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Prenatal Stress: Effects on Brain Biogenic Amine and Plasma Corticosterone Levels

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PETERS, D. A. V. *Prenatal stress: Effects on brain biogenic amine and plasma corticosterone levels.* PHARMAC. BIOCHEM. BEHAV. 17(4) 721–725, 1982.—Pregnant rats were subjected to once daily stress treatments consisting of handling and a saline injection. The offspring showed region-specific changes in brain 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine (NE) levels in infancy but only the hypothalamus still showed significant changes at 60 days of age. In a reaction-to-stress test 23-day-old offspring in the prenatal stress group showed a greater elevation in plasma corticosterone level but smaller changes in hypothalamic NE and 5-HIAA levels than control offspring suggesting that prenatal stress may have altered the functioning of the hypothalamic-pituitary-adrenal axis. It is suggested that changes in the development of specific monoamine-containing neurons may be associated with the reported behavioral deficits in offspring of female rats stressed during pregnancy.

Prenatal stress Brain Norepinephrine Serotonin Hypothalamus Plasma corticosterone

MANY studies of the effects of maternal prenatal stress on offspring behavior in the rat have been published. Several different types of stress have been employed including daily handling [1, 2, 19], anxiety [15, 23, 26], electric shocks [10,24] and restraint combined with bright lights and an elevated temperature [6, 8, 16, 17]. Prenatal maternal stress has been reported to alter several behaviors including male sexual activity [7, 15, 16, 28] and "emotionality" or "reactivity" [1, 9, 15, 26] of the offspring. These studies have provided some contradictory data on changes in offspring behavior (reviewed by Chapman and Stern [6]). However, it is probable that differences in experimental design, such as the use of radically different types of stress, have been responsible for some of the inconsistencies while Chapman and Stern [5,6] have suggested that failure to control for litter variables in some studies may have resulted in reports of significant treatment effects when none actually existed.

Prenatal stress also appears to modify the levels of 5-hydroxytryptamine (5-HT) in whole brain [19] and norepinephrine (NE) in brain regions [17] and some evidence suggests a link between changes in brain biogenic amine levels and alterations in endocrine function in the adult offspring [8,17]. It is conceivable that both changes in endocrine function and the altered behaviors are related to changes in the functioning of specific monoaminergic neurons. We therefore designed a study to investigate the effect of prenatal stress on the development of central NE and 5-HT-containing neurons using an experimental design which controlled for possible litter variables and which separated prenatal from postnatal influences by fostering the offspring to control dams at birth.

We now report that prenatal stress results in significant changes in the levels of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and NE in several brain regions at 9 and/or 16 days of age although only the hypothalamus showed persistant changes. Consistent with the hypothesis of an altered reactivity, an elevated plasma corticosterone response to stress was found in 23-day-old offspring following prenatal stress treatment.

METHOD

Animals

Forty-eight female Sprague-Dawley rats (150–170 g) were housed in groups of 2 during a 1 week acclimatization period. One male Sprague-Dawley rat was then placed in each cage and five days later the females were removed and randomly assigned to control or prenatal stress groups in a ratio of 2:1. Vaginal smears were not used to determine pregnancy to avoid unnecessary stress to the control rats [24]. All rats were housed in pairs until shortly before giving birth and food and water were available ad lib. To avoid unnecessary disturbance of the control rats during the daily stress treatments the two groups were housed in separate identical cubicles. The entire study was repeated with the cubicle assignment during pregnancy reversed to control for possible differences between the cubicles and to provide sufficiently large group sizes.

Stress Treatment

The choice of a suitable stress presented some difficulty. There appeared to be no advantage in using very severe or

TABLE 1

EFFECT OF PRENATAL MATERNAL STRESS ON OFFSPRING BODY WEIGHT AND MEAN LITTER SIZE AT BIRTH

Treatment	Number of litters	Birth Weight (g)		Litter Size	
		Male	Female	Male	Female
Control	56	6.29 ± 0.06	6.05 ± 0.06	6.27 ± 0.28	6.55 ± 0.24
Maternal Stress	26	6.10 ± 0.08 (97)*	5.93 ± 0.07 (98)	7.23 ± 0.56 (115)	5.96 ± 0.48 (91)

Results are Mean ± S.E.M.
*Percent control.

complex forms of stress when even "mild" stress such as handling has been reported to affect offspring behavior [1, 2, 19]. A preliminary study showed that a daily period of handling combined with a subcutaneous saline injection was an adequate stress since the typical plasma corticosterone response to stress was present even on the 14th consecutive day of treatment.

