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Opiate Effects on Social Investigatory Behavior of Male Mice

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LANDAUER, M. R. AND R. L. BALSTER. *Opiate effects on social investigatory behavior of male mice.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1181–1186, 1982.—The effects of morphine and naloxone, alone and in combination, on social investigatory behavior and motor activity was examined in CD-1 male mice. Tests were conducted in a Plexiglas apparatus in which a center area was separated from two adjacent stimulus compartments by wire mesh screens. One compartment housed a female conspecific while the other remained empty and served as a control for non-specific investigatory responses. A photocell bisected the center compartment and recorded motor activity. Male mice were placed individually into the center area and the time spent investigating each screen was recorded using contact circuits during the 15-min test. In Experiment 1, males (N=11) received saline, 0.1, 1.0 and 10.0 mg/kg morphine sulfate IP 20 min prior to testing. The high dose significantly decreased investigation of the female compartment while investigation of the uninhibited chamber and motor activity were not significantly affected. In a second experiment (N=16), 3, 10 and 30 mg/kg naloxone administered 30 min prior to testing had no significant effect on any of the measures recorded. In a third group of subjects (N=16), 3 mg/kg naloxone reversed the decrease in female investigation time observed with 10 mg/kg morphine, indicating an opiate mechanism for these results. These data provide further evidence that an animal model can be used to study the disruption of socio-sexual behavior produced by opiates.

Socio-sexual behavior Morphine Naloxone Locomotor activity Mice

IT is well established that opiate drugs such as morphine, heroin and methadone adversely affect sexual motivation as well as sexual performance in both men and women [7, 10, 24, 25, 31, 32] while withdrawal from these drugs renews sexual interest [31]. Recently, there has been considerable interest in establishing animal models for the investigation of the effects of exogenous and endogenous opiates on sexual behavior. In general, the opiates have been found to disrupt mating behavior in all mammalian species studied to date. Thus, acute administration of morphine to monkeys [5] or rats [18, 23, 27, 33] has been shown to impair reproductive activity. In addition, rats administered morphine chronically [33,42] or hamsters given acute doses of methadone [34] show reductions in reproductive performance. Similar alterations in copulatory behavior have been observed following administration of the endogenously occurring opioid, β-endorphin [23, 27, 29] or the synthetic enkephalin, D-Ala^2-Met-enkephalinamide [13, 39].

Administration of the opiate antagonists (naloxone and naltrexone) has been shown to block the reduction in sexual behavior following injections of opiates or opiate peptides to rodents, suggesting that this response is mediated by opioid receptors [13, 18, 23, 29, 34, 39]. Interestingly, the narcotic antagonists given alone have been reported to enhance sexual performance in copulating rats [13, 18, 23, 35, 36, 39, 40] and to initiate mating behavior in rats that had previously been sexually inactive [13,23]. In addition, spontaneous penile erections have been observed following administration of naloxone to rats [1] and naltrexone to normal adult men [26]. Naloxone, however, failed to increase sexual arousal in sexually unresponsive women [2] and had no effect on sexual arousal, or orgasm, in a single male subject [14].

The purpose of the present study was to extend our knowledge of the effects of morphine and naloxone alone and in combination on the socio-sexual behavior of the laboratory mouse. This measure has previously been shown to be sensitive to drug and hormonal manipulations in both rats [19,27] and mice [22]. To circumvent some of the problems with observational studies of social behavior, we have devised an automated apparatus for recording investigatory behavior and spontaneous motor activity in mice. The concurrent assessment of investigatory behavior and locomotor activity allows an evaluation of the specificity of drug effects. The measure utilizes the amount of time a male subject spends investigating a compartment housing a female conspecific compared to the investigation of an uninhabited compartment. We [22] have previously shown that sexual experience by the male subject increases the amount of investigation of the female in this apparatus indicating that some aspect of socio-sexual behavior is measured. Three separate experiments on the effects of various doses of morphine alone, various dose of naloxone alone, and combinations of morphine and naloxone were carried out.

