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Prenatal Naltrexone Facilitates Male Sexual Behavior in the Rat

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COHEN, E., G. KESHET, Y. SHAVIT AND M. WEINSTOCK. *Prenatal naltrexone facilitates male sexual behavior in the rat.* PHARMACOL BIOCHEM BEHAV **54**(1) 183–188, 1996. – The involvement of endogenous opiates in the differentiation of sexual behavior was tested by exposing rat fetuses to continuous naltrexone during the last 9 days of gestation. Time-mated female rats received oral naltrexone, 40 mg/kg/day, via their drinking water, from gestational day 13 until parturition. Early motor development, measured by swimming ability in 7-, 9-, and 11-day-old offspring of the treated dams, was unaffected by prenatal naltrexone. Adult male offspring were given three tests of male sexual behavior, then castrated, primed with ovarian hormones, and given two tests of feminine receptivity (lordosis quotient). Prenatal naltrexone facilitated masculine behavior and suppressed feminine receptivity: latencies to first mount and to ejaculation were shorter, mount rate was higher, and lordosis quotient was lower in naltrexone-treated rats, compared with control animals. These findings implicate endogenous opiates in prenatal organization of sex-specific behavioral dispositions.

Prenatal development Oral naltrexone Endogenous opiates Sexual differentiation Masculinization Defeminization

ENDOGENOUS opiate systems (EOS) have been shown to play a regulatory role in neural development (2,31,33). EOS may also be implicated in the differentiation of brain mechanisms related to reproduction. Thus, prenatal exposure to opiates interferes with male sexual development, as demonstrated by the suppression of the activity of Δ^5-3β-ol-steroid dehydrogenase (an enzyme critical for the synthesis of testosterone) and the reduction of plasma testosterone in male fetuses (1,21). Excess activity of endogenous opiates at a critical period during fetal development may also be responsible for the partial feminization and demasculinization of rats exposed prenatally to stress (16,24,28). This hypothesis is supported by the finding that the opiate antagonist naltrexone, injected to pregnant rats before each stress session, decreased the feminizing effect of prenatal stress (27). When administered continuously by minipumps to stressed pregnant rats (from day 17 of gestation to parturition) naltrexone also prevented the reduction in ano-genital distance in the male offspring (15). Furthermore, the feminizing effect of prenatal stress could be mimicked by injection of β-endorphin to pregnant dams (14).

In studies that have investigated the role of EOS in growth, a high dose of naloxone administered pre- or postnatally, enhanced neural and organ growth, and accelerated motor development in the offspring, while a lower dose delayed growth and slowed development (22,32).

The effects of opiate antagonists, repeatedly injected to pregnant dams or developing pups, are often hard to interpret, because they are confounded by the daily variation in drug levels, and the stress of frequent handling and injecting. Also, it has been argued that low doses of naloxone, which block the opiate receptors for less than 12 h a day, elevate the level of endogenous opiates and increase the density of opiate receptors, due to upregulation. Therefore, their ultimate effect is enhancement, rather than blockade of EOS, which accounts for the bidirectional effect of naloxone (32).

In the present study, we investigated the involvement of endogenous opiates in brain sexual differentiation, using orally administered naltrexone. The drug was administered to pregnant rats during the last 9 days of pregnancy, via their drinking water. This procedure was used to avoid the stress of daily handling and to ensure continuous blockade of opiate receptors.

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METHOD

Albino Sabra rats were maintained in controlled temperature rooms (23°C), on a 12 L : 12 D cycle, with lights on 0700–1900 h.

Animals were housed in group cages of four. Food and water were provided ad lib.

Assessment of the Required Dose of Oral Naltrexone

The dose of continuous oral naltrexone required to block opiate receptors in pregnant rats was assessed in a preliminary series of experiments. Fifty-eight female rats were intramuscularly (IM) injected with either 1 ml/kg saline or one of the following doses of morphine HCl (Teva Ltd.), 0.75, 2.0, or 4.0 mg/kg. Pain threshold was assessed 30 min later, using the tail-pressure test with a Randall-Selitto apparatus (Hugo Basile Ltd.) (19). The pressure, applied to the tail of the tested animal, was expressed in arbitrary units ranging between 0–25. Mean values (±SEM) of pain threshold, observed following saline or the above doses of morphine, were 3.5 (±1.2), 5.6 (±1.2), 13.4 (±1.2), and 24.0 (±1.2), respectively. The 4 mg/kg dose was chosen for subsequent experiments.

