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Opioid Receptor Blockade During Prenatal Life Modifies Postnatal Behavioral Development

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MCLAUGHLIN, P. J., S. W. TOBIAS, C. M. LANG AND I. S. ZAGON. *Opioid receptor blockade during prenatal life modifies postnatal behavioral development.* PHARMACOL BIOCHEM BEHAV **58**(4) 1075–1082, 1997.—The ontogeny of physical characteristics, spontaneous motor, and sensorimotor behaviors of preweaning rats, as well as ambulation and emotionality at weaning (day 21) were studied in rats exposed to 50 mg/kg naltrexone (NTX) or saline (controls) daily throughout gestation by maternal administration; all animals were cross-fostered to untreated mothers at birth. Morphine challenge tests and nociceptive measures revealed that this dosage of opioid antagonist blocked opioid receptors for 24 h. At birth and weaning, animals in the NTX group weighed 12 and 20%, respectively, more than control offspring. The age at which a specific physical characteristic, spontaneous motor behavior, or reflex initially appeared and the age at which 100% of the animals demonstrated a particular characteristic/behavior often were accelerated in animals prenatally exposed to NTX. The frequency of ambulation was subnormal in the NTX group, and the frequency and/or incidence of rearing, grooming, wet-dog shakes, and defecation were reduced from normal levels in these opioid antagonist-exposed rats. These results imply that interactions of endogenous opioid systems during embryogenesis are determinants of somatic, physical, and behavioral development in postnatal life. © 1997 Elsevier Science Inc.

Opioids Naltrexone Development Prenatal Postnatal Maternal Behavior Growth factors Opioid antagonist

IN addition to serving as neuromodulators (1), endogenous opioids serve to regulate the growth of developing, renewing, healing, and neoplastic tissues, and function in both prokaryotes and eukaroytes (2,3,9,15,17,18,24,27,32–40,42). One native opioid peptide, [Met^5]-enkephalin, has been identified as a negative regulator of growth that acts in vivo and in vitro directly on cells and tissues; this peptide has been termed opioid growth factor (OGF) because of its nonneurotransmitter properties (i.e., growth) and distribution (i.e., neural and nonneural cells and tissues). OGF serves as a modulatory agent in cell proliferation as well as in cell migration, differentiation, and survival. Moreover, blockade of OGF activity results in a stimulatory response, indicating that this peptide exerts a continuous tone with respect to growth events. OGF interacts with an opioid receptor, zeta (ζ), to influence growth.

One experimental paradigm utilized to understand the function of opioid peptides during development has been to study the consequences of opioid antagonist administration (5–7,9,11–14,16,19-21,23,25,26,28,29,33–40). However, because the duration of receptor blockade is crucial to comprehending opioid action (35,37), interpretation of the experiments rests on knowledge about the efficacy and extent of receptor blockade. Continuous blockade of opioids from opioid receptors imposed by administration of naltrexone (NTX) during postnatal life has been shown to result in rat pups that are larger in size than control animals, and to have enhanced brain and organ weights, increased body weights, early acquisition of some physical characteristics, and spontaneous motor and reflexive behaviors, and an acceleration in the proliferation and development of brain cells (including differentiation of spines and dendrites in the cerebellum and hippocampus) with respect to control subjects (9,14,33–38,40).

A few studies have begun to examine the effects of administering opioid antagonists during pregnancy (5,11–13,20,21, 23,25,26,28,29). Unfortunately, none of these investigations disrupted opioid–receptor interaction continuously from fertilization to parturition and, with some exceptions (5,11), did not examine whether the opioid antagonist treatment utilized was sufficient to block opioid receptors. Therefore, it is difficult to discern from these reports whether the response(s) ob-

