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Naltrexone's Influence on Neurobehavioral Development

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ZAGON, I. S. AND P. J. McLAUGHLIN. *Naltrexone's influence on neurobehavioral development.* PHARMACOL BIOCHEM BEHAV 22(3) 441–448, 1985.—The ontogeny of spontaneous motor and sensorimotor behaviors of preweaning rats, as well as ambulation, emotionality, and nociception at weaning (day 21), were studied in rats given chronic administration of 1 or 50 mg/kg naltrexone from birth to day 21. The age at which a specific spontaneous motor behavior or performance initially appeared and the age at which 100% of the animals demonstrated a particular behavior were accelerated in animals given 50 mg/kg naltrexone, but delayed in rats injected with 1 mg/kg naltrexone. In general, ambulation, emotionality, and nociceptive responses were not affected by naltrexone treatment, although the frequency of face-washing in both naltrexone groups and activity cage performance in the 50 mg/kg naltrexone group deviated from control levels. Observations of head-shake and wet-dog shake behaviors in naltrexone-treated animals at 2 hr and 10 hr post-drug injection were similar to controls with the exception of an abnormal increase in the 1 mg/kg naltrexone group at 10 hr. Although these results may imply that endogenous opioid systems play a role in regulating neurobehavioral development, further study is needed to distinguish whether these changes are a consequence of the somatic and morphological alterations known to occur with naltrexone administration or if the timetable of behavioral ontogeny is governed by endorphin-opiate receptor interaction.

Naltrexone Opiate antagonists Endorphins Behavioral development Motor activity Opioids Nociception Rats

THE adverse effects of exogenous opioids such as methadone, morphine, and heroin on somatic and neurobiological development of humans and laboratory animals are well known [20, 21, 24–27]. Studies conducted in animals, and in cell cultures, reveal that these actions are stereospecific and blocked by concomitant administration of opioid antagonists [21,36]. Investigations in our laboratory have documented that opioid antagonists also influence normal growth as well as tumor development [31–35, 38, 39]. Administration of naltrexone, a potent opioid antagonist, to preweaning rats has both stimulatory and inhibitory effects on body and brain development that are dose-dependent. Preweaning rats, receiving daily injections of a relatively high dosage of naltrexone (i.e., 50 mg/kg) which blocked the opiate receptor for 24 hr each day, were found to have an increase in body and organ weights, acceleration in the appearance of physical characteristics (e.g., eye opening), and were able to walk earlier than control counterparts [32]. Macroscopic, histological, and morphometric studies revealed marked increases in brain size and cellular growth [33]. Daily administration of 1 mg/kg naltrexone, a dosage that blocked the receptor for 4–6 hr each day, had the opposite effects on development.

The relationship between growth processes, drug dosage, and the pharmacological properties of naltrexone recently has been clarified [39]. Studies utilizing dosages of naltrexone ranging from 0.1 to 100 mg/kg showed that dosages below 10 mg/kg, which blocked the opiate receptor for less than 12 hr/day, had an inhibitory effect on growth. Dosages of 20 mg/kg naltrexone or greater, which blocked the opiate receptor for 24 hr/day, had a stimulatory action. However, low dosages (3 mg/kg) of naltrexone, given three times each day, which in effect blocked the receptor for 24 hr/day, also resulted in notable increases in body and brain development. A cumulative dosage of 9 mg/kg given once daily retarded growth. Thus, developmental events appear to be governed by the duration of opiate receptor blockade. These results, in conjunction with previous reports of endorphin immunoreactivity in fetal and neonatal cells [13,16], the presence of endorphins in the brain and plasma of developing organisms [2,17], and the identification of opiate receptors in brain and body tissues of young animals [5, 9, 14] provide a strong argument that endogenous opioid systems play a crucial role in growth.

The dramatic alterations in development induced by naltrexone, and presumably acting by modulation of the endogenous opioid systems, merits further consideration. In view of the changes in body and brain growth, along with our provocative finding concerning the timetable for walking, the present study was designed to investigate in a comprehensive manner the effects of naltrexone on neurobehavioral ontogeny. The functional status of the developing nervous system in young rats chronically exposed to 1 or 50 mg/kg naltrexone was assessed by examining the ontogeny of spontaneous motor activity and sensorimotor reflexes during the preweaning period, as well as by determining motor activity and nociceptive response at weaning.

