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Behavioral and Developmental Changes Associated With Prenatal Opiate Receptor Blockade

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SHEPANEK, N. A., R. F. SMITH, L. A. ANDERSON AND C. N. MEDICI. *Behavioral and developmental changes associated with prenatal opiate receptor blockade.* PHARMACOL BIOCHEM BEHAV **50**(3) 313-319; 1995. Pregnant Long-Evans hooded rats were dosed with 1, 5, or 10 mg/kg per day naloxone from gestational day 7 (GD7) through GD20. The control groups included both uninjected animals and injected animals pairfed to the 10-mg dose animals. At birth, all litters were culled to four males and four females, and fostered to undosed surrogate dams. Prenatal naloxone exposure produced changes in body weight development, pain sensitivity, and motor behavior in the offspring. Five and 10 mg/kg naloxone increased adult body weights in females only, as did the pairfeeding condition. The 10 mg/kg naloxone altered pain sensitivity (in males only) as measured by the tail flick test. Animals in the 1 mg/kg dose condition habituated more rapidly than uninjected (UN) subjects in the open field, and showed less activity than UNs as they matured. Bar pressing rates were reduced in the 10 mg/kg dose males in a visual discrimination task, while 10 mg/kg males and females showed reduced bar pressing rates on differential reinforcement of low rates of responding (DRL). These findings confirm that prenatal exposure to naloxone alters some aspects of neurobehavioral development in the rat, and are consistent with the hypothesis that 1 mg/kg prenatally may increase opiate function in offspring, while 10 mg/kg prenatally may decrease opiate functioning in the offspring.

Naloxone Pregnancy Prenatal drug effects Pain sensitivity development Motor behavior Opiate receptors

OPIATE receptor blockade has become an increasingly important tool in medicine and research. It is now widely used in cases of hypotension associated with various types of shock, as a treatment in cases of morphine addiction, and in obstetrical practice to reverse some of the effects of opioid drugs administered during labor and delivery (15,21). Since 1987, the opiate receptor blockers have been used to treat autism in children (11,16,17,19). Some of the behaviors of these children are similar to those exhibited by opiate addicts, opiate-treated laboratory animals, and human infants prenatally exposed to opiates. Behavioral changes include attentional dysfunction, insensitivity to pain, irritability, and withdrawal (18). Naltrexone seems to have a calming effect on the social behaviors of autistic children and also improves their appropriate language production. Furthermore, there is growing evidence that naltrexone is helpful in suppressing the self-mutilating behavior that is exhibited by many autistic children (4). Sandman, et al. (20) recently confirmed that naltrexone reduces self-injurious behavior in a mixed population which included autistic children.

The use of opiate receptor blockers such as naloxone and naltrexone continue to provide insight into the effects of the endogenous opiates, which influence control of pain, sexual and social behaviors, learning and memory, locomotor activities, stress-induced analgesia, modulation of release of some neurotransmitters and hormones and tissue growth in the central nervous system (2,3,6,7,26,27,31,32).

Although there is a considerable amount of data regarding effects of opiate receptor blockade in adulthood, there is much less on its effects during development. Zagon and McLaughlin (31,32) have found that 50 mg/kg per day naltrexone (which they hypothesize to produce total blockade of opiate receptors) in the early postnatal period resulted in growth enhancement in both cerebellum and cortex, while 1 mg/kg per day (hypothesized to produce temporary blockade) retarded growth of these areas in 21-day-old animals as mea-

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sured by cell size and number, and amount of dendritic branching. This dose also decreased body weights. Accompanying these changes were similar effects on behavioral development, which was accelerated by 50 mg/kg per day and slowed by 1 mg/kg per day (30). Through [^{3}H]-thymidine radioautography, Zagon and McLaughlin (33) have shown that acute 50 mg/kg naltrexone facilitates cell proliferation on postnatal day 6 (PND 6), while 1 mg/kg per day inhibits proliferation.

Other authors have confirmed that postnatal opiate receptor blockade can alter neurobehavioral development. Seatriz & Hammer (22) reported that 10 mg/kg per day naltrexone appeared to enhance neuronal maturation in somatosensory cortex. Finally, Najam and Panksepp (14) reported that 5 mg/kg per day naloxone during the postnatal period accelerated some indices of behavioral development, including eye and vaginal opening, and homing behavior.

