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Prenatal Stress Increases the Behavioral Response to Serotonin Agonists and Alters Open Field Behavior in the Rat

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PETERS, D. A. V. *Prenatal stress increases the behavioral response to serotonin agonists and alters open field behavior in the rat.* PHARMACOL BIOCHEM BEHAV **25**(4) 873–877, 1986.—Female rats were exposed to mild stress throughout pregnancy and the offspring tested at 60 days of age. In an open field test the prenatal stress group showed increased locomotion and increased rearing compared to control rats confirming that the prenatal stress treatment was sufficient to produce persistent behavioural changes in the offspring. The prenatal stress offspring also showed an increased behavioural response to injections of 5-hydroxy-L-tryptophan (wet-dog shakes) and an enhanced 5-HT syndrome following treatment with the 5-HT agonist 5-methoxy-N,N- dimethyltryptamine. These results provide further evidence that maternal stress produces long-lasting changes in the functioning of central 5-HT neurons in the offspring.

Open field Prenatal Serotonin Stress 5-HT syndrome

OFFSPRING of female rodents exposed to stresses such as crowding [6,14], handling [1,2], electric shocks [13] or daily saline injections [19] during pregnancy show behavioral abnormalities relative to control offspring. Little is known of the underlying biochemical changes but some data suggest that altered functioning of serotonin (5-HT)-containing neurons may be associated with the behavioral changes. For example, the stress of daily vehicle injections was reported to accelerate the onset of neuronal differentiation in brain regions known to contain 5-HT terminals or to have a high 5-HT content [15]. Our data also suggest that exposure of pregnant rats to the stress of daily saline injections may affect the development of central sertonergic neurons in the offspring. Thus, brain 5-hydroxyindole levels showed a short-lasting elevation relative to age matched controls during infancy [18] while both 5-HT_1 and 5-HT_2 receptor binding in several brain regions were persistently altered [19].

The question of whether the biochemical changes that we observe are associated with functional changes is of primary importance. To examine this possibility we studied the ability of 5-hydroxy-L-tryptophan (5-HTP) and the 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) to elicit behavioural responses in offspring of female rats subjected to mild stress during pregnancy. We now report that both the whole-body shakes ("wet-dog shakes," WDS) produced by injections of 5-HTP in the presence of a peripheral decarboxylase inhibitor [4,17] and the "5-HT syndrome" produced by 5-MeODMT [9, 11, 16] were more pronounced in the prenatal stress group. Adult offspring of stressed female rats were also more active and reared more than control offspring in the open field confirming that the stress procedure was sufficient to produce persistent behavioural changes in the offspring.

These findings strengthen the view that adverse environmental conditions during pregnancy can significantly affect offspring behaviour. Moreover, our results suggest that an altered sensitivity of central serotonergic neurons may play a role in the biochemical basis of the behavioral changes.

METHOD

Litters

Thirty-two female Sprague-Dawley rats (200–225 g) were kept in pairs for a 7 day acclimatization period after arrival. One male rat (400–450 g) was then placed in each cage for a 4 day period and on the 5th day the females were randomly assigned to either control or stress groups. Rats in the stress group were kept under crowded conditions (5 rats in a 20×40 cm polycarbonate cage) while control rats were maintained in groups of 2 in similar cages. The stress group also received a single saline injection once daily as before [18]. Preliminary studies had provided evidence that this combination of

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TABLE 1
EFFECT OF MATERNAL STRESS ON BODY WEIGHT OF OFFSPRING

Offspring age (days)	Control		Stress	
	Male	Female	Male	Female
1	6.02 ± 0.22	5.56 ± 0.21	5.99 ± 0.16 (100)*	5.38 ± 0.26 (97)
15	27.8 ± 0.6	27.4 ± 0.5	30.5 ± 0.6 (110)	29.2 ± 0.5 (107)
30	88.5 ± 2.0	76.3 ± 1.3	98.6 ± 1.9 (111)	81.1 ± 1.2 (106)
60	236 ± 4	179 ± 3	241 ± 3 (102)	181 ± 3 (101)

Results are mean ± S.E.M. for 12 control and 11 prenatal stress litters. *Percent control.