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served is(are) due to blockade of opioid receptors and/or reflects events following opioid-receptor intervention. Recently, McLaughlin and colleagues (16) showed that pregnant rats receiving daily injections of 50 mg/kg NTX throughout gestation, a dosage found to block opioids from interacting with opioid receptors for 24 h, had no alteration in the course of pregnancy. However, body weights, crown–rump lengths, and wet and dry weights of the brain, heart, kidney, liver, and skeletal muscle in neonates delivered by NTX-treated rats and crossfostered to untreated mothers at birth were substantially elevated compared to control offspring. Moreover, at weaning (day 21), the body weights of NTX-exposed rats were 36% greater than controls, and organ weights were increased from 18 to 246% in this group. These results suggested that native opioids are important growth-inhibiting, tonically active regulators of prenatal ontogeny, and that events occurring in prenatal life are determinants (at least in part) of postnatal outcome insofar as somatic development.

In view of the changes in body and brain growth in postnatal animals continuously exposed prenatally to an opioid antagonist, this study was designed to learn about the ontogeny of physical characteristics, spontaneous motor activity, and sensorimotor reflexes during the preweaning period, as well as motor activity and emotionality at weaning, in offspring maternally subjected to NTX during gestation and crossfostered to untreated mothers at birth.

METHOD

Animals

Nulliparous female (195–230 g) and male (250–300 g) Sprague–Dawley rats (Charles River Labs, Wilmington, MA) were used in this study. Animals were housed in an environment of 21 ± 0.5°C with a relative humidity of 50 ± 10%. The room had a complete exchange of air 15–18 times per hour and a 12-h light–dark cycle with no twilight; water and Harlan Teklab Purina 8604 Rodent Chow were continuously available.

Animals were mated (one male to two females) and the presence of sperm indicated pregnancy (= day 1 of gestation); all animals were maintained in stainless steel, wire-bottom cages except where noted. Three days prior to parturition, the pregnant females were separated and placed individually into solid-bottom cages to deliver their pups.

Within 6 h of birth, litters were culled to 10 pups/mother and all animals were cross-fostered to lactating, noninjected control females. Inasmuch as possible, fostered litters consisted of pups from two or three NTX-treated or sterile water-treated females. At least four litters representative of equal numbers of male and female rats exposed to either NTX or sterile water were formed for each treatment; approximately 40 pups per treatment were observed for behavioral development.

Drug Injections

On day 1 of pregnancy, animals were randomly assigned to two groups, and received daily intraperitoneal (IP) injections of either 50 mg/kg NTX (Sigma, St. Louis, MO) or an equivalent volume (0.2 ml) of saline (control). Pregnant rats were weighed daily and the dose of NTX adjusted. All injections were given between 0900 and 0930 h. NTX was prepared weekly and stored at 4°C. All injections were terminated at parturition.

Behavioral Apparatus and Procedures

Spontaneous motor and reflexive tests. Spontaneous motor capabilities and developmental reflexes were determined using methodology reported previously by Zagon and McLaughlin (38). A brief description of each behavior is presented in Table 1. Spontaneous motor behaviors were measured by placing each animal in the center of an examination area (60 × 60 cm) covered by a laboratory underpad. All movements made by the animal were recorded for 2 min/day. After completion of the spontaneous motor tests, rat pups were subjected to a battery of sensorimotor tests. For these tests, each animal was observed for 5–10 min/day depending on the level of performance.

TABLE 1

CATEGORIES OF BEHAVIOR EXAMINED IN PREWEANING RATS EXPOSED TO NALTREXONE THROUGHOUT GESTATION

A. Spontaneous motor behavior
   1. Unilateral head turn and no return
   2. Unilateral head turn with return
   3. Simultaneous movement of head and forelimbs
   4. Pivoting less than 360°
   5. Crawling: forward progression for the distance of the animal's body
   6. Walking: forward progression for the distance of the animal's body with the abdomen lifted from the testing surface