Less attention has been paid to the effects of opiate receptor blockade during prenatal development. Vorhees (29) administered 20 mg/kg naloxone twice daily from GD7-GD20. The results of this study revealed a slight increase in postnatal weight gain of offspring, as well as accelerated development of righting reflex, incisor eruption, auditory startle, and olfactory orientation. These changes were accompanied by increased errors on the Biel water maze when subjects were tested as adults.

Recently, Shepanek et al. (24) conducted a limited behavioral and neuroanatomical evaluation of prenatal opiate receptor blockade, using a much lower dose range (1–5 mg/kg per day naloxone) to determine whether doses more similar to those used by humans would produce postnatal changes in behavior and/or neuroanatomy. Results indicated that prenatal exposure (GD4–GD18) to 5 mg was followed by accelerated development in both righting reflex and negative geotaxis. Changes following exposure to the low dose were limited to increased errors on a spatial learning task (Warden maze) in females only. These behavioral changes were accompanied by increased concentration of granule cells in the curvature of the dentate gyrus in the high dose group.

The purpose of the present study was to perform a broader behavioral screen and expand the range of doses used. We anticipated, based on our earlier prenatal study, that the animals in the low dose condition would not differ substantially from our control group, although our high dose animals would show accelerated development. Specific behaviors were chosen to be representative of the classes recommended by Geyer and Reiter (9), and included behaviors that involved development from the early postnatal period well into adulthood. The specific tests included in the battery included physical growth and maturation (body weights and negative geotaxis), reflexes (negative geotaxis, tail flick, and foot shock sensitivity), motor development/activity (open field), sensory/attentional (visual discrimination), affective (tail flick and foot shock sensitivity), and cognitive functioning (DRL-20 and water maze).

METHOD

Animals and Drug Protocol

Long-Evans hooded female rats were purchased from Blue Spruce Farms (Altamont, NY). Virgin females were housed with breeder males until discovery of a sperm plug. Pregnant dams were randomly assigned to either 1, 5, or 10 mg/kg per day, uninjected or injected pairfed (PF) control conditions; an equal number of surrogate mothers were also bred. All drugs were administered in 1 ml/kg volume sterile saline, with dosing occurring from 1000–1200. Since some degree of anorexia was expected as a result of exposure to 10 mg/kg per day (8), the injected controls were PF to the 10 mg/kg per day condition, although earlier studies with neural and behavioral effects of prenatal opiate receptor blockers did not include PF controls. All animals except the uninjected controls (UNs) were treated with SC injections from GD7–GD20 (GD1 = gestational day 1 = day found sperm positive). At delivery, all litters were fostered to uninjected dams and randomly culled to four males and four females. Dams with litters were maintained in breeding boxes with BetaChip bedding (Northeastern Products, Warrensburg, NY) until weaning on postnatal day 21 (PND 21). Dams and postweaning offspring were housed individually in hanging wire mesh cages. Animals were maintained on a 12 : 12 light/dark schedule, with lights on from 0700 to 1900; testing occurred between 1000 and 1600. Food and water were available ad lib, except when food restriction was required during visual discrimination, differential reinforcement of low rates of responding, and for pair feeding.

Developmental Testing Procedures

Birth and growth. Maternal weight gain, litter weight and number at delivery, and number of females and males in each litter were recorded.

Early development. Subjects from all litters in each drug condition were tested on negative geotaxis from PND 4 to PND 10. Infant body weights were recorded twice weekly from PND1 to PND 21. Males and females were weighed separately and litter means recorded.

Postweaning body weights. Animals were weighed biweekly from PND 21 to PND 77.

Adult Behavioral Measures

Testing regimen. At weaning, the four males and four females from each litter were randomly assigned to sex pairs, with four sex pairs in each litter. Each sex pair was assigned to a different testing regimen. Table 1 illustrates specific testing assignments.

Spontaneous alternation. To assess development of spontaneous alternation, animals were tested on PNDs 25, 30, 35, 40, and 45. Each subject was given two unreinforced trials per day in a T-maze, and both latency and choice of side recorded.

