Pharmacology Biochemistry and Behavior, Vol. 58, No. 2, pp. 387–393, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00254-2

Effect of Prenatal Stress on Opioid Component of Exploration in Different Experimental Situations

TATYANA POLTYREV AND MARTA WEINSTOCK[1]

Department of Pharmacology, School of Pharmacy, Hebrew University Hadassah Medical Centre, Ein Kerem, Jerusalem, Israel

Received 8 August 1996; Revised 28 November 1996; Accepted 28 November 1996

POLTYREV, T. AND M. WEINSTOCK. *Effect of prenatal stress on opioid component of exploration in different experimental situations.* PHARMACOL BIOCHEM BEHAV **58**(2) 387–393, 1997.—Prenatal stress interferes with the expression of opioid systems in rats. The present study determined the effect of prenatal stress on the opioid-influenced component of exploratory behavior, defined as the difference between the behavior of vehicle-treated and naloxone-treated rats, in three novel situations previously shown to cause different degrees of arousal. Pregnant rats were stressed three times weekly on a random basis by noise and flashing lights. Experiments were performed on 60–70-day-old offspring (male and female) of control and stressed dams. Fifteen minutes after injection of vehicle or naloxone (1 mg/kg), the proportion of time spent in eight different behavioral parameters, including locomotion, rearing, sniffing, hole poking, pivoting, and grooming, was assessed during 4 min of exposure to an open field, either with or without prior exposure to a hole box. The magnitude of the depressant effect of naloxone on exploration depended on the nature of the environment, previous experience of the animal in another situation, and the parameter of exploration assessed. The opioid-influenced component of locomotion and rearing was significantly reduced by prenatal stress, particularly in female rats. Further studies using a cross-fostering design are needed to assess the relative contributions of pre- and postnatal factors to the reduction of opioid activity in prenatally stressed rats. More specific opioid antagonists could be used to determine the nature of the opioid receptors involved. © 1997 Elsevier Science Inc.

Rats Gender differences Prenatal stress Naloxone Open field Hole box

PRENATAL stress has been shown to interfere with the normal expression of opioid systems in offspring. The number of μ-opioid receptors in the striatum, nucleus accumbens, and lateral amygdala is decreased in rats of each gender (12), the analgesic effect of morphine is reduced in males and increased in females (19), and the levels of pro-opiomelanocortin (the precursor of β-endorphin) are reduced in the hypothalamus of females (33). Prenatal stress also suppresses saccharin preference (an opioid-dependent behavior) (20) in females but strengthens it in males (18). These data show that the direction of the effect of prenatal stress depends on the particular opioid system involved and on the gender of the animal.

The mechanisms involved in the stress-induced alterations in the opioid systems in the offspring are not clear, but are similar to those seen after maternal administration of exogenous opiates during gestation (29,30,31,34). Moreover, several of the behavioral abnormalities induced in the offspring by prenatal stress can be prevented by administration of an opioid receptor antagonist to the stressed pregnant rat during the last week of gestation (18,32). This finding suggests that excess opioid activity in the mother and foetus induced by stress (7) at a critical time during development may result in a permanent alteration in the opioid systems of the offspring.

Endogenous opioid peptides, β-endorphin (13,26) and enkephalins (4), are released when animals are exposed to novel environments. Exogenous opioids influence exploratory behavior by modulating dopamine release in the ventral tegmental area of the brain (17,28). The direction of their influence depends on the nature of the opioid system involved, the degree of stress or arousal induced by the environment, and the

[1]To whom requests for reprints should be addressed. E-mail: martar@cc.huji.ac.il

particular measure of exploration (1,4). Both stimulation and inhibition of exploration by exogenous opioids can be antagonised by opioid receptor antagonists (4).

Some studies (8,25) but not others (1,6) have reported that spontaneous exploratory activity can also be reduced by opioid antagonists, supporting a role of endogenous opioid systems in novelty-induced exploration. Failure to detect a significant effect of such antagonists may have been due to the experimental conditions, including the level and type of illumination, task novelty, degree of stress (1,15), the method by which exploration was assessed (6), or the dose of drug (25). The effect of prenatal stress on exploratory behavior is also dependent on the experimental situation in which it is assessed (25) and could be linked to an altered sensitivity of the endogenous opioid systems.