Starting on the day of removal from the males, all cages containing females in the prenatal stress group were removed to an adjacent laboratory and starting 10 minutes later each rat was given a single 0.1 ml subcutaneous saline injection in random order. When all rats in the stress group had been injected the cages were returned to the cubicle. The stress treatment was repeated once daily at the same time until the pregnant rats showed signs of given birth. Control rats remained undisturbed throughout pregnancy except for the minimum routine animal care. Since the exact time of conception was unknown the period of treatment could not be accurately determined for each rat. However, the average time of birth (the number of days post day 1 of mating) did not differ significantly between the control and stress group suggesting that the stress treatment had no effect on the gestation period.

Litters

The pregnant rats were checked for litters 3 times daily, at 0830, 1330 and 1830 hr. Within 12 hours of birth all litters in the stress group were fostered to control dams and an equal number of control litters was obtained by cross-fostering between control dams. Surplus litters were discarded. At this time all litters were reduced to 10 pups with as close to equal numbers of male and female pups as possible. Several litters lost 1 or 2 pups during the first postnatal week and all litters were reduced to 8 pups at the end of that week to maintain uniform litter numbers. A total of 20 control and 20 prenatal stress litters were available for the offspring experiments.

Offspring Procedures

One male and 1 female pup were randomly selected from each of 10 control and 10 prenatal stress litters at 9 and 16 days of age. The rats were removed from the cubicles to an adjacent laboratory in groups of 2 or 3 and killed by decapitation within 1 minute of leaving the cubicle. Blood was collected in heparinised tubes while the brain was removed, dissected into cortex, cerebellum, pons-medulla, corpus striatum, hippocampus, hypothalamus and remainder and the parts weighed and frozen in liquid nitrogen. Plasma samples, brain parts and adrenals were finally stored in individual plastic capsules in a freezer kept at −60° until the collection of all tissues was complete. Since the litters were born over a 4 day period, a maximum of 7 pairs of pups were processed in a day. All pups could therefore be killed and dissected within the period of 0800 to 0900 hr, approximately corresponding to the trough of the plasma corticosterone diurnal cycle [21] when the rats were maintained on a 12 hour light 12 hour dark cycle with lights-on at 0900 hr.

The remaining pups were weaned at 22 days of age and kept in groups of 4–5 separated by treatment group and sex. At 23 and 60 days of age 1 male and 1 female were taken from each of 10 control and 10 prenatal stress litters and treated as before. An additional male and female from each litter were used in a response-to-stress test at 23 days only. For this test half of the pups were given a 0.1 ml subcutaneous saline injection immediately after removal from the holding room and exactly 10 minutes before killing. The response to stress was calculated from differences between injected and non-injected litter-mates of the same sex and treatment group.

Biochemical Assays

The brain tissues were analysed for 5-HT, 5-HIAA and NE by a fluorometric procedure combining the methods of Maickel, Cox, Saillant and Miller [14] and Laverty and Taylor [13]. Plasma corticosterone levels were assayed by the method of Silber, Busch and Oslapas [22].

RESULTS

Once daily exposure of pregnant rats to stress during pregnancy had only a slight effect on litter size and birth weight (Table 1). No significant differences were found by t-test whereas an analysis of variance showed significant sex, $F(1,1057)=11.02$, $p<0.001$, and treatment, $F(1,1057)=4.43$, $p<0.05$, effects on birth weight and a significant treatment × sex effect for the average number of pups in a litter, $F(1,160)=4.53$, $p<0.05$. There were no significant treatment or treatment × sex effects on body, brain or adrenal weights when studied at 9, 16, 23 and 60 days of age. The mortality rate was less than 10% between days 2 and weaning in both groups.