Saccharin preference. On PND 75, subjects were moved to double hanging wire cages to accommodate two water bottles. Subjects were given 4 days of preference testing between tap water and 0.25% saccharin. Bottle positions were randomly alternated. Consumption was measured by weighing each bottle before and after each 24-h consumption period.

Visual discrimination. Gerbrands G7321 Skinner boxes (Gerbrands Co., Arlington, MA) housed in BRS/LVE sound attenuation chambers (BRS/LVE, Beltsville, MD) were controlled by IBM personal computers (Armonk, NY) using the OPN operating system (Med Associates, Georgia, VT) (26). Beginning on PND 90, animals in this testing regimen were food deprived, shaped to bar press, given 4 days of continuous reinforcement (100 reinforcements per day), and trained on a visual discrimination task for 10 days, which consisted of 20 min per day of light-cued continuous reinforcement schedule, alternating at semirandom intervals (mean = 20 s, SD = 5 s) with a dark-cued extinction. The dependent measure was the number of responses.

Open field. A 76 × 76 cm open field was trisected in each

TABLE 1

OVERVIEW OF BEHAVIORAL TESTING SEQUENCE AND LITTER SPLITTING PROCEDURE*

Sex Pair/Test	Age at Testing
Sex Pair 1	
Spontaneous alternation	PND 25–40
Saccharin preference	PND 75–78
Visual discrimination	PND 90–100
Sex Pair 2	
Open field	PND 28–63
DRL-20 s acquisition	PND 90–120
Water maze	PND 130
Sex Pair 3	
Shuttle box testing	PND 76–80
Tail flick	PND 85
Footshock sensitivity	PND 85
Sex Pair 4	
Tail flick	PND 85
Foot shock	PND 85

**Note*: Sex pairs 3 and 4 were designed to allow specific comparison of pain sensitivity following repeated shocks with pain sensitivity at the same age, but in animals not previously shocked.

horizontal dimension by two photocell beams. To assess development of activity, animals were placed individually in the open field for 30 min on PNDs 28, 35, 42, 48, 55, and 63. On each of these days, counts of photocell beam breaks for ten consecutive 3-min periods were recorded on an IBM PC interfaced with the open field apparatus.

Differential reinforcement of low rates of responding. Beginning on PND 90, subjects were food deprived, shaped to bar press, and given continuous reinforcement as described above for visual discrimination. They were then given 30 days of training on a schedule of 30 min per day. The dependent measures were the number of reinforced responses and the total responses made.

Water maze. Water temperature was maintained at 27°C. The multiple alley water maze has been described previously (25). Subjects were tested for ten trials in a single session. On each trial the subject was placed in the water, and the latency to reach the goal box and the number and direction of errors during the trial were recorded, with a 300 s limit per trial.

Shuttle box avoidance. Animals received 5 days of 30 Sonalert-cued two-way avoidance trials per day in a shuttle box on PND 76–PND 80. BRS/LVE shuttle cages controlled by the OPN system were mounted in sound attenuating chambers. For each trial, a 10-s conditional stimulus (Sonalert plus shift of the cue light) preceded shock. If the subject did not cross within 10 s, a 0.6-Ma shock (BRS/LVE SGS-003) was delivered for a maximum of 40 s. Each trial ended when the animal crossed the barrier.

Tail flick testing. Five days following the conclusion of the shuttle box testing, two sex pairs from each litter received tail flick and foot shock sensitivity testing, separated by 1 h. One sex pair from each litter had received shuttle box testing; the other had not. Tail flick testing consisted of immersion of approximately 2.5 cm of the tail in 50°C water at a 30° angle. The latency to lift the tail from the water was recorded as the dependent measure.

Foot shock sensitivity. One hour following the tail flick test, animals underwent testing for foot shock sensitivity, which consisted of a series of ten ascending intensities (0.2 to 2.0 Ma) followed by the same in descending order (ISI = 10 sec), then a second entire series after a 2-min delay. For each trial, subject movement was monitored by a Lafayette 86010 activity monitor (Lafayette Instruments, Lafayette, IN); the dependent measure (millimeter pen deflection to each shock) was recorded on an Esterline-Angus miniservo (Esterline-Angus, Indianapolis, IN).

Shock history and pain sensitivity. For tail flick and foot shock sensitivity, note that one sex pair was assessed 5 days after shuttle box training (see Table 1), while a second sex pair was tested without any history of shock exposure, to assess the effects of prior shock history on these measures.

