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Opiate Regulation of Maternal Behavior in the Rat[1]

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GRIMM, C. T. AND R. S. BRIDGES. *Opiate regulation of maternal behavior in the rat.* PHARMACOL. BIOCHEM. BEHAV. 19(4) 609–616, 1983.—The effects of the opiate agonist morphine, the opiate antagonist naloxone and the weak opiate nonanalgesic dextrorphan on the expression of maternal behavior were investigated in a series of three experiments. In the first experiment treatment of rats with morphine (5 mg/kg, subcutaneously) after ovariectomy and hysterectomy on day 17 of gestation resulted in a disruption in the onset and quality of maternal responsiveness in the homecage and in a T-maze test. The duration of morphine's acute disruptive action was 2–4 hours. In the second experiment concurrent treatment of morphine-injected rats with naloxone prevented the disruptive effects of morphine in both the homecage and T-maze tests. The effects of morphine did not appear to result from a severe alteration in activity levels as measured in an open-field test, although morphine did increase activity slightly by the fifth day of treatment. In the third experiment treatment of rats after ovariectomy plus hysterectomy on day 17 of gestation with dextrorphan failed to disrupt maternal behavior. These results indicate that morphine disrupts maternal behavior through an opiate receptor mechanism, and suggests to us that endogenous opiates may mediate the expression of maternal behavior under certain physiological conditions.

Dextrorphan Maternal behavior Morphine Naloxone Opioids

THE physiological events associated with pregnancy prime the female to respond maternally to her young at the time of delivery. One important set of biological events that account for the rapid onset of maternal behavior at birth is the hormonal changes of pregnancy and parturition. In rats, as well as mice, rabbits and sheep, estradiol (E_2) and progesterone (P), two steroid hormones found in large amounts in blood during pregnancy, can stimulate mother-young interactions [4, 15, 16, 19, 21, 29, 36, 37].

One mechanism through which these steroid hormones affect maternal behavior may involve alterations in endogenous opioid activity. Hypothalamic β-endorphin content is elevated during pregnancy in rats [32] at a time when serum E_2 and P concentrations are high [12, 30, 34]. Furthermore, β-endorphin content in the CNS of female rats is influenced by the levels of circulating steroids [32]. In addition to these biochemical studies, behavioral experiments have shown that pain thresholds, which are opiate-mediated, change during pregnancy and lactation in rats [11]. Specifically, sensitivity to pain decreases as pregnancy advances and then increases during lactation.

These findings led us to investigate the possibility that the onset of maternal behavior in the rat might result in part from a decrease in CNS opiate activity around the time of parturition. To test this hypothesis we attempted to maintain high levels of opiates by administering morphine to pregnancy-terminated rats. We found that morphine administration delayed the onset of maternal behavior after pregnancy-termination, and that concurrent treatment of rats with morphine plus the opiate antagonist, naloxone hydrochloride, prevented this disruption from occurring [3].

In the present study we have attempted to characterize the behavioral and biochemical effects of morphine's disruptive action on maternal behavior. In a series of three experiments, we have examined the affects of morphine injections on the duration and intensity of disruption of maternal behavior, the effects of morphine and naloxone treatments on maternal behavior and activity, and the actions of dextrorphan, an inactive stereoisomer of the morphine-like levorphanol, on maternal behavior in rats.

GENERAL METHOD

Animals

Adult virgin female Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were housed in a light (on 0500–1900 hr) and temperature (22–23° C) controlled room upon their arrival in our laboratory. Food (Purina Rat Chow) and water were available ad lib throughout the study.

Approximately 2–4 weeks after their arrival in our laboratory, experimental females (225–250 g) were bred to males. The day after mating was observed or the day sperm was detected in the vaginal lavage was designated day 1 of pregnancy. At this time pregnant females were housed individually in opaque polypropylene cages (45×25×20 cm). In addition to the experimental females, a large group of lactating

[1]Some of the data presented in Experiment 2 were included in an earlier published report [3].

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donor rats was maintained throughout the study to provide a source of rat test young.

Surgery and Injections

On day 17 of pregnancy females were ovariectomized and hysterectomized under ether anesthesia as previously described [2]. Treatments were initiated immediately after surgery when animals were injected subcutaneously with morphine, morphine plus naloxone, or dextrorphan, each dissolved in 0.9% NaCl, or with 0.5 ml saline alone. Morphine (morphine sulfate, courtesy of Eli Lilly Co.) and dextrorphan (dextrorphan tartrate, courtesy of Hoffman-LaRoche, Inc.) were administered at a dose of 5 mg/kg. Naloxone (naloxone hydrochloride, courtesy of Endo Labs) was given at a dose of 0.5 mg/kg. Animals received a second injection 20–23 hr after surgery, and behavioral testing began one hour later. On subsequent days, unless otherwise specified, rats received a single injection after the pre-test, one hour before the test session. All solutions were prepared daily prior to injections.