The effect of maternal stress on 5-HT, 5-HIAA and NE levels is summarized in Table 2. A preliminary analysis showed no significant treatment × sex effects and the data for males and females were therfore combined. A series of 2-way analyses of variance were conducted on treatment and age for the different brain regions. The hypothalamus

TABLE 2

EFFECT OF PRENATAL MATERNAL STRESS ON 5-HT, 5-HIAA AND NE LEVELS IN BRAIN REGIONS OF THE OFFSPRING

		ng/g wet weight					
		5-HT		5-HIAA		NE	
Region	Age (days)	Control	Stress	Control	Stress	Control	Stress
Hypothalamus	9	642 ± 37	580 ± 57	248 ± 27	176 ± 17*	757 ± 92	551 ± 50*
	16	808 ± 59	599 ± 61†	328 ± 18	352 ± 32	925 ± 77	791 ± 95
	23	1078 ± 112	1248 ± 150	349 ± 14	408 ± 25*	887 ± 48	919 ± 53
	60	1239 ± 51	1397 ± 62*	456 ± 15	479 ± 28	966 ± 29	1266 ± 91†
Cortex	9	223 ± 8	241 ± 16	353 ± 18	376 ± 26	227 ± 11	264 ± 8†
	16	302 ± 17	354 ± 17*	484 ± 21	558 ± 28*	219 ± 6	254 ± 6†
	23	358 ± 19	399 ± 26	498 ± 17	477 ± 21	253 ± 12	251 ± 12
	60	377 ± 16	389 ± 20	483 ± 18	490 ± 16	244 ± 11	241 ± 7
Pons-medulla	9	529 ± 25	513 ± 19	338 ± 16	351 ± 20	284 ± 16	272 ± 14
	16	775 ± 23	906 ± 38†	344 ± 8	366 ± 18	324 ± 13	357 ± 16
	23	857 ± 23	871 ± 44	378 ± 17	332 ± 18	365 ± 15	346 ± 27
	60	901 ± 19	890 ± 35	370 ± 9	355 ± 22	372 ± 15	366 ± 14
Cerebellum	9	216 ± 38	216 ± 49	153 ± 15	153 ± 19	204 ± 24	186 ± 14
	16	334 ± 60	325 ± 27	240 ± 9	256 ± 7	281 ± 27	295 ± 17
	23	328 ± 10	324 ± 23	293 ± 16	287 ± 13	353 ± 31	365 ± 20
	60	322 ± 21	333 ± 24	378 ± 15	395 ± 25	353 ± 17	382 ± 36

Results are Mean ± S.E.M. for groups of 10 male and 10 famale rats.
*$p < 0.05$, †$p < 0.01$ by Newmans-Keuls test.

TABLE 3

RESPONSE TO STRESS IN 23-DAY OLD OFFSPRING OF CONTROL AND STRESSED FEMALE RATS

			Hypothalamus	
Prenatal Treatment	Treatment at 23 days	Plasma Corticosterone (μg%)	NE (ng/g)	5-HIAA (ng/g)
Control	None	6.0 ± 0.4	836 ± 78	349 ± 14
	Stress	13.1 ± 0.9‡	743 ± 39	403 ± 17‡
	Difference‡	+7.2 ± 0.6	−93 ± 24	+54 ± 14
Maternal Stress	None	6.7 ± 0.6	926 ± 56	442 ± 44*
	Stress	18.5 ± 1.5†*	891 ± 45*	436 ± 20
	Difference	+11.8 ± 1.3*	−35 ± 26*	−8 ± 21*

Results are Mean ± S.E.M. for groups of 5 male and 5 female rats. One rat from a pair of littermates was subjected to stress (0.5 ml saline SC) 10 minutes before killing.
*Effect of prenatal stress, $p < 0.05$ by Newmans-Keuls test.
†Effect of saline injection at 23 days of age, $p < 0.05$ by Newmans-Keuls test.
‡Between injected and non-injected littermates of the same sex.

showed significant treatment × age interactions for 5-HT, $F(3,152)=3.10$, $p<0.05$, 5-HIAA, $F(3,152)=4.03$, $p<0.05$, and NE, $F(3,152)=5.03$, $p<0.05$, but no main treatment effects. In contrast, in the cerebral cortex there were significant main treatment effects for 5-HT, $F(1,152)=5.84$, $p<0.05$, and NE, $F(1,152)=6.28$, $p<0.05$, but no significant interactions. Pons-medulla showed only a significant treatment × age interaction for 5-HT, $F(3,152)=3.01$, $p<0.05$.