The aim of the present study was to determine the effect of prenatal stress on the opioid component of several parameters of exploratory activity in rats of both sexes. Because the nature of the novel environment may determine both the degree of activation and the particular opioid system involved, we used two situations previously shown to cause different degrees of arousal (23) and response to opioids (4): a large, brightly lit open field and a relatively small, dark container covered with a lid and with holes placed in the floor, designed to encourage hole poking. To see whether previous experience in one novel environment altered opioid-dependent activity in another, two experiments were performed. In one, rats were exposed to the open field, and in the other they were placed in the same open field after they had been in the hole box. Naloxone was injected at a dose of 1 mg/kg because both smaller and larger doses were shown to be less effective (25). Prenatally stressed (PS) rats may show less exploratory activity than control (C) rats in a novel situation because of a lack of either a stimulatory opioid component or a nonopioid component of this behavior. It was postulated that a deficit in the opioid component of exploratory behavior in PS rats would be expressed as a smaller reduction in locomotion, hole poking, and rearing after pretreatment with naloxone than in control offspring.

MATERIALS AND METHODS

Animals

Virgin female (28) Sprague–Dawley rats were mated with stud males and randomly allocated to equal numbers of stressed and control groups. From day 1 of pregnancy, they were housed singly in small acrylic cages (22 × 17 × 13 cm) at an ambient temperature of 22 ± 1°C and a 12 L:12 D cycle (lights on at 0700 h), with food and water ad lib. Stressed dams were housed in a special acoustic chamber, having the same temperature, light cycle, and humidity, but in which noise and flashing light stress were applied on an unpredictable basis, three times weekly, as previously described (10). None of the dams was handled except for routine cage cleaning. Within 24 h of birth, litters were culled to eight pups, with equal numbers of males and females whenever possible. The pups were weaned at 21 days of age and housed in groups of four by litter and sex. Only two pups of each sex were used from each litter; one pup was given vehicle and the other was given naloxone.

All experiments were performed on C and PS offspring aged 60–70 days, in a room in the animal house that was maintained under the same conditions of temperature, light cycle, and humidity as those in which the rats were housed. The contents of the room remained in identical positions throughout the experimental procedures to provide the same visual cues for all animals.

Experimental Procedures

Eight groups consisting of 8–14 C and PS rats of both sexes were injected intramuscularly (1 ml/kg) with either vehicle (ascorbic acid, 1 mg/kg) or naloxone HCl (Taro, Israel Ltd.; 1 mg/kg + 1 mg/kg ascorbic acid, to prevent oxidation). The composition of the experimental groups and the tests to which they were subjected are shown in Table 1. The rats were then placed individually in holding cages for 15 min before being exposed singly for 4 min either to the open field (OF1) or first to the hole box, followed immediately by the open field (OF2). The relative durations of several spontaneous paradigms of exploratory, recuperative, and intermediate behaviors were assessed by an uninformed observer by means of a computer program. In the open field and hole box, exploratory behavior included locomotion, rearing (with and without support by forepaws), and hole poking; recuperative behaviors comprised immobility (interruption of any activity, including movement of vibrissae), pivoting (circular movement of body by forepaws only), and grooming (11,27); sniffing (interruption of any of the above activities and their replacement by movement of the nose and vibrissae) was regarded as being intermediate between exploration and recuperation (5). The number of transitions from one type of behavior to another during the observation period was assessed in all situations, and the latency to begin hole poking was measured in the hole box only. This method of recording enabled us to show how naloxone affected the whole continuum of behavior in response to a novel environment and not just locomotor activity, as in previous studies.

Open field test. The open field (OF) consisted of a circular wooden arena 100 cm in diameter designed to discourage the rats from sitting in a corner, which often occurs in a rectangular open field. The floor of the arena was covered with dark brown Formica and had 16 holes 3 cm in diameter evenly spaced in it. It was surrounded by a 30-cm-high wall and illuminated by two 60-watt bulbs placed 120 cm above it, in addition to the fluorescent light in the room.

Hole box test. The hole box, modified from that described by File and Wardill (9), consisted of a wooden chamber, 30 × 30 × 25 cm, with a lid, three black inside walls, and one clear Plexiglas wall. The floor, illuminated from below, had four holes 3 cm in diameter equally spaced in it. Head dipping into any hole presented a view of a diffusely lit white field. All the experiments were carried out between 1300 and 1800 h. Each piece of apparatus was carefully cleaned with detergent and

TABLE 1

NUMBERS OF RATS USED IN THE EIGHT EXPERIMENTAL GROUPS OF THE STUDY

Prenatal Treatment	Drug	Open Field 1	Hole Box	Open Field 2
C males	Vehicle	8	13	13
C males	Naloxone	10	13	13
C females	Vehicle	10	14	14
C females	Naloxone	10	14	14
PS males	Vehicle	10	14	14
PS males	Naloxone	10	13	13
PS females	Vehicle	10	13	13
PS females	Naloxone	9	13	13

The same rats were exposed to the hole box and to open field 2, and different animals were exposed to open field 1. C, control rats; PS, prenatally stressed rats.

