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Inhibitory Effects of Naltrexone on the Induction of Parental Behavior in Juvenile Rats

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ZAIAS, J., L. OKIMOTO, A. TRIVEDI, P. E. MANN AND R. S. BRIDGES. *Inhibitory effects of naltrexone on the induction of parental behavior in juvenile rats.* PHARMACOL BIOCHEM BEHAV **53**(4) 987–993, 1996. – Juvenile rats are rapidly responsive to pups soon after weaning, displaying maternal-like behaviors such as licking, retrieving, grouping, and crouching over pups. As juveniles reach 30 days of age, they become less responsive to pups and show increased latencies to display the same parental behaviors. In light of previous data implicating opiates in the display of ongoing maternal behavior, we administered naltrexone, a long-acting opiate antagonist, beginning 5 and 9 days prior to and continuing throughout the period of behavioral testing, which started at 26 or 30 days of age. Male and female juveniles treated with 10 mg/kg of naltrexone SC for 9 days (days 21 to 29 of age) prior to and during behavioral testing (days 30 to 37) showed longer latencies to retrieve, group, and crouch over pups than did the vehicle-injected controls. These results suggest that opioids may have a stimulatory role in parental behavior during this prepubertal period.

Juvenile rats Naltrexone Opiate antagonist Opioid Parental behavior Prepubertal

THE ONSET OF maternal behavior is hormonally regulated in rats. Estradiol, progesterone, and prolactin have been shown to have a stimulatory effect on maternal behavior in adult female rats (2,3,5). Further studies have shown that endogenous opiate concentrations change during the course of pregnancy and lactation and may play a role in regulating the expression of maternal behavior (17–20). In support of this hypothesis, systemic administration of morphine to female rats disrupts maternal behavior, and concurrent treatment with opiate antagonists reverses this disruption (4,9).

Findings from studies on the expression of parental behavior in juvenile rats in general are similar to those in adults. Interestingly, juvenile rats (18–25 days of age) are rapidly responsive to younger pups, exhibiting maternal-like behaviors (i.e., retrieving, grouping, and crouching over pups) (6,8,24). Male juveniles (25 days of age) respond more quickly and have higher concentrations of serum prolactin than do female juveniles, which is in sharp contrast to latencies in adult males and females: adult females respond faster than adult males (13,24). As in adults, treatment of juvenile male rats with bromocriptine, a dopamine agonist that inhibits prolactin release, disrupts parental behavior (13). By 30 days of age, however, the parental behavior of juvenile rats shifts from being rapidly to slowly responsive (6).

Opioids appear to regulate maternal-like behavior in juveniles as they do in adult female rats. Disruption of parental behavior and reversal of disruption occurs following administration of morphine and morphine plus naloxone (a short-acting opiate antagonist), respectively (14). Other studies support the concept that an endogenous opioid system is developing at this time in juvenile rats (10). Hence, it is possible that increasing endogenous opiate activity during this prepubertal period from 24 to 30 days of age accounts for the decreased responsiveness of juveniles to pups by 30 days of age.

The present experiments are designed to evaluate the role of opioids in the induction of parental behavior in juvenile (prepubertal) rats by blocking opiate receptors in male and female rats with naltrexone, a long-acting competitive opiate antagonist. Based on previous findings that indicate an inhibitory role of opioids on parental behavior, we hypothesize that juvenile rats treated with naltrexone will not display the expected inhibition of parental behavior that normally occurs at 30 days of age.

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METHODS

Animals

Nulliparous female Sprague-Dawley Norway rats (*Rattus norvegicus*, [Crl : CD(SD)BR virus-free]) were obtained from Charles River Laboratories, Kingston, NY. All animals were housed in translucent plastic cages (45 × 25 × 20 cm) and maintained in temperature (21-25°C)- and light-controlled (on between 0500–1900 h) rooms. Purina Rat chow and water were available ad lib.

Females were mated to males in our colony and then housed two females/cage until 3 days prior to expected delivery day, at which time they were individually housed. The females were observed daily for parturition (day 0) and litters were culled to four female and four male pups the following day (day 1). Initial litter sizes averaged 15.0 ± 2.5 pups. Pups remained with their mother until lactation day 21, at which time pups were weighed, housed individually, and randomly assigned to a treatment group. Donor female rats were bred to provide stimulus pups with which to test the experimental juveniles.