TABLE 2
EFFECT OF PRENATAL STRESS ON THE BEHAVIOURAL SYNDROME PRODUCED BY 5-HYDROXY-L-TRYPTOPHAN IN 60 DAY-OLD FEMALE RATS

5-HTP (mg/kg)	"Wet-Dog Shakes"		
	Control	Prenatal Stress	% Control
50	1.1 ± 0.5	5.4 ± 2.2	491
75	3.0 ± 1.7	13.0 ± 3.9	433
100	4.0 ± 0.6	18.0 ± 1.1	450
150	19.4 ± 2.9	16.6 ± 4.6	86

Carbidopa (25 mg/kg) was given 30 min before the 5-HTP injection. The animals were observed for "wet-dog shakes" during a 10 min period starting 60 min after injection of 5-HTP. Results are mean ± S.E.M. for groups of 6 rats.

crowding and daily saline injections produced a more consistent effect on open field behaviour in the adult offspring than either stress procedure alone. Shortly before giving birth all rats were transferred to individual breeding cages. Within 12 hr of birth the litters were weighed and reduced to 10 pups. Other than during routine cage changing operations and weighing at 15 days of age the litters were undisturbed until weaning at 21 days. The animals were weighed again at 30 and 60 days of age. The 60-day-old offspring were assigned to various experiments in such a way that the maximum number of different litters was represented in each experiment. Naive animals were used in all experiments.

For the present study we did not cross-foster offspring to control mothers at birth since at this stage we were interested in examining the overall effect of maternal stress on the offspring rather than determining the separate prenatal and postnatal influences.

5-HT Behavioural Syndromes

Female offspring (60–65 days of age) were individually housed and given an injection of carbidopa (25 mg/kg, IP) followed 30 min later by an injection of 5-HTP (50–150 mg/kg, IP). The number of WDS [4] seen during a 10 min period starting 60 min after the 5-HTP was recorded. Other rats were given a single injection of 5-MeODMT (2 or 4 mg/kg, IP) and observed for the 5-HT syndrome during the first 10 min following the injection. The presence of Straub tail, head weaving, forepaw treading and hindlimb abduction [16] were each rated on a scale of 0–5. In both experiments neither the group identity nor the drug dose were known to the observer.

Open Field Behaviour

An automated 1 meter square open field was used for this study. A total of 64 focused infra-red light-emitting diodes were arranged in 2 evenly spaced rows 5 and 15 cm above the floor along 2 adjacent sides of the field. A matched detector was located directly opposite each emitter. The computer-based control unit switched each of the 64 emitter-detector pairs on and off in sequence so that 2 complete scans of the field were made each second. The location of the rat in the field was calculated automatically at the end of each scan by analysis of the pattern of intact and obstructed infra-red beams. Rearing was detected by the upper row of beams. The data were recorded on magnetic disc for later processing. Rats used for the study were maintained on a reverse light cycle for at least 20 days prior to the experiment and were tested during the dark phase of the cycle. The total number, location and duration of rearing episodes, the total distance traveled, the total number of timing intervals during which motion was detected, and the time spent in each part of the field were calculated.

All rats were tested once daily on 4 consecutive days. Immediately after removal from the open field on the final day of testing each rat was decapitated and a blood sample collected in a heparinised tube. A plasma sample was obtained from each and stored at −80°C until assayed for corticosterone by the method of Glick, von Redlick and Levine [8].

RESULTS

Litters

A total of 12 control and 11 experimental litters were obtained over a 4-day period. Prenatal stress had no effect on birth weight but the offspring in the stress group were

TABLE 3

EFFECT OF PRENATAL STRESS ON THE BEHAVIOURAL SYNDROME PRODUCED BY 5-METHOXY-N,N-DIMETHYLTRYPTAMINE (5-MeODMT) IN 60 DAY-OLD RATS

	5-HT Syndrome			
	Straub Tail	Head Weaving	Forepaw Treading	Hindlimb Abduction
Males				
Control	0.10 ± 0.04	0.43 ± 0.13	0.90 ± 0.23	1.43 ± 0.34
Stress	0.33 ± 0.09	1.43 ± 0.36	1.67 ± 0.30	1.95 ± 0.30
% Control	330	333	186	136
Females				
Control	0.19 ± 0.06	0.34 ± 0.10	0.86 ± 0.21	1.42 ± 0.22
Stress	0.29 ± 0.10	0.68 ± 0.21	1.19 ± 0.29	1.86 ± 0.35
% Control	153	200	138	131

The rats were given an injection of 5-MeODMT (4 mg/kg IP) and observed for the 5-HT syndrome for a period of 10 min. Each behaviour was rated on a scale of 0–5. The results are mean ± S.E.M. for groups of 8 rats.