Twenty-four experimentally naive female rats were randomly assigned to four groups and given tap water or naltrexone solution of either 10, 20, or 40 mg/kg/day, as their drinking fluid. Drug concentration was computed for each rat according to body weight, daily fluid consumption, and the assigned dose. The analgesic effect of morphine, 4 mg/kg, was evaluated in water- and naltrexone-treated rats, using the tail-pressure test. An additional group of water-drinking, saline-injected rats served as controls. The results, obtained following a 3-day treatment, are shown in Fig. 1.

The minimal dose of oral naltrexone, required to block completely morphine analgesia, is 40 mg/kg/day. The time of onset of naltrexone effect was studied in nine naive female rats that were provided naltrexone solution, 40 mg/kg/day, and given the morphine analgesia test at different times. Naltrexone fully antagonized morphine-induced analgesia 6 h after it was presented. Daily fluid intake was not affected by the addition of naltrexone: female rats consumed either 18.8 (±1.7) ml of tap water or 18.8 (±1.9) ml of naltrexone solution per day.

Preparation and Treatment of Pregnant Dams

Virgin female rats were placed with sexually vigorous males for a brief test of sexual receptivity. The test took place during the first 2 h after darkness. Estrus was determined by the occurrence of lordosis behavior. Estrous females were left overnight with the males, and separated on the following day, which was designated gestational day 1. Pregnant dams were randomly assigned to naltrexone-treated (NTX) or control group. Animals of the NTX group received a daily dose of 40 mg/kg naltrexone in their drinking water from gestation day 13 until parturition. Ascorbic acid, 40 mg/kg/day, was added to the naltrexone solution as an antioxidant. Control dams drank tap water with the same amount of ascorbic acid. Solutions were individually prepared according to daily fluid consumption and body weight. To minimize the stress of handling, animals were weighed just once, on gestation day 13; a weight-gain of 5 g/day was presumed on subsequent days.

Assessment of Early Motor Development

Litters were culled to eight pups on day 1 after delivery. Tests of swimming ability were performed on days 7, 9, and 11, using two male and two female pups from each litter. Pups were individually placed into a tank of 30 × 60 cm, 30 cm high, containing 20 cm deep water at 27°C, for 5–10 s. Swimming ability was assessed by the nose position and movements of the front legs, and scored on a scale of 1–5 [see (7)]. Pups were weaned at 22 days of age and housed in groups of three to four, according to sex and prenatal treatment.

Assessment of Sexual Behavior

At the age of 4 months, male offspring were switched to a reversed dark/light cycle, with light on between 2000 and 0800

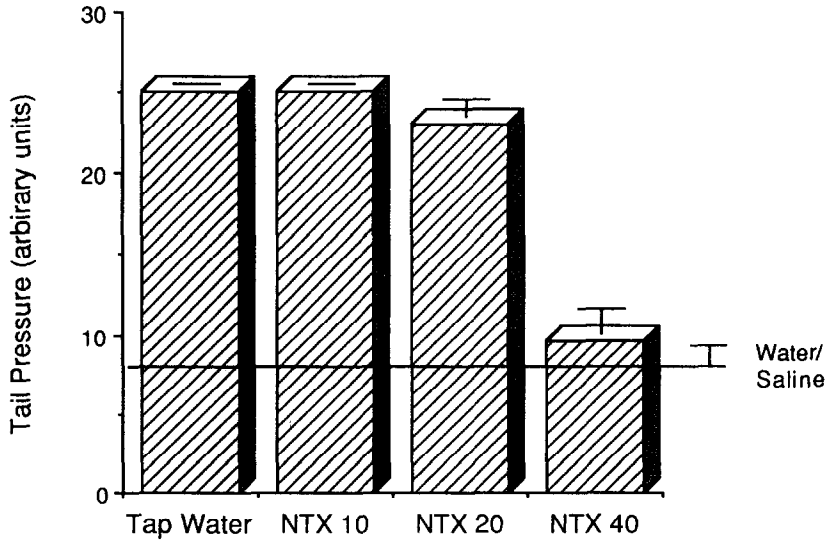

FIG. 1. Pain threshold (expressed as tail pressure scores) in female rats given different doses of continuous oral naltrexone, and injected with morphine, 4 mg/kg, IM, or saline. Values represent means (±SEM).

h. Female and male partners for the respective tests of masculine or feminine behavior were housed under the same conditions. Tests of sexual behavior started after 2 weeks of habituation. All animals were given three weekly tests of masculine behavior, followed by two tests of feminine receptivity. Tests were conducted during the dark phase of the day (1400–1700 h), in a dimly lighted room, using four semicircular boxes (60 × 30 × 30 cm) with woodshave bedding and Plexiglas front walls.

