies and short-term drug studies make it difficult to predict what differences might emerge during brief daily access to the running wheel. In rats, it has been reported [10] that females have higher levels of locomotor activity than males, but only during the last half of fourteen minute test sessions, suggesting that males and females differ in perseverative behavior.

In addition to comparing baseline locomotor activity of male and female hamsters, Experiment 1 was designed to investigate possible sex differences in the effects of naltrexone and morphine on hamster locomotion. Whether or not opiate antagonists, such as naloxone and naltrexone, influence locomotor activity is a matter of some dispute [1, 2, 4, 7], but according to one hypothesis, naloxone enhances responsivity and/or sensitivity to environmental stimuli [2, 11, 14, 15]. To the extent that a rotating running wheel provides stimulus support for running [3], one might anticipate that the potent, long-acting antagonist naltrexone would enhance running wheel activity in animals that otherwise habituate quickly and thus fail to perseverate. Although our previous work has not revealed sex differences in the behavioral effects of morphine, the present experiment is the first to systematically compare the effects of morphine on male and female hamsters. Daily running wheel activity of male and female hamsters was monitored for two hours following either no injection or following an injection of saline, naltrexone or morphine.

METHOD

Subjects

Eight male and eight female experimentally naive, golden Syrian hamsters, with mean weights of 99.5 and 104.5 g, respectively, were used. The hamsters were descended from animals obtained from Sasco, Inc. (Omaha, NE). They were housed individually in stainless steel cages, maintained on a 12:12 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, and maintained at an

ambient noise level of 79 dB (re: 0.0002 dynes/cm^2, A scale). Running wheels were fitted with microswitches and interfaced to Canon printing calculators (Model TP-8), modified [5] to record the number of wheel revolutions.

Morphine injections consisted of 15 mg/kg doses of morphine sulfate (Lilly), expressed as the salt, dissolved in 1 ml of physiological saline. Naltrexone injections consisted of 1 mg/kg doses of naltrexone hydrochloride (Endo), also expressed as the salt, and dissolved in 1 ml of physiological saline. All injections were administered in 1 ml/kg volumes.

Procedure

Eleven test sessions, spaced 24 hours apart, were conducted. At daily intervals animals were removed from their home cages, weighed, and transported to an adjoining room where they received either no injection, or a subcutaneous injection in the dorsal surface of the neck of saline, morphine, or naltrexone. The animals were then left undisturbed in their cages for 15 minutes before being placed in the running wheels for a 2-hour test session. The number of wheel revolutions was recorded every 20 minutes for each animal. All animals received the following treatments: Days 1–3, no injection; Days 4–6, saline injection; Day 7, naltrexone injection; Days 8–10, morphine injection; Day 11, no injection.

RESULTS AND DISCUSSION

The top panel in Fig. 1 shows mean activity (number of wheel revolutions) as a function of days for males and females. The mean activity of both sexes increased on the first three test days, but the females maintained a higher daily level of activity than the males. During the next three days (saline), activity levels diverged as the females showed an increase and the males showed a decrease in locomotor behavior. On Day 7, neither group reacted to the naltrexone, and males and females ran at previously established rates. On Days 8–10, the relative activity levels of males and females shifted in response to morphine. Females exhibited a dramatic decrease in activity on Day 8, followed by a gradual return to high levels of activity on Days 9–10. Males, however, exhibited increased activity during the three days of morphine administration. Thus, the daily activity of the males was higher than that of the females during this period. Finally, on Day 11, when injections were discontinued, both sexes returned to earlier patterns of responding and females displayed significantly higher levels of activity than the males. These conclusions are substantiated by a 2 (Sex) × 6 (Time Blocks) × 11 (Days) mixed factorial analysis of variance (ANOVA) which indicated that the effect of sex was not significant, $F(1,14)=2.03$, but that the effect of days, $F(10,140)=2.79$, $p<0.005$, and the interaction between sex and days, $F(10,140)=2.74$, $p<0.005$, were significant. Further, a simple effects ANOVA indicated that females maintained higher activity levels than males on Day 5, $F(1,77)=7.60$, $p<0.01$, Day 6, $F(1,77)=3.42$, $0.05<p<0.10$, Day 7, $F(1,77)=7.23$, $p<0.01$, and Day 11, $F(1,77)=4.27$, $p<0.05$.