Behavioral Testing

Maternal behavior. Rats were tested for maternal behavior in the homecage as previously described [1]. On the first test day, about 21–24 hours after surgery, three foster rat pups, 3–8 days of age, were placed in each test animal's homecage. Rats were then observed continuously for 15 minutes and checked at 30, 45 and 60 minutes to determine latencies in minutes to carry a pup, retrieve pups to the nest, group the pups together and crouch over any of the young in a position characteristic of nursing mothers. The pups remained with the test animals overnight. The next day about 1 hour prior to testing the positions of the females and pups were again recorded. This 23 hour checkpoint was termed the "pre-test." The test subjects' pups were then returned to lactating donors and the test subjects were injected with the designated solutions. An hour later homecage testing was conducted with freshly fed pups. This procedure was repeated until the test animal responded fully, that is retrieved, grouped, and crouched over all three pups on two consecutive test days, or for 11 to 14 days, as designated in each experiment.

In addition to measuring the rate of onset of maternal behavior in the homecage, in Experiments 1 and 2 the intensity of maternal responsiveness was measured using a T-maze apparatus [5,31]. The test apparatus and procedure has previously been described in detail [7]. Briefly, the day after each animal reached a level of full maternal responsiveness in the homecage, it was tested for responsiveness to young in a T-maze. Animals were tested once daily for 4 consecutive days. One hour before testing on each test day, foster pups were removed from the test cage and the test animal was injected with the appropriate solution. At the start of T-maze testing a wire cage lid devoid of food and water bottle was attached atop the test animal's cage. The test began when the cage door (adapted test cages, see [7]) was opened and a T-maze with one 3–5 day old pup at the end of each maze arm was inserted into the homecage opening. The frequencies and latencies to enter the maze, contact the pups and retrieve the test young to the homecage were recorded during 10 minute test sessions on each of the 4 test days. After a test session, three recently fed foster young were placed in the subject's homecage in order to maintain homecage maternal responsiveness between T-maze tests.

Open-field activity. In Experiment 2 activity was measured in morphine-treated and control animals in an open-field apparatus. Twenty-three hours after surgical termination of pregnancy and drug treatment, females were reinjected with the designated solution. Testing commenced one hour later using an open field test similar to that described by Hall [14]. Testing consisted of placing animals on a designated spot in a square wooden enclosure that measured 3×3′, within which a grid of 6″ squares had been demarcated, and recording the number of times rats reared and crossed the grid lines into different squares with all four feet during the next 5 minutes. Each rat was injected with the designated solution 1 hr prior to open field testing on each of four consecutive days.

Statistical Analysis

Statistical analyses were preformed on two measures of homecage responsiveness. First, we compared the latencies, in days, to full homecage responses. For this analysis, the first day of behavioral testing was counted as day 0. If a female responded on the first and second days of testing, her latency was thus scored as zero days. Second, we compared the latencies, in minutes, to retrieve one pup and to retrieve, group and crouch over all three pups within the homecage test session on the first and second days of full maternal responsiveness ("response days"). Rats which did not fulfill the criterion for full homecage behavior were not included in response day comparisons.

The behavioral responses of the test animals in the T-maze apparatus were compared on each of the four test days. Comparisons of the incidences of retrieval responses, pup contact and maze entrance were made among and between experimental groups.

In the open-field test the number of rears and grids crossed were compared between experimental groups over the four test days.

Data were analyzed using an analysis of variance (AOV), Mann-Whitney U-test, Fisher Exact Probability Test and Chi Square Test.

EXPERIMENT 1: MORPHINE ADMINISTRATION AND MATERNAL BEHAVIOR: BEHAVIORAL EFFECTS AND DURATION OF ACTION

This experiment was designed to characterize the effects of morphine administration on maternal behavior in rats, and to determine the duration of morphine's action. In preliminary studies it was found that morphine-injected animals often failed to respond behaviorally within the first two hours after treatment, but appeared behaviorally responsive 24 hr later at the pretest rating. In the present study, therefore, we decided to monitor the behavioral responses of the treated females at more frequent intervals after morphine injection and to increase the frequency of morphine injections.

Method

A total of twenty rats had their pregnancies terminated on day 17 of gestation. At this time 10 females were assigned to a morphine group (M1) and the remaining 10 animals assigned to a saline control group (S1). All females were injected with the designated solutions at the time of surgery and then twice daily thereafter, once at the pretest period (10:30 hr) and again about 12 hours after the pretest injection.

Behavioral testing for maternal responsiveness began the day after surgery and was performed as previously described. However, in addition to scoring the position of the pups during the pretest session and the active responses of the rats during the 1 hour behavioral test, the positions of the pups and test animals were noted at two other times: 4 hours after the morning injection (3 hours after introduction of the test pups) and again 12 hours after the morning treatment, immediately prior to the second injection. After this later injection the test young were placed throughout the homecage in a manner similar to that used during the 1 hour morning test session.

Animals were tested in the homecage for maternal behavior until they responded fully during the test session hour on two consecutive days. The following 4 days these maternal rats were tested for maternal responsiveness in a T-maze apparatus (see the General Method section). During the four days of T-maze testing test animals were injected twice daily at the same intervals and with the same dose of morphine or saline used during homecage testing.

Rats which failed to respond maternally in the homecage after 14 test days were assigned a homecage latency of 14 days and were not tested in the T-maze apparatus.