TABLE 2

PROPORTION OF TIME SPENT BY C AND PS MALE RATS TREATED WITH VEHICLE OR NALOXONE IN VARIOUS BEHAVIORAL PARADIGMS IN WHICH SIGNIFICANT DIFFERENCES WERE FOUND IN DIFFERENT TESTS

Behavior	C Males, Vehicle	C Males, Naloxone	PS Males, Vehicle	PS Males, Naloxone
		Open Field 1		
Locomotion	0.36 ± 0.03	0.31 ± 0.04	0.32 ± 0.02	0.33 ± 0.04
Sniffing	0.41 ± 0.02	0.43 ± 0.03	0.45 ± 0.02	0.47 ± 0.04
Pivoting	0.06 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.13 ± 0.02
		Hole Box		
Sniffing	0.42 ± 0.01	0.49 ± 0.01*	0.44 ± 0.01	0.42 ± 0.02†
Supported rearing	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.05 ± 0.01†
Grooming	0.010 ± 0.006	0.004 ± 0.004	0.010 ± 0.008	0.02 ± 0.01†
Immobility	0.06 ± 0.03	0.09 ± 0.02	0.08 ± 0.03	0.19 ± 0.04†*
		Open Field 2		
Locomotion	0.32 ± 0.03	0.17 ± 0.03*	0.32 ± 0.03	0.17 ± 0.03*
Rearing	0.021 ± 0.003	0.006 ± 0.002*	0.009 ± 0.002	0.013 ± 0.006
Supporting rearing	0.03 ± 0.005	0.015 ± 0.005*	0.025 ± 0.005	0.014 ± 0.003
Immobility	0.05 ± 0.02	0.24 ± 0.05*	0.08 ± 0.02	0.30 ± 0.08*

*Significantly different from vehicle-treated rats; †significantly different from control rats with naloxone. C, control rats; PS, prenatally stressed rats.

water and dried after each animal. Experiments were performed according to the guidelines set out by the Institutional Committee for Care and Use of Animals.

Data Analysis

The proportion of time spent in any of the above behavioral paradigms, number of behavioral transitions, and latency to begin hole poking were subjected to analysis of variance (ANOVA) for factors test situation (OF1 or OF2), prenatal treatment, pretest treatment, and gender. If a significant effect was found for one or more of these factors, pairwise comparisons were made by Duncan's range test. A difference at the level of $p < 0.05$ was considered statistically significant. All data represent the mean ± SEM.

RESULTS

The duration of a number of behavioral patterns in the open field varied widely according to whether or not the rat had

TABLE 3

PROPORTION OF TIME SPENT BY C AND PS FEMALE RATS TREATED WITH VEHICLE OR NALOXONE IN VARIOUS BEHAVIORAL PARADIGMS IN WHICH SIGNIFICANT DIFFERENCES WERE FOUND IN DIFFERENT TESTS

Behavior	C Females, Vehicle	C Females, Naloxone	PS Females, Vehicle	PS Females, Naloxone
		Open Field 1		
Locomotion	0.43 ± 0.03	0.41 ± 0.01	0.34 ± 0.02‡	0.32 ± 0.02†
Sniffing	0.35 ± 0.03	0.41 ± 0.02	0.46 ± 0.02‡	0.43 ± 0.03
Pivoting	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.02
		Hole Box		
Sniffing	0.42 ± 0.01	0.40 ± 0.02	0.44 ± 0.01	0.43 ± 0.02†
Rearing	0.021 ± 0.004	0.010 ± 0.004	0.008 ± 0.003	0.025 ± 0.007*
Supporting rearing	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Grooming	0.002 ± 0.001	0	0.003 ± 0.003	0.002 ± 0.002
Immobility	0.06 ± 0.03	0.23 ± 0.05*	0.06 ± 0.02	0.12 ± 0.03†
		Open Field 2		
Locomotion	0.34 ± 0.03	0.21 ± 0.02*	0.33 ± 0.03	0.30 ± 0.03
Rearing	0.011 ± 0.004	0.003 ± 0.001*	0.010 ± 0.003	0.025 ± 0.009†
Supported rearing	0.030 ± 0.005	0.016 ± 0.005*	0.025 ± 0.005	0.010 ± 0.004
Immobility	0.05 ± 0.01	0.24 ± 0.04*	0.08 ± 0.02	0.09 ± 0.02†

*Significantly different from vehicle-treated rats of same prenatal treatment group; †significantly different from control rats with naloxone; ‡significantly different from vehicle-treated controls. C, control rats; PS, prenatally stressed rats.