Data analysis. For litter measures, including negative geotaxis and infant body weights, litter means per sex were the unit of analysis. For other measures, one sex pair per litter was tested on each test, and the individual animal was used as the unit of analysis. The SPSSX statistical package (Carey, NC) was used for the analyses of variance. For repeated measures, significance was determined using the Greenhouse-Geisser correction for repeated measures. Significant effects were further analyzed by post hoc analyses (Tukey's HSD) to determine specific differences.

RESULTS

Developmental Measures

Maternal and litter measures. There were no differences in litter size, sex distribution, or infant body weights. Analysis of variance of maternal weight gain revealed a significant difference between treatment groups [$F(4, 52) = 3.75$, $p < 0.01$]. However, the only significant pairwise comparison was that the PF dams gained less weight (89.39 g) than UN dams (125.44 g). The number of litters delivered in each dose condition were as follows: UN, 11; PF, 10; 1 mg/kg, 10; 5 mg/kg, 13; and 10 mg/kg, 10.

Negative geotaxis. Analysis of variance of the litter means revealed a trend toward significance [$F(4, 96) = 2.20$, $p < 0.08$], with the 10 mg/kg dose group tending to show shorter latencies to turn 180° on PNDs 8, 9, and 10.

Adult body weights. Analyses of variance of adult body weights revealed a significant main effect of dose on body weights [$F(4, 93) = 4.45$, $p < 0.01$], and a trend toward a significant interaction of dose by age [$F(16, 372) = 1.64$, $p < 0.06$]. Separate one-way analyses of variance on males and females indicated that changes in body weights due to dosing were restricted to females [$F(4, 47) = 2.92$, $p < 0.05$], with the 5 and 10 mg/kg dose conditions gaining more weight than the 1 mg/kg and UN groups (Fig. 1). PF animals gained slightly more weight than 10 mg/kg subjects. There were no significant differences in male body weights as a result of dosing.

Behavioral Measures

Spontaneous alternation. There were no significant dose related effects in spontaneous alternation.

Open field activity. The analysis of variance for open field activity revealed significant main effects of dose [$F(4, 89) = 3.52$, $p < 0.01$], day, [$F(4, 445) = 141.95$, $p < 0.01$], and period (5-min time period within each 30-min daily session) [$F(20, 445) = 1.90$, $p < 0.01$]. There were also significant interaction effects of dose by period [$F(20, 445) = 1.90$, $p < 0.01$], sex by day [$F(5, 445) = 6.67$, $p < 0.01$], and sex by period [$F(5, 445) = 1.90$, $p < 0.05$], and a trend toward a

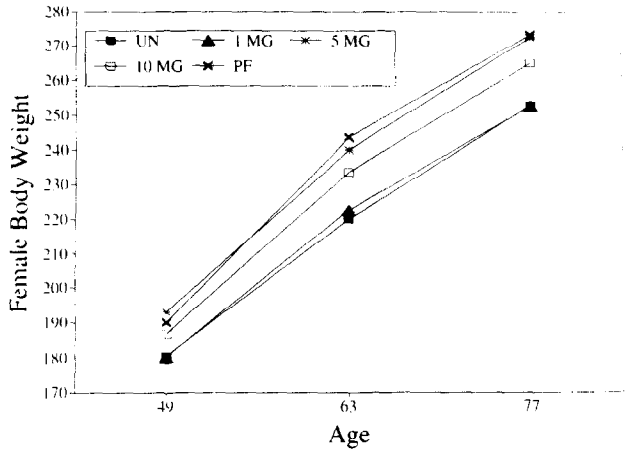

FIG. 1. Body weight gain of female offspring. There was a significant main effect [$F(4, 93) = 4.45, p < 0.01$], with 5 mg Ss differing significantly from controls. No comparable effect was seen in males. Group sizes: UN (11), PF (10), 1 mg (11), 5 mg (9), 10 mg (11).

significant dose by day interaction [$F(20, 445) = 1.55, p < 0.06$]. Analysis of variance of the overall amount of activity in the open field revealed a trend toward lower activity in the 1 mg/kg condition, [$F(3, 75) = 2.22, p < 0.09$]. The dose by period interaction revealed that animals in the 1 mg/kg dose condition habituated to the open field more quickly; that is, the 1 mg/kg animals became less active after the first 5 min in the open field than animals in the UN control condition. Finally, the PF controls showed significantly less activity than the animals in the other two dose conditions (Fig. 2).