Results

Morphine treated rats had significantly longer latencies to show full maternal behavior in the 1 hour homecage test session than did controls. The latency of the M1 group was 8.2 ± 1.6 days, while that of the saline controls was 2.0 ± 0.6 days, $F(1,18) = 12.64$, $p < 0.001$, Fig. 1). At the 3 hour checkpoint, 4 hours after morphine treatment, there were no differences in the responses of the groups as judged by pup and test animal positions. The mean latencies to have all test young in the nest with the females at the 3 hour checkpoint were 3.2 days for the morphine-treated rats and 1.8 days for controls (see Fig. 1). Similarly, no differences were found between the responses of the groups at the 11 or 23 hour checkpoints. Thus, it appears that the duration of the morphine disruption of maternal behaviors is greater than 2 hours, but less than 4 hours.

Although the disruption of maternal behavior in the homecage test resulting from the morphine injection did not appear to be long lasting, it was quite profound. When the morphine treated rats ($N = 7$) responded maternally during the homecage test session, they were found to retrieve more slowly than did the saline treated controls. The difference in retrieval latency within the test session was significant on the first response day, but not on the second response day (Table 1).

The T-maze testing revealed further differences in the quality of maternal behavior exhibited by the two groups. All but one of ten maternal saline-treated rats retrieved at least one pup at least once during the four test days, while only 1 of 7 morphine-treated rats ever retrieved over the four test days. The differences in the incidences of retrieval between the morphine and saline treated animals reached statistical significance on T-maze test days 3 ($p < 0.02$) and 4 ($p < 0.05$), when more saline-treated rats retrieved test young during the 10 minute test session (see Fig. 2).

EXPERIMENT 2: EFFECTS OF THE OPIATE ANTAGONIST NALOXONE HYDROCHLORIDE AND MORPHINE ON MATERNAL BEHAVIOR AND ACTIVITY

In Experiment 1 morphine treatment was found to disrupt the onset and quality of maternal behavior in rats after pregnancy termination. The second experiment examines whether the disruptive effects of morphine treatment on maternal behavior can be reversed by treatment with the receptor antagonist, naloxone hydrochloride, and whether morphine treatment at the given dose affects other behavioral responses, i.e., activity in an open field test paradigm.

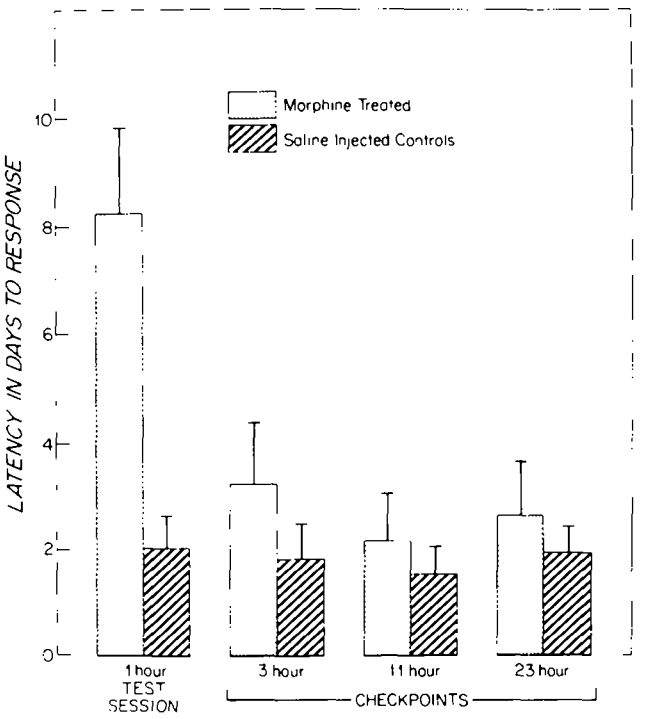

FIG. 1. Effects of morphine treatment on maternal behavior after surgical termination of pregnancy in rats. Females were injected SC with morphine sulfate (5 mg/kg) or saline twice daily, 1 hour before the onset of behavioral testing and again after the 11 hour checkpoint. An animal was assigned a positive response score during 1 hr test session, if she retrieved all 3 test young, grouped them in the nest and crouched over them. A positive score was given to a female at the 3, 11 or 23 hr checkpoints, if the test animal was found crouching over the 3 test young in the nest at the specified time. The latency to response during the 1 hour test session was significantly longer in the morphine-treated than the saline-treated rats ($p < 0.001$). The means plus SEM represent the response latencies in days of the 10 morphine- and 10 saline-injected rats.

Method

Thirty-seven primigravid rats had their pregnancies surgically terminated on day 17 of gestation. At this time females were assigned to two separate studies. In the first study, 8 rats were treated with morphine (group M2), 9 females were administered morphine plus naloxone hydrochloride (group MN2), and 8 animals were injected with saline (group S2). All rats were injected immediately after surgery and once a day thereafter, at 1000 hr, one hour prior to behavioral testing. Test procedures for maternal behavior were identical to those employed in Experiment 1 except that homecage testing was extended only through day 11. Non-responding rats were, therefore, assigned homecage latencies of 11 days.