TABLE 4

EFFECT OF PRENATAL STRESS ON OPEN FIELD BEHAVIOR AND PLASMA CORTICOSTERONE LEVEL IN 60 DAY-OLD RATS

	Males			Females		
	Control	Stress	%	Control	Stress	%
Distance (cm)	1397 ± 95	1659 ± 105	119	1569 ± 131	1788 ± 94	114
Rearing:						
Number	17.9 ± 1.9	24.8 ± 2.4	139	19.7 ± 1.9	24.2 ± 1.7	123
Duration (sec)	6.3 ± 0.7	6.4 ± 0.6	102	7.0 ± 0.7	7.3 ± 0.4	104
Location (sec):						
Center	27 ± 4	39 ± 4	145	22 ± 3	23 ± 3	103
Sides	143 ± 5	141 ± 8	99	138 ± 6	149 ± 8	108
Corners	130 ± 6	121 ± 10	93	139 ± 8	128 ± 8	92
Plasma Corticosterone (μg/100 ml)	27.0 ± 2.1	34.5 ± 5.2	128	43.5 ± 3.3	59.9 ± 5.1	138

Each rat was tested for a 5 min period on 4 consecutive days and the 4 sets of readings averaged. Blood samples were taken for corticosterone assay following decapitation within 1 min of removal from the open field on the final day of testing. Results are mean ± S.E.M. for groups of 8 rats.

slightly but significantly heavier at 15 and 30 days of age (ANOVA: $F(1,226)=18.7$ and 18.8 respectively, $p<0.001$). The results are summarized in Table 1. Litter size was unaffected by prenatal stress (controls: males 6.4±0.4, females 6.2±0.4; stress group: males 6.0±0.6, females 6.5±0.6).

5-HT Behavioral Syndromes

In a preliminary experiment the effect of 5-HTP injections on 50–70-day-old control female rats was examined. As observed by others [4], administration of 5-HTP (50, 100 or 150 mg/kg) and carbidopa (25 mg/kg) produced WDS as the dominant behaviour with only occasional indications of stereotypic behavior (forepaw treading or hindlimb abduction) at the 150 mg/kg level while increasing the dose to 200 mg/kg resulted in marked stereotypic behavior and a drop in the number of WDS. For the main study 24 control and 24 prenatal stress females received an injection of carbidopa (25 mg/kg) followed 30 min later by an injection of 5-HTP (50–150 mg/kg). Prenatal stress greatly increased the number of WDS seen following low doses of 5-HTP (50–100 mg/kg) although the number of shakes seen after 150 mg/kg 5-HTP was not increased (Table 2). A 2-way ANOVA (treatment × dose) showed a significant treatment effect, $F(1,40)=11.97$, $p<0.005$, and a significant treatment × dose interaction, $F(3,40)=3.92$, $p<0.025$. The failure to show an increased frequency of WDS in the prenatal stress group receiving 150 mg/kg 5-HTP most likely reflects increased stereotypic behavior in this group and a resulting decrease in WDS.

A 4 mg/kg injection of 3-MeODMT produced a typical 5-HT syndrome [11,16] consisting of hindlimb abduction, forepaw treading and, in some animals, head weaving and/or Straub tail. In both male and female rats the syndrome occurred more frequently and with higher intensity in the pre-

natal stress group than in the control group (Table 3). Two-way analyses of variance showed a significant treatment effect for all components of the syndrome with the exception of the hindlimb abduction [Straub tail, $F(1,28)=4.87$, $p<0.05$; head weaving, $F(1,28)=8.95$, $p<0.01$; forepaw treading, $F(1,28)=4.76$, $p<0.05$; hindlimb abduction, $F(1,28)=2.53$, $p>0.05$]. There was no significant sex effect or treatment × sex interaction. The lower dose of 5-MeODMT (2 mg/kg) produced a syndrome in less than 50% of the control group and although the syndrome occurred in a higher percentage of the prenatal stress group the difference was not statistically significant (data not presented).

Open Field

Each rat was individually tested in the open field for a 5 min period once daily on 4 consecutive days. On day 1 of testing the prenatal stress group appeared to be more active than the control group and both rearing activity and the distance traveled were increased. Similar results were obtained on subsequent test days with a slight increase in the intergroup differences between the first and second day. When the data were tested by 3-way ANOVA (treatment × sex × days) both the distance travelled and the number of rearing movements were significantly increased [distance, $F(1,112)=8.70$, $p<0.005$; rearing, $F(1,112)=8.07$, $p<0.01$]. On each of the 4 test days the male stress group appeared to spend more time in the center of the field than male control group but a 3-way ANOVA found no significant treatment effect or treatment × sex interaction. A 2-way ANOVA (treatment × sex) showed a significant increase in plasma corticosterone level in the prenatal stress animals, $F(1,28)=8.36$, $p<0.01$. As the results were qualitatively similar for the 4 days of the study the data were averaged for presentation (Table 4).

DISCUSSION

Our previous studies have provided evidence that maternal stress may affect the development of central monoaminergic neurons. For example, offspring of stressed rats showed transient increases in brain 5-hydroxyindole levels relative to control rats at about 21 days of age [18] while 5-HT receptor binding was persistently altered [19]. In the present study we find evidence of a behavioural hyperactivity of the central serotonergic system.