When analyzed by Newmans-Keuls test 5-HT, 5-HIAA and NE levels were all decreased at 9 or 16 days but increased at 23 or 60 days of age in hypothalamus. Cerebral cortex and the pons-medulla showed early increases in levels but no effect beyond 16 days of age. The remaining 3 regions, cerebellum, corpus striatum and hippocampus showed no statistically significant changes and only data for the cerebellum are included in Table 2 as representative of this group.

The results of the response-to-stress test in 23-day-old offspring are summarized in Table 3. Since 5-HT levels

showed no significant main effects or interactions the 5-HT data are omitted from the table. As before, a preliminary analysis of the results showed no significant treatment × sex interactions and the data for males and females were therefore combined. Two-way analyses of variance were conducted on prenatal maternal stress and postnatal stress (saline injection at 23 days) for plasma corticosterone and hypothalamic 5-HT, 5-HIAA and NE. There were significant prenatal stress effects for plasma corticosterone, $F(1,36)=16.4$, $p<0.001$, 5-HIAA, $F(1,36)=15.8$, $p<0.001$, and NE, $F(1,36)=4.45$, $p<0.05$, but not for 5-HT, $F(1,36)=2.01$. Although only corticosterone showed a significant postnatal stress effect, $F(1,36)=161.0$, $p<0.001$, both plasma corticosterone and hypothalamic 5-HIAA showed a significant prenatal stress × postnatal stress interaction, $F(1,36)=9.70$, $p<0.005$ and $F(1,36)=5.35$, $p<0.05$, respectively.

Matched pairs of rats (same litter and gender) were used for the response-to-stress test. An analysis of the within-pair differences showed that prenatal stress increased the plasma corticosterone response-to-stress but reduced the 5-HIAA and NE responses-to-stress.

DISCUSSION

Exposure of pregnant rats to daily stress treatments resulted in a small (2–3%) but statistically significant decrease in birth weight but had no effect on body, brain or adrenal weights when studied at 9 to 60 days of age. The small and transient effect on body weight is not inconsistent with the suggestion that low levels of stress decrease and high levels of stress increase birth weight with saline injections occupying an intermediate position [10]. The increase in the male-female ratio at birth is of doubtful significance since the average number of pups in a litter in the prenatal stress group did not differ from control values for either males or females separately.

Plaut, Graham and Leiner [19] reported that prenatal maternal handling produced a small decrease in wholebrain 5-HT level (approximately 5% on a μg/g basis) in the 21-day-old offspring. Although we found no significant change in 5-HT level in any of the brain regions studied at a similar age (23 days) a change of only 5% would not have been detectable in the small brain parts used in our study. The failure of Sobrian [24] to find prenatal stress-related changes in 5-HT, 5-HIAA and NE in the brainstem of offspring of electrically shocked females at 1, 12, and 25 days of age may have been due to the different type of stress used. However, we did not study 1-day-old animals and we similarly found no change in most brain regions post-weaning. Even at intermediate ages we found significant changes in only a few brain regions and these changes might have been masked if large brain parts had been studied. Moyer, Herrenkohl and Jacobowitz [17] found significant pre-natal stress-related changes in NE level in 3 of 24 discrete brain regions of adult offspring but a comparison of results is diffficult to make since there were marked differences in technique between their study and the present one. Thus, Moyer *et al.* [17] used a combination of heat, restraint and bright lights as the stress, administered the treatment only on days 14–21 of pregnancy, did not foster the offspring to control mothers at birth and studied the offspring only as adults.