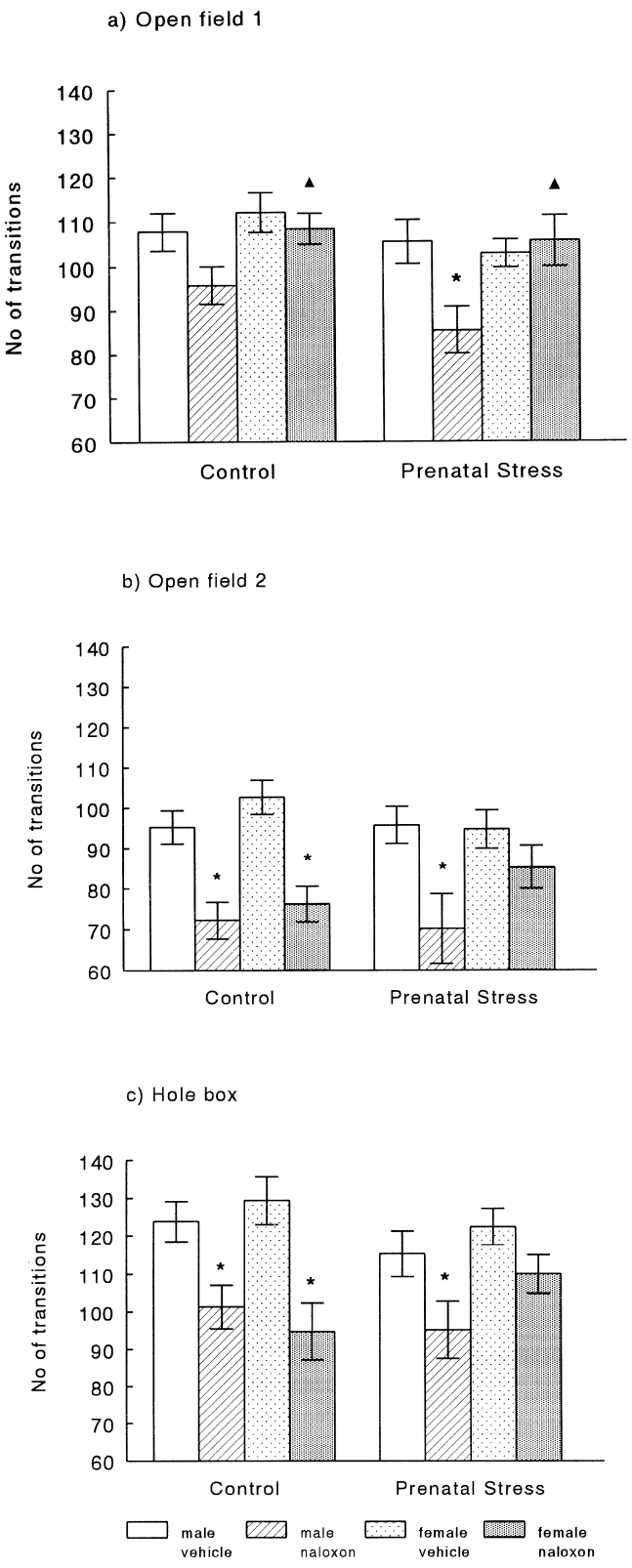

FIG. 1. Effect of naloxone on the number of behavioral transitions in the three experimental situations. *$p < 0.05$, significantly different from vehicle; ▲$p < 0.05$, significantly different from control rats with naloxone.

previously been exposed to the hole box. The rats spent more of their time in locomotion [$F(1, 183) = 20.15, p < 0.0001$] and in rearing (unsupported) [$F(1, 183) = 8.4, p < 0.005$] and rearing (supported) [$F(1, 183) = 79.03, p < 0.0001$] in OF1 than in OF2, whereas the duration of immobility [$F(1, 183) = 41.44, p < 0.0001$] was greater in OF2. The proportion of time spent in those paradigms of behavior in which significant differences were found between PS and C rats, or between vehicle and naloxone treatment in the different situations for males and females, are shown in Tables 2 and 3, respectively. These do not include the data found in Fig. 2.