Behavioral Testing

Plexiglas dividers (5 cm high), which separated the cages into four quadrants, were placed into clean home cages one day before testing. Thus, juveniles had 24 h to acclimate to the dividers before testing began. One hour following injection, testing started. Three 3- to 8-day-old pups were placed in each juvenile's cage in the three quadrants other than that in which the juvenile nested. A 60-min testing session then began. Latencies to first contact with stimulus pups, first retrieval of a pup (R1), subsequent retrievals, grouping of the pups (G), and finally, crouching over the pups were recorded. Juveniles were observed continuously for the first 15 min for these behaviors, and then spot checked at 30, 45, and 60 min, at which time juvenile and pup positions were recorded.

The criterion for induction of full parental behavior (FPB) consisted of observing the juvenile crouching over all three pups in two consecutive test sessions. Testing was terminated after the eighth day of testing, if the juvenile did not reach the criteria for successful induction of parental behavior.

Juveniles were assigned a latency score (for each behavior) corresponding to the number of days that they were exposed to pups prior to performing that behavior. For example, if a juvenile first retrieved a pup on the first test day, its retrieval score would be "0." If a subject first retrieved on the second day of testing, that juvenile would receive a score of "1" for retrieval (1 day of previous exposure). Juveniles not responding at all by the eighth test session received a score of "8" for that behavior.

Drugs

Naltrexone HCl, obtained from Sigma Chemical Co., St. Louis, MO, was dissolved in physiological saline. The volume of injection used for both naltrexone treatments and saline vehicle treatment was 0.2 ml/100 g body weight.

Experimental Treatments

Experiment 1: unmanipulated juveniles tested at 24 and 30 days of age and juveniles injected from 25 days and tested at 30 days of age. This experiment was designed to compare the parental behaviors demonstrated by unmanipulated (i.e., not injected) 24- and 30-day-old juveniles and 30-day-old juveniles after injection with either a high (10 mg/kg) dose of the opiate antagonist naltrexone, a low (1 mg/kg) dose of naltrexone, or saline vehicle starting at 25 days of age. In the first part of this experiment, unmanipulated 24-day-old juveniles were tested for parental behaviors to verify the previously reported short latencies at this age (6,8). In addition, to document a behavioral shift, the responses of these 24-day-old unmanipulated rats were compared with a separate group of unmanipulated juveniles first tested at 30 days of age.

In the second part of this experiment, the experimental juveniles receiving naltrexone and the controls receiving vehicle were injected (subcutaneously) twice daily, once between 0830–0930 h and once between 1630–1730 h. Injections continued throughout the period of behavioral testing until the juvenile had displayed the criterion for full parental behavior (see above) or 8 test days had elapsed. Juveniles were weighed each afternoon, and saline and naltrexone dosages for that afternoon and the next morning were derived from that weight. (The afternoon prior to the first day of injections, juveniles were weighed, but not injected.)

Experiment 2: juveniles injected from 21 days and first tested at 26 days of age. In Experiment 2, male and female juveniles were injected with either the high dose of naltrexone (10 mg/kg) or vehicle beginning at weaning, i.e., 21 days of age. Behavioral testing began 5 days later at 26 days of age. Behavioral testing and injection protocols were as described above.

Experiment 3: juveniles injected from 21 days and first tested at 30 days of age. In Experiment 3, male and female juveniles were injected with either the high dose of naltrexone (10 mg/kg) or vehicle beginning at weaning. These juveniles were first tested for parental behaviors 9 days later, at 30 days of age. Behavioral testing and injection protocols were as described above.

Statistical Analysis

Nonparametric statistics were utilized because of the nonnormality of the behavioral data. Kruskal-Wallis tests were used for comparisons of three or more groups. The Mann-Whitney U-statistic and Fisher Exact Probability Test were used for all pair-wise comparisons.