Stimulus-females, used in the tests of masculine behavior, had been ovariectomized under ether anesthesia prior to the beginning of the experiment. Sexual receptivity was induced in the stimulus-females by SC injections of 10 μg estradiol benzoate (Sigma Ltd.), 72 and 48 h prior to testing, and 1 mg progesterone (Sigma Ltd.), 4 h before testing. Both hormones were dissolved in olive oil at volume of 0.1 ml/animal. Experimental rats were each placed in a separate arena and allowed 5 min of habituation, following which a stimulus-female was introduced. Instances of mounting, intromission, and ejaculation were recorded on a computerized event recorder. The test lasted until ejaculation was observed, with a cutoff time of 30 min on the first test and 20 min on the two subsequent tests. Masculine performance was assessed using the following parameters: a) latency to first mount (LFM), the time from introduction of the stimulus-female until a mounting or intromission behavior first occurred; b) ejaculation latency (EL); and c) mount rate (MR), the average count of mounting and intromission per minute, during the time from introduction of the female until ejaculation occurred.

Upon completion of the third test, males were castrated under ether anesthesia. Tests of feminine receptivity were conducted about 2 weeks later. Experimental males were primed with ovarian hormones, 40 μg estradiol benzoate, 72 and 48 h before testing, and 2 mg progesterone, 4 h before testing. A sexually vigorous stud was placed in the testing arena with a stimulus-female that had been hormonally pretreated as previously described. Following the beginning of a sexual interaction the female was quickly replaced by an experimental male. The stud was allowed 10 mounts on the experimental animal, and the number of lordosis responses was recorded. Feminine receptivity was expressed as lordosis quotient (LQ), the number of lordosis responses per number of mounting attempts by the stud, ×100.

Statistical Analysis

Data of early motor behavior, at each age level, were analyzed using the Mann–Whitney test. Measures of sexual behavior were each analyzed using analysis of variance with repeated measures according to group and testing session.

RESULTS

Early Motor Behavior

Mean scores of swimming ability of NTX and control pups are presented in Table 1. As no appreciable sex differences were detected, data of male and female pups were combined.

No statistically significant differences were observed between NTX and control pups at any of the ages tested.

TABLE 1

SWIMMING ABILITY SCORES IN RAT PUPS OF THE NTX (PRENATALLY TREATED WITH NALTREXONE) AND CONTROL GROUPS

Age (days)	Control (n = 12)	NTX (n = 14)
7	2.5 (±0.3)	2.5 (±0.3)
9	3.8 (±0.3)	3.3 (±0.2)
11	4.2 (±0.3)	3.8 (±0.3)

Values are means (±SEM).

Sexual Behavior

Group means (±SEM) of the three indices of male sexual behavior, LFM, EL, and MR, as measured in three sessions, are shown in Fig. 2. Male sexual behavior was clearly enhanced in rats prenatally exposed to naltrexone: NTX animals approached the stimulus-female more quickly, reached ejaculation faster, and exhibited higher mount rates than control animals. Analyses of variance with repeated measures (according to group and testing session) resulted in significant group differences ($p < 0.01$) in all measures of sexual behavior [LFM, $F(1, 31) = 8.05$; EL, $F(1, 31) = 14.35$; and MR, $F(1, 31) = 13.50$].

To assess the efficiency of copularory performance, an additional measure was computed, intromission ratio (IR), the ratio of intromission to the total count of mount and intromission, observed during a test session, ×100. Average IR over the three sessions was 38.97 (±4.85) in NTX animals, and 23.31 (±4.92) in controls. Thus, the efficiency of copularory performance was significantly higher following prenatal naltrexone, compared with control animals, $F(1, 31) = 4.92$ ($p < 0.05$.

Data of the two LQ tests are shown in Table 2. Feminine receptivity following castration and ovarian hormone priming was significantly lower in NTX animals, as compared to controls: $F(1, 30) = 12.90$, $p < 0.01$.

DISCUSSION

This study demonstrated that continuous administration of naltrexone to pregnant rat dams during the last 9 days of gestation enhanced masculinization and defeminization in the male offspring. The findings clearly implicate endogenous opiates in prenatal organization of sex-specific behavioral dispositions. In contrast, we have recently found that prenatal morphine, administered via slow-release injections during gestational days 12–18, suppressed mating behavior and enhanced lordosis responses in male offspring (Gagin et al., unpublished observation).

