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Both Prenatal and Postnatal Factors Contribute to the Effects of Maternal Stress on Offspring Behavior and Central 5-Hydroxytryptamine Receptors in the Rat

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PETERS, D. A. V. *Both prenatal and postnatal factors contribute to the effects of maternal stress on offspring behavior and central 5-hydroxytryptamine receptors in the rat.* PHARMACOL BIOCHEM BEHAV 30(3) 669–673, 1988.—Litters from stressed and control females were cross-fostered at birth to determine whether the effects of maternal stress on the offspring originated prenatally or during the neonatal period. Offspring of stressed females reared by control mothers from birth showed a reduced behavioral response to injections of the 5-hydroxytryptamine (5-HT) agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), increased 5-HT_2 receptor binding in cerebral cortex and increased open field activity when tested at 60 days of age. In contrast, control litters reared by previously stressed females showed an increased behavioral response to 5-MeODMT, increased 5-HT_2 receptor binding and only minor changes in open-field activity. These results provide further evidence that adult rat behavior can be significantly altered by exposure to the effects of maternal stress in utero. However, the effect of maternal stress on central 5-HT receptors is also strongly influenced by the postnatal rearing conditions.

Prenatal Stress Brain development 5-Hydroxytryptamine 5-HT syndrome Open field

THERE is now a substantial body of evidence that exposure of pregnant animals to stressful conditions can have long-lasting effects on the offspring. Even relatively mild stresses such as handling [1,2] or overcrowding [4,11] appear to affect fetal development. We have previously reported that offspring of female rats exposed to mild stress conditions during pregnancy show both changes in brain 5-hydroxytryptamine (5-HT) receptor binding and increased behavioral responses to 5-HT agonists [16,17] suggesting that prenatal stress may have a permanent effect on the functioning of central serotonergic neurons. The prenatally stressed animals also showed increased locomotion and rearing in an open field which we have suggested may be associated with the increased 5-HT receptor binding [17].

In our previous studies the litters were left with the original mothers until weaning since at that time we were only interested in establishing whether maternal stress adversely affected the offspring. The present study addresses the further question of whether the effects originate entirely prenatally or whether postnatal mother-offspring interactions play a significant role. Litters from stressed and control females were cross-fostered shortly after birth. The litters transferred from stressed to control mothers were used to investigate the effects of stress in utero while the control litters transferred to previously stressed females were used to study the postnatal influences. The offspring were studied at 60 days of age. Two methods of assessing changes in the functioning of central serotonergic neurons were used; in vitro measurements of 5-HT_2 receptor binding in cerebral cortex and the behavioral responses to injection of the 5-HT receptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT). Locomotion and rearing were measured in an automated open field.

METHOD

Thirty female Sprague-Dawley rats (200–225 g) were maintained in groups of 3 for a 1 week acclimatisation period after arrival. A male rat (400–450 g) was placed in each cage for a 4-day period and on the 5th day the females were randomly assigned to either control or stress group. As before [17] the stress treatment consisted of a combination of

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TABLE 1

EFFECT OF MATERNAL STRESS ON THE 5-HT SYNDROME PRODUCED BY INJECTION OF 5-METHOXY-N,N-DIMETHYLTRYPTAMINE (5-MeODMT) TO 60–70-DAY-OLD OFFSPRING

		Exposure to Stress Effects			
	Control	Prenatal	(%)	Postnatal	(%)
Straub Tail					
Males	0.31 ± 0.07	0.11 ± 0.08	35	1.14 ± 0.36	368
Females	0.38 ± 0.08	0.12 ± 0.07	32	0.90 ± 0.25	237
Head Weaving					
Males	0.56 ± 0.14	0.16 ± 0.08	29	1.34 ± 0.35	239
Females	0.31 ± 0.07	0.14 ± 0.07	45	0.49 ± 0.17	158
Forepaw Treading					
Males	1.06 ± 0.20	0.61 ± 0.12	58	1.90 ± 0.40	179
Females	1.18 ± 0.18	0.79 ± 0.20	67	2.48 ± 0.22	210
Hindlimb Abduction					
Males	1.54 ± 0.23	1.06 ± 0.22	68	2.50 ± 0.31	162
Females	1.90 ± 0.10	1.11 ± 0.24	58	2.66 ± 0.29	140

Each animal was given an injection of 5-MeODMT (4 mg/kg) and observed for a 10 min period during which the behaviors associated with the 5-HT syndrome were rated on scales of 0 (not present) to 4 (severe). The results are presented as mean ± s.e.m. for groups of 16 rats.

crowding (5 female rats in a 20×40 cm breeding cage) and once-daily saline injections from the day of separation of the sexes until shortly before the females gave birth. Although this was a relatively mild stress we have previously found that once-daily saline injections alone were sufficient to produce a marked elevation in plasma corticosterone level in pregnant rats even after more than 2 weeks of treatment [15]. Control females were kept in pairs in similar breeding cages and were undisturbed except for routine animal care.