Visual discrimination. The analyses of variance revealed a significant main effect of dose on overall rate of responding in visual discrimination [$F(4, 68) = 3.24, p < 0.05$]. There was also a significant main effect of sex [$F(1, 68) = 14.91, p < 0.01$]. Post hoc analyses of variance and Tukey's HSD test indicated that the dose effect was restricted to males only, with the 10 mg/kg animals responding significantly less than their PF controls [$F(4, 41) = 3.23, p < 0.05$].

DRL. The analyses of variance on both total responding and reinforced responses revealed significant main effects of dose [$F(4, 81) = 3.60, p < 0.01$; $F(4, 81) = 2.55, p < 0.05$, respectively], and significant dose by day interactions [$F(116, 2349) = 1.38, p < 0.01$; $F(116, 2349) = 1.35, p < 0.01$]. Post hoc testing with Tukey's HSD test indicated that animals in the 10 mg/kg dose condition demonstrated facilitated performance; that is, the 10 mg/kg animals responded the least and obtained the most reinforcements for their responses, indicating that they were able to learn to pace themselves on this learning task more efficiently than UN control animals. The significant dose by day interaction revealed that in addition to performing more efficiently, the 10 mg/kg animals also improved performance at a faster rate than the UN controls (see Figs. 3 and 4).

Shuttle box avoidance. The analysis of variance on shuttle box avoidance indicated a main effect of sex [$F(1, 86) = 4.82, p < 0.05$], and a main effect of day [$F(4, 344) = 36.18, p < 0.01$]. There were no significant effects of dose on shuttle box avoidance behavior.

Tail flick latency. The overall analysis of variance on tail flick sensitivity indicated a significant main effect of dose [$F(4, 164) = 3.24, p < 0.01$], and a significant dose by history interaction (prior shuttle box exposure) ($F(4, 164) = 2.57, p < 0.05$). One-way analyses of variance and Tukey's HSD test revealed that the significant main effect of dose and the dose by history interaction were due specifically to failure of the 10 mg/kg males to exhibit a desensitizing effect after prior shuttle box experience. In other words, prior shuttle box exposure normally has a desensitizing effect on pain sensitivity in the tail flick test. (This is the case with the UN controls).

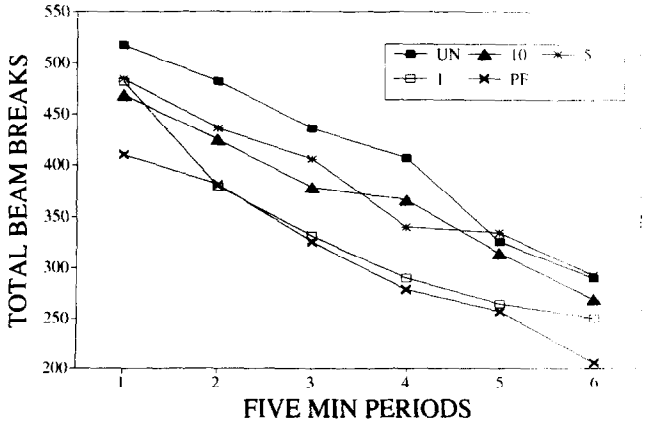

FIG. 2. Open field activity. Subjects were tested at six ages in the open field. Across test ages, there was a dose × time period effect [$F(4, 89) = 3.52, p < 0.01$]. The 1-mg Ss habituated more rapidly to the apparatus than did their controls. The 10-mg Ss also differed from the UN group, but so did their PF controls. For clarity, male and female data are combined in this figure. Group sizes (M + F): UN (10 + 10), PF (10 + 10), 1 mg (11 + 11), 5 mg (9 + 8), 10 mg (9 + 11).