The bottom panel of Fig. 1 shows mean activity for males and females as a function of time (collapsed across Days 1–3). During the first hour, males and females exhibited nearly identical activity levels. However, during the second hour, females maintained a high level of activity while males did not. Figure 2 shows that this pattern was evident also following saline injections (Days 4–6) and following naltrexone injections (Day 7). Thus, there was no evidence in

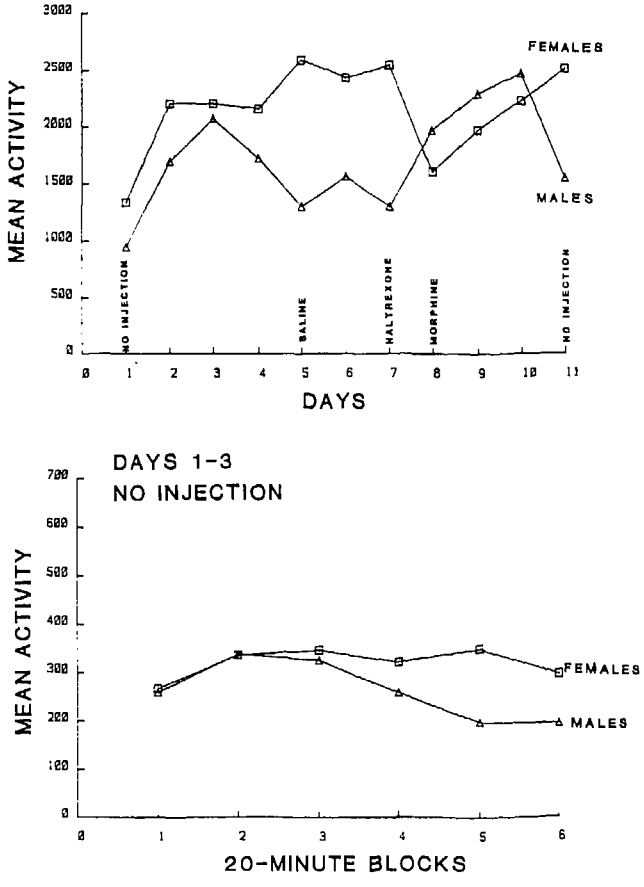

FIG. 1. Top panel shows mean activity (number of wheel revolutions) in Experiment 1 as a function of days and treatment for both sexes. Bottom panel shows mean activity (number of wheel revolutions) in Experiment 1 among males (n=8) and females (n=8) following no injection as a function of 20-minute blocks of time (collapsed across Days 1–3).

this experiment for an effect of naltrexone on activity. Naltrexone neither depressed activity in animals running at high rates (i.e., females) nor enhanced activity in animals running at low rates (i.e., males). The effects of naltrexone on hamster locomotion are investigated further in Experiment 2 below.

Figure 3 (top) shows morphine's time-effect curve for males and females: Mean activity (collapsed across Days 8–10) is shown for the two hours following drug administration. Consistent with other observations [16,17], morphine produced a biphasic time-effect pattern. Compared with performance on any of the previous days, morphine elicited an initial period of hypoactivity, followed by recovery and then by a phase of hyperactivity. Here the biphasic pattern is seen separately for males and females, animals that had very different pre-morphine baselines. Morphine's stimulant effects were particularly noticeable among male hamsters: They responded with a five- or six-fold increase in running wheel activity above their pre-morphine baseline. The bottom panel in Fig. 3 shows mean activity for males and females as a function of time on Day 11. It is clear that, 24 hours after morphine administration, females were running at higher