In the second study two groups of 6 females each were injected with morphine (group M2') or saline (S2'). The injection doses and schedules were as described in the General

TABLE 1

LATENCIES IN MINUTES (Mean ± SEM) FOR MORPHINE (M), MORPHINE PLUS NALOXONE (MN), AND SALINE (S) TREATED RATS TO RETRIEVE, GROUP AND CROUCH OVER THE TEST YOUNG IN THE NEST AREA ON RESPONSE DAYS 1 AND 2 IN EXPERIMENTS 1 AND 2

	Experiment 1 Treatments		Experiment 2 Treatments		
	M	S	M	MN	S
Day 1 of Full Maternal Responsiveness	(7)	(10)	(6)	(9)	(8)
Retrieval of First Pup	22.4 ± 10.5*	2.3 ± 1.4	27.7 ± 5.4†	1.0 ± 0.4‡	1.4 ± 0.7
Retrieval, Grouping and Crouching over Young	49.3 ± 4.3†	8.9 ± 3.7	41.3 ± 6.7†	3.9 ± 1.2‡	12.6 ± 5.8
Day 2 of Full Maternal Responsiveness					
Retrieval of First Pup	13.3 ± 8.8	0.4 ± 0.1	2.0 ± 0.5*	0.3 ± 0.1§	0.3 ± 0.1
Retrieval, Grouping and Crouching over Young	19.6 ± 8.1*	2.4 ± 0.8	30.3 ± 11.4†	1.2 ± 0.3‡	5.2 ± 3.6

In Experiment 2 significant overall differences among the three treatments were present on response day 1 (both $p<0.05$) and on response day 2 ($p<0.05$ and <0.01) for overall latencies to retrieve the first pup and to retrieve, group and crouch over young, respectively.

*$p<0.05$ when compared with saline controls.

†$p<0.01$ when compared with saline controls.

‡$p<0.01$ when compared with morphine-treated rats.

§$p<0.05$ when compared with morphine-treated rats.

Method section. Behavioral testing began an hour after the daily injection and measured activity using an open-field test apparatus [14,33]. Testing was conducted daily for 4 days between 1100 and 1500 hr starting 1 day after surgery.

Results

Significant overall differences were found among the groups in the first study in Experiment 2 for latencies to exhibit complete homecage responsiveness, $F(2,22)=12.04$, $p<0.001$, with the M2 latency significantly longer than both the MN2 latency, $F(1,15)=12.52$, $p<0.001$, and the S2 latency, $F(1,14)=11.99$, $p<0.001$. Again, the morphine treated rats showed deficits in maternal behavior. These deficits were totally reversed by naloxone, for no differences were found between the MN2 and S2 groups in any aspect of maternal behavior studied.

In the homecage test the latency to show full maternal behavior was 5.0±1.3 days for M2 rats, 0.6±0.2 days for MN2 animals, and 0.4±0.2 days for S2 females. All eight morphine treated rats retrieved, grouped and crouched over the pups on at least one test day, although only six of these rats did so for two consecutive days. On response days, group M2 rats retrieved pups significantly slower than did group MN2 or S2 animals. These latter two groups did not differ from one another (Table 1). There were no differences among the groups in latencies to respond at the pre-test session.

In the T-maze test the morphine treated rats failed to retrieve pups, while the morphine plus naloxone group retrieved as readily as did saline-injected controls (see Table 2). Only 1 of 6 M2 rats retrieved a pup from the T-maze, and this only occurred on the fourth day of T-maze testing. In contrast, all 8 S2 rats and 8 of 9 MN2 rats retrieved a pup at least once over the 4 test days. There were no differences in the number of S2 and MN2 animals retrieving pups on any of testing. Each day, however, significantly fewer M2 rats retrieved ($p<0.05$–0.005, Fisher Exact Probability) pups than did S2 controls. As in Experiment 1, the failure of the morphine injected rats to retrieve pups was not associated with failure to enter the maze and contact the young. One striking difference noted among groups was that the morphine treated females repeatedly entered the maze, sniffed the young, but then returned to the homecage without the young. MN2 and S2 rats usually retrieved the pups from the arms of the T-maze upon the initial contact with the young.

Measurement of activity in an open field test in the second study of Experiment 2 revealed that morphine injected animals entered more squares than did saline injected rats over the four test days ($p<0.05$, see Table 3). This difference was statistically significant on the fourth test day ($p<0.05$). There were no differences in the number of rears between the two groups in the open field apparatus (see Table 3).