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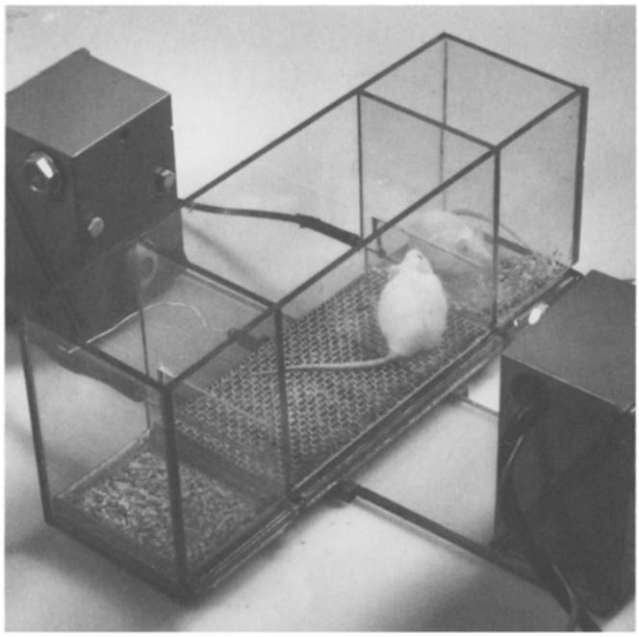

FIG. 1. Plexiglas apparatus used for the evaluation of social investigatory behavior. A male subject was placed into the center compartment and the amount of time spent investigating each of the adjacent stimulus compartments was automatically recorded using contact circuits. One compartment housed a female conspecific and the other remained uninhabited, serving as a control for non-social investigatory responses. A photocell unit placed outside the apparatus was used to record locomotor activity of the male subject. The entire apparatus as shown was placed into a sound and light attenuating chamber during tests.

EXPERIMENT 1

METHOD

Subjects

Eleven sexually naive adult male CD-1 mice weighing between 29 and 37 g were purchased as adults from Charles River Breeding Labs, Wilmington, MA. The animals were weaned at 21 days of age and caged in all-male groups prior to being shipped to our laboratory. After a two week acclimization period the mice were individually housed in clear plastic cages (28.5×17.5×12 cm) with wire mesh tops (Wahmann Manufacturing Co., Timonium, MD) and wood-chip bedding (Beta-chip, Northeastern Products, Warrensburg, NY). The animals were kept under a reversed 12 hr light-dark cycle with lights off at 9:00 A.M. and maintained at 21±1° C. They had free access to water and Purina Laboratory Chow #5001, except during experimental sessions.

Apparatus

Social investigatory behavior and spontaneous motor activity were recorded in a rectangular Plexiglas apparatus divided into three compartments (Fig. 1). The larger middle compartment (19×10×15cm) was separated from the two adjacent stimulus compartments (8×10×15 cm) by clear Plexiglas partitions with 3×8 cm windows of wire mesh metal screen (11.8×11.8 meshes per cm). These windows were located 1.5 cm above the wire mesh (2.4×2.4 meshes per cm) floor of the center compartment. Each time a subject placed into the center compartment touched a screen window separating the stimulus chambers, the amount of time was automatically recorded using contact circuits connected between the floor and the windows. A photocell (Autotron, Danville, IL) bisecting the middle compartment and positioned 1.3 cm above the wire mesh floor recorded spontaneous motor activity as the number of beam interruptions. All compartments were equipped with removable trays so that bedding could be easily changed between trials. A Plexiglas cover with air holes prevented subjects and stimulus animals from leaving the compartments. The entire apparatus was placed into a sound and light attenuating chamber (Coulbourn Instruments, Lehigh Valley, PA) during testing. Timing of the session and data recording were accomplished by solid-state electronic circuitry.