METHOD

Animals and Drug Treatment

Female (180–200 g) and male (400–450 g) Sprague-Dawley rats (Charles River Labs, Wilmington, MA) and their offspring were used in this study. Animals were housed in steel solid-bottom cages (5 animals/cage) in an environment at 21±0.5% with a relative humidity of 50±10%. The room had a complete exchange of air 15 to 18 times per hour and a 12-hour light-dark cycle with lights turned on at 0700. Water and Agway Lab Chow were available ad lib. All animals were allowed at least 6 days to acclimate to their surroundings prior to the beginning of experimentation.

Animals were mated 1 male to 1 female. At birth (Day 0), litters were culled to 8 pups/mother with an equal number of males and females. Beginning on Day 1, pups were injected subcutaneously with either 1 mg/kg or 50 mg/kg naltrexone hydrochloride (Endo Laboratories, Garden City, NY), or an equivalent volume of sterile water. Naltrexone was prepared in sterile water; dosages are expressed as salts. Animals were weighed every 3 days and appropriate dosage adjustments made. Injections were terminated at weaning (Day 21).

Behavioral Apparatus and Procedures

Spontaneous motor and reflexive tests. Developmental reflexes and spontaneous motor capabilities were determined using methodology described by Zagon and McLaughlin [28,29], and the apparatus and procedures used in these tests, as well as the criteria for positive responses, are fully explained in these earlier studies. A brief description of behaviors tested in naltrexone-treated and control rats is presented in Table 1. Spontaneous motor behavior was measured by placing each animal in the center of a 55×55 cm examination table covered by a Durasorb laboratory underpad (Parke-Davis, Jessup, MD); all movements were recorded for 2 min/day. After completion of the spontaneous movement tests, rat pups were subjected to a battery of sensorimotor tests. For these tests, each animal was observed 5–10 min/day depending on the level of performance.

Animals were tested within 30 min to 2 hr of drug injection. This enabled us to examine the cumulative effects of naltrexone at a time when naltrexone was present from the daily injection of each drug dosage. Pups were marked, individually removed from their mother, and observed for spontaneous and reflexive behaviors. Sixteen animals per group, chosen from at least 4 litters and equally representative of both sexes, were observed every day from postnatal days 2 to 19. Each animal was scored as either exhibiting the behavior or unable to perform the task.

The age at which a specific movement or behavior initially appeared for any group member (=the age of initial appearance) and the age at which 100% of the animals demonstrated a particular behavior (=the age of maximal response) were recorded. The range of maturational performance was calculated as the number of days between the age at which a positive response was initially observed and the age at which 100% of the rats displayed a positive response.

Motor activity. Motor activity was assessed in 21-day old rats using a darkened activity cage and an open field. Groups of 10 to 18 rats per group (equal number of males and females), chosen from at least 4 litters, were evaluated within 1 hr of drug or vehicle injection. These rats were littermates of those examined earlier. Animals were randomly rotated through the behavioral procedures in order to control for time of day and sequence of test presentation.

Activity cage. A cylindrical activity cage (Lehigh Valley Electronics, Model 145-03), 60 cm in diameter and 38 cm high and containing 6 banks of infrared photobeams connected to counters that were activated whenever a photobeam was interrupted, was utilized to assess locomotor activity in a darkened area. Inside walls were flat black to minimize ambient light reflections. An animal's movement was measured as the total number of photobeam interruptions during a 5 min period.

Open field. The open field was a 52.5×52.5 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, which displayed the following behaviors while in the open field were also recorded: rearing, face washing, defecation, head shakes and wet-dog shakes. Head shakes and wet-dog shakes [30] were scored for all groups of rats 2 and 10 hr after the injection of drug or vehicle on day 21.

Nociception. The hot-plate technique of Woolfe and MacDonald was utilized to monitor nociceptive response in 21-day old rats [30]. At 2 hr, 6 hr, and 24 hr after injection of drug or vehicle, and utilizing at least 10 rats per group, animals were placed on an Analgesia Meter (Technilabs Instrument, Pequannock, NJ) maintained at 55±0.5°C and the latency (±0.1 sec) of their reflex response to heat was recorded [30]. Animal responses employed as end-points included the licking of a paw or withdrawal of one of the hindlimbs from the plate; any subject not responding within 45 sec was removed from the hot-plate. Animals were tested only once.