B. Reflexive tests
   1. Pain: the hindpaw was quickly grasped, squeezed with blunt-edged surgical forceps, and the pup was required to turn its head toward the hindpaw and/or withdraw the paw.
   2. Olfactory response: a negative olfactory stimulus (cedar wood oil) was placed on a cotton applicator and moved within 5–10 cm of the rat's nose. A positive response was withdrawal from the direction of the stimulus.
   3. Righting reflex: the ability of an animal to turn itself over instantly.
   4. Cross-extensor reflex: pinching the dorsal surface of the hindpaw with resultant flexion of the stimulated limb and simultaneous extension of the contralateral hindlimb.
   5. Edge aversion: pups were placed on the edge of a table with forelimbs and head extending over the edge. A positive response was turning and withdrawing from the edge.
   6. Tail hanging: pups were held by the tail with their head hanging down for 15 seconds. Within that period of time, pups must twist or flex the torso to produce head elevation above the body.
   7. Bar grasping: a wooden dowel was simultaneously placed against the ventral surface of both forepaws as the animal was held in a vertical position. Curvature of both paws around the dowel and the ability to support oneself from the dowel for 5 s was considered a positive response.
   8. Negative geotaxis: a rat was placed head down on an inclined plane (45°). The ability to turn around (180°) within 10 s was required.
   9. Falling: rats were held in a supine position 30 cm above a soft cloth and dropped. Turning in midair and landing with all four limbs under the body was a positive response.
   10. Visual orientation: a visual stimulus was passed through both visual fields at a distance of 1 cm from each eye. Five passes through each field in both naso-temporal and temporal-nasal directions were carried out and tracking (turning head) toward the stimulus was noted.
   11. Auditory reflex: a stimulus of a pen clicking was presented three times at 1-s intervals at each level and 20 cm from the head. Turning the head toward the sound was an end point.

Animals were examined between 1300 and 1630 h each day and remained with their mother prior to being tested. Pups were individually removed from their mother, marked, and observed for spontaneous and reflexive behaviors. At least 30 animals for each treatment group, obtained from eight mothers and equally representative of both sexes, were observed every day from postnatal days 1 to 19. Three separate experiments were conducted. Each animal was scored as either exhibiting the behavior or unable to perform the task. Tests were randomized among treatment groups and within litters.

Physical Characteristics

During analysis of spontaneous motor and reflexive behaviors, the time of appearance of four physical features were noted: (a) incisor eruption—visible protrusion of upper incisors; (b) hair covering–appearance of a complete coat of white hair; (c) ear opening–opening of the external auditory meatus; and (d) eye opening—visible break in eye lid and membrane covering eye. At least 30 animals/treatment group, representing eight mothers and equally representative of both sexes, were utilized.

Motor Activity

Motor activity was assessed in 21-day-old rats using an open field. Groups of 10 rats per treatment chosen from at least three litters were evaluated. The open field was a 52.5 $\times$ 52.5 cm masonite surface divided into 25 squares by painted lines; the walls were 20 cm high. Illumination during the test period was provided by standard fluorescent ceiling lights. During testing each rat was placed in the center square of the field and allowed to explore for 5 min. Locomotion was scored as the total number of squares entered with all four paws.

The number of animals, as well as the frequency of activity, associated with the following behaviors in the open field also were recorded: face washing, rearing, grooming, wet-dog shakes, and defecation. Face washing was defined as rubbing the facial area with the forepaws. Rearing was recorded if the rat lifted its body off the surface and stood on its hindpaws. Grooming consisted of rubbing and licking the torso and hindlimbs. Shaking was recorded if the rat pup stopped and shook itself in the manner of a wet dog shaking water from its coat. Each fecal bolus was counted during the 5-min period.

Data Analysis

Body weights for neonates were analyzed using a one-factor analysis of variance. On day 21, sexes were analyzed separately with a two-factor analysis of variance. Subsequent planned comparisons were made using Newman–Keuls tests.

The age at which a specific characteristic, behavior, or movement appeared for any member of each group was considered the age of initial appearance. The range of maturational performance was calculated as the number of days between initial appearance and the age at which 100% of the rats displayed a positive response or characteristic. In comparing groups for each behavior or characteristic, the initial day of appearance for every animal in a group was analyzed using the Mann–Whitney *U*-test.