RESULTS

Experiment 1: Unmanipulated Juveniles Tested at 24 and 30 Days of Age and Juveniles Injected from 25 Days and Tested at 30 Days of Age

Twenty-four-day-old males and females showed similar latencies to retrieve, group, and exhibit FPB (Mann-Whitney *U*-tests, all *ps* > 0.05; Table 1). Thirty-day-old male and female juveniles did not differ in their latencies to retrieve pups; however, males grouped pups and showed FPB more rapidly than did females (grouping: $U = 28.5$, $p < 0.01$; FPB: $U = 38$, $p < 0.05$; Table 1).

There were no differences across the eight 30-day-old treatment groups (i.e., unmanipulated, vehicle, low, and high dose naltrexone, for each sex) in latency to retrieve one pup [Kruskal-Wallis, $H(7) = 5.6$, $p > 0.05$]. Furthermore, there were no differences in retrieval latencies across treatments when the sexes are analyzed separately [males: $H(3) = 0.40$; females: $H(3) = 0.52$; both *ps* > 0.05].

Overall, there was a significant treatment effect on grouping behavior, $H(7) = 22.9$, $p < 0.05$ (Table 1). Among the males, treatment differences in grouping behavior approached,

TABLE 1

MEDIAN LATENCIES (DAYS) TO EXHIBIT PARENTAL BEHAVIORS IN UNMANIPULATED JUVENILES AND JUVENILES INJECTED AT 25 DAYS AND TESTED AT 30 DAYS (EXPERIMENT 1)*

	Control (24 days)		Control (30 days)		Vehicle		Naltrexone (1 mg/kg)		Naltrexone (10 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Retrieve	0	0	0.5	1	1	1	1	2	1	2
Group	3	2	2	6	2	3	3	5.5	7	6
FPB	6	3	3	8	6	4	6.5	6.5	8	8
N	11	11	12	12	11	13	12	12	11	13

*See text for differences between the groups.

but did not reach significance, $H(3) = 5.7$, $0.05 < p < 0.1$; male juveniles injected with the high dose of naltrexone tended to exhibit longer latencies to group than did either control (unmanipulated or vehicle) treatment or the low dose naltrexone group. There was a significant treatment effect among females, $H(3) = 12.9$, $p < 0.05$; vehicle-injected females had shorter latencies to group pups than did the unmanipulated, low dose, or high dose treatments (M-W pairwise comparisons, all $ps < 0.05$).

There was no difference in latency to show FPB (i.e., crouching over pups for 2 consecutive days) across all eight treatments, $H(7) = 10.7$, $p > 0.05$. However, when FPB was analyzed by sex, significant treatment effects were present among females, $H(3) = 9.5$, $p < 0.05$, but not among males, $H(3) = 1.8$, $p > 0.05$. Females from the vehicle-injected group had the shortest latencies to exhibit FPB relative to females who were unmanipulated ($U = 33$, $p < 0.01$), injected with the low dose of naltrexone ($U = 49.5$, $0.05 < p < 0.1$), or injected with the high dose of naltrexone ($U = 33.5$, $p < 0.01$). The cumulative percentage of females that displayed FPB was significantly greater in the vehicle group than in the high dose naltrexone treatment starting on day 5 and continuing through day 8 of testing (Fisher Exact, all $ps < 0.05$; Fig. 1). There were no other significant differences between female treatments, nor between male treatments (Fisher Exact, all $ps > 0.05$; Fig. 1).

Latencies of 24- and 30-day-old males to respond to pups are shown in Table 1. There were no significant differences in the latencies to retrieve, group, and show FPB to pups as males aged. A significantly greater percentage of 24-day-old males were parental on the second day of testing than were 30-day-old males (4 out of 11 vs. 0 out of 12, respectively, Fisher Exact, $p < 0.05$); however, there were no differences for any other test day as the percentage that were parental for both age groups varied between 40 to 60%. In contrast to male juveniles, latencies for all three behaviors increased with age in females (R1: $U = 39$, $p = 0.06$; grouping: $U = 16$, $p < 0.001$; FPB: $U = 29.5$, $p < 0.05$). There was a longer initial sensitization period in 30- vs. 24-day-old females, with fewer 30-day-old females being parental than 24-day-old females throughout the full testing period. Significant differences were present on test days 3 and 4 when 24-day-old females were 38% and 62% responsive (respectively) vs. 0% of 30-day-old females parental on both days (Fisher Exact, $p < 0.05$). On the fifth test day, the percentage of 30-day-old females behaving parentally rose from 0 to 38%, and percentages of females exhibiting FPB thereafter did not significantly differ from 24-day-olds.