Within 6 hours of birth all litters were reduced to 10 pups and cross-fostered. The offspring control group consisted of litters transferred between control mothers while 2 experimental groups of litters were obtained by cross-fostering litters between stressed and control mothers. The stress litters transferred to control mothers at birth were used to study the prenatal effects of maternal stress while the control litters reared by previously stressed mothers were used to investigate the postnatal influences. At least 8 litters in each treatment group were available for the offspring studies. The pups were weaned at 22 days and maintained in groups of 4 separated by sex and treatment group until 60 days of age when testing was initiated. The entire procedure was repeated with a new group of adult rats to provide a total of at least 16 litters in each treatment group. All rats were maintained in a temperature and humidity controlled room on a 12 hr light-12 hr dark reverse light cycle. For each experiment the subjects were selected so that not more than 1 rat was taken from each litter and no animal was used in more than 1 experiment.

Behavioral Response to 5-MeODMT

Administration of 5-HT agonists to rats produces a characteristic syndrome consisting of hyperactivity, hyperpyrexia, head weaving, forepaw treading, hindlimb abduction and Straub tail [9]. The syndrome is believed to involve a population of central 5-HT_2 receptors and probably a subgroup of 5-HT_1 receptors [9, 10, 20]. As in our previous studies [17] 60-day-old offspring in random order were given intraperitoneal injections of 5-MeODMT (4 mg/kg) and placed individually in polycarbonate cages. Each rat was continuously observed during the first 10 min after the injection and rated for the presence of Straub tail, head weaving, forepaw treading and hindlimb abduction on scales of 0 (not present) to 4 (severe). The observer was not aware of the group membership of the animals during testing.

5-HT_2 Receptor Binding

Sixty-day-old male offspring were killed by decapitation and the brains removed and dissected. The cerebral cortex from each rat was quickly weighed and homogenised in 40 volumes of ice-cold 50 mM pH 7.4 tris-HCl buffer using a Brinkman Polytron. The homogenates were centrifuged at 35000×g for 20 min and the pellet washed twice more in the same volume of buffer. The final pellet was suspended in 80 volumes of 50 mM pH 7.4 tris-HCl buffer containing ascorbic acid (5.7 mM) and $CaCl_2$ (4 mM). 5-HT_2 receptor binding to the suspended membranes was measured by the method of Creese and Snyder [5] using [^{3}H]spiperone as the ligand and cinanserine (1 μM) to define nonspecific binding. Bound ligand was isolated by rapid filtration through Whatman GF/B glass fiber filters followed by two washes of 5 ml ice-cold 50 mM tris-HCl, pH 7.4. The filters were dried, placed in scintillation vials containing PCS (Amersham) and counted in a Beckman LS 8100 liquid scintillation counter. Ten concentrations of the tritiated ligand in the concentration range of 0.02–5 nM were used to determine the number of binding sites (B_{max}) and the apparent dissociation constant (K_d) by Scatchard method [18]. The procedure of Lowry *et al.* [14] was used to measure the protein content of the membrane fraction.

Open-Field Study

A 1 meter-square automated open field was used as previously described [17]. Briefly, movement of a single rat in

TABLE 2

EFFECT OF MATERNAL STRESS ON [^{3}H]SPIPERONE BINDING IN CEREBRAL CORTEX

	Kd (nM)	Bmax (fmole/mg protein)
Control	3.20 ± 0.22	581 ± 50
Prenatal	3.11 ± 0.31	770 ± 38*
(% control)	(97)	(133)
Postnatal	3.01 ± 0.29	733 ± 51*
(% control)	(94)	(126)

[^{3}H]Spiperone binding to 5-HT$_2$ receptors was calculated from the difference in binding in the presence and absence of 1 μM cinanserine. Values for Kd and Bmax were obtained from Scatchard plots using 10 ligand concentrations in the range 0.02-5 nM. Results are mean ± s.e.m. for groups of 6 male rats, 60–70 days of age.
*Denotes $p<0.05$ by Anova.

the field was measured with a computer-controlled system of 64 infra-red light beams arranged in two horizontal levels. The beams were switched on and off in sequence so that 2 complete scans of the field were made each second. The position of the animal within the field was calculated automatically after each scan from the pattern of obstructed beams. The data were processed to give the total distance travelled, the duration of activity, the time spent in each part of the field and the number, location and duration of rearing events. Testing was carried out under a dim red light between 0900 and 1000 hr. Each rat was exposed to the field for a single 5 min period.