The median age was the time when 50% of all animals in the combined experimental and control groups first exhibited a given behavior or positive response/characteristic. The percentage of rats demonstrating a behavior/characteristic (incidence) was recorded on the initial day of appearance and on the median day. The incidence of median age was analyzed using the chi-square test. Observations on male and female rats were combined within each group.

The number of squares traversed in the open field (i.e., frequency) was analyzed using a two-way analysis of variance with sex and treatment as independent variables; no differences were noted between sexes; thus, the means reflect scores of both male and female rats. All subsequent comparisons were made using Newman–Keuls procedures. The percentage of animals that traversed at least one square in the open field reflected the incidence of activity.

The frequencies of face washing, rearing, grooming, wet-dog shakes, and defecation (i.e., boli) were analyzed using analysis of variance. All subsequent comparisons were made using Newman–Keuls tests. The incidence of these behaviors was the percentage of animals in the NTX and control groups displaying a behavior; incidences were compared using chi-square analyses.

All analyses utilized Stats-Plus or Statistica software.

RESULTS

Body Weights

The birth weights of neonates prenatally exposed to NTX were 12% greater than their control counterparts with the mean control weight being 6.1 $\pm$ 0.1 g. At weaning, offspring delivered by mothers receiving NTX weighed 20% more than corresponding control animals (39.4 $\pm$ 1.6 g).

Physical Characteristics

In regard to the timing of appearance of certain physical characteristics, the rat pups exposed to NTX prenatally often exhibited a significantly earlier appearance than controls (Table 2). The appearance of hair covering and eye opening began 1 day earlier in the pups exposed to NTX prenatally than in the control animals. Moreover, when 100% of the animals had hair covering in the NTX-treated group, and 70% of these rats had their eyes open, no control animal had exhibited either of these traits. Comparison of the rank order of the age of initial appearance of incisor eruption, hair covering, ear opening, and eye opening revealed that the pups born of NTX-subjected mothers demonstrated a significantly earlier appearance than control offspring. Finally, computation of the age (median age) when all the animals in both groups exhibited incisor eruption, ear opening, and eye opening showed that animals exposed to NTX during prenatal life demonstrated these characteristics markedly earlier than the control animals.

Spontaneous Motor Behaviors

The ontogeny of spontaneous motor development in rat pups prenatally exposed to NTX and saline is presented in Table 3. In general, the spontaneous motor abilities in rats born of mothers given NTX often were accelerated in comparison to controls. Although the day of initial appearance of a motor behavior for a single member in each group was comparable between the NTX-treated and control animals, computation of the rank order of appearance for unilateral head turn and no return, unilateral head turn with return, pivoting, and walking revealed a significantly earlier appearance in offspring exposed prenatally to NTX than for control subjects. Analysis of the number of rats with each spontaneous behavior on the median day of appearance indicated that notably more NTX-exposed

TABLE 2

EFFECTS OF MATERNAL EXPOSURE TO NALTREXONE THROUGHOUT GESTATION ON THE DEVELOPMENT OF PHYSICAL CHARACTERISTICS OF THE OFFSPRING

	Group	Range (days)	Incidence of Initial Appearance	Median Age (day)	Incidence at Median Age
Incisor eruption	Control	7–12	21%	8	37%
	Naltrexone	7–13†	60%		90%*
Hair covering	Control	10–10	100%	10	100%
	Naltrexone	9–9†	100%		100%
Ear opening	Control	13–15	5%	14	58%
	Naltrexone	13–13†	100%		100%*
Eye opening	Control	13–15	16%	14	53%
	Naltrexone	12–13†	70%		100%*

Range indicates the age (days) at which a characteristic was initially observed for any rat in a given group and the age at which 100% of the animals in each group displayed a characteristic. Analysis of the day of initial appearance using Mann–Whitney *U* rank order tests; $p < 0.05$ (†) relative to control. Incidence of initial appearance is the percentage of animals in each group that displayed the characteristic on the initial day of appearance for the treatment group. Median age (days) is the time when 50% or more of rats from both groups displayed a physical characteristic. Incidence at the median age represents the proportion of rats with a characteristic in each treatment group at the median age (day) of all animals (i.e., includes rat pups from both the naltrexone-treated and saline-injected groups). Significantly different from controls at $p < 0.05$ (*) by chi-square. Each group contained 40 animals representative of 12 litters.

rats demonstrated unilateral head turn and no return, unilateral head turn with return, and pivoting than control animals.