In our study, prenatal stress treatment produced short-lasting increases in 5-HT, 5-HIAA and NE levels in cerebral cortex. The increases appeared to be greatest at about 16 days of age and were no longer present at 23 days of age. Pons-medulla showed a similar pattern. The finding of short-lasting increases in monoamine levels may be consistent with the suggestion that stress (saline injections) accelerates the onset of differentiation of nerve cells in regions known to contain 5-HT terminals [12]. An accelerated development of monoamine neurons might explain the elevated 5-HT, 5-HIAA and NE levels during the period of rapid increase in levels with no effect when near-adult levels had been reached. Furthermore, evidence of an altered time-table of behavioral development during the first 2–3 postnatal weeks is provided by the report [24] that prenatal maternal stress (electric shocks) accelerated the appearance of the peak in spontaneous locomotor activity, peak activity occurring at about 10 days of age compared to approximately 17 days of age in control animals.

In the hypothalamus a different pattern of monoamine changes was found. At 9 or 16 days of age the levels were reduced and showed significant elevations only at 23 or 60 days. It is possible that prenatal stress has a specific effect on the development of the hypothalamic-pituitary-adrenal axis, a suggestion which is supported by our finding of an altered plasma corticosterone response to stress and reported evidence that there may be changes in the hypothalamic control mechanisms for endocrine release following prenatal stress [8, 17, 20]. It is perhaps significant that several parameters of the hypothalamic-pituitary-adrenal axis mature at about 16 days of age [3], approximately the age at which we found a change from reduced to increased monoamine levels.

Further evidence that the postnatal stress treatment had produced a modification of the postnatal stress response came from measurements of the effect of stress on hypothalamic NE, 5-HT and 5-HIAA levels. Both NE and 5-HT-containing neurons appear to be involved in the stress response and subjecting adult rats to stress has been reported to lower 5-HT [18, 25, 27] and NE [4,11] and elevate 5-HIAA [4] levels in hypothalamus in several studies. In our study, a comparatively mild stress at 23 days of age increased 5-HIAA and decreased NE but had no apparent affect of 5-HT in the control offspring. However, the application of stress to the prenatal stress group at the same age appeared to produce smaller changes than in the control group, both when the effects of prenatal and postnatal stress were in the same direction (5-HIAA) and when the effects were opposite (NE). These data suggest that there may be an altered functioning of hypothalamic 5-HT and/or NE neurons in the prenatal stress group which may be associated with the increased plasma corticosterone response to stress.

Our study provides further evidence that prenatal stress can affect the neurological development of the offspring and suggests that the results cannot be attributed to either "litter effects" or to altered postnatal mother-offspring interactions. However, the conclusions from this study are not necessarily applicable to totally different forms of stress and it remains to be established whether other types of maternal stress affect offspring development in a similar way.