EXPERIMENT 3: EFFECT OF DEXTRORPHAN ON THE RATE OF ONSET OF MATERNAL BEHAVIOUR IN PREGNANCY TERMINATED RATS

The objective of this experiment was to determine if administration of dextrorphan, a compound which has low affinity for the opiate receptor [17,25], would affect the ex-

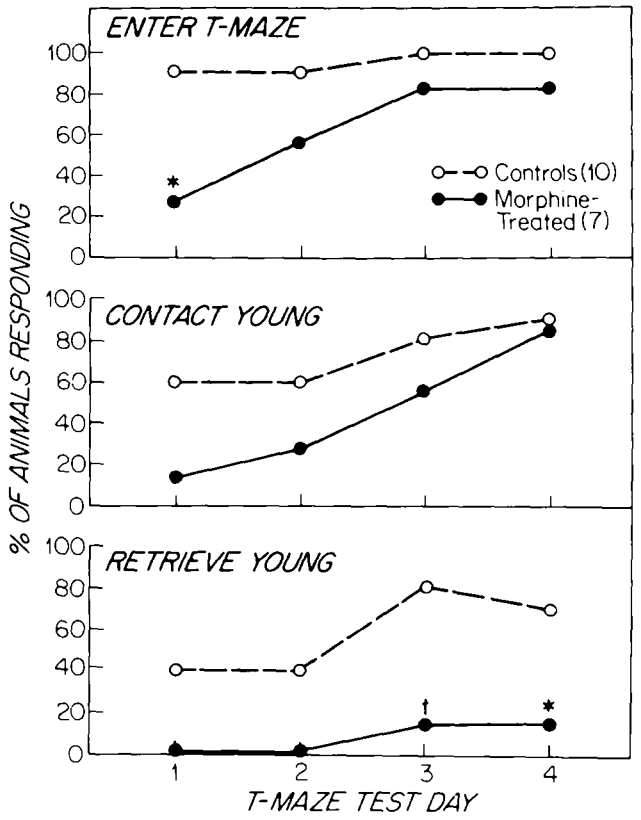

FIG. 2. Maternal responsiveness in the T-maze test after morphine treatment. Maternal saline-injected controls and morphine-treated rats were tested once daily for 4 days after exhibiting maternal behavior in the home cage. Significantly more control rats entered the T-maze on test day 1 than did morphine-treated females. Fewer morphine-injected animals retrieved pups from the T-maze on test days 3 and 4. *$p < 0.05$ versus controls, Fisher Exact Probability; †$p < 0.025$ versus controls, Fisher Exact Probability.

pression of maternal behavior. Disruption of maternal behavioral by dextrorphan would indicate that morphine's disruptive action on the behavior was not solely regulated by a receptor mechanism, but rather would suggest that morphine's action was a general nonspecific effect.

Method

Twenty females whose pregnancies had been surgically terminated on day 17 of gestation were assigned to 1 of 2 treatments. Rats were either injected subcutaneously with dextrorphan tartrate (lot Ro 1-6794) at a dose of 5 mg/kg or 0.5 ml saline immediately after surgery. The following day between 1000 and 1100 hr rats were reinjected with the designated solutions. One hour later testing for maternal behavior in the homecage was initiated. Daily injections and testing continued for 11 days or until a female showed full responsiveness during the 1 hour test on 2 consecutive test days.

Results

The effects of dextrorphan administration on maternal behavior are shown in Fig. 3. Dextrorphan treatment, unlike morphine administration, did not disrupt the rate of onset of maternal behavior in the homecage test. The mean latency to

TABLE 2

BEHAVIORAL RESPONSIVENESS (PERCENTAGE RESPONDING) OF MORPHINE (M2), MORPHINE PLUS NALOXONE (MN2), AND SALINE (S2) TREATED RATS IN THE T-MAZE TEST

	T-Maze Test Day			
Group	1	2	3	4
M2 (6)				
Contact Young	50‡	67	67	67
Retrieve Pup	0*†	0*§	0*¶	16*†
MN2 (9)				
Contact Young	67	89	89	100
Retrieve Pup	56	89	78	78
S2 (8)				
Contact Young	100	100	100	100
Retrieve Pup	100	100	100	100

All rats showed complete maternal responsiveness towards foster young in the home cage for 2 consecutive days prior to the initiation of daily T-maze testing.

*$p < 0.005$ when compared with S2 females (all tests, Fisher Exact Probability).

†$p < 0.05$ when compared with MN2 females.

§$p < 0.005$ when compared with MN2 females.

¶$p < 0.01$ when compared with MN2 females.

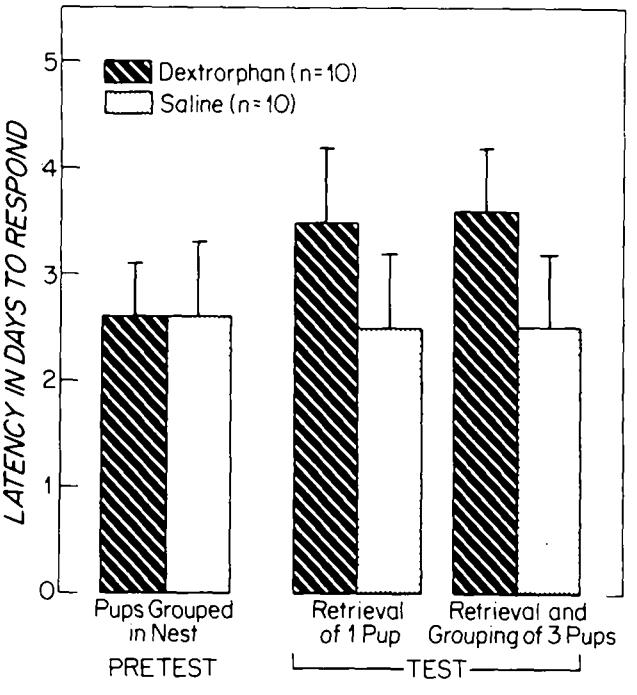

FIG. 3. Maternal behavior in the rats after pregnancy termination and dextrorphan treatment (5 mg/kg, SC). Females were injected after surgery and after the pretest rating each day, 1 hour prior to daily behavioral testing. There were no statistical differences found between the two groups on any of the behavioral measures. Values are expressed as mean days to response plus SEM.