Two different behavioural models of 5-HT receptor activation have been described, the head twitch or WDS behaviour and the 5-HT syndrome. Administration of 5-HTP to the rat produces a series of intermittent head and body shaking movements which begin about 15 min after the 5-HTP injection and last at least 4 hr [4,22]. The number of shakes in a set observation period is dose dependent up to approximately 150 mg/kg while higher doses elicit the 5-HT syndrome accompanied by a drop in the number of WDS [4,22]. Similar head and body shakes can be observed but are less prominent following activation of central serotonergic receptors by treatment with 5-HT agonists or L-tryptophan with a monoamine oxidase inhibitor [4,17] or by direct intracerebral injection of 5-HT [7]. Higher doses of 5-HT agonists produce a different behavioral response usually referred to as the 5-HT syndrome. This syndrome includes forepaw treading, head weaving, hindlimb abduction, piloerection, hyperactivity, Straub tail and resting tremor [9–11, 16]. Both the WDS behavior and the 5-HT syndrome are blocked by 5-HT antagonists [4, 10, 17, 20] but studies of the relative potencies of different 5-HT antagonists in blocking the two responses led to spectulations that different populations of receptors may be involved. Most reports are consistent with the view that the WDS or head twitch response is likely to be associated with 5-HT_2 receptors [10, 16, 22] but the receptor sub-type linked to the 5-HT syndrome has not yet been conclusively identified. Both 5-HT_1 [16] and 5-HT_2 [10] receptors have been proposed as mediators of the syndrome while later data suggest that a 5-HT_1 sub-type, the 5-HT_{1A} sites, may be involved [21]. Green [10] has recently concluded that both 5-HT_{1A} and 5-HT_2 receptor sub-types may be involved in mediating the forepaw treading, head weaving and hind-limb abduction produced by 5-HT agonists. Our present finding of increases in both the number of WDS and in the severity of the 5-HT syndrome is therefore interesting in view of our previous report of increases in 5-HT_2 receptor binding in several brain regions following mild prenatal stress [19]. This evidence of an apparent link between increased 5-HT_2 receptor binding and increased behavioral responses to 5-HT agonists is strengthened by our previous data on the distribution of prenatal stress-induced changes in 5-HT receptor binding. Whereas 5-HT_2 receptor binding was increased in all brain regions studied, including hindbrain, the 5-HT_1 binding was increased only in cerebral cortex [19]. Evidence from lesion and brain section studies indicate that the 5-HT syndrome is primarily mediated by the brainstem or spinal cord [12] while the WDS response appears to be localized in the lower diencephalon and brainstem [4]. Thus, our data is consistent with the veiw that 5-HT_2 receptors may be associated with both 5-HT receptor-mediated behaviors.

We previously reported evidence of increased night-time locomotor activity which we attributed at least partly to increased rearing [19]. For the present study we examined adult offspring of control and stressed rats in a 1 meter automated open field capable of measuring both vertical and horizontal movements. Male and female rats from the two groups were tested in the field once daily on 4 consecutive days. A 3-way ANOVA showed that mild prenatal stress significantly increased the total number of rearing events and the total distance traveled. The major difference between the two groups appeared to be that the prenatal stress group spent less time inactive in the corners of the field and correspondingly more time in active movement, both ambulation and rearing. This evidence of a behavioural arousal was supported by data indicating that the plasma corticosterone response to the stress of open field exposure was also greater in the prenatal stress group than in controls.

Administration of 5-HT precursors is associated with behavioural hyperactivity [9] and it is possible that there is a link between the increased open field activity and the enhanced sensitivity of 5-HT neurons in prenatally stressed rats. However, an involvement of other neurotransmitter systems cannot be excluded. For example, it is possible that dopaminergic neurons may play a role in the increased rearing activity. Rearing activity has been shown to be enhanced in rats withdrawn from long-term treatment with haloperidol, an effect which was attributed to supersensitivity of dopamine receptors [5]. Moreover, several reports suggest an involvement of dopamine neurons in some 5-HT-dependent "stereotypic" behaviours [3] although not in WDS behaviour [12,17]. Thus, it is possible that prenatal stress produces changes in the functioning of central dopaminergic neurons in addition to those we observed for 5-HT neurons and this possibility is presently under investigation.

In summary, the evidence presented suggests that expo-

sure of pregnant rats to chronic stress alters the behavior of the offspring. The altered behavior is accompanied by an increased sensitivity of central 5-HT neurons which can be demonstrated both by receptor binding changes and by an increased behavioral response to 5-HT receptor activation.

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