The mechanism underlying these effects is yet unknown. Given the role of testosterone in sexual differentiation (8) and the hypothalamic and pituitary control of testosterone release,

TABLE 2

LORDOSIS QUOTIENT IN MALE RATS OF THE NTX PRENATAL NALTREXONE, (n = 19) AND CONTROL (n = 13) GROUPS

Session	Control	NTX
I	32.73 (±10.45)	3.54 (±1.85)
II	53.85 (±9.46)	27.37 (±7.91)

Values are means (±SEM) of data recorded in two test sessions. Differences between the NTX and control groups are significant at $p < 0.01$.

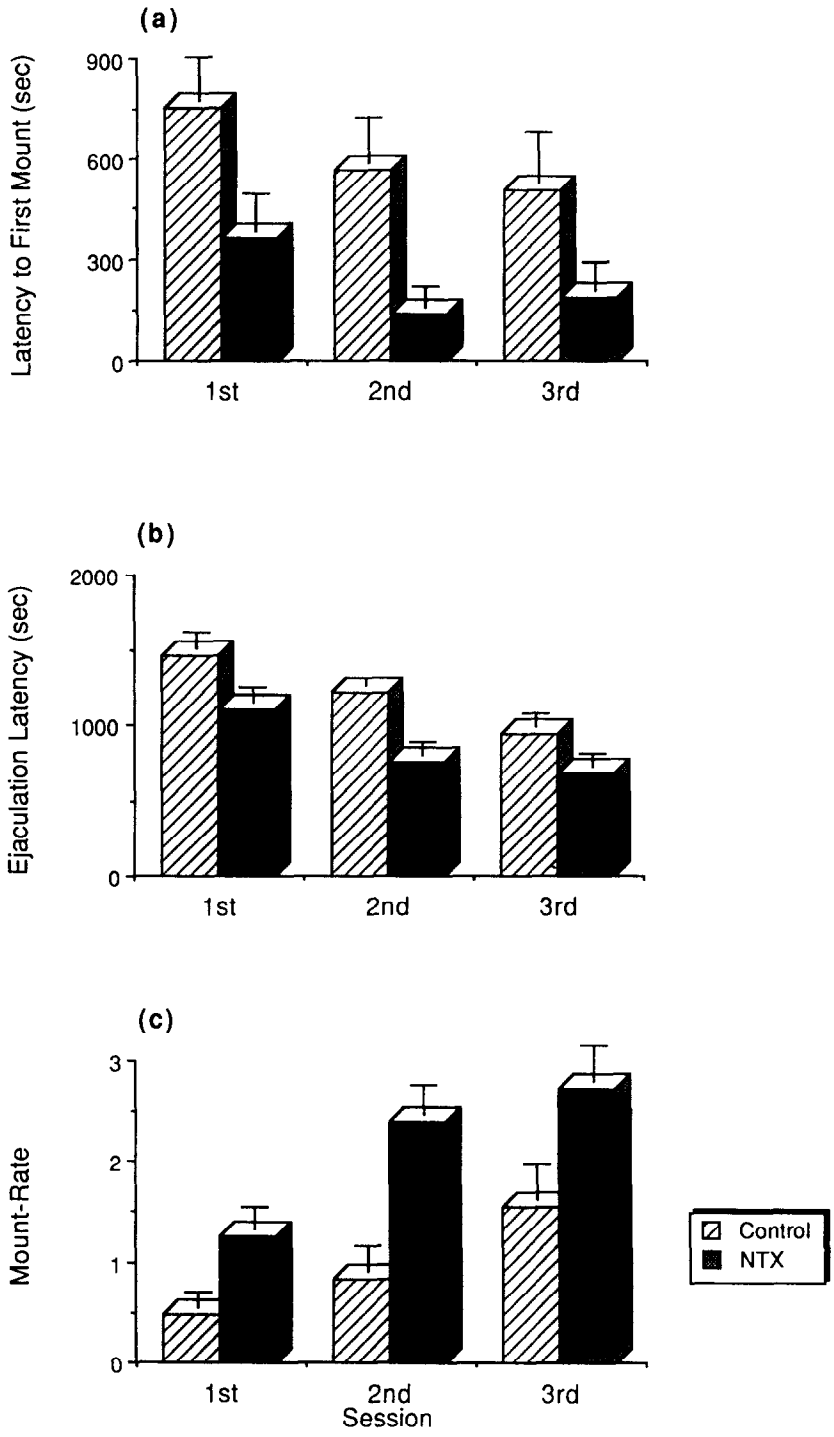

FIG. 2. Mating performance in male rats of the NTX (prenatal naltrexone, $n = 19$) and control ($n = 14$) groups. (a) Latency to first mount; (b) ejaculation latency; (c) mount rate per minute. Values represent means (±SEM) of data recorded in three test sessions.