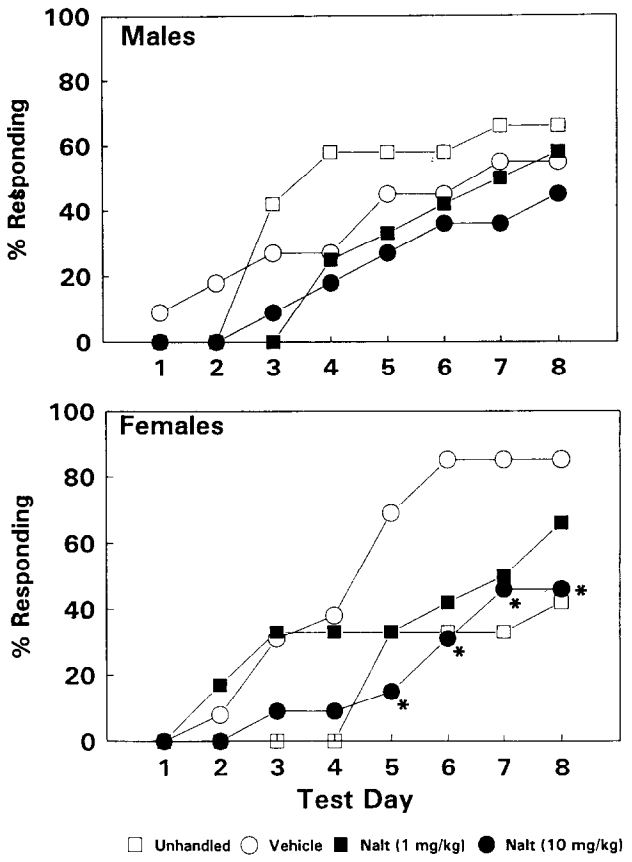

FIG. 1. Cumulative percentage of male and female juvenile rats injected from 25 days and tested at 30 days of age (Experiment 1) exhibiting FPB over the 8-day test period. Asterisks denote significant differences between treatments on that test day.

Experiment 2: Juveniles Injected from 21 Days and First Tested at 26 Days of Age

Although there were tendencies for the naltrexone-injected juveniles to respond slower as in the previous experiment, there were no statistically significant differences observed between treatments in latencies for any of the behaviors, nor between sexes within treatments (Table 2). Nor were differ-

TABLE 2

MEDIAN LATENCIES (DAYS) TO EXHIBIT PARENTAL BEHAVIORS IN JUVENILES INJECTED FROM 21 DAYS AND TESTED AT 26 DAYS OF AGE (EXPERIMENT 2)

	Vehicle		Naltrexone (10 mg/kg)	
	Male	Female	Male	Female
Retrieve	0	1	1	1
Group	2	3	2	4
FPB	4	3	8	6
N	9	9	9	11

TABLE 3

MEDIAN LATENCIES (DAYS) TO EXHIBIT PARENTAL BEHAVIORS IN JUVENILES INJECTED FROM 21 DAYS AND TESTED AT 30 DAYS OF AGE (EXPERIMENT 3)

	Vehicle		Naltrexone (10 mg/kg)	
	Male	Female	Male	Female
Retrieve	0	1	1	3*†
Group	0.5	3†	7*	5*
FPB	2	4	8*	6*
N	10	10	10	9

*Significantly different from vehicle within sex ($p < 0.05$).

†Significantly different from males within treatment ($p < 0.05$).

ences in percentages of animals displaying FPB between treatments evident (Fisher Exact, all $ps > 0.05$; Fig. 2). Thus, treatment with an opiate antagonist starting on day 21 did not affect parental behavior in 26-day-old males and females.