Gender differences in the duration of some behavioral patterns were found in each test situation. In OF1, the duration of locomotor activity [$F(1, 76) = 10.25, p < 0.0025$] and the number of behavioral transitions [$F(1, 76) = 7.32, p < 0.01$] were greater in females, whereas the duration of pivoting was greater in males [$F(1, 76) = 6.73, p < 0.025$]. The gender differences were significant only in the naloxone-treated rats. In OF2, the duration of locomotion was also greater in females [$F(1, 106) = 5.8, p < 0.025$] only after naloxone treatment. In the hole box, the times spent in rearing [$F(1, 106) = 9.23, p < 0.005$] and grooming [$F(1, 106) = 5.36, p < 0.05$] were longer in males.

An inhibitory effect of prenatal treatment on exploration was much more pronounced in OF1 than in the other situations (Tables 1, 2). PS rats spent less time than C rats in locomotion [$F(1, 76) = 13.66, p < 0.0005$] but more time sniffing [$F(1, 76) = 5.85, p < 0.025$] and pivoting [$F(1, 76) = 6.57, p < 0.025$]. In OF2 [$F(1, 106) = 4.09, p < 0.05$] and the hole box [$F(1, 106) = 7.11, p < 0.01$], the only significant effect of prenatal stress on exploration was to reduce the time spent in supported rearing. The duration of pivoting was increased in the hole box [$F(1, 106) = 4.84, p < 0.05$].

In contrast, the effect of naloxone on exploratory behavior was more evident in the hole box and OF2, where there was little depressant effect of prenatal stress. In OF1, ANOVA revealed only a small reduction by naloxone in locomotion [$F(1, 76) =$

TABLE 4

SUMMARY OF BEHAVIORAL PARAMETERS ON WHICH NALOXONE HAD A SIGNIFICANT EFFECT IN DIFFERENT EXPERIMENTAL SITUATIONS

	Control		Prenatal Stress	
Parameter	Male	Female	Male	Female
Hole Box				
Hole poking	↓	↓	↓	0
Rearing	0	0	0	↑
Immobility	0	↑	↑	0
Transitions in behavior	↓	↓	↓	0
Latency to hole poke	↑	0	0	0
Open Field 1				
Transitions in behavior	0	0	↓	0
Open Field 2				
Locomotion	↓	↓	↓	0
Supported rearing	↓	↓	0	0
Rearing	↓	↓	0	↑
Immobility	↑	↑	↑	0
Transitions	↓	↓	↓	0

↓, Significant reduction by naloxone, $p < 0.05$; ↑, significant increase by naloxone, $p < 0.05$; 0, no significant effect of naloxone.

4.23, $p < 0.05$], but in post hoc tests, the differences from the respective vehicle-treated animals did not reach statistical significance. There was a significant reduction by naloxone in the number of transitions from one behavior to another in males only [gender × pretest treatment, $F(1, 76) = 6.20$, $p < 0.025$] (Fig. 1a). The effect of naloxone on behavioral parameters in the three tests is summarized in Table 4.

In OF2, naloxone decreased the time spent in locomotion [$F(1, 106) = 32.8$, $p < 0001$], supported rearing [$F(1, 106) = 17.0$, $p < 0001$], and number of transitions in behavior [$F(1, 106) = 32.8$, $p < 0001$] (Fig. 1b), and increased the duration of immobility [$F(1, 106) = 27.1$, $p < 0001$] (Tables 2, 3). The effect of naloxone on these behaviors was seen in C rats of both genders and in PS males. There was a significant interaction of prenatal treatment × pretest treatment [$F(1, 106) = 10.4$, $p < 0.0025$] and gender × prenatal treatment [$F(1, 106) = 4.26$, $p < 0.05$] for the duration of rearing. Post hoc tests revealed that this parameter was significantly reduced by naloxone in C rats, unchanged in PS males, and increased in PS females. This was the only significant effect of naloxone in PS females (Table 3).

In the hole box, naloxone also significantly reduced the number of behavioral transitions [$F(1, 106) = 25.9$, $p < 0.0001$] (Fig. 1c) and the time spent in hole poking [$F(1, 106) = 24.15$, $p < 0.0001$] (Fig. 2), and increased the duration of immobility [$F(1, 106) = 11.1$, $p < 0.0025$] (Tables 2, 3). There was a significant interaction of gender × prenatal treatment [$F(1, 106) = 4.7$, $p < 0.05$] for hole poking, with PS females showing a significantly greater duration of this activity after naloxone (Fig. 2) and less immobility [$F(1, 106) = 4.83$, $p < 0.05$] than PS males (Tables 2, 3).