Reflexive Tests

Pups exposed prenatally to NTX displayed many sensory and motor reflexive behaviors prior to the initial appearance in the control group (Table 4). Some pups exposed to NTX prenatally had positive responses to righting, falling, visual orientation, auditory orientation, and edge aversion 1 to 4 days earlier than animals in the control group. In fact, 40–50% of the rats in the NTX group exhibited these behaviors at a time when no offspring in the control group demonstrated any of these reflexive responses. However, olfactory orientation and tail hanging were observed to begin in rats delivered by NTX-treated mothers 1 day later than control animals.

TABLE 3

SUMMARY OF SPONTANEOUS MOTOR BEHAVIORAL DEVELOPMENT IN RATS PRENATALLY EXPOSED TO NALTREXONE

	Group	Range (days)	Incidence of Initial Appearance	Median Age (day)	Incidence at Median Age
Unilateral head turn, no return	Control	1–4	63%	1	63%
	Naltrexone	1–1†	100%		100%*
Unilateral head turn with return	Control	1–5	21%	1	21%
	Naltrexone	1–3†	80%		80%*
Simultaneous movement of head, forelimbs	Control	1–3	63%	1	63%
	Naltrexone	1–4	90%		90%
Pivoting <360°	Control	2–8	10%	4	32%
	Naltrexone	3–3†	100%		100%*
Crawling	Control	4–11	21%	9	89%
	Naltrexone	4–9	39%		100%
Walking	Control	11–15	5%	13	89%
	Naltrexone	9–13†	5%		100%

Range indicates the age (days) at which a characteristic was initially observed for any rat in a given group and the age at which 100% of the animals in each group displayed a characteristic. Analysis of the day of initial appearance using Mann–Whitney *U* rank order tests; $p < 0.05$ (†) relative to control. Incidence of initial appearance is the percentage of animals in each group that displayed the characteristic on the initial day of appearance for the treatment group. Median age (days) is the time when 50% or more of rats from both groups displayed a physical characteristic. Incidence at the median age represents the proportion of rats with a characteristic in each treatment group at the median age (day) of all animals (i.e., includes rat pups from both the naltrexone-treated and saline-injected groups). Significantly different from controls at $p < 0.05$ (*) by chi-square. Each group contained 40 animals representative of 12 litters.

TABLE 4
SUMMARY OF SENSORIMOTOR DEVELOPMENT IN RATS PRENATALLY EXPOSED TO NALTREXONE

	Group	Range (days)	Incidence of Initial Appearance	Median Age (day)	Incidence at Median Age
Pain–withdrawal	Control	1–1	100%	1	100%
	Naltrexone	1–1	100%		100%
Olfactory orientation	Control	2–9	10%	6	84%
	Naltrexone	3–9†	10%		40%
Righting	Control	3–9	32%	4	53%
	Naltrexone	2–4	50%		100%*
Cross extensor	Control	3–11	10%	8	58%
	Naltrexone	3–3†	100%		100%*
Edge aversion	Control	4–10	16%	7	63%
	Naltrexone	3–9†	30%		90%
Tail hanging	Control	4–9	16%	6	47%
	Naltrexone	5–7	10%		80%
Bar grasping	Control	6–11	5%	8	53%
	Naltrexone	6–9	20%		90%*
Negative geotaxis	Control	7–13	32%	10	68%
	Naltrexone	8–11	10%		50%
Falling	Control	14–16	10%	15	84%
	Naltrexone	11–14†	50%		100%
Visual orientation	Control	17–18	47%	17	47%
	Naltrexone	14–17†	40%		100%*
Auditory orientation	Control	17–17	100%	17	100%
	Naltrexone	13–16†	50%		100%