Experiment 3: Juveniles Injected from 21 Days and First Tested at 30 Days of Age

For all behaviors, naltrexone-injected juveniles (both males and females) showed significantly longer latencies than did their counterparts injected with vehicle (retrieval: $U = 26.5$, $p < 0.05$ in males, $U = 12.5$, $p < 0.01$ in females; grouping: $U = 24$, $p < 0.05$ in males, $U = 19.5$, $p < 0.05$ in females; FPB: $U = 12.0$, $p < 0.01$ in males, $U = 26$, $0.05 < p < 0.1$ in females; Table 3).

Male juveniles treated with naltrexone retrieved pups significantly sooner than did females treated with naltrexone ($U = 17.5$, $p < 0.05$). Males in the vehicle treatment grouped pups sooner than did vehicle females ($U = 26$, $p < 0.05$). There were no sex differences in latencies to show FPB within treatments.

Overall, the percentage of juveniles behaving parentally was low for both male and female juveniles injected with naltrexone (Fig. 3). By the third and fourth test day (females and males, respectively), the percentage of animals displaying FPB was significantly lower in the naltrexone vs. vehicle treatment groups (Fisher Exact: males—days 4–7, $ps < 0.05$; females—days 3–6, $ps < 0.05$).

Weaning Weights and Growth

Mean weaning weight at 21 days of age for male and female juveniles over all treatments was 64.4 ± 6.2 g ($n = 95$) and 61.6 ± 5.9 g ($n = 100$), respectively. There were no differences in weaning weights between treatment groups within experiments.

Body weight/growth data for each treatment within each experiment were obtained. By and large, there were no differences between treatment groups in absolute weights, nor in the

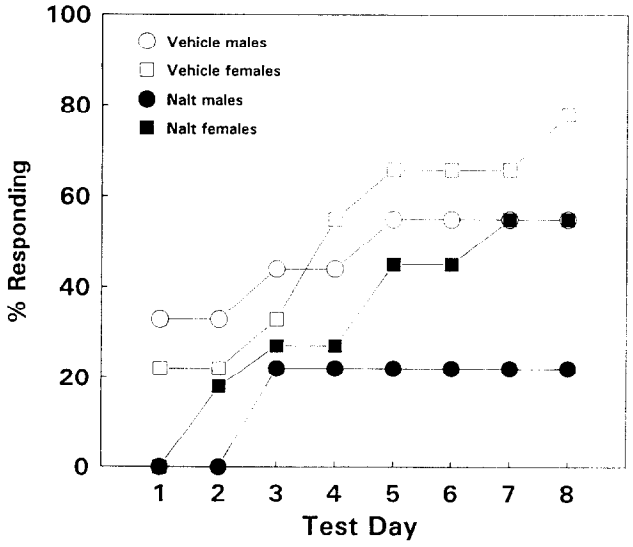

FIG. 2. Cumulative percentage of male and female juvenile rats injected from 21 days and tested at 26 days of age (Experiment 2) exhibiting FPB over the 8-day test period.

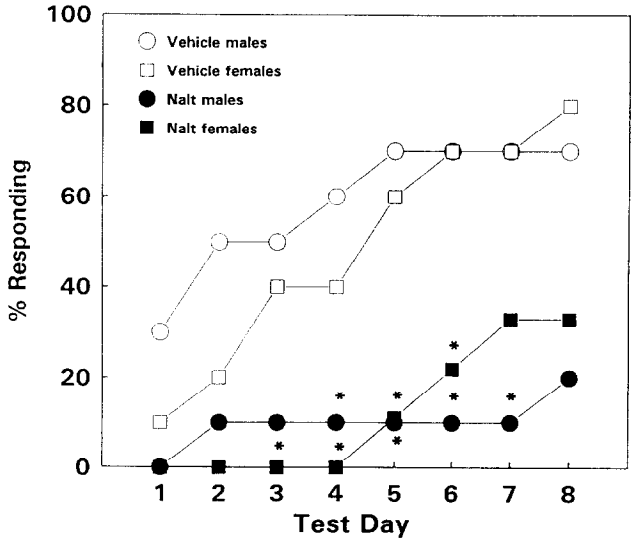

FIG. 3. Cumulative percentage of male and female juvenile rats injected from 21 days and tested at 30 days of age (Experiment 3) exhibiting FPB over the 8-day test period. Asterisks denote significant differences between treatments within the same sex on that test day.