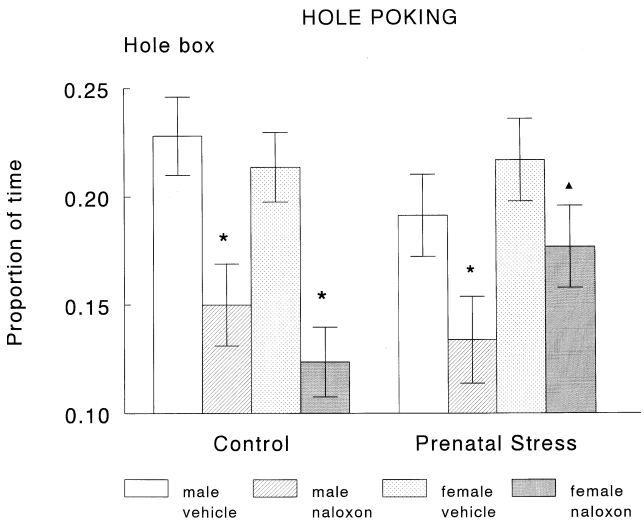

FIG. 2. Effect of naloxone on the time spent hole poking in the hole box. *$p < 0.05$, significantly different from vehicle; ▲$p < 0.05$, significantly different from control females with naloxone.

DISCUSSION

Opioid peptides can increase or decrease exploratory behavior in rats after parenteral administration (15) or local injection into the ventral tegmental area (4,28). The direction of their effect depends on the type of opioid receptor activated and the degree of stress generated by the test situation (4,14). Exposure of a rat to a novel environment causes the release of β-endorphin (13) and other opioid peptides, which may also increase or decrease exploration according to the situation, as seen by the direction of effect of ketalorphan, a specific inhibitor of enkephalin-degrading enzymes, on this behavior (4).

Previous studies have shown that prenatal stress can interfere with the development of endogenous opioid systems (12,33) and alter the expression of opioid-dependent behaviors (18,19). Thus, it might be expected that the opioid component of exploratory behavior could be altered by prenatal stress. This possibility was investigated in the present study, which assessed the opioid-influenced component of exploratory behavior, defined as that which is sensitive to naloxone, in male and female rats in unfamiliar experimental situations causing different degrees of arousal.

Naloxone had little inhibitory effect on the time spent by the C or PS rats in any pattern of exploratory behavior in a large open field when this was the first novel situation to which the rats were exposed. This suggests that opioid systems were not activated to any significant extent in this environment and supports other data showing that naloxone had no effect in unstressed controls, but decreased locomotor activity in a similar environment if the rats were first subjected to noise stress (15).

In contrast to the open field, exposure to a small dark hole box activated opioid systems, because naloxone produced a significant reduction in hole poking and in the number of transitions from one behavior to another, in accordance with data from previous studies (8). A new finding in the present study was that exposure of the rats to the same open field immediately after they had been in the hole box resulted in a highly significant inhibitory effect of naloxone on the duration of a number of exploratory patterns. These included supported rearing and locomotion, as well as the number of behavioral transitions. Thus, a preceding stressful experience, or that of a contrasting unfamiliar situation and/or of the additional handling procedure, appears to stimulate endogenous opioid systems. These systems were probably still active when the rats were exposed to the open field and enabled us to detect an inhibitory effect of naloxone on exploration.

Prenatal stress reduced the duration of locomotor activity of vehicle-treated rats exposed only to the open field, but not after prior experience in the hole box. If the suppression of locomotion by prenatal stress in OF1 had been due to a loss of the opioid-influenced component of this behavior, one would have expected it to have been decreased by naloxone in C rats but not in PS. Thus, it is unlikely that the smaller duration of locomotion in PS rats resulted from an inability to activate opioid systems in this situation. However, in the other unfamiliar situations, the behavior of vehicle-treated PS rats did not differ significantly from that of controls, but the opioid-influenced component of exploratory activity was suppressed, especially in females. Thus, in PS males, naloxone did not decrease the amount of time spent in rearing, with and without support, but had an effect similar to that in C males on the other behavioral patterns. In PS females, naloxone did not significantly reduce any of these behaviors. This suggests that, perhaps, opioid peptides do not stimulate exploratory behavior in PS females. Alternatively, these peptides may interact with different receptor subtypes to produce both a stimulatory and an inhibitory effect on exploration in PS females, both of which are blocked by naloxone, in contrast to a production of a preponderance of stimulatory activity in C rats. The latter possibility is supported by the observation that the duration of rearing was reduced by naloxone in C rats, unchanged in PS males, but increased in PS females. The finding

is in accordance with those of a previous report, in which selective activation of μ receptors decreased whereas that of δ receptors increased rearing and locomotion in experimental situations similar to those used in the current study (4,21). Thus, in PS rats, particularly females, the situation could have induced a more μ-receptor-mediated opioid activity, in contrast to a preponderance of δ-receptor activation in controls. These data support other observations that showed an increase in μ-receptor-mediated analgesia by morphine (19) and a decrease in saccharin preference (18), which is believed to be mediated by δ receptors (2), in PS females. This issue could probably be resolved by the use of antagonists that are specific for the different receptor subtypes.