Procedure

Adult males were isolated 10 days prior to testing in order to increase their sexual arousal [9]. Each mouse was adapted to an empty test apparatus for 15 min, 24 hr prior to their first preference test. A preference test involved placing the subject into the apparatus for 15 min and recording the amount of time investigating each stimulus compartment. One stimulus compartment always remained empty except for clean wood-chip bedding and served as a control for non-social investigation; the other housed a bilaterally ovariectomized female and bedding from her home cage. Ovariectomized females were chosen as stimulus animals since they could be used every 72 hours without changes in endogenous hormones, and are as effective as intact females in eliciting some aspects of courtship behavior from male conspecifics [37]. Moreover, although sexually naive mice have been shown to exhibit a preference for an ovariectomized female over an intact male conspecific (Landauer and Balster, unpublished], they do not show a preference for estrous vs. non-estrous females [16]. Each male received IP injections of the saline vehicle and 0.1, 1.0 and 10.0 mg/kg morphine sulfate (Mallinckrodt, St. Louis, MA) in a volume of 10 ml/kg, 20 min prior to each of four test sessions. Tests were conducted every 72 hr and the sequence of drug dosages was determined by a Latin-Square design. All males received a different stimulus female during each of the four tests, the left-right position of the female being counterbalanced. Bedding from the subjects' home cage was placed beneath the screen in the center compartment. The entire apparatus was cleaned with a 70% ethanol solution and allowed to dry between trials. All tests were conducted during the dark period of the light-dark cycle.

Data Analysis

Investigation time for each of the stimulus compartments was recorded in tenths of seconds. The data was submitted to a two-way analysis of variance with repeated measures on both factors (compartment and dose) and was required to meet the assumption of compound symmetry [8]. When the assumption of symmetry is not met an approximate F-test [15] can be used. The BMDP Statistical Software (UCLA Department of Biomathematics) was utilized for these analyses. Selected planned individual comparisons were also made. The effects of each dose of morphine were compared to vehicle control using a two-tailed Dunnett's test [45]. An

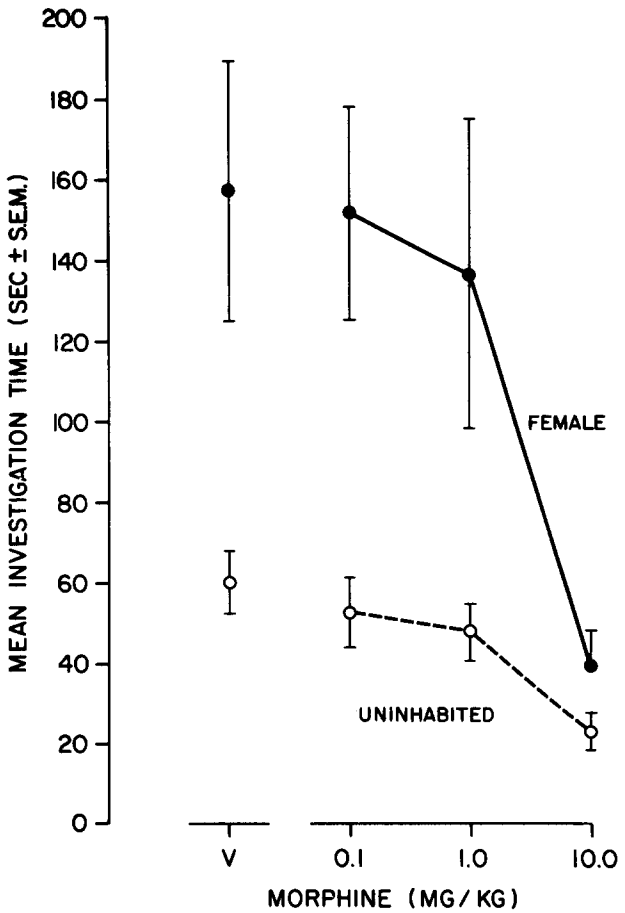

FIG. 2. Mean time spent investigating female and uninhabited compartments by male subjects (N=11) following acute IP injections of saline vehicle (V), 0.1, 1.0 and 10.0 mg/kg morphine sulfate administered 20 min prior to the 15 min test session.