Data Analysis

Body weights of 21-day old offspring were analyzed using a two-factor analysis of variance with Treatment Groups and Sex as independent variables. Subsequent planned comparisons were performed using Newman-Keuls tests.

Overall group differences in the age of appearance of a given behavior were analyzed using the chi square test. The point at which these comparisons were made was the age at which 50% of the animals in the combined experimental and control groups first exhibited a given behavior or positive response. If the results of the chi square test revealed significant overall group differences, subsequent comparisons were then made between each experimental group and the control animals using the Fisher Exact Probability test. This analysis thus provided a test of differences in the proportion of animals within each group that had exhibited the behavior by the time of its median age of appearance.

Activity in the open field and activity cage were evaluated separately using analysis of variance. Latencies on the hot-plate test, number of boluses, head shakes, wet-dog shakes, and rearing and grooming frequencies also were analyzed using analysis of variance. All subsequent comparisons were made using Newman-Keuls procedures. In addition, the number of animals per group that displayed head shakes, grooming, rearing, and boluses (one or more) was analyzed using chi square tests. Analyses were performed with the Stats-Plus statistical package adapted for the Apple II Plus computer.

TABLE 1

CATEGORIES OF BEHAVIORAL DEVELOPMENT TESTED IN RATS POSTNATALLY EXPOSED TO NALTREXONE

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. Simultaneous movement of head, forelimbs, and hindlimbs
5. Pivoting less than 360°
6. Crawling: forward progression for the distance of the animal's body
7. Walking: forward progression or the distance of the animal's body with the abdomen lifted from the testing surface

B. Reflex tests

1. Righting reflex: the ability of an animal to turn itself over instantaneously with all four feet on the ground after being placed on its back.
2. Falling reflex: rats were held in a supine position 30 cm above a bed of litter and dropped; turning in midair and landing with all four limbs under the body was considered a positive response.
3. 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 performed. Tracking (turning head) toward the stimulus as it passed through the field was a positive response.
4. Auditory reflex: a stimulus of a pen clicking was presented 3 times at 1 sec intervals, 20 cm from head at ear level; turning the head to the side of the stimulus sound was considered a positive response.
5. Olfactory reflex: olfactory stimuli (warm chocolate or cedarwood oil) were placed on cotton swabs and held 5–10 cm from nose. A positive response consisted of movement toward the stimulus (in the case of the chocolate) or movement toward the odorant tip (in the case of the cedarwood oil) and withdrawal (approach-avoidance).
6. Vibrisseal stroking: vibrissae on alternate sides of the head were stroked at 10 sec intervals with a cotton swab; turning the head and attempting to grasp the stimulus was a positive response.
7. Edge aversion: the rat was placed on the edge of the table with forepaws and head extending over the edge; a positive response was considered to be turning, withdrawing, and walking away from the edge of the table.
8. Pain: the hindpaw was quickly grasped, squeezed with surgical forceps, and the rat was observed for 10 sec. The procedure was repeated on both hindpaws, alternating the side of the initial stimulation every day. Turning the head or withdrawing the paw was considered a positive response for pain.
9. Cross extensor reflex: pinching the dorsal surface of the hindpaw causes flexion of the stimulated limb and simultaneous extension of the contralateral hindlimb.
10. Bar grasping: a wooden dowel (4 mm diameter) 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 hang from the dowel for 5 sec was considered a positive response.
11. Tail hanging: pups were held by the tail (head down) for 15 sec. Twisting and flexing of the torso to produce head elevation was considered a positive response.
12. Negative geotaxis: pups were placed on a 45° inclined plane of fine-holed wire mesh with head pointing down. Ability of animal to turn around and begin crawling up the incline was a positive response.

RESULTS

Body Weights

The preweaning growth of rats subjected to naltrexone has been reported earlier [32]. In general, the 1 mg/kg and 50 mg/kg naltrexone groups exhibited decreases and increases, respectively, in body weight in comparison to control animals. At 21 days, male (mean±SE=52.13±0.38 g) and female (mean±SE=51.10±0.49 g) control rats weighed significantly more than their counterparts given 1 mg/kg naltrexone (males=45.09±0.43 g; females=42.38±0.48 g) but markedly less than 50 mg/kg naltrexone-treated animals (males=62.65±0.68 g; females=57.14±1.40 g).