In conclusion, the present data show that the nature of an unfamiliar environment and the previous experience of the rat determine the degree of involvement of opioid systems in the exploratory activity of rats. Prenatal stress can modify the opioid-influenced component of exploratory activity in novel environments, particularly in the female rat. This could result from an interference by maternal stress hormones with the development of the normal afferent input to fetal hypothalamic and limbic structures (3). This interference may then be followed by a long-lasting change in the response of the target tissue that is revealed by appropriate environmental stimulation of the adult offspring. The more marked effect of prenatal stress in females is seen in other opioid-dependent behaviors, such as saccharin preference (18) and stress-induced analgesia (19). The mechanism of this preferential effect is not clear, but it may be related to a sex difference in the time of the appearance of the opioid systems, or to a greater sensitivity of the developing female brain to maternal stress hormones.

Our findings do not enable us to conclude that the changes induced in the opioid system are entirely mediated by events occurring during the prenatal period. Prenatal stress has been shown to alter the amount of maternal attention given to the male offspring (24), and this is not affected by fostering PS pups onto nonstressed mothers (22). Moreover, maternal attention may also modulate opioid systems in the developing offspring, because, like morphine, it can cause a marked reduction in ultrasonic vocalizations emitted by the pups when separated from the mother (16). Thus, changes in fetal opioid systems induced by maternal stress hormones may be modified by maternal behavior during the early neonatal period. Studies in which PS and C pups are cross-fostered onto stressed and control dams may help to ascertain the relative contributions of pre- and postnatal factors to the opioid-influenced components of exploratory and other behaviors. However, in view of the findings of Moore and Power (22,24), maternal behavior should be monitored during the neonatal period to ascertain whether behavior toward PS pups differs from that toward control pups, by either a stressed or a control dam.

ACKNOWLEDGEMENTS

T.P. gratefully acknowledges the financial support provided by the Israel Ministry of Absorption and the technical assistance of Ms. Rachel Malool.