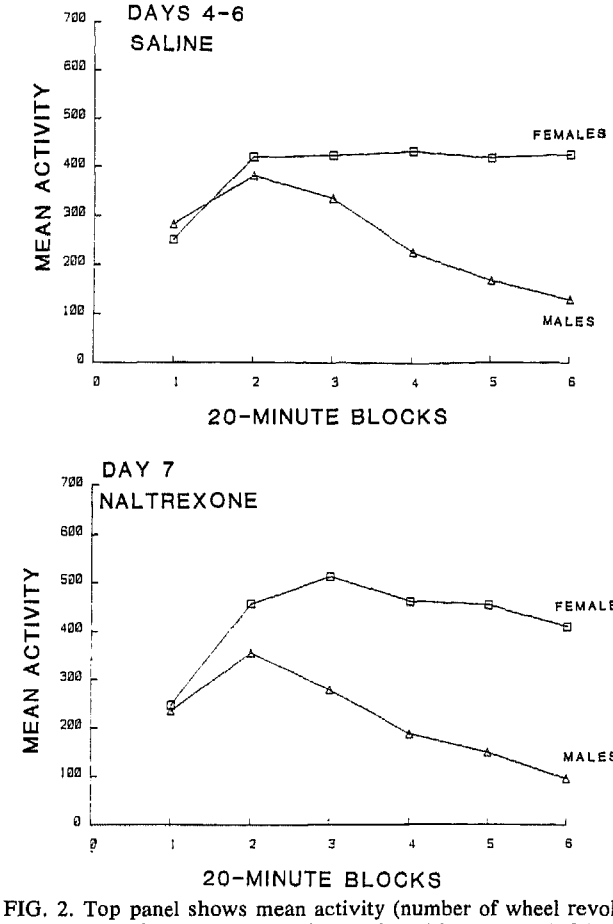

FIG. 2. Top panel shows mean activity (number of wheel revolutions) in Experiment 1 among males (n=8) and females (n=8) following saline injections as a function of 20-minute blocks of time (collapsed across Days 4–6). Bottom panel shows mean activity (number of wheel revolutions) in Experiment 1 among males (n=8) and females (n=8) following a naltrexone (1 mg/kg) injection as a function of 20-minute blocks of time on Day 7.

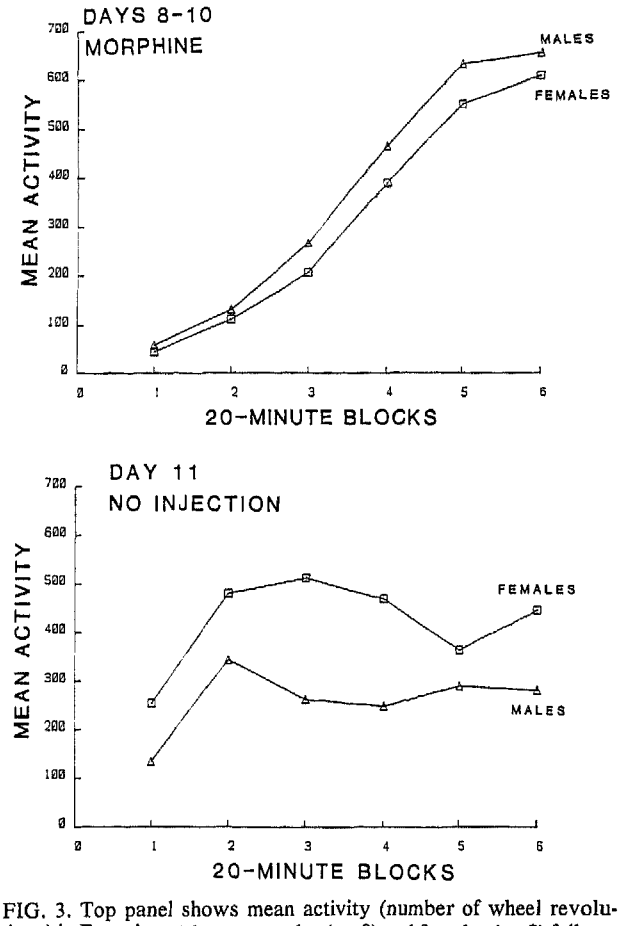

FIG. 3. Top panel shows mean activity (number of wheel revolutions) in Experiment 1 among males (n=8) and females (n=8) following morphine (15 mg/kg) injections as a function of 20-minute blocks of time (collapsed across Days 8–10). Bottom panel shows mean activity (number of wheel revolutions) in Experiment 1 among males (n=8) and females (n=8) following no injection as a function of 20-minute blocks of time on Day 11.

rates than males, much as they had on each of the pre-morphine days. These conclusions are corroborated by the ANOVA which indicated that the effects of time blocks, $F(5,70)=19.28$, $p<0.001$, the interaction between sex and time blocks, $F(5,70)=3.31$, $p<0.01$, the interaction between days and time blocks, $F(50,700)=16.85$, $p<0.001$, and the interaction between sex, days and time blocks, $F(50,700)=1.71$, $p<0.001$, were all significant.