RESULTS

There was no significant between-group differences in body weights at birth, weaning or at the time of testing.

5-HT Syndrome

The effects of maternal stress on the 5-HT syndrome produced by injection of 4 mg/kg 5-MeODMT are summarised in Table 1. A 2-way ANOVA (treatment × sex) showed a significant treatment effect for Straub tail, $F(2,90)=12.4$, $p<0.001$, head weaving, $F(2,90)=9.58$, $p<0.001$, forepaw treading, $F(2,90)=21.13$, $p<0.001$ and hindlimb abduction, $F(2,90)=16.04$, $p<0.001$. Similar results were obtained when the data for prenatal and postnatal treatments were analysed separately using the same 2-way ANOVA [Prenatal: $F(1,60)=9.36$, $p<0.005$; 9.08, $p<0.005$; 5.56, $p<0.05$; and 9.55, $p<0.005$ for the Straub tail, head weaving, forepaw treading and hindlimb abduction respectively. Postnatal: $F(1,60)=8.96$, $p<0.005$; 5.24, $p<0.05$; 16.31, $p<0.001$; and 9.39, $p<0.005$, for the same 4 behaviors]. There was no significant sex effects or treatment × sex interactions.

5-HT$_2$ Receptor Binding

The effects of maternal stress on the number of 5-HT$_2$ binding sites (B_{max}) and the apparent dissociation constant (K_d) in cerebral cortex are summarised in Table 2. Analysis by ANOVA showed a significant treatment effect on B_{max}, $F(1,15)=4.91$, $p<0.005$, with no change in K_d, $F(1,15)=0.12$, $p<0.05$. When the data for prental and postnatal exposure were analysed separately both treatments were found to increase B_{max} [Prenatal: $F(1,10)=9.39$, $p<0.05$; Postnatal: $F(1,10)=5.00$, $p<0.05$] and again the K_d was not significantly affected.

Open-Field Activity

Table 3 summarises the effects of maternal stress on open field activity in 60–70-day-old offspring. The data for each of the 5 measures (distance travelled, duration of rearing and time spent in the center, sides and corners of the field) were analysed by 2-way ANOVA (treatment × sex). When the data for all 3 treatment groups were analysed there proved to

TABLE 3

EFFECT OF MATERNAL STRESS ON OPEN FIELD ACTIVITY IN 60–70-DAY-OLD OFFSPRING

	Distance (cm)	Rearing (sec)	Location in Field (sec)		
			Center	Sides	Corners
			Males		
Control	1010 ± 125	66 ± 5	8.1 ± 3.4	107 ± 11	185 ± 14
Prenatal	1379 ± 213	79 ± 13	13.4 ± 3.3	117 ± 9	169 ± 11
	(137)	(120)	(165)	(109)	(91)
Postnatal	1120 ± 119	72 ± 7	9.0 ± 2.4	90 ± 3	201 ± 6
	(111)	(109)	(111)	(84)	(109)
			Females		
Control	1340 ± 164	86 ± 9	8.2 ± 2.3	87 ± 9	205 ± 11
Prenatal	1928 ± 98	118 ± 11	21.8 ± 4.4	112 ± 5	166 ± 6
	(144)	(137)	(214)	(129)	(81)
Postnatal	1692 ± 107	128 ± 9	10.8 ± 2.9	103 ± 4	186 ± 15
	(126)	(149)	(132)	(118)	(91)

Each animal was exposed to the open field for a single 5 min period. The data are mean ± s.e.m. for groups of 8 rats. The percentages of control values are given in parentheses.