REFERENCES

1. Arnstein, A. F. T.; Berridge, C.; Segal, D. S.: Stress produces opioid-like effects on investigatory behavior. Pharmacol. Biochem. Behav. 22:803–809; 1985.
2. Beczkowska, I. W.; Koch, J. E.; Bostock, M. E.; Leibowitz, S.; Bodnar, R. J.: Central opioid receptor subtype antagonists differentially reduce intake of saccharine and maltose dextrin solutions in rats. Brain Res. 618:261–270; 1993.
3. Beyer, C.; Feder, H. H.: Sex steroids and afferent input: Their roles in brain sexual differentiation. Annu. Rev. Physiol. 49:349–364; 1987.
4. Calenco-Choukroun, G.; Dauge, V.; Gacel, G.; Feger, J.; Roques, B. P.: Opioid δ agonists and endogenous enkephalins induce different emotional reactivity than μ agonists after injection in the rat ventral tegmental area. Psychopharmacology 103:493–502; 1991.
5. Clarke, S.; Trowill, J. A.: Sniffing and motivated behavior in the rat. Physiol. Behav. 6:49–52; 1971.
6. Dokla, C. P. J.: Naloxone reduced social locomotor activity in rats. Pharmacol. Biochem. Behav. 43:1183–1193; 1992.
7. Falconer, J.; Chan, E.-C.; Madsen, G.; Thomson, M.; Davies, J.; Smith, R.: Secretion of β-endorphin into the maternal circulation by utero placental tissues in response to hypoglycaemic stress. J. Endocrinol. 118: R5–R8; 1988.
8. File, S. E.: Naloxone reduces social and exploratory activity in the rat. Psychopharmacology 71:41–44; 1980.
9. File, S. E.; Wardill, A. G.: Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacologia 44:53–59; 1975.
10. Fride, E.; Weinstock, M.: The effects of prenatal exposure to predictable or unpredictable stress on early development in the rat. Dev. Psychobiol. 17:651–660; 1984.
11. Hendrie, C. A.: Hyperalgesia following agonistic encounters in DBA/2 intruder mice is not associated with recuperative behaviors. Physiol. Behav. 45:453–457; 1989.
12. Insel, T. R.; Kinsley, C. H.; Mann, P. E.; Bridges, R. S.: Prenatal stress has long-term effects on brain opiate receptors. Brain Res. 511:93–97; 1990.
13. Izquierdo, I.; Netto, C. A.: Role of β-endorphin in behavioral regulation. Ann. N.Y. Acad. Sci. 444:162–177; 1985.
14. Katoh, A.; Nabeshima, T.; Kameyama, T.: Behavioral changes induced by stressful situations: Effects of enkephalins, dynorphin, and their interactions. J. Pharmacol. Exp. Ther. 253:600–606; 1990.
15. Katz, R. J.; Manik, C. P.: Endogenous opioids and stress. Behavioral activation elicited in the rat and effects of naltrexone, diprenorphine and morphine. Neuropharmacology 23:1425–1430; 1984.
16. Kehoe, P.; Blass, E. M.: Opioid-mediation of separation distress in 10-day-old rats: Reversal of stress with maternal stimuli. Dev. Psychol. 19:385–398; 1986.
17. Kelley, A. E.; Cador, M.: Behavioral evidence for differential neuropeptide modulation of the mesolimbic dopamine system. Ann. N.Y. Acad. Sci. 537:415–434; 1988.
18. Keshet, G. I.; Weinstock, M.: Maternal naltrexone prevents morphological and behavioral alterations induced in rats by prenatal stress. Pharmacol. Biochem. Behav. 50:413–419; 1995.
19. Kinsley, C. H.; Mann, P.; Bridges, R. S.: Prenatal stress alters morphine and stress-induced analgesia in male and female rats. Pharmacol. Biochem. Behav. 30:123–128; 1988.
20. Lynch, W. C.: Opiate blockade inhibits saccharin intake and blocks normal preference acquisition. Pharmacol. Biochem. Behav. 24:833–836; 1986.
21. Michael-Titus, A.; Dourmap, N.; Costentin, J.: Mu and delta opioid receptors control differently the horizontal and vertical components of locomotor activity in mice. Neuropeptides 13:235–242; 1989.
22. Moore, C. L.; Power, K. L.: Prenatal stress affects mother–infant interaction in Norway rats. Dev. Psychobiol. 19:235–245; 1986.
23. Poltyrev, T.; Keshet, G. I.; Kay, G.; Weinstock, M.: Role of experimental conditions in determining differences in exploratory behavior of prenatally-stressed rats. Dev. Psychobiol. 29: 453–462; 1996.
24. Power, K. L.; Moore, C. L.: Prenatal stress eliminates differential maternal attention to male offspring in Norway rats. Physiol. Behav. 38:667–671; 1986.
25. Rodgers, R. J.; Deacon, R. M. J.: Effect of naloxone on the behav-

ior of rats exposed to a novel environment. Psychopharmacology 65:103–105; 1979.
26. Siegfried, B.; Netto, C. A.; Izquierdo, I.: Exposure to novelty induces naltrexone-reversible analgesia in rats. Behav. Neurosci. 101:36–438; 1987.
27. Spruijt, B. M.; Van Hooff, J. A. R. A. M.; Gispen, W. H.: Ethology and neurobiology of grooming behavior. Physiol. Rev. 72: 825–852; 1992.
28. Stinus, G. F.; Koob, G. F.; Ling, N.; Bloom F. E.; LeMoal, M.: Locomotor activation induced by infusion of endorphins into the ventral tegmental area: Evidence for opiate dopamine interactions. Proc. Natl. Acad. Sci. USA 77:2323–2327; 1980.
29. Tsang, D.; Ng, S. C.: Effects of antenatal exposure to opiates on the development of opiate receptors in rat brain. Brain Res. 188: 196–206; 1980.
30. Vathy, I. U.; Etgen, A. M.; Barfield, R. J.: Effects of prenatal exposure to morphine on the development of sexual behavior in rats. Pharmacol. Biochem. Behav. 22:227–232; 1985.
31. Ward, I. L.: Effects of maternal stress on the sexual behavior of male offspring. Monogr. Neural Sci. 9:169–175; 1983.
32. Ward, O. B.; Monaghan, E. P.; Ward, I. L.: Naltrexone blocks the effects of prenatal stress on sexual behavior differentiation in male rats. Pharmacol. Biochem. Behav. 25:573–576; 1986.
33. Weinstock, M.; Matlina, E.; Maor, G. I.; Rosen, H.; McEwen, B. S.: Prenatal stress selectively alters the reactivity of the hypothalamic–pituitary adrenal system in the female rat. Brain Res. 595: 195–200; 1992.
34. Zagon, I. S.; McLaughlin, P. J.: Behavioral effects of prenatal exposure to opiates. Monogr. Neural Sci. 9:159–168; 1983.