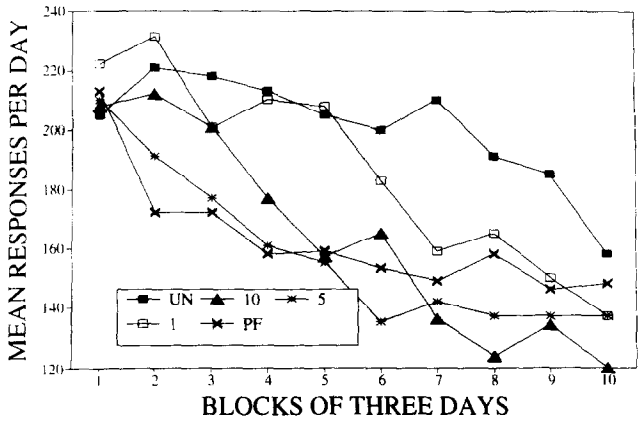

FIG. 3. Total bar presses per day on a DRL-20 schedule. After shaping, Ss were maintained at 85% ad lib body weight and were given 30-min/day DRL training for 30 days. A significant dose main effect [$F(2, 50) = 4.84, p < 0.05$] indicated that 10-mg Ss emitted fewer bar presses per daily session than controls. A similar effect of lower bar pressing rate was seen (for males, but not for females) on a light-cued operant discrimination task (not shown). A specific comparison of 10-mg subjects to PF controls revealed a significant dose × day interaction ($p < 0.05$), indicating that DRL response reduction was not completely attributable to the tendency of 10-mg animals to gain less weight during pregnancy. For clarity, male and female data are combined in this figure. Group sizes (M + F): UN (9 + 9), PF (10 + 9), 1 mg (9 + 9), 5 mg (8 + 9), 10 mg (8 + 11).

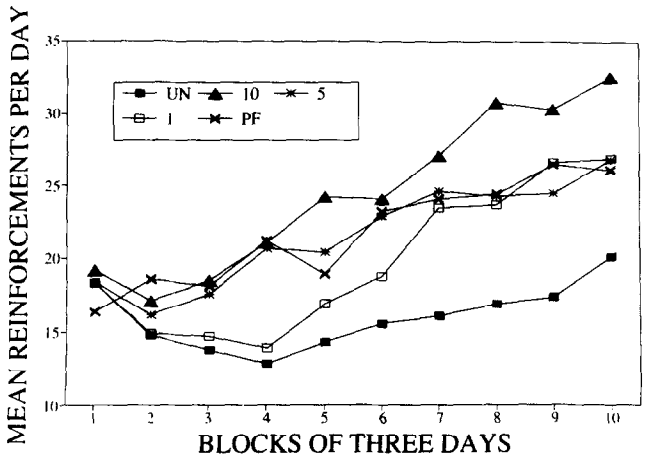

FIG. 4. Reinforced bar presses per day on a DRL-20 schedule. A significant dose main effect [$F(2, 50) = 5.31$, $p < 0.01$], indicated that 10-mg Ss generated more reinforced bar presses than UN subjects per daily session. Although 10-mg and PF subjects differed on responses on DRL (Fig. 3), they did not differ significantly on reinforcements obtained. Group sizes same as Fig. 3.

Prenatal exposure to 10 mg/kg of naloxone reverses this desensitizing effect of prior shuttle box exposure and causes the males to become more sensitive to pain (Fig. 5).

Footshock sensitivity. The analysis of variance on foot shock sensitivity included the usual dose and sex factors, ten levels in an intensity factor, and series (the four repetitions of the ten levels). This analysis revealed a significant main effect of series, [$F(3, 408) = 32.80$, $p < 0.01$], a significant main effect of sex [$F(1, 136) = 28.98$, $p < 0.01$], and a significant interaction effect of dose by series [$F(12, 408) = 2.03$, $p < 0.05$], indicating that the dose conditions reacted differently to the different series of shocks. There was also a trend toward significance for the main effect of dose [$F(4, 136) = 2.10$, $p < 0.08$]. Post hoc testing indicated that these effects were attributable to PF animals reacting more to shock in the final series than the UN subjects (not shown); naloxone dosing per se did not significantly alter foot shock sensitivity.