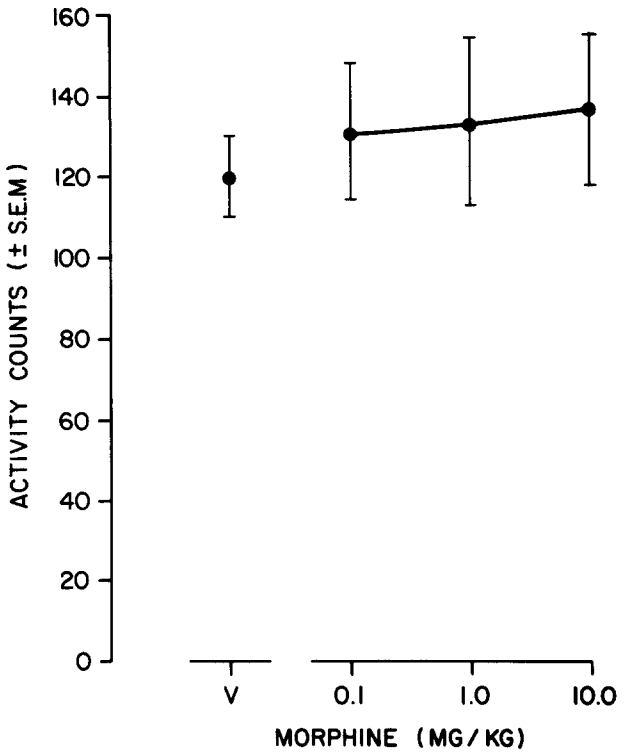

FIG. 3. Mean number of photobeam interruptions (activity counts) by male subjects (N=11) following acute IP administration of saline vehicle (V), 0.1, 1.0 and 10.0 mg/kg morphine sulfate.

independent set of comparisons was made for investigation of the female and the uninhabited side with $\alpha<0.05$ for each set. Additional comparisons of the investigation time spent with the female vs. uninhabited side for the vehicle and each drug dose were also made. Since each of these four comparisons is statistically independent, t-tests were performed with each one-tailed $\alpha<0.013$ resulting in an overall $\alpha<0.05$ for the set.

Activity measures were obtained as total photocell beam interruptions and submitted to a one-way analysis of variance with repeated measures. The effect of each drug dose was compared to the vehicle control using a two-tailed Dunnett's test with $\alpha<0.05$ for the set of comparisons.

RESULTS

The effects of vehicle and morphine on mean investigation time is presented in Fig. 2. Administration of morphine resulted in a significant dose-dependent decrease in total investigation time directed towards both the female and uninhabited compartments, $F(3,30)=8.51$, $p<0.001$. Dunnett's test revealed that, compared to vehicle conditions, only the dose of 10 mg/kg morphine significantly decreased female investigation time, while none of the morphine doses significantly affected investigation of the uninhabited chamber. Overall, the female compartment was investigated significantly more than the uninhabited compartment, $F(1,10)=12.56$, $p<0.005$, but with increasing doses of morphine this difference was decreased resulting in a significant dose × compartment interaction, $F(3,30)=3.70$, $p<0.02$. The males significantly preferred the female to the uninhabited side under vehicle, 0.1 and 1.0 mg/kg morphine, but this preference was eliminated at the high dose of morphine (10 mg/kg). As shown in Fig. 3, the doses of morphine tested failed to significantly affect motor activity.

EXPERIMENT 2

Several studies have reported a facilitation in copulatory performance following the administration of opiate antagonists to male rats [13, 18, 23, 34–36, 40], although Sachs [41] failed to observe these effects. Experiment 2 was conducted to determine the effects of naloxone on sociosexual investigatory behavior in sexually naive adult male mice.