TABLE 3

FREQUENCIES (MEAN ± SEM) THAT MORPHINE (M) AND SALINE (S) INJECTED PREGNANCY-TERMINATED RATS ENTERED SQUARES AND REARED DURING A 5 MINUTE OPEN FIELD ACTIVITY TEST ON FOUR CONSECUTIVE DAYS IN EXPERIMENT 2

Group	Response	Test Day 1	2	3	4	Total 1–4
M	Squares entered	103 ± 27.3	116.2 ± 20.7	117.7 ± 19.3	125.3 ± 24.0*	472.5 ± 46.0*
	Rears	18.0 ± 4.5	14.8 ± 2.5	18.8 ± 4.0	17.8 ± 3.5	69.5 ± 6.8
S	Squares entered	66.8 ± 17.9	86.7 ± 24.4	70.2 ± 22.5	44.3 ± 21.3	268.0 ± 60.3
	Rears	12.2 ± 3.6	15.5 ± 4.7	13.0 ± 4.7	10.8 ± 5.6	51.5 ± 16.0

*$p < 0.05$ when compared with saline-injected controls.

retrieve one pup was 3.5 days for dextrorphan-treated rats and 2.5 days for saline controls. Similarly, the latencies to retrieve and group the three test young during the one hour test session were 3.6 and 2.5 days for the dextrorphan and saline groups, respectively. The pretest response latencies were identical for the two groups (mean=2.6 days).

GENERAL DISCUSSION

The data for the present set of experiments indicate that moderate doses of morphine can interfere with the expression of maternal behavior in the rat. This morphine effect persists for between 2 and 4 hour and appears to act through an opiate receptor mechanism. These findings suggest to us that under physiological conditions endogenous opiates may be involved in the regulation of maternal behavior.

Morphine treatment after surgical termination of pregnancy in rats has previously been shown to disrupt the onset of maternal behavior in the rat [3]. The results of the present study indicate that the effect of morphine is not absolute, however, but wanes both between injections and with repeated injections. Each injection inhibits the expression of maternal behavior for less than four hours (Experiment 1), as evidenced by the fact that morphine-injected animals, like saline-injected rats, were found in their nests crouching over the young at both 3 and 11 hour checkpoints by about the second test day. This time course of morphine's action is consistent with published reports of the duration of other behavioral effects of morphine. A dose of 3.2 mg/kg morphine sulfate was without effect on activity 4 hours after SC administration of the drug [10].

It is not surprising that most of the morphine-injected rats eventually responded to pups during the homecage test session. Continuous exposure to young is a strong stimulus for promoting the induction of maternal behavior in rats. Inexperienced females as well as males will respond maternally to young, if exposed to them for a period of 5–7 days [8,26]. It is possible that the continued exposure to young overcame the disruptive effects of morphine after 4 or 5 days. It seems less likely that the animals habituated to the drug, since the quality of maternal responsiveness in the T-maze test was poorer for morphine-injected rats. Furthermore, the rats injected twice daily with morphine in Experiment 1 responded, if anything, more slowly than the once injected rats in Experiment 2. Were the response due to habituation, the more frequently injected rats would have habituated, and so responded, more quickly than the singly injected animals.

The behavioral specificity of morphine's action on the disruption of maternal behavior is not completely known. When we measured activity levels of morphine-treated rats in an open field activity test, we failed to detect major impairments or alterations in activity 1 hour after drug administration. In Experiment 2 we found a slight increase in activity in the open field after morphine injection on the fourth test day. However, this effect of morphine on activity was temporally dissociated from its effect on maternal behavior; the activity effect was most pronounced on the fourth day of repeated injection and testing, while the maternal behavior effect began to wane by this time. The disruption of maternal behavior by morphine, therefore, cannot be explained solely on the basis of a severe disruption in motor activity. Rather, it seems likely that morphine treatment in some manner alters the animal's perception of the young.

Another approach employed to help elucidate the specificity of morphine's action is to attempt to localize its site of action in the central nervous system, and thereby associate or exclude certain neural structures with the behaviors. We have recently localized one site of morphine's disruptive effect on maternal behavior in the CNS [27]. Specifically, bilateral placement of morphine sulfate into the medial preoptic area of maternal rats disrupted ongoing materal behavior. This area of the brain has been shown to be involved in the expression of maternal behavior in rats [23,24]. Placement of morphine into the ventromedial nucleus (VMN) of the hypothalamus, in contrast, failed to disrupt maternal behavior, although open field activity scores in VMN rats given morphine were actually suppressed [27].