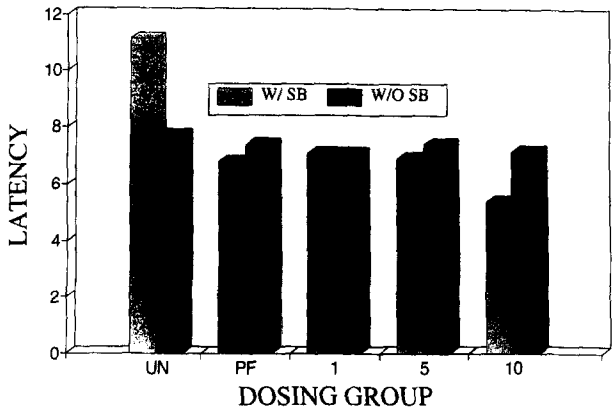

FIG. 5. Tail flick latencies for male offspring. Some Ss had recent exposure to repeated shuttle box testing (designated W/) and others had not (W/O). A dose by history interaction [$F(4, 164) = 2.57$, $p < 0.05$] indicated that prior shuttle box history increases latency in UN Ss, but decreases latency in 10-mg Ss. A specific comparison of 10-mg and PF subjects indicated that these groups did differ significantly ($p < 0.05$). Group sizes (W/ SB, W/O SB): UN (11, 11), PF (10, 7), 1 mg (11, 10), 5 mg (8, 8), and 10 mg (9, 7). Females did not exhibit these effects.

Other Behavioral Measures

There were no significant effects of dosing on saccharin preference or water maze.

DISCUSSION

These data indicate that prenatal naloxone administration results in changes in the general areas of development, pain sensitivity, and adaptive motor behavior, some of which persist into adulthood. Several of these changes appear to be restricted to one sex or the other, and they did not occur in a linear dose-dependent manner.

There were also changes peculiar to the PF control group, whose dams were allotted a food consumption equal to that of the 10 mg/kg dams during the dosing period. The PF/vehicle injection procedure, intended as an additional 'control,' produced some effects on maternal weight gain, open field activity, visual discrimination, DRL, and adult body weight gain. However, there were no differences between the PFs and the 10 mg/kg animals (for whom they were controls) on any maternal or litter measures taken. We suggest that although PF apparently produces some changes by itself, it may be inappropriate as a control procedure in cases where maternal and litter indices are unaffected by the dosing regimen.

Developmental Measures

Weight gain. Female offspring of 5 and 10 mg/kg dams gained weight more rapidly than UN controls. Vorhees (29) reported a similar effect of 40 mg/kg/d naloxone; however, he found this effect in both males and females. Najam and Panksepp (14) found a similar effect of postnatal 5 mg/kg naloxone. In our data, there was a trend toward decreased maternal weight gain in the 10 mg/kg condition, but this maternal trend did not reach significance and was actually in the opposite direction than the weight gain of the offspring. While there were no (naloxone-induced) changes in maternal weight gain in this study, others have reported reductions of food and water intake with doses as low as 1 mg/kg (1,8,10,12,23).

Negative geotaxis. Ten mg/kg offspring tended to exhibit shorter latencies for negative geotaxis, particularly in the later days of testing. These results are consistent with the findings of our earlier study (24), although our findings in the present study did not reach significance. Vorhees (29) found more rapid development of surface righting, acoustic startle reflex, and olfactory orientation after 40 mg/kg per day naloxone.

Pain Sensitivity

Tail flick. Subjects in the UN condition exposed to chronic shock exhibited an analgesic effect on the tail flick test, which was not seen in male 10 mg/kg subjects. Although acute naloxone in adults can reverse some types of stress-induced analgesia, no previous report of an effect of prenatal opiate receptor blockade on this measure has appeared in the literature. However, prenatal morphine has been shown to increase stress-induced analgesia in the offspring (5); our data suggest that 10 mg/kg naloxone during the prenatal period decreases stress-induced analgesia in male offspring.

Adaptive Motor Activity

Open field. Low dose animals habituated more quickly than UN subjects in the open field, and showed less activity as they matured. Shepanek et al. (24) previously found that dosing with 1 or 5 mg/kg naloxone during pregnancy resulted in a significant drug × day × sex interaction, which could not be decomposed. Meyerson, et al. (13) found a morphine-induced reduction, after neonatal naltrexone, in several aspects of adult exploratory behavior. Environmental stress, which might accompany placement in the open field, is often accompanied by analgesia and the release of endogenous opioid peptides (16). It may be that our 1 mg/kg animals are more responsive to the opiates thus released, in response to the novel environment of the open field, and therefore, become less active more quickly. No direct confirmation exists in the literature, but we note that Castellano and Ammassari-Teule (5) reported enhanced offspring responsiveness to morphine in an activity measure, after prenatal morphine.