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REFERENCES

1. Ader, R. and P. M. Conklin. Handling of pregnant rats: effects on emotionality of their offspring. *Science* **142:** 411–412, 1963.
2. Ader, R. and S. M. Plaut. Effects of prenatal maternal handling and differential housing on offspring emotionality, plasma corticosterone levels and susceptibility to gastric erosions. *Psychosom. Med.* **30:** 267–268, 1968.
3. Allen, C. and J. W. Kendall. Maturation of the circadian rhythm of plasma corticosterone in the rat. *Endocrinology* **80:** 926–930, 1967.
4. Bliss, E. L., J. Ailion and J. Zwanziger. Metabolism of norepinephrine, serotonin and dopamine in the rat brain with stress. *J. Pharmac. exp. Ther.* **163:** 122–134, 1968.
5. Chapman, R. H. and J. M. Stern. Maternal stress and pituitary-adrenal manipulations during pregnancy in rats: effects on morphology and sexual behavior of male offspring. *J. comp. physiol. Psychol.* **92:** 1074–1083, 1978.
6. Chapman, R. H. and J. M. Stern. Failure of severe maternal stress or ACTH during pregnancy to affect emotionality of male rat offspring. Implications of litter effects for prenatal studies. *Devl Psychobiol.* **12:** 255–267, 1979.
7. Dunlap, J. L., J. E. Zadina and G. Gougis. Prenatal stress interacts with prepuberal social isolation to reduce male copulatory behavior. *Physiol. Behav.* **21:** 873–875, 1978.
8. Herrenkohl, L. R. and J. A. Politch. Effects of prenatal stress on the estrous cycle of female offspring as adults. *Experientia* **34:** 1240–1241, 1978.
9. Hockman, C. H. Prenatal maternal stress in the rat: its effects on emotional behavior in the offspring. *J. comp. Physiol.* **54:** 679–684, 1961.
10. Joffe, J. M. Modification of prenatal stress effects in rats by dexamethasone and adrenocorticotrophin. *Physiol. Behav.* **19:** 601–606, 1977.
11. Keim, K. L. and E. B. Sigg. Plasma corticosterone and brain catecholamines in stress: Effect of psychotropic drugs. *Pharmac. Biochem. Behav.* **6:** 79–85, 1977.
12. Lauder, J. M. and H. Krebs. Serotonin as a differentiation signal in early neurogenesis. *Devl Neurosci.* **1:** 15–30, 1978.
13. Laverty, R. and K. M. Taylor. The fluorometric assay of catecholamines and related compounds. *Analyt. Biochem.* **22:** 629–676, 1968.
14. Maickel, R. P., R. H. Cox, J. Saillant and F. P. Miller. A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. *Int. J. Neuropharmac.* **7:** 275–281, 1968.
15. Masterpasqua, F., R. H. Chapman and R. K. Lore. The effects of prenatal psychological stress on the sexual behavior and reactivity of male rats. *Devl Psychobiol.* **9:** 403–411, 1976.
16. Meisel, R. L., G. P. Dohanich and I. L. Ward. Effects of prenatal stress on avoidance acquisition, open-field performance and iordotic behavior in male rats. *Physiol. Behav.* **22:** 527–530, 1979.
17. Moyer, J. A., L. R. Herrenkohl and D. M. Jacobowitz. Stress during pregnancy: effect of catecholamines in discrete brain regions of offspring as adults. *Brain Res.* **144:** 173–178, 1978.
18. Palkovits, M., M. Browstein, J. S. Kizer, J. M. Saavedra and I. J. Kopin. Effect of stress on serotonin concentration and tryptophan hydroxylase activity of brain nuclei. *Neuroendocrinology* **22:** 298–304, 1976.
19. Plaut, S. M., C. W. Graham and K. Y. Leiner. Effects of prenatal maternal handling and rearing with aunts on behavior, brain weight and whole-brain serotonin levels. *Devl Psychobiol.* **5:** 215–221, 1972.
20. Politch, J. A., L. R. Herrenkohl and R. R. Gala. Effects of ether stress on prolactin and corticosterone levels in prenatally stressed male rats as adults. *Physiol. Behav.* **20:** 91–93, 1978.
21. Seggie, J. A. and G. M. Brown. Stress response patterns of plasma corticosterone, prolactin and growth hormone in the rat following handling or exposure to novel environment. *Can. J. Physiol. Pharmac.* **53:** 629–637, 1975.
22. Silber, R. H., R. D. Busch and R. Oslapas. Practical procedure for estimation of corticosterone or hydrocortisone. *Clin. Chem.* **4:** 278–285, 1958.
23. Smith, D. J., J. M. Joffe and G. F. D. Heseltine. Modification of prenatal stress effects in rats by adrenalectomy, dexamethasone and chlorpromazine. *Physiol. Behav.* **15:** 461–469, 1975.
24. Sobrian, S. K. Aversive prenatal stimulation. Effects on behavioral, biochemical and somatic ontogeny in the rat. *Devl Psychobiol.* **10:** 41–51, 1977.
25. Telegdy, G. and I. Vermes. Changes induced by stress in the activity of the serotonergic system in limbic brain structures. In: *Catecholamines and Stress*, edited by E. Usdin, R. Kvetnansky and I. J. Kopin. New York: Pergamon Press, 1976, pp. 145–156.
26. Thompson, W. R. Influence of prenatal maternal anxiety on emotionality in young rats. *Science* **125:** 698–699, 1957.
27. Vermes, I., G. Telegdy and K. Lissak. Effect of midbrain raphe lesion on diurnal and stress-induced changes in serotonin content of discrete regions of the limbic system and the adrenal function in the rat. *Acta physiol. hung.* **45:** 217–224, 1974.
28. Ward, I. L. Prenatal stress feminises and demasculinises the behavior of males. *Science* **175:** 82–84, 1972.