Spontaneous Motor Behavior

The ontogeny of gross motor development in naltrexone-exposed and control pups is presented in Table 2. Overall, spontaneous motor abilities in rats given 1 mg/kg naltrexone often were delayed in comparison to controls whereas 50 mg/kg naltrexone-treated rats were accelerated. Although these observations applied to simple spontaneous movements (e.g., unilateral head turn with return), complex behaviors such as crawling and walking were also affected. The age of initial appearance for a behavior was altered by up to 3 days in both naltrexone-treated groups. For example, rats in the 50 mg/kg group began to walk on day 8 in contrast to day 10 in the control rats and day 13 in the 1 mg/kg naltrexone

TABLE 2

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

	Median Age (days)	Control	1 mg/kg Naltrexone	50 mg/kg Naltrexone
Unilateral head turn and no return	3	100% (3–3)	80% (3–4)	100% (3–3)
Unilateral head turn with return	5	50% (3–6)	20% (3–6)	100%* (3–5)
Simultaneous movement of head, forelimbs	5	100% (4–5)	100% (3–5)	100% (4–5)
Simultaneous movement of head, forelimbs, and hindlimbs	7	50% (6–10)	40% (6–10)	100%* (6–7)
Pivoting <360°	8	50% (5–10)	20% (5–10)	100%* (5–8)
Crawling	9	100% (8–9)	30%* (9–11)	100% (5–8)
Walking	12	50% (10–13)	0%* (13–15)	100%* (8–11)

Values (percentages) represent the proportion of positive response by each treatment group at the median age for all animals from both experimental and control groups. Significantly different from controls at $p<0.05$ (*). Values in parentheses indicate the range of maturational performance (days), and includes the age at which an initial response was observed and the day when the maximal number of animals displayed a positive response.

group. Thus, rats in the 50 mg/kg naltrexone group began to walk almost a week earlier than pups receiving the low dosage of naltrexone. The age at which 100% of the animals demonstrated a particular behavior also differed between control and naltrexone-treated offspring. In 2 cases (i.e., crawling and walking), the 1 mg/kg naltrexone group took up to 2 days longer than controls for all of the animals to achieve this behavior. In contrast to the 1 mg/kg group, the 50 mg/kg group had 3 instances (simultaneous movement of the head, forelimbs, and hindlimbs, pivoting and walking) in which 100% of the animals achieved a particular behavior 2 to 3 days earlier than the controls. Maturational range was often noted to be slightly extended (i.e., 1 day) in the 1 mg/kg naltrexone group, and was shortened up to 3 days in the 50 mg/kg naltrexone group.

Statistical evaluation of each spontaneous motor activity was performed and those behaviors with response most notably different from controls are listed in Table 2. Significant delays in the median age of appearance of crawling and walking were seen in the 1 mg/kg naltrexone groups. In the 50 mg/kg naltrexone group, the median ages of appearance for unilateral head turn with return, simultaneous movement of head, forelimbs, and hindlimbs, pivoting, and walking were earlier than controls. The most notable differences between all 3 groups were recorded with regard to walking, often considered to be a milestone of behavioral achievement. Thus, while no animal in the 1 mg/kg group could walk on day 12, the day when 50% of the controls could walk, 100% of the animals in the 50 mg/kg naltrexone group demonstrated this behavior.

Reflexive Tests

As with spontaneous motor behaviors, the 1 mg/kg naltrexone group was characterized by delays in the achievement of a battery of sensory and motor reflexive tests, and the 50 mg/kg naltrexone an acceleration (Table 3). In the age of initial appearance and maximal appearance of a reflex action, the 1 mg/kg naltrexone group exhibited delays of 1 to 5 days in 11 of the 13 parameters examined. Less dramatic changes from control values occurred for the ages of initial and maximal response of a reflex action in the 50 mg/kg group, with 4 behaviors initially appearing up to 2 days earlier relative to control levels. Although changes were encountered in the range of maturation development for both naltrexone groups, no clear direction was recorded.

Statistical analysis comparing the median age of appearance of sensorimotor behavior for control and naltrexone-treated animals is presented in Table 3. The 1 mg/kg naltrexone group exhibited significant delays in 7 of the 13 categories of behavior examined, whereas the 50 mg/kg group were accelerated in 5 of the 13 behaviors. These deviations from the normal timetable of behavioral ontogeny included those for motor reflexes, but were most prominent in sensory challenges.