Visual discrimination. Overall rate of bar pressing was lower in the 10 mg/kg males as compared to their PF controls. This effect appeared to be primarily a motor effect, as accuracy of discrimination and rate of acquisition of the task were unaffected by dosing. Ten mg/kg males exposed to total prenatal receptor blockade simply bar press less when compared to their PF control group.

This effect is similar to that found in a previous (unpublished) study with acute 10 mg/kg naloxone dosing in adulthood on this same task. Acute naloxone dosing in that study also reduced bar pressing rates (Shepanek, unpublished thesis, 1986).

DRL. For DRL, the high dose animals exhibited a lower rate of bar pressing, a higher rate of reinforcement (probably secondary to the lower bar press rate), and more rapid improvement than uninjected controls. Tripp & McNaughten (28) found a similar effect after acute 3 mg/kg naloxone dosing in adult animals.

Speculation on Mechanisms of Action

Prenatal naloxone induces long-lasting behavioral changes in the offspring, which presumably are secondary to long-lasting CNS changes. The nature of the behavioral effects offers some clues as to what those long-lasting changes may be. Although we did not directly measure opiates or opiate receptors in the offspring, there are behavioral similarities between our offspring of naloxone-dosed dams, and rats with explicit pharmacological modification of opiate receptor availability. In particular, several aspects of our data are consistent with enduring changes in opiate functioning (either changes in endogenous opiates or changes in opiate receptors) after prenatal naloxone. Some aspects of the data are consistent with the notion that high and low doses of naloxone produce different effects on development, as proposed for neonatal naltrexone by Zagon and McLaughlin (31,32). In addition, behavioral effects are consistent with decreased opiate functioning after 10 mg/kg prenatal naloxone in the maturing offspring, and increased opiate functioning after 1 mg/kg.

In the data, 10 mg/kg given prenatally produced decreased responding on DRL and a visual discrimination task; the first of these effects has also been found after acute administration of 3 mg/kg naloxone to adult animals (28), and the second after acute 10 mg/kg dosing in adults (Shepanek, unpublished thesis, 1986).

In addition, 10 mg/kg produced hypersensitivity to thermal stimuli, compared to the PF controls. Although both opiate and nonopiate mechanisms regulate pain sensitivity, this finding is also consistent with a reduction in some aspect of opiate function.

Finally, changes in open field activity after 1 mg/kg naloxone are consistent with effects of increasing opiate function via administration of opiates. As noted above, data of Meyerson et al. (13) suggest that developmental opiate receptor blockade may alter opiate-induced activity in the maturing offspring. Our open field data may reflect a similar effect.

Only our weight gain data are inconsistent with this notion; 5 and 10 mg/kg produced increased weight gain in the female offspring, which is consistent with increased opiate functioning [Najam and Panksepp (14)]. Our behavioral data on adult offspring, but not weight gain data during development, then, are consistent with the notion that 1 mg/kg naloxone during prenatal development leads to a slight increase in opiate functioning in the offspring at the ages tested, while 10 mg/kg leads to a decrease in opiate functioning in the offspring. Further work is clearly required to determine whether this hypothesis is correct.

CONCLUSION

In summary, the present study found that prenatal naloxone produces a number of developmental and behavioral changes that extend into adulthood. These include some changes such as body weight gain, pain sensitivity, activity, and rate of bar pressing on two operant tasks.

In addition, our data appear to be consistent with a biphasic dose-effect curve for prenatal naloxone, as suggested by Zagon and McLaughlin (31,33) for neonatal naltrexone. Some of the effects of the prenatal naloxone were sex specific, and we suggest that more research is necessary to determine the mechanism of these sex specific effects, including the possibility of endocrine changes mediating the effects.

We note in closing that pain sensitivity and motor behavior, two aspects of behavior altered after prenatal naloxone, appear grossly related to the apparent effectiveness of opiate receptor blockers currently being evaluated for management of autistic behavior. Further research might best be directed at examining these types of effects, as well as determining the mechanisms which underlie them.

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