growth rates. Sex differences were evident in all experiments, however. Statistical analyses were conducted on weight data only from Experiment 1 (these juveniles were injected from 25 days of age and first tested at 30 days of age). By 24 days of age, males were heavier than females, with the differences being significant starting at day 26 and thereafter ($p < 0.05$). These data are presented in Fig. 4 and are representative of Experiments 2 and 3 as well.

DISCUSSION

Juvenile rats (males and females) in this study responded rapidly when presented with 3- to 8-day-old pups, showing little inhibition to approach them upon first presentation. Latencies to display FPB were shorter in juveniles than in adults, confirming previous reports (13,24); however, 24-day-old male juveniles showed somewhat longer latencies than previously recorded (6,13,24). Both sexes showed a greater reluctance to retrieve, group, and crouch over young by day 30 (vs. day 24), with the most striking differences apparent in female juveniles. Although this pattern in females is consistent with previous reports (6), in contrast to earlier reports, 30-day-old male juveniles in this study maintained a more rapid response to pups than did 30-day-old females. Like Bridges et al. (6), but unlike Mayer et al. (21), sex differences at day 30 were found in the grouping and FPB categories.

The most striking and consistent differences between the naltrexone and control treatments in this study are seen in Experiment 3 where juveniles are pretreated with 10 mg/kg of SC naltrexone (or vehicle) for 9 days prior to and continuing throughout behavioral testing. Virtually all behavioral comparisons in this experiment revealed significantly longer latencies for the naltrexone-treated juveniles relative to the control juveniles. This was not the case, however, for Experiments 1 and 2 where the juveniles were only pretreated for 5 days. These differences were most marked when comparing females across experiments, suggesting perhaps some sex difference in endogenous opioid system and/or receptor mechanism (10).

Rather than supporting our initial hypothesis that opioids may be inhibitory to the expression of parental care in the 30-day-old juvenile rat, the results of the present study support the general hypothesis initially proposed by Panksepp that opioids facilitate social attachments and play a stimulatory role in the establishment of parental care (22,23). In the present study, chronic high dosages of naltrexone increased behavioral latencies in juveniles relative to those of the unmanipulated or vehicle-injected juveniles. This effect was most evident in males and females pretreated with naltrexone for the longest time (9 days) before behavioral testing (i.e., Experiment 3). It is possible, therefore, that the inhibitory actions of morphine and opioid agonists in earlier studies of the induction of parental behavior (4,9,14) was due in part to the dose of morphine administered, and that lower doses of opiates might exert a facilitatory action. This possibility merits further study.

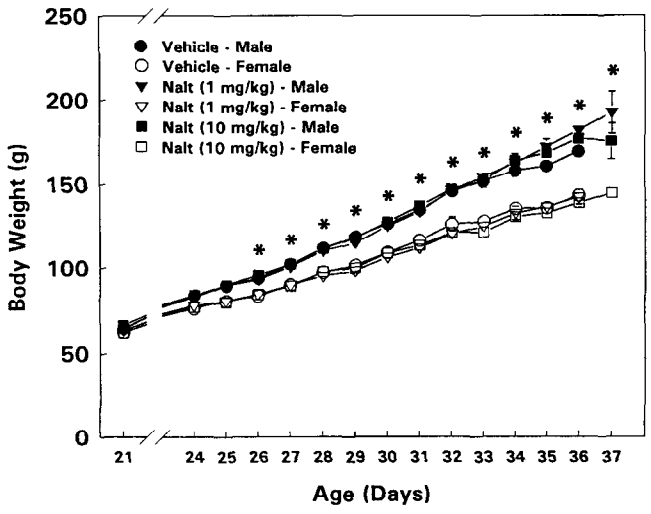

FIG. 4. Body weight vs. age data for Experiment 1. Asterisks indicate significant differences between males and females.