Motor Activity

Open field. No significant differences between 21-day old naltrexone-treated and control animals were recorded in motor activity (i.e., number of squares entered), rearing, number of boluses, and head-shake and wet-dog shake be-

TABLE 3

SUMMARY OF SENSORIMOTOR BEHAVIORAL DEVELOPMENT IN RATS POSTNATALLY EXPOSED TO NALTREXONE

	Median Age (days)	Control	1 mg/kg Naltrexone	50 mg/kg Naltrexone
Pain-withdrawal	2	80% (2–3)	0%* (4–5)	100% (2–2)
Righting reflex	4	80% (3–5)	90% (4–6)	90% (3–5)
Cross extensor	4	100% (3–4)	0%* (5–9)	100% (2–4)
Grasping	5	50% (4–6)	50% (4–9)	70% (4–8)
Falling	7	100% (4–7)	60% (5–9)	90% (4–8)
Edge aversion	7	100% (4–7)	10%* (6–9)	100% (5–7)
Negative geotaxis	8	50% (4–9)	20% (8–9)	100%* (5–8)
Tail hanging	8	80% (6–9)	0%* (9–10)	100% (5–8)
Vibrissael stroking	8	40% (8–9)	10% (8–10)	100%* (6–8)
Olfactory orientation to cedar wood oil	5	100% (4–5)	0%* (6–8)	100% (4–5)
Olfactory orientation to Hershey's chocolate	6	40% (5–7)	10% (6–9)	90%* (5–7)
Auditory orientation	10	50% (10–12)	0%* (11–12)	100%* (10–10)
Visual orientation	16	50% (15–16)	0%* (17–19)	100%* (14–15)

Values (percentages) represent the proportion of positive response by each treatment group at the median age (days) of all animals (i.e., includes rat pups from both experimental and control groups). Significantly different from controls at $p<0.05$ (*). Values in parentheses indicate the range of maturational performance (days), and includes the age at which an initial response was observed and the age when the maximum number of animals displayed a positive response.

haviors at 2 hr following drug injection (Table 4). However, examination of rats given daily injections of 1 mg/kg naltrexone did have abnormal increases in the number of head shakes and wet-dog shakes at 10 hr post-drug injection; no differences between control and 50 mg/kg naltrexone rats were detected in this regard. Face-washing was the only category of behavior that was altered in both drug-exposed groups, with significantly more face washing noted in the 1 mg/kg naltrexone group and subnormal activity recorded in grooming behavior for 50 mg/kg naltrexone rats.

Activity cage. The control and 1 mg/kg naltrexone groups were comparable in the number of photobeam interruptions made in the activity cage (Table 4). However, an increase of 32% in activity was recorded in animals of the 50 mg/kg naltrexone group and this difference was statistically reliable from control values.

Nociception

Naltrexone-treated and control offspring examined for nociceptive responsiveness on the hot-plate test at a variety of intervals following drug administration on day 21 were comparable (Table 4).

DISCUSSION

The results of this investigation show that rats receiving chronic administration of naltrexone during preweaning life have an altered timetable of behavioral maturation. In general, animals given a dosage of 50 mg/kg naltrexone were accelerated in the appearance of gross motor development and the maturation of both simple and complex sensory motor behaviors, whereas rats given 1 mg/kg naltrexone exhibited some delays. These alterations included those involv-

TABLE 4

ACTIVITY LEVELS OF 21-DAY OLD RATS TREATED WITH NALTREXONE

	Controls	1 mg/kg Naltrexone	50 mg/kg Naltrexone
Open Field			
Number of Squares entered	62.6 ± 1.6	60.1 ± 4.6	51.7 ± 5.3
Rearing	4.2 ± 1.2	6.4 ± 1.2	4.6 ± 1.3
Face washing	2.5 ± 0.8	4.9 ± 0.8*	1.5 ± 0.3*
Number of boli	2.0 ± 0.6	0.7 ± 0.5	1.1 ± 0.4
Head shakes: 2 hr	0.4 ± 0.1	1.8 ± 0.8	0.6 ± 0.9
10 hr	2.5 ± 0.3	7.8 ± 0.7†	3.8 ± 1.6
Wet-dog shakes: 2 hr	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
10 hr	0.0 ± 0.0	3.0 ± 0.4†	0.0 ± 0.0
Activity Cage			
Number of photobeam interruptions	105.5 ± 5.2	102.0 ± 8.4	139.2 ± 7.9*
Hot-Plate Test			
Latency: 2 hr	15.1 ± 0.5	15.6 ± 1.9	14.6 ± 1.3
6 hr	14.3 ± 0.6	16.6 ± 0.9	14.6 ± 1.0
24 hr	14.5 ± 0.7	16.9 ± 1.1	14.9 ± 0.6