METHOD

Sixteen experimentally naive adult male CD-1 mice were maintained under the same conditions as the animals used in Experiment 1. All animals received IP injections of 3, 10, and 30 mg/kg naloxone hydrochloride (obtained from the National Institute on Drug Abuse), or the saline vehicle in a volume of 10 ml/kg body weight 30 min prior to the social

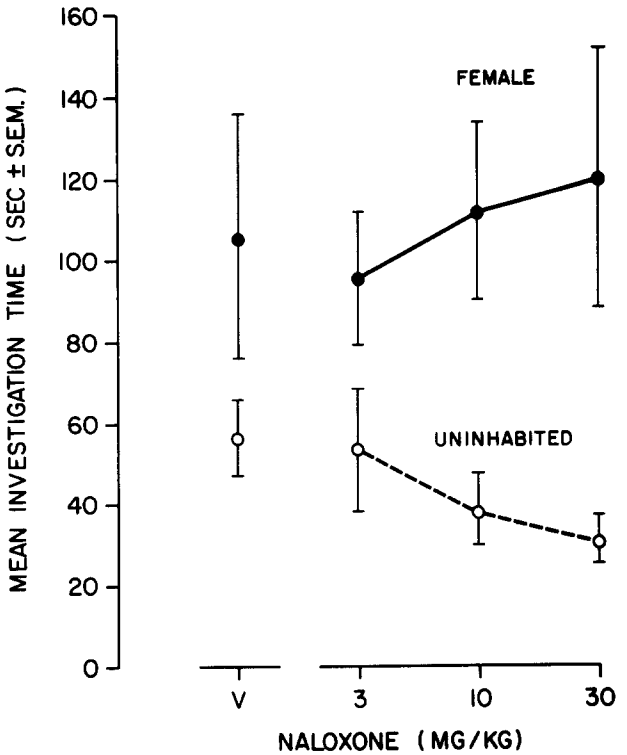

FIG. 4. Mean time spent investigating female and uninhabited compartments by male subjects (N=16) after acute IP injections of saline vehicle (V), 3, 10 and 30 mg/kg naloxone hydrochloride administered 30 min prior to the 15 min social preference test.

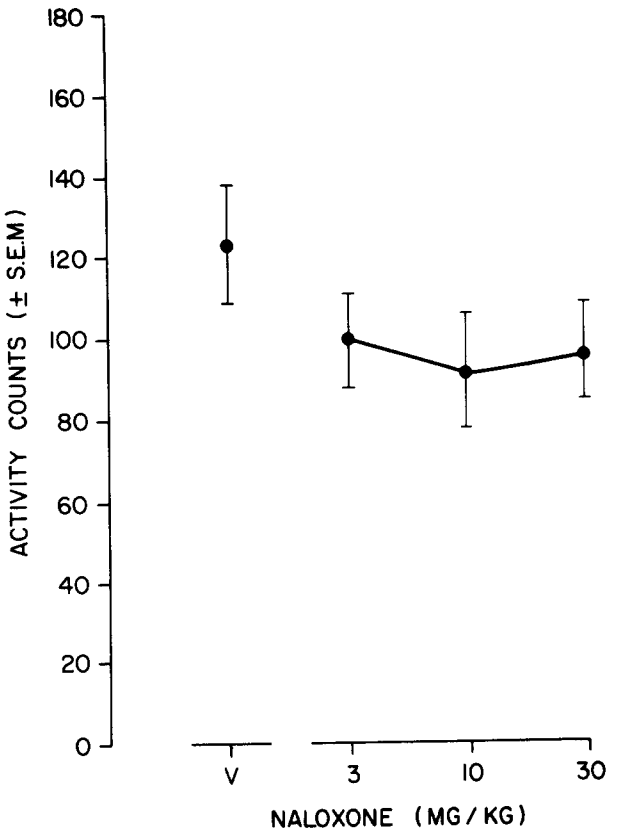

FIG. 5. Mean number of photobeam interruptions (activity counts) by male subjects (N=16) following acute IP administration of saline vehicle (V), 3, 10 and 30 mg/kg naloxone hydrochloride.

preference test. The procedure was identical to that followed in Experiment 1.