The findings from the present experiments are in agreement with other studies that have shown a facilitory role for opiates and an inhibitory role for opiate antagonists in the expression of maternal behavior. Thompson and Kristal (26) injected various doses of morphine sulfate into the ventral tegmental area (VTA) of the brain of virgin (nulliparous) rats and quaternary naltrexone into the VTA of parturitional rats. They found a dose-dependent decrease in maternal latencies exhibited by morphine-injected virgin rats when presented with stimulus pups. Conversely, parturitional rats injected with naltrexone showed significantly longer latencies to respond maternally than did control rats. Mayer et al. (21) reported a significant decrease in placentophagia and licking/cleaning of pups by recently parturient females that were implanted with naltrexone pellets. Panksepp et al. (23), likewise, observed that naloxone caused increased retrieval latencies in Swiss-Webster mice, while low doses of morphine facilitated retrieval. In sheep, Keverne and Kendrick (11,12) demonstrated increased maternal acceptance of lambs in multiparous ewes and decreased rejection of lambs in both nulliparous and multiparous ewes following treatment with morphine. In addition, naltrexone significantly inhibited maternal behavior in multiparous ewes. Taken together, these data suggest that endogenous opioids do occupy a regulatory role in the expression of parental behavior. However, previous reproductive and behavioral histories (e.g., nulliparous, parturient, etc.) and endogenous or experimental opioid concentrations may determine whether parental behavior is facilitated or inhibited by opioids. One possible explanation to account for the apparent differential effects of opioids on maternal behavior is that opioids help facilitate the establishment of parental behavior, yet tend to quiet the female or "inhibit" active maternal behavior once suckling is initiated in lactating dams.

Naltrexone is a potent, relatively long-acting (half-life = 4.6 h vs. 15–40 min for naloxone) nonspecific opiate receptor antagonist (29). Among the proposed effects of opiate antagonism are increased luteinizing, follicle-stimulating, and adrenocorticotropin hormone, reversal of morphine-induced increases of growth hormone, prolactin, and thyroid-stimulating hormone, and decreased food intake (1,16). The response to administration of naltrexone, however, varies with dosage and duration of treatment. For example, Zagon and McLaughlin (30) showed that daily SC administration of 1 mg/kg naltrexone hydrochloride in rat pups beginning on day one and continuing through weaning resulted in 15–19% lower weaning weights than control pups. In contrast, those pups injected with 50 mg/kg of naltrexone hydrochloride had a 19–22% percent increase in weaning weight than did controls. A similar pattern was noted in dry organ weights. Zagon and McLaughlin concluded that once daily dosages of < 10 mg/kg

of naltrexone effectively blocked opiate receptors for < 12 h and inhibited growth while daily injections of > 20 mg/kg or multiple injections at a lower dosage (e.g., 3 mg/kg) resulted in continuous (24 h) receptor blockade and increased growth.

The effect of chronic treatment and naltrexone dosage is not limited to growth effects. Yoburn et al. (28) demonstrated an increase in analgesic potency of morphine in rats implanted with naltrexone (30 mg) for 8 days, while no such effect was observed in rats implanted for only 24 h. The increased analgesia (functional supersensitivity) and increases in receptor binding (upregulation) demonstrated with naltrexone in rats (23,27,28) and mice (29) and also shown with chronic naloxone treatment (15) may be due to increased pre- and posttranslational activity of proopiomelanocortin (POMC) mRNA, as has been demonstrated in the arcuate nucleus (7). Taken together, these studies indicate that dosage and duration of exposure to naltrexone are important considerations when designing and interpreting experiments involving the endogenous opioid system. In the present study, although 10 mg/kg is a relatively high dose, there were no differences in body weight gain between juveniles treated with high dose of naltrexone and control juveniles (Fig. 4); therefore, it is not likely that the behavioral results were due to the pharmacological effects of naltrexone.

In summary, the results of this study suggest that opioids may influence (at least in part) the expression of parental behavior in juvenile rats. Future studies evaluating the possible stimulatory effects on parental behavior of various doses of exogenous morphine administered to 24- to 30-day-old juveniles should help clarify the precise role of opioids in regulating the onset of parental behavior.

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