Values represent means ± SE. Significantly different from controls at $p<0.05$ (*) and $p<0.01$ (†). An explanation of procedures and statistical methodology is included in the Methods section.

ing the age of initial appearance of a particular behavior, as well as the ages at which 100% of the animals expressed the behavior. More instances of change in spontaneous motor measures were noted in the 50 mg/kg naltrexone group than the 1 mg/kg naltrexone group, whereas more categories of sensorimotor behavior were disrupted in the 1 mg/kg naltrexone group. The magnitude of temporal change was often greatest in the 1 mg/kg naltrexone group, with alterations ranging from 1 to 5 days noted. This was in contrast to ranges of 1 to 3 days for rats in the 50 mg/kg naltrexone group. Although data on median age, and ages of initial response and maximal response, were clearly directional in respect to dosage (i.e., delays in the 1 mg/kg naltrexone group, acceleration in the 50 mg/kg naltrexone group), data in regard to maturation range were difficult to interpret. Analysis of the time difference between the age at which a behavioral characteristic was first observed and the age at which 100% of the rats displayed a specific activity revealed instances of prolongations and contractions of this interval for both the 1 and 50 mg/kg naltrexone groups.

The measures of preweaning behavioral ontogeny were based on Blanck *et al.* [3], Fox [6], and Almli and Fisher [1] and were designed to determine when the ability to perform a given behavior was attained, irrespective of the velocity and/or coordination of response. It should be emphasized that permanent behavioral deficits were not encountered in animals of either the naltrexone-treated or control groups but, in fact, every animal was observed to eventually express all of the behavioral responses by the conclusion of the observation period on postnatal day 19.

It is important to note that the present study addressed the cumulative effects of daily naltrexone administration on behavior, but did so from the standpoint of examining animals soon after receiving daily drug administration. Therefore, at both high and low dosages, naltrexone was present during evaluation. This timetable of testing was devised in order to avoid problems of interpretation when rats were examined at later times each day (e.g., just prior to daily injections) and drug was still present in the 50 mg/kg group but not in the 1 mg/kg group. Subsequent studies conducted at later times each day would certainly be valuable and complementary to the results of the present investigations.

Subsequent examination of naltrexone-treated offspring at weaning was concerned with measures of behavior including locomotion, emotionality, and nociceptive response. The methodology utilized in these studies generally followed that employed previously in our laboratory [28–30, 40]. Behavioral measures such as performance in the activity cage or the number of squares traversed in the open field were used to assess locomotor activity [7,23], whereas the frequency of such activities as rearing, face washing, and defecation provided an index of "emotionality" [8, 10, 23]. With the exception of some alterations in face-washing in both naltrexone-treated groups, and some increased activity recorded in the 50 mg/kg naltrexone group in the activity cage, no alterations in the general profile of behavior were established.

A fascinating observation in our studies is that concerned with behavior (wet-dog shakes or head-shakes) resembling drug withdrawal [30]. Two hours following adminstration of either 1 or 50 mg/kg naltrexone, withdrawal-like behavior was not recorded. However, at 10 hr after naltrexone injection, an abnormal number of head shakes and wet-dog shakes were encountered in the 1 mg/kg group, but not in the animals of the 50 mg/kg naltrexone group. These results appear to correspond to data obtained in previous reports [32] in which the duration of opiate receptor blockade was evaluated. At 2 hr, both 1 and 50 mg/kg naltrexone dosages block morphine-induced nociceptive response but at 10 hr, the opiate receptor block is only in the 50 mg/kg naltrexone group.