EXPERIMENT 2

The purpose of Experiment 2 was to investigate the effects of four doses of naltrexone on hamster locomotor behavior. As pointed out above, there are conflicting findings concerning the effects of the opiate antagonist naloxone on locomotor behavior, with some investigators reporting that naloxone reduces locomotion [2, 7, 14, 15], but others reporting no effect [1,4]. In the hamster, naloxone has been reported to be without reliable effects on several indices of female sexual behavior [13]. Since morphine has reliable effects on locomotor behavior in the hamster, it is of interest to determine whether opiate antagonists affect that behavior. The present study investigates the dose-effect relationship of the long-acting opiate antagonist naltrexone (0.3, 1, 3, 10 mg/kg) on hamster locomotor activity.

METHOD

Subjects

Forty adult female golden Syrian hamsters with a mean weight of 97.8 g were used. All animals were experimentally naive when obtained from Sasco, Inc. (Omaha, NE). They were housed individually in stainless steel cages, maintained on a 12:12 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. Running wheels were fitted with microswitches and interfaced (Lafayette minicomputer interface, Model No. 1180) to an Apple II Plus computer to record the number of wheel revolutions. An

ambient noise level of 79 dB (re: 0.0002 dynes/cm^2, A scale) was maintained.

Naltrexone injections consisted of 0.3, 1, 3, or 10 mg/kg doses of naltrexone hydrochloride, expressed as the salt, dissolved in 1 ml of physiological saline. All 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 four 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. These sessions served to accustom the animals to the running wheel and to the handling/injection procedures. Baseline data were used also to create five groups which had approximately equal activity levels. These groups were then randomly assigned to five drug treatment conditions (n=8): Group SAL (saline controls); Group NTX-0.3 (0.3 mg/kg naltrexone hydrochloride); Group NTX-1 (1.0 mg/kg naltrexone hydrochloride); and Group NTX-3 (3.0 mg/kg naltrexone hydrochloride); and Group NTX-10 (10.0 mg/kg naltrexone hydrochloride). On day 4, all animals were weighed, injected with the appropriate dose of saline or naltrexone and, following a 15-minute interval, placed in the running wheels for a two hour test session.

RESULTS AND DISCUSSION

Figure 4 shows mean activity as a function of 20-minute blocks for all groups. Although there was a small increase in locomotor activity during the two hour session, there were no reliable differences among groups. That is, at the doses tested here, naltrexone had no effect on hamster locomotor activity compared with saline controls. A 5 (Dose) × 6 (Time Blocks) repeated measures mixed factorial ANOVA indicated that the effects of time blocks was significant, $F(5,175)=4.73$, $p<0.001$, but that neither the effect of naltrexone dose ($F<1$) nor the Dose×Time Blocks interaction ($F<1$) was significant.

Thus, this experiment confirms the lack of effect of a 1 mg/kg dose of naltrexone observed in Experiment 1 and extends that finding to doses of 0.3, 3 and 10 mg/kg. This result is consistent with the reported failures of naloxone to affect locomotor activity in rats and, therefore, is inconsistent with data indicating that opiate antagonists reduce locomotor activity. Although naltrexone was without effect in these experiments, it is still expected to block morphine's effects on locomotor behavior. The role of naltrexone as a morphine antagonist is investigated in Experiment 3.

EXPERIMENT 3

Experiment 3 was designed to investigate the effects of naltrexone (1 mg/kg) on morphine (15 mg/kg) elicited changes in hamster locomotor behavior. Since morphine produces a biphasic time effect pattern on locomotor activity in hamsters [16,17], it is of interest to determine whether naltrexone antagonizes morphine elicited sedation as well as morphine elicited excitation.