Range indicates the age (days) at which a characteristic was initially observed for any rat in a given group and the age at which 100% of the animals in each group displayed a characteristic. Analysis of the day of initial appearance using Mann–Whitney U rank order tests; $p < 0.05$ (†) relative to control. Incidence of initial appearance is the percentage of animals in each group that displayed the characteristic on the initial day of appearance for the treatment group. Median age (days) is the time when 50% or more of rats from both groups displayed a physical characteristic. Incidence at the median age represents the proportion of rats with a characteristic in each treatment group at the median age (day) of all animals (i.e., includes rat pups from both the naltrexone-treated and saline-injected groups). Significantly different from controls at $p < 0.05$ (*) by chi-square. Each group contained 40 animals representative of 12 litters.

Comparison of the rank order for the initial appearance of reflexes related to falling, visual orientation, auditory orientation, olfactory orientation, edge aversion, and cross-extension revealed that pups born to mothers receiving NTX throughout pregnancy demonstrated significantly earlier appearance than control offspring. Calculation of the age (median age) when all the animals in both groups exhibited reflexes associated with righting, visual orientation, cross-extension, and bar grasping showed that rats prenatally exposed to NTX exhibited these reflexes significantly earlier than controls.

Motor Activity and Related Behaviors

At weaning, rats prenatally exposed to NTX entered 25% fewer squares in the open field than control animals (Table 5). The frequency and incidence of face washing, rearing, grooming, wet-dog shakes, and boli in rats exposed to NTX or saline during gestation are presented in Table 5. Prenatal exposure to NTX decreased the frequency of rearing by 45%, and the number of boli by 71%, compared to control subjects. The frequency of face washing, grooming, and wet-dog shakes was comparable between offspring exposed prenatally either to NTX or saline. Analysis of the incidence of these activities showed subnormal levels in rearing, grooming, wet-dog shakes, and boli for animals in the NTX group.

DISCUSSION

The results of this investigation show that rats delivered by females given the opioid antagonist NTX throughout gestation at a dosage invoking a continuous opioid receptor blockade, and cross-fostered to untreated mothers at birth, have an acceleration in the timetable of physical and behavioral maturation. These alterations included those involving the age of initial appearance of a particular behavior, as well as the age at which 100% of the animals expressed the behavior. These data would lead us to suggest that removal of the interplay between opioids and opioid receptors during embryogenesis has remarkable implications on physical and behavioral maturation in the postnatal period. Further reasoning would indicate that if blockade of opioid–opioid receptor interaction accelerates the development of physical and behavioral characteristics, then opioids must serve actively to regulate the ontogeny of these modalities by repressive mechanisms.

Observations of rats at weaning that were maternally exposed to NTX also showed that prevention of opioid–opioid receptor interfacing during gestation had considerable impact

TABLE 5

ACTIVITY LEVELS AND RELATED BEHAVIORS OF 21-DAY-OLD RATS EXPOSED PRENATALLY TO NALTREXONE

Activity	Group	Frequency (Range/Animal)	Incidence of Activity
Open field	Control	67.7 ± 5.6 (5–131)	100%
	Naltrexone	50.7 ± 6.4† (3–141)	100%
Face washing	Control	2.4 ± 0.3 (1–6)	97%
	Naltrexone	1.9 ± 0.2 (1–7)	90%
Rearing	Control	4.7 ± 0.7 (1–21)	93%
	Naltrexone	2.6 ± 0.4‡ (1–9)	69%*
Grooming	Control	1.4 ± 0.0 (3–5)	60%
	Naltrexone	0.0 ± 0.0 (0–0)	0%*
Wet-dog shakes	Control	0.2 ± 0.0 (1–1)	10%
	Naltrexone	0.0 ± 0.0 (0–0)	0%*
Boli	Control	0.7 ± 0.2 (1–2)	37%
	Naltrexone	0.2 ± 0.1‡ (1–2)	7%*