the involvement of the hypothalamic-pituitary-gonadal (HPG) axis seems plausible. As is well established, endogenous opiates suppress the release of gonadotrophic hormones, whereas opiate antagonists enhance the secretion of LHRH and LH (5,13). Thus, a naltrexone-induced increase in LH release and the subsequent rise in plasma testosterone levels may account for the masculine shift in fetal development observed in the present study. It should be noted that the timing of naltrexone administration in this study coincided with a critical stage in brain sexual differentiation, which is characterized by a testosterone surge on gestational day 19 (26).

Evidence against this hypothesis is the finding that the

HPG axis is naloxone-insensitive until the onset of puberty in male rats: naloxone failed to affect the secretion of LH (4,17) or LHRH (17) in prepubescent male rats, nor did it even reverse the inhibitory effect of morphine on LH release in such animals (4). Thus, maturation of the opiatergic control of the HPG axis appears to take place at puberty, which questions the blockade of this particular system as mediating naltrexone prenatal influence.

On the other hand, the above claim (namely, naloxone insensitivity of the prepubertal HPG axis) has been demonstrated in postweaning, prepubertal rats, and does not necessarily hold during early development. LH response to naloxone has been observed in 5-day-old female rats (3) and in neonatally castrated male pups (20,23). It might be speculated that the opiate control of LH secretion is inactivated following the critical period for brain sexual differentiation, but is functional pre- or perinatally. To the best of our knowledge, the specific examination of the effect of naloxone on fetal secretion of LH has not yet been performed.

A sexually dimorphic distribution of opiate receptors has been described in the medial preoptic area (MPOA). The density of MPOA μ-opiate receptors varies in the adult rat with sex and the phase of the estrous cycle, being lowest in males and in estrous and proestrous females, and significantly higher in met- and diestrous females (9,11). A greater density of MPOA μ-opiate receptors is evident in 6-day-old female pups than in male pups of the same age (10,12). Perinatal morphine treatment (via osmotic minipumps, from gestational day 12 to postnatal day 5) induced receptor downregulation, measured on postnatal day 6 (12). However, neither long-lasting changes in receptor density, nor any other effect of perinatal morphine on sexual differentiation were studied in the above research. It has been demonstrated that the masculine development of MPOA opiate receptors is determined by the presence of androgen during early postnatal period (10). At present, there is no evidence supporting an opiate effect on sexual differentiation that is not mediated by the HPG axis.

Early motor development, as measured in the present study by the swimming ability test, was not influenced by prenatal naltrexone. This negative result is incongruent with earlier reports by Zagon and McLaughlin (29,32) and by Shepanek and co-workers (22): changes in neural and motor development were found by both groups following perinatal naloxone treatment. It is possible that a higher degree of opiate receptor blockade, or a more prolonged treatment than presently employed, is required for the modification of motor development. For example, the period of drug exposure in the present study (gestational days 13–22) was different from that used by Shepanek and colleagues [gestational days 4–19 (22)]. Alternatively, the earlier gestational period may be more sensitive to the effect of opiate blockade on development.

Oral administration of naltrexone was found to be a useful method for prenatal blockade of opiate receptors. In treating the pregnant dams, the least disturbing procedure was employed: naltrexone was administered via the water voluntarily consumed, and the quantity applied each time was determined using approximate estimates of daily weight gain, to minimize the stress involved in handling, weighing, and injecting the dams. Thus, stress factors, which could affect fetal development (18,24,25,28) and confound the experimental effects, were minimized. Furthermore, because naltrexone was taken with the drinking water, a continuous presence of the drug was ensured. Zagon and McLaughlin (29) obtained a long-term inhibition of opiate receptors either by using a high dose of naltrexone or by injecting their subjects three times a day. The present procedure achieved a similar outcome with a significantly smaller stress. The dose of oral naltrexone employed was apparently within the appropriate range: it was small enough to cause no detectable change in food and water intake of the treated dams [see (6)], yet sufficient to counteract the analgesic effect of a moderate dose of morphine and to affect fetal development, as is evident from the sexual behavior of the adult offspring.

In conclusion, continuous opioid receptor blockade by naltrexone during the last 9 days of gestation in the rat can enhance masculine behavior.

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