METHOD

Subjects

Twenty-four adult female golden Syrian hamsters with a mean weight of 100.0 g were used. The hamsters were obtained from Sasco, Inc. (Omaha, NE), housed individually in stainless steel cages, maintained on a 12:12 hour light-dark cycle, and given free access to food and water throughout the experiment. All animals had served in the naltrexone dose-response experiment one week prior to the present experiment and thus had previous exposure to the running wheel and to naltrexone.

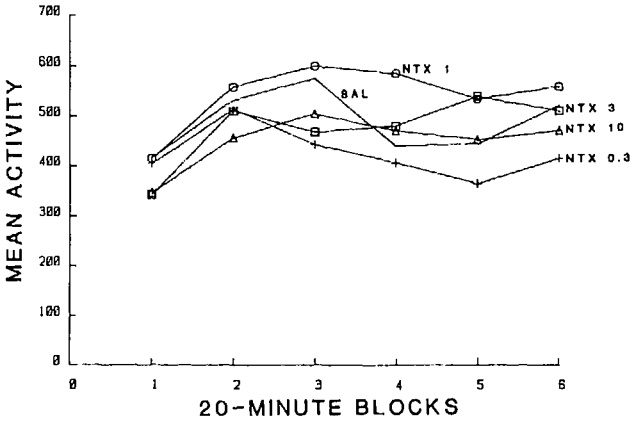

FIG. 4. Mean activity (number of wheel revolutions) in Experiment 2 as a function of 20-minute blocks of time in Groups (n=8) SAL, NTX-0.3, NTX-1, NTX-3, NTX-10.

Apparatus and Materials

The apparatus was the same as that used in the previous experiment. Naltrexone injections consisted of a 1 mg/kg dose of naltrexone hydrochloride, expressed as the salt, dissolved in 1 ml of saline. Morphine injections consisted of a 15 mg/kg dose of morphine sulfate, also exprssed as the salt, and dissolved in 1 ml of physiological saline. Morphine, naltrexone, and saline injections were administered subcutaneously in the dorsal surface of the neck in 1 ml/kg volumes.

Procedure

One test session was conducted. Three groups, matched for prior drug treatment in Experiment 2, were randomly assigned to one of three drug treatment conditions to receive a series of two injections: Group SAL/SAL (two saline injections), Group SAL/MS (saline followed by morphine injection), Group NTX/MS (naltrexone followed by morphine injection). The two injections were separated by an interval of approximately ten minutes. Five minutes after the second injection, animals were placed in the running wheels for a two hour test session. The number of wheel revolutions was recorded at 20-minute intervals for each animal.

RESULTS AND DISCUSSION

Figure 5 shows mean activity as a function of 20-minute blocks for all groups. It is evident that compared with saline controls, morphine produces an initial decrease in locomotor activity followed by a gradual recovery that is complete within two hours. Under the conditions of this experiment, a 15 mg/kg dose and a single two hour test session, morphine

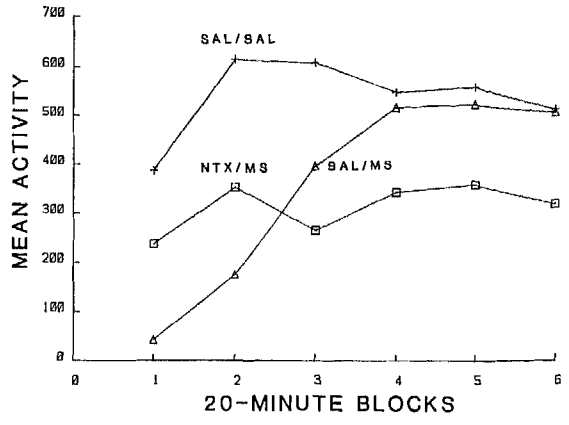

FIG. 5. Mean activity (number of wheel revolutions) in Experiment 3 as a function of 20-minute blocks of time. Group SAL/SAL (n=8) received two saline injections, Group SAL/MS received a saline followed by a morphine (15 mg/kg) injection, and Group NTX/MS received a naltrexone (1 mg/kg) injection followed by a morphine (15 mg/kg) injection.