Several other studies have examined the effects of early opiate antagonist exposure on preweaning development. Two important studies concerned with naltrexone are those of Paul *et al.* [18] and Harry and Rosencrans [11]. Paul and colleagues [18] administered naltrexone to rats (litter size was not stated to be controlled) on postnatal days 3–20 (20 mg/kg days 3–14; 60 mg/kg days 15–20; subcutaneous injections) and these animals did not differ from controls in growth or on a battery of reflex tests that included cliff avoidance, righting, negative geotaxis, and open field, nor on nociceptive responses evaluated with tail-flick and hot-plate tests. However, an abnormal increase in analgesic response to a challenge of morphine was recorded on day 29, but not on day 42. Harry and Rosencrans [11] administered naltrexone (1 mg/ml in drinking water) to pregnant female rats throughout gestation and, in some cases, during lactation. Activity cage measurements did not differ between naltrexone-treated and control offspring at postnatal day 28, although as adults some differences in nociceptive response and ability to habituate to a novel environment were noted between naltrexone-treated and control offspring. A number of studies have begun to explore the effects of perinatal exposure to naloxone on development. Hetta and Terenius [12] showed that maternal naloxone treatment (using implanted pellets) for 7 days beginning on either day 11 or 17 of pregnancy or 3 days postpartum, had no effect on offspring nociception as measured with the hot-plate test on day 30. Challenge with morphine on day 40 revealed a heightened analgesic response in animals perinatally exposed to naloxone. Monder *et al.* [15] found that female mice exposed to naloxone (subcutaneous implants) during the last 5 days of pregnancy showed hyperalgesia on a hot-plate test at 50 days in females, but not in males. Sandman *et al.* [19] administered naloxone to rats (100 mg/rat) during days 2 to 7 of postnatal life and found elevated thresholds to thermal stimulation when the rats reached 90 days of age. In a detailed behavioral study of the effects of twice daily injections of naloxone (20 mg/kg per injection) to female rats on gestation days 7–20, Vorhees [22] found that naloxone offspring were accelerated in postweaning growth, upper incisor eruption, righting development, startle development, home scent discrimination, and in directional swimming. As adults, these animals showed impaired Biel water maze learning. No significant differences between naloxone and control groups on open field, running wheel, M-maze, spontaneous alternation, active or passive avoidance, rotorod, food grasping, or tail flick could be discerned.

The changes in the timetable of behavioral maturation of naltrexone-treated animals that are documented in this study are accompanied by a number of other somatic and neurobiological alterations. Body weights during preweaning growth are consistently higher in the 50 mg/kg naltrexone group and subnormal in the 1 mg/kg naltrexone group [32]. At 21 days, brain weights and macroscopic dimensions follow a similar pattern [32, 33, 39]. Histological and morphometric studies [33] conducted on the 50 mg/kg naltrexone group reveal that these 21-day old rats have a somatosensory cortex that was 18% thicker than controls. Moreover, the cerebellum of rats in the 50 mg/kg naltrexone group was 41% larger in total area and contained at least 70% more glial cells and 30% more granule neurons. Neurons derived prenatally, however, were unaffected by drug treatment, suggesting that only cells with the potential to divide are affected by preweaning naltrexone administration. The present data reveal that an entire array of functional changes also takes place along with these morphological alterations.

The specific mechanisms as to how naltrexone modulates growth processes has yet to be determined. The dosages needed to alter ontogeny are extraordinarily low, being 0.05% to 2.5% of the LD_{50} in adult rats [4]. It does not appear that naltrexone's effects are a reflection of nutritional status, nor are hormonal changes believed to be responsible for naltrexone's actions on development [39]. Naltrexone's influence on growth has been shown to depend on its pharmacological activity to block the opiate receptor [32,39], and the presence of opiate receptors in developing (but not necessarily adult) tissues and organs [9,14], along with reports of endorphins in the plasma and brains of young animals [2,17] has provided the framework for a novel idea concerning biological development [32, 33, 39]. It would appear that endorphins, along with exogenous opioids such as morphine and methadone [26,27], serve to inhibit growth through interaction with the opiate receptor complex located on developing cells. Complete prevention of this interaction by administration of relatively high dosages of naltrexone given once daily, or low dosages given repeatedly, allows growth to proceed in an "unimpeded" fashion that produces larger animals. Enhancement of this interaction such as that observed with temporary opiate receptor blockade results in smaller rats. Presumably, this retardation in growth occurs because low dosages of naltrexone provide some interval each day when the inhibitory action of basal or elevated levels of endorphins can act on cells possessing a greater complement of opiate receptors.

Regardless of the precise mechanisms involved, the present study clearly demonstrates that opioid antagonists markedly alter neurobehavioral ontogeny. Although our data imply that endogenous opioid systems serve an important role in regulating behavioral development, further study is needed to distinguish whether these changes are reflective and consequential to the alterations in somatic growth that occur, or whether the timetable of behavioral ontogeny is dictated by endorphin-opiate receptor interaction.

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