be significant overall treatment effects for distance, $F(2,42)=5.58$, $p<0.05$, rearing, $F(2,42)=3.98$, $p<0.05$ and time spent in the center, $F(2,42)=4.95$, $p<0.05$, sides, $F(2,42)=3.79$, $p<0.05$, and corners, $F(2,42)=3.90$, $p<0.05$, of the field. The distance travelled and duration of rearing also showed a significant sex effect with females showing greater activity than males at the $p<0.001$ level of significance. The treatment effect proved to be due mainly to differences between control and prenatal exposure groups. Thus a separate analysis of the prenatal and control data showed significant treatment effects for all measures [distance $F(1,28)=9.39$, $p<0.005$; rearing, $F(1,28)=5.11$, $p<0.05$; center, $F(1,28)=7.58$, $p<0.05$; sides, $F(1,28)=3.98$, $p<0.05$, and corners, $F(1,28)=6.38$, $p<0.05$] while a similar comparison of the control and postnatal exposure groups showed that only the total duration of rearing was significantly different between the two groups, $F(1,28)=9.04$, $p<0.01$.

DISCUSSION

Several lines of evidence suggest that the development of central serotonergic neurons may be affected by the prenatal or neonatal environment. For example, a single cold exposure of neonatal rats was found to produce a delayed increase in brain 5-HT levels [8] while we have previously found evidence of changes in brain 5-hydroxyindole levels [15] and 5-HT receptor binding [16] and increased behavioral responses to 5-HT agonists [17] following prenatal stress.

In our previous studies the litters were left with the stressed mothers until weaning and there was a possibility that the development of the offspring had been modified by the presence of the previously stressed parent. We now report that this is indeed the case with respect to the 5-MeODMT-induced 5-HT syndrome in adult offspring. The effect of maternal stress on the 5-HT syndrome in the adult offspring appears to be complex. One mechanism, which operates in utero, produces a significantly reduced behavioral response to 5-HT receptor activation. A second mechanism, which appears to involve an early postnatal interaction between previously stressed mothers and the offspring, results in a marked increase in the 5-HT syndrome.

The finding of increased 5-HT_2 receptor binding in cerebral cortex when the 5-HT syndrome was enhanced as well as when it was reduced provides further evidence that 5-HT_2 receptors play a relatively minor role in the mediation of the 5-HT syndrome. A similar lack of correlation between 5-HT_2 receptor numbers and the intensity of the 5-HT syndrome has recently been reported following prenatal administration of several antidepressant drugs [7]. Although earlier studies suggested that the intensity of the 5-HT syndrome was closely linked to the numbers of 5-HT_2 receptors there is now clear evidence that other receptors including 5-HT_{1a} sites are also involved (see discussion in [10,20]). We had previously shown that the maternal stress also altered 5-HT_1 receptor binding in several brain regions of the offspring and it is possible that changes in the 5-HT_{1a} subgroup may correlate with the altered intensity of the 5-HT syndrome.

The mechanism which mediates the effects of prenatal stress on fetal serotonergic neurons is presently unknown but one possibility is that glucocorticoids may be involved. This proposal is supported by evidence that high levels of glucocorticoids produce a general delay in cellular maturation of several tissues and may interfere with synaptic development in the central nervous system [19]. The maternal circulation is a likely source of the glucocorticoids since corticosterone has been shown to cross the placenta in several species including the rat [21], while stress produces an unusually high plasma level of unbound (i.e., biologically active) corticosterone in pregnant rodents [3,6].

The report by Lauder, Sze and Krebs [13] that neither daily restraint stress nor daily hydrocortisone injections altered embryonic rat brain tryptophan hydroxylase activity suggests that there may be no direct effect on the early development of the 5-HT neurons. However, there is some evidence that prenatal stress may affect the development of 5-HT target neurons. Thus, the stress of vehicle injections appeared to cause an earlier onset of differentiation of neurons which later receive 5-HT innervation and a mechanism involving 5-HT as an intermediate humoral signal for cell differentiation has been proposed [12]. It is possible that the changes in the intensity of the 5-HT syndrome that we find in prenatally stressed rats may be related to changes in the 5-HT target cells such as alterations in the numbers of postsynaptic 5-HT receptors.

In the previous study in which the offspring were not cross-fostered at birth we found increases in open field rearing and locomotion in the prenatal stress group. We now find that cross-fostering of the prenatal stress litters to control mothers at birth did not significantly modify these changes confirming the prenatal origin for the maternal stress-induced changes in open field behavior.

In summary, our results show that the effects of maternal stress on the offspring are produced both prenatally and during the neonatal period. We confirm that exposure to stress in utero produces both behavioral and neurochemical changes and also demonstrate the importance of litter-parent interactions as a factor in the overall effects of maternal stress on offspring development.

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