elicited hyperactivity was not evident. Nevertheless, the antagonistic effects of naltrexone can be discerned. That is, a 1 mg/kg dose of naltrexone blocked morphine's sedative effects: Group NTX/MS was less sedated than Group SAL/MS. However, at the dose tested naltrexone did not completely reverse morphine-elicited sedation: Group NTX/MS was less active than Group SAL/SAL. Furthermore, it appears that naltrexone blocked the increase in locomotor activity that characterizes recovery from morphine sedation: Whereas Group SAL/MS recovered from its initial sedation to achieve a level of activity comparable to that of saline controls, Group NTX/MS showed no increase in locomotion during the session. These conclusions are corroborated by a 3 (Drug Treatment × 6 (Time Blocks) mixed factorial ANOVA which indicated that the effect of time blocks, $F(5,105)=10.71$, $p<0.001$, and the interaction between time blocks and drug treatment, $F(10,105)=4.59$, $p<0.001$, were significant. In addition, post-hoc comparisons using Tukey's HSD test indicated that during the first 20-minutes, Group SAL/MS was less active than Group SAL/SAL ($p<0.01$), and Group NTX/MS was more active than Group SAL/MS ($p<0.05$). During the last 20 minutes, Group NTX/MS was less active than Groups SAL/SAL and SAL/MS ($p<0.05$).

GENERAL DISCUSSION

The results of the present study indicate that female hamsters run more than males during two hour test sessions. This difference is due principally to the fact that females maintain a high level of activity for two hours, whereas males slow down considerably during the second half of each session. Similar findings have been reported in rats [10,18]. In addition, the higher female activity level persists for several days and survives various treatments (i.e., no injection, saline, naltrexone, morphine injections). Indeed, there is some suggestion in the data that the sex difference increases with exposure to the running wheel (cf., [16]). For example, inspection of Figs. 1, 2, and 3 indicates that the decline in male activity starts progressively earlier and reaches a lower level during the course of the experiment. These changes, however, cannot be attributed unambiguously to exposure per se, since treatment varied during that same exposure. Additional research is needed to determine if the sex difference in locomotor activity found in Experiment 1 is related to similar differences that are under circadian control [6].

The present data also indicate that naltrexone, at doses of 0.3, 1, 3, and 10 mg/kg, has no effect on hamster locomotor activity. This finding is consistent with the reported failure of naloxone to affect locomotor activity in rats [1,4], but it is inconsistent with other data indicating that naloxone reduces locomotor activity in rats [2, 7, 14, 15]. The reasons for the discrepancies in the literature are not clear, but it is likely that the effects of opiate antagonists on behavior are both species [12] and situation dependent [7]. One situational variable that has been identified as important in mediating naloxone's effect is environmental novelty. That is, opiate antagonists may influence behavior in novel but not familiar surroundings [2, 11, 14, 15]. If that is the case, then naltrexone's failure to influence locomotion in the present experiments might be due to the fact that, following several baseline days of exposure to the running wheel, the environment was not sufficiently novel to activate naltrexone's effects.

Finally, the present experiments confirm our earlier observations [16,17] concerning morphine's biphasic effects and suggest that both phases are naltrexone reversible. In Experiment 1, morphine, compared with either a no-injection baseline or a saline baseline, first inhibited, then stimulated locomotor activity. Experiment 1 demonstrated this pattern separately for females and males and indicated that, even when baselines are suppressed, morphine's effects, particularly its excitatory effects, are evident. The hyperactivity among male hamsters on Days 8–10 is especially impressive documentation of morphine's stimulant effects. That both hypoactivity and hyperactivity were the result of morphine administration (and not, for example, a consequence of continued exposure to the wheel) is indicated by the return of behavioral baselines on Day 11. In Experiment 3, morphine elicited sedation and gradual recovery were seen again, but hyperactivity was not evident. Nevertheless, both sedation and the subsequent recovery were shown to be naltrexone reversible.

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