RESULTS

The effects of naloxone on investigation time are shown in Fig. 4. Total investigation time was unaffected by naloxone, $F(3,45)=0.14$, $p>0.05$, and although there was a trend for naloxone to increase investigation of the female side and decrease that of the uninhabited side, the interaction term in the analysis of variance was not significant, $F(3,45)=2.15$, $p>0.05$. Individual comparisons also revealed that none of the doses of naloxone produced a significant difference from control investigation time for either side. Overall, there was considerably more investigation of the female side, $F(1,15)=6.3$, $p<0.05$, and this difference was also significant following each treatment. Spontaneous motor activity was also not significantly affected by these doses of naloxone (Fig. 5).

EXPERIMENT 3

The decrease in sexual performance of male rodents following administration of endogenous or exogenous opiates is readily reversed by the opiate antagonists, naloxone and naltrexone [13, 18, 23, 29, 34, 39]. The following experiment was performed to determine whether a behaviorally inactive dose of naloxone could antagonize the decrease in female investigation observed when male mice were administered 10 mg/kg morphine (Experiment 1).

METHOD

Sixteen experimentally naive CD-1 adult male mice were housed under identical conditions as the subjects used in the previous two experiments. These males all received four social investigatory tests according to the procedures followed in Experiments 1 and 2. Subjects received IP injections of saline (S) or 3 mg/kg naloxone hydrochloride (N) 30 min before testing and IP administration of saline (S) or 10 mg/kg morphine sulfate (M) 20 min prior to test sessions. Thus, all animals received the following vehicle and drug combinations: S-S, N-S, S-M, N-M. As in Experiments 1 and 2, tests were administered every 72 hr according to a Latin-Square design.

RESULTS

The results of Experiment 3 are presented in Fig. 6. The male subjects spent significantly more time investigating the female compartment compared to the uninhabited chamber for the S-S and N-S groups, replicating the effects produced in Experiment 2. Administration of 10 mg/kg morphine (group S-M) eliminated the preference for the female, confirming the findings of Experiment 1. Naloxone was found to antagonize the effects of morphine, reinstating the preference for the female compartment. Dunnett's procedure revealed that the activity counts (mean±SEM) for groups N-S

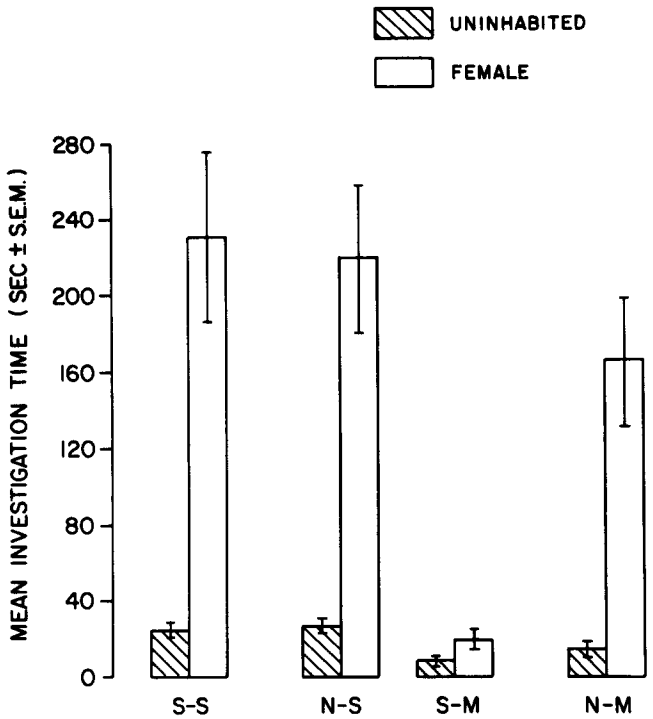

FIG. 6. Mean time spent investigating female and uninhabited compartments by male subjects (N=16). Each male was tested four times. Subjects received IP injections of saline (S) or 3 mg/kg naloxone (N) 30 min before the test session and saline (S) or 10 mg/kg morphine (M) 20 min prior to testing.