Data from Experiments 2 and 3 show that the disruptive effects of morphine appear to be mediated by an opiate receptor mechanism. In Experiment 2 treatment of rats with both morphine and opiate antagonist naloxone reversed the disruptive effects of morphine in both the homecage and T-maze tests. Since naloxone competes strongly with morphine for the opiate receptor, these findings argue in favor of the hypothesis that a significant opiate occupation of these receptors interferes with the onset and maintenance of maternal behavior. In Experiment 3 treatment of pregnancy-terminated rats with the weak opiate dextrorphan, a stereo-

isomer of the more active opiate agonist levorphanol, failed to disrupt maternal behavior. These results lend support to the proposal that morphine's action is receptor mediated.

A second interpretation of these data is that morphine might not act through a receptor-mediated mechanism. It is possible the naloxone masked a non-specific effect of morphine by stimulating maternal behavior through an unrelated non-opiate mechanism. It is known that naloxone itself can effect behavioral processes, presumably through an action not associated with the opiate system [28]. This explanation of the data, however, seems unlikely since the dose of naloxone needed for these non-opiate effects is usually higher than the dose used in the present study. The failure of dextrorphan to duplicate the action of morphine also makes this interpretation of the results unlikely. Dextrorphan lacks the stereospecificity for the opiate receptor, and so will not mimic a receptor-mediated opiate effect. However, it should be noted that while dextrorphan did not have the same action as morphine on maternal behavior, this finding does not necessarily prove that morphine is acting via a specific opiate receptor. In cultured spinal cord neurons, administration of morphine and of levorphanol appears to act in a non-specific manner on membrane polarization, effects not found after treatment with dextrorphan [13]. Thus, while neither antagonism of the morphine effect with naloxone nor the lack of an effect with dextrorphan is sufficient evidence to prove that the opiate actions of morphine are receptor mediated, these two findings together strongly indicate that the morphine disruption of maternal behavior is a specific opiate effect.

While the mechanism of morphine's action on maternal behavior is only partially understood, it seems feasible that morphine might be disrupting maternal behavior through morphine's effect on thermoregulation [6,35]. In rats morphine treatment at the dose of 5 mg/kg used in the present study induces hyperthermia [9]. Recently, it has been shown that lactating rats tend to leave their nests and the young when the female's body temperature becomes elevated [18]. This finding has led Leon and his colleagues to propose that shifts in the mother's body temperature may regulate the expression of maternal care [18]. Furthermore, anatomical studies indicate that similar regions of the brain in the rat (i.e., MPOA-anterior hypothalamus) may affect both maternal behavior [23] and temperature regulation [20,22]. These findings when taken together make the proposal that morphine's action on maternal behavior is mediated by thermoregulatory processes an attractive hypothesis. We are presently investigating this possibility.

Finally, we do not know whether endogenous opioids, i.e. β-endorphin, or met-enkephalin, have behavioral actions similar to those found for the drug morphine sulfate. In addition, the effects of opiate antagonists on maternal behavior have yet to be investigated. Studies are presently being conducted that investigate possible physiological roles of opioids in the normal regulation of maternal behavior.

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REFERENCES

1. Bridges, R. S. and H. H. Feder. Inhibitory effects of various progestins and deoxycorticosterone on the rapid onset of maternal behavior induced by ovariectomy-hysterectomy during late pregnancy. *Horm Behav* **10:** 30–39, 1978.
2. Bridges, R. S., H. H. Feder and J. S. Rosenblatt. Induction of maternal behavior in primigravid rats by ovariectomy, hysterectomy, or ovariectomy and hysterectomy: effect of length of gestation. *Horm Behav* **9:** 156–169, 1977.
3. Bridges, R. S. and C. T. Grimm. Reversal of morphine disruption of maternal behavior by concurrent treatment with the opiate antagonist naloxone. *Science* **218:** 166–168, 1982.
4. Bridges, R. S. and D. W. Russell. Steroidal interactions in the regulation of maternal behaviour in virgin female rats: effects of testosterone, dihydrotestosterone, oestradiol, progesterone and the aromatase inhibitor, 1,4,6-androstatriene-3-17-dione. *J Endocrinol* **90:** 31–40, 1981.
5. Bridges, R. S., M. X. Zarrow, R. Gandelman and V. H. Denenberg. Differences in maternal responsiveness between lactating and sensitized rats. *Dev Psychobiol* **5:** 123–127, 1972.
6. Clark, W. G. Influence of opioids on central thermoregulatory mechanisms. *Pharmacol Biochem Behav* **10:** 609–613, 1979.
7. Cohen, J. and R. S. Bridges. Behavioral differences in maternal responsiveness exhibited by nonlactating primiparous and re-sensitized nulliparous rats. *J Comp Physiol Psychol* **95:** 450–459, 1981.
8. Cosnier, J. and C. Couturier. Comportment maternal provique chez les adults castrees. *CR Seances Soc Sci (III)* **160:** 789–791, 1966.
9. Cox, B., T.-F. Lee and M. J. Vale. Effects of morphine and related drugs on core temperature of two strains of rat. *Eur J Pharmacol* **54:** 27–36, 1979.
10. Domino, E. F., M. R. Vasko and A. E. Wilson. Minireview: mixed depressant and stimulant actions of morphine and their relationship to brain acethycholine. *Life Sci* **18:** 361–376, 1976.
11. Gintzler, A. R. Endorphin mediated increases in pain threshold during pregnancy. *Science* **210:** 193–195, 1980.
12. Grota, L. J. and K. B. Eik-Nes. Plasma progesterone concentrations during pregnancy and lactation in rats. *J Reprod Fertil* **13:** 83–91, 1967.
13. Gruol, D. L. and T. G. Smith. Opiate antagonism of glycine-evoked membrane polarizations in cultured mouse spinal cord neurons. *Brain Res* **223:** 355–365, 1981.
14. Hall C. S. Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. *J Comp Psychol* **18:** 385–403, 1934.
15. Keverne, E. B., F. Levy, P. Poindron and D. R. Lindsay. Vaginal stimulation: an important determinant in maternal bonding in sheep. *Science* **219:** 81–83, 1983.
16. Koller, G. Hormonale and psychische steuerung beim nestbau weiber mause. *Zool Anz (suppl.) Vehr Dt Zool Ges* **19:** 125–132, 1955.
17. Law, P.-Y., H. H. Loh and C. H. Li. Properties and localization of β-endorphin receptor in the rat brain. *Proc Natl Acad Sci USA* **76:** 5455–5459, 1979.
18. Leon, M., P. G. Croskerry and G. K. Smith. Thermal control of mother-young contact in rats. *Physiol Behav* **21:** 793–811, 1978.