Frequency is the average number of squares entered in the open field, or the average number of occurrences for an activity. Data are expressed as means ± SEM and were analyzed using ANOVA and the Newman–Keuls tests; $p < 0.05$ (†) or $p < 0.01$ (‡). The incidence of activity is the percentage of animals in each group that displayed the activity; significantly different from controls at $p < 0.05$ (*) by chi-square. Each group contained 40 animals representative of 12 litters.

on measures of behavior that included locomotion and emotionality. Behavioral measures such as the number of squares traversed in the open field were used to assess locomotor activity, and we found that offspring delivered by mothers receiving NTX entered fewer squares than control subjects. This would suggest that the NTX-treated rats were less active than control animals. Given that motor activity in the open field decreases with age in rats (41), our results could suggest that the NTX-exposed pups were more advanced in this behavioral measure. This study also monitored the frequency of such activities as rearing, face washing, and defecation, providing an index of "emotionality" (8,30). Although face washing was comparable in animals subjected prenatally to NTX or saline, rats in the NTX group did exhibit a lower frequency and/or incidence of activities related to rearing, grooming, wet-dog shakes, and defecation. The reason(s) for this reduced activity in the NTX-exposed offspring is(are) unclear, but once again, may be related to the advanced maturational state of these rats.

Several other studies have examined the effects of dispensing opioid antagonists during gestation on postnatal outcome with respect to behavior. Voorhees (28) administered the short-acting, low potency opioid antagonist naloxone to rats at 20 mg/kg once or twice daily on days 7–20 of gestation. Naloxone-treated offspring were accelerated in postweaning growth, upper incisor eruption, righting and startle development, home scent discrimination, and directional swimming, but as adults showed impaired Biel water maze learning. Shepanek and colleagues (25,26) gave injections of naloxone to rats from gestation day 7 to 20 and found that animals receiving 1 mg/kg habituated more rapidly in the open field and showed less activity than control pups. Bar pressing rates in a visual discrimination task were subnormal in male rats of the 10 mg/kg group, while 10 mg/kg males and females showed reduced bar pressing rates on differential reinforcement of low rates of responding. Keshet and Weinstock (11) reported that maternal NTX administration (10 mg/kg/day) using minipumps implanted in rats on day 17 of gestation prevented some behavioral alterations induced in the offspring by prenatal stress. Cohen et al. (5) found that NTX given to mothers during the last 9 days of gestation resulted in offspring with no changes in swimming ability during preweaning life, but facilitated masculine behavior and suppressed feminine receptivity. Comparison of these studies with the present is difficult because we have blocked opioid receptors throughout gestation, and the antagonist was presented in such a manner (i.e., a dosage of 50 mg/kg) as to continuously block opioid–receptor interaction in the mother as tested by morphine challenge and measures. Thus, our paradigm eliminated confounding variables introduced by opioid antagonist exposure and response that makes interpretation of other data difficult. Studies by Sandman and Yessaian (22) in which fetal exposure of rats to β-endorphin resulted in mild developmental delays supports our findings that blockade of the endogenous opioids enhances neurobiological growth.