(119.0±9.5), S-M (114.6±18.1), and N-M (70.2±11.4) did not differ significantly from the S-S control group (100.9±10.9).

GENERAL DISCUSSION

Morphine resulted in a dose-dependent decrease in investigatory behavior in male mice, however the effect on investigation of a female conspecific was larger than the effect on the investigation of an uninhabited compartment. Greater sensitivity of social investigation to the effects of morphine can be argued based on the following results. The statistical analysis revealed a significant dose by compartment interaction demonstrating that the effect of morphine depended upon the occupancy of the compartment. Individual comparisons revealed that only the effect of 10 mg/kg morphine on female investigation was statistically significant, whereas this dose did not have a significant effect on investigation of the uninhabited compartment. The normal preference for investigation of the female compartment observed after vehicle administration was abolished by the high dose of morphine. Finally, none of the doses of morphine (0.1–10 mg/kg) had any effect on motor activity under these conditions. Thus, there is specificity for the effects of morphine on socio-sexual investigatory behavior.

Naloxone at doses of 3–30 mg/kg failed to significantly affect investigation of either the female or the uninhibated compartment or alter locomotor activity compared to control levels. The failure of 3 mg/kg naloxone to affect investigation and the reduction in female investgatory behavior in mice administered 10 mg/kg morphine was replicated in Experiment 3. Naloxone (3 mg/kg), however, readily antagonized the reduction in female investigation time produced by the 10 mg/kg dose of morphine.

The decrease in female investigation observed with 10 mg/kg morphine in the mouse is in agreement with related studies using rats. Morphine has previously been shown to disrupt male-female socio-sexual behavior [18, 23, 27, 33, 42] as well as male-male interactions in rats [38] and mice [17]. Similarly, both β-endorphin [23, 27, 29] and D-Ala^2-Met-enkephalinamide [13,39] have been shown to reduce male-female interactions in rats. The decrease in female investigation time produced by morphine in the present situation appears to be directly mediated by an opiate mechanism since it can be readily reversed by low doses (3 mg/kg) of the opiate antagonist naloxone.

Although there was a trend for high doses of naloxone to increase female investigation, this was not statistically significant. This non-significant increase in socio-sexual behavior may be due to naloxone's enhancement of mating behavior in sexually inactive animals [13,23]. One must be cautious of this interpretation, however, since (1) these studies have been reported only for rats, and (2) the mice used in the current study were all sexually naive and never evaluated for copulatory performance. Naloxone, moreover, has also been reported to reduce active social interaction between two male rats [12] although Panksepp *et al.* [38] have reported equivocal results.

When compared to vehicle control levels there was no change in locomotor activity following administration of morphine (0.1–10mg/kg). Effects of morphine on activity appear to differ widely across strains of mice [44]. Approximately 30–45 min following administration of 10 mg/kg morphine, locomotor activity has been reported to increase [3,6], decrease [11,17], or like the present study, remain unaffected [3, 6, 43].

The effects of naloxone on locomotor activity also appear to differ across strains of laboratory mice. Like Holtzman [20], we found that naloxone produced no significant effect on activity levels when administered in doses ranging from 3 to 30 mg/kg. Doses of 3 mg/kg [30] and 8 mg/kg [21] however, have been reported to decrease locomotor activity in some strains of mice.

Taken together, the results of the present study provide additional evidence for the role of opiates in the control of mammalian socio-sexual behavior. Moreover, they demonstrate the feasibility of an automated procedure for studying this phenomenon in mice.

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