19. Lisk, R. D., R. A. Pretlow and S. M. Friedman. Hormonal stimulation necessary for elicitation of maternal nest-building in the mouse (mus musculus). *Anim Behav* **17:** 730–737, 1969.
20. Martin, G. E. and J. E. Morrison. Hyperthermia evoked by the intracerebral injection of morphine sulfate in the rat: the effect of restraint. *Brain Res* **145:** 127–140, 1978.
21. Moltz, H. M. Lubin and M. Numan. Hormonal induction of maternal behavior in the nulliparous rat. *Physiol Behav* **5:** 1373–1377, 1970.
22. Myers, R. D. Temperature regulation: neurochemical systems in the hypothalamus. In: *The Hypothalamus*, edited by W. Haymaker, E. Anderson and W. J. H. Nauta. Springfield, IL: Charles C. Thomas, 1969, pp. 506–523.
23. Numan, M. Medial preoptic area and maternal behavior in the female rat. *J Comp Physiol Psychol* **87:** 746–759, 1974.
24. Numan, M., J. S. Rosenblatt and B. R. Komisaruk. Medial preoptic area and the onset of maternal behavior in the rat. *J Comp Physiol Psychol* **91:** 146–164, 1977.
25. Pert, C. B. and S. H. Snyder. Properties of opiate-receptor binding in rat brain. *Proc Natl Acad Sci USA* **70:** 2243–2247, 1973.
26. Rosenblatt, J. S. Nonhormonal basis of maternal behavior in the rat. *Science* **156:** 1512–1514, 1967.
27. Rubin, B. S., C. T. Grimm and R. S. Bridges. Disruption of maternal behavior after systemic or central administration of morphine in rats. *Abstract, Society for Neurosciences*. Minneapolis, MN 1982.
28. Sawynok, J., C. Pinsky and F. S. LaBella. Minireview on the specificity of naloxone as an opiate antagonist. *Life Sci* **25:** 1612–1631, 1979.
29. Siegel, H. I. and J. S. Rosenblatt. Estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats. *Physiol Behav* **14:** 465–471, 1975.
30. Soloff, M. S., M. Alexandrova and M. J. Fernstrom. Oxytocin receptors: triggers for parturition and lactation? *Science* **204:** 1313–1314, 1979.
31. Stern, J. M. and D. A. MacKinnon. Postpartum, hormonal and nonhormonal induction of maternal behavior in rats: effects on T-maze retrieval of pups. *Horm Behav* **7:** 305–316, 1976.
32. Wardlaw, S. L., L. Thoron and A. G. Frantz. Effects of sex steroids on brain β-endorphin. *Brain Res* **245:** 327–331, 1982.
33. Whimbey, A. E. and V. H. Denenberg. Two independent behavioral dimensions in open-field performance. *J Comp Physiol Psychol* **63:** 500–504, 1967.
34. Wiest, W. G. Progesterone and 20 α -hydroxypregn-4-en-3-one in plasma, ovaries and uteri during pregnancy in the rat. *Endocrinology* **87:** 43–48, 1970.
35. Yehuda, S. and A. J. Kastin. Peptides and thermoregulation. *Neurosci Biobehav Rev* **4:** 459–471, 1980.
36. Zarrow, M. X., A. Farooq, V. H. Denenberg, P. B. Sawin and S. Ross. Maternal behavior in the rabbit: endocrine control of maternal-nest building. *J Reprod Fertil* **6:** 375–383, 1963.
37. Zarrow, M. X., R. Gandelman and V. H. Denenberg. Prolactin: Is it an essential hormone for maternal behavior in mammals? *Horm Behav* **2:** 343–354, 1971.