Comparison of the behavioral effects of prenatal exposure to NTX as presented in this study with those occurring with postnatal exposure (35,38) to NTX as previously reported is valuable to decipher the role of opioids in somatic and neurobiological development. In general, continuous interruption of opioids from opioid receptors during prenatal life often had more ramifications in terms of physical and behavioral measures than exposure after birth. For example, in regard to physical characteristics only eye opening was accelerated in rats given NTX postnatally, but incisor eruption, ear opening, and eye opening were all markedly advanced in initial appearance in animals exposed to NTX throughout gestation if one uses changes in the incidence of median age as a measure. Prenatal or postnatal exposure to NTX often had the same effects on the appearance of many spontaneous motor and sensorimotor responses (e.g., acceleration of pivoting, no effect on crawling), but differences in behaviors altered due to the timing of opioid receptor blockade were apparent. Thus, increases in the median age of righting, bar grasping, and cross-extensor reflex only were noted in the group exposed prenatally to NTX, yet auditory and olfactory orientation and negative geotaxis only were influenced in the group receiving injections of NTX postnatally. Analysis of open-field behavior showed that rats exposed to NTX either prenatally or postnatally were less active at weaning, but rearing, wet-dog shakes, and defecation (but not face washing) were markedly decreased only in animals of the prenatal NTX group compared to controls. Thus, the expression of physical traits and behavioral measures in developing rats is related to opioid–receptor interaction, and both prenatal and postnatal periods of neurogenesis contribute individually to postnatal development of behavior.

Few studies have focused on the influence of exogenously applied native opioid peptides during gestation, and all of the studies (10,22,31) have been concerned with β-endorphin and exposure during the last semester of pregnancy. Kashon and coworkers (10) administered β-endorphin three times daily (33 μg/injection) and reported alterations in the differentiation of some sexually dimorphic traits. Zadina et al. (31) gave daily injections of 100 μg of β-endorphin and found that the timetable of eye opening and open-field rearing behavior in adults were comparable in offspring from opioid-treated and control females. In a study performed by Sandman and Yessaian (22), 100 μg of β-endorphin was administered daily and the time of eye opening, "activity," and startle reflex were normal in all progeny. Although these studies indicate that prenatal exposure to β-endorphin is only of some consequence to the sexual traits of offspring, the limited exposure (i.e., third semester of

pregnancy) and dosages of this native peptide do not allow a full understanding of β-endorphin activity. Moreover, placed into the perspective of the present investigation in which receptor blockade was employed throughout pregnancy, it is premature to draw any conclusions about the role of NTX on β-endorphin's (or any other endogenous opioid peptide's) potential modulatory capabilities on developmental events.

The changes in the timetable of physical and behavioral ontogeny in rats born to mothers receiving NTX that are documented in this study are accompanied by a number of somatic and neurobiological-related alterations. The body weights of neonatal and weaned rats in the NTX-exposed group were increased 12 and 20%, respectively, from control levels in this study; these changes compare favorably with those in an earlier investigation (8 and 36%, respectively) (16). Rats delivered by mothers receiving daily injections of NTX have been reported (16) to have increases in brain weights at birth and weaning of 43 and 18%, respectively. Whether the functional changes seen in the behavior of these animals recorded in this study are related to structural alterations in the nervous system requires clarification.

The specific mechanism(s) as to how NTX modulates growth needs to be elucidated. A dosage of 50 mg/kg is 2.5% of the LD_{50} in adult rats (4), and was not deemed detrimental to any aspect of the present experiments. Moreover, administration of a 50 mg/kg dosage of NTX in this and other studies (35) is known to produce an opioid receptor blockade for 24 h; after this point NTX's efficacy to block opioid receptors is dissipated, indicating that drug is no longer present. We do know that opioids are tonically active negative regulators of DNA synthesis, and that the opioid responsible for these activities, [Met^5]-enkephalin, is an autocrine produced growth factor in both neural and nonneural tissues. Given the marked effects on somatic and neurobehavioral development in offspring exposed to NTX only during embryogenesis, and the well-documented direct effects of NTX in influencing growth (e.g., organ and tissue culture studies), it may be postulated that daily interruption of opioid–opioid receptor interaction by NTX prevents opioids from their normal trophic activities in the developing nervous system of the prenatal rat. Behavioral alterations witnessed during preweaning ontogeny and at weaning in animals maternally exposed to NTX only throughout gestation indicates that marked changes have occurred in the nervous system during early life that are manifested at later stages (and when drug is no longer present). Whether these changes are reflective and consequential to the alterations in somatic growth that occur, or if the timetable of physical and behavioral ontogeny is dictated by opioid–receptor interaction needs to be elucidated.

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