Pharmacology Biochemistry & Behavior, Vol. 35, pp. 943–947. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Maternal Stress Increases Fetal Brain and Neonatal Cerebral Cortex 5-Hydroxytryptamine Synthesis in Rats: A Possible Mechanism by Which Stress Influences Brain Development

DAVID A. V. PETERS

Department of Pharmacology, Faculty of Health Sciences, University of Ottawa
451 Smyth Road, Ottawa, Ontario, Canada, K1H 8M5

Received 17 November 1988

PETERS, D. A. V. *Maternal stress increases fetal brain and neonatal cerebral cortex 5-hydroxytryptamine synthesis in rats: A possible mechanism by which stress influences brain development.* PHARMACOL BIOCHEM BEHAV **35**(4) 943–947, 1990.— Previous studies have shown that maternal stress modifies 5-hydroxytryptamine (5-HT) receptor binding in several brain regions of the adult offspring and alters the intensity of the behavioral responses to 5-HT receptor agonists. We now report that the same stress, crowding combined with daily saline injections during the final week of pregnancy, elevates maternal plasma free tryptophan level without significantly affecting total tryptophan. The increased maternal plasma free tryptophan was associated with significantly increased fetal brain levels of tryptophan, 5-HT and 5-hydroxyindoleacetic acid. These increases were maintained after birth until at least postnatal day 10. Since 5-HT is recognised as having a role in the control of neuron development during the perinatal period, we suggest that the stress-induced increase in fetal brain 5-HT synthesis may play a part in the mechanisms by which prenatal stress alters adult behavior.

5-Hydroxytryptamine Tryptophan Brain development Stress Fetus

SEVERAL authors have suggested that maternal stress can adversely affect human fetal brain development resulting in later behavioral deficits. Animal studies have supported this hypothesis by providing evidence that prenatal stress is capable of significantly altering offspring behavior. Many investigators have reported that periods of restraint under bright lights at an elevated ambient temperature modified several sex-related behaviors in the offspring: it feminized male sexual activity (4, 5, 22, 36, 37), reduced maternal (15,30) and intermale (14) aggression and eliminated the sex difference normally observed in pup-induced maternal behavior (13). However, this stress also reduced maternal and neonatal body weight and increased neonatal mortality (15,30), whereas we have previously reported that even stress procedures which had no effect on body weight or neonatal mortality were capable of producing persistent alterations in offspring behavior. Thus, a prenatal stress consisting of a combination of crowding and once daily saline injections was sufficient to increase open field activity, both the total distance travelled and the total duration of rearing (27). Prenatal crowding alone has also been reported to produce significant behavioral effects in several studies. For example, group housing of pregnant mice influenced sexual behavior of both male and female offspring (1), while offspring of crowded female rats elicited different maternal care than control offspring (23,31).

We have also reported evidence that daily saline injections alone or crowding combined with daily injections during pregnancy altered the development of central serotonergic neurons in the offspring. Transient neonatal changes in 5-hydroxyindole levels in several brain regions (25) were followed by persistent changes in serotonin (5-HT) receptor binding (26,28). The 5-HT receptor alterations appear to be functionally significant since prenatal stress also significantly decreased the intensity of the head weaving, forepaw treading, hindlimb abduction and straub tail produced by treatment of 60–70-day-old offspring with the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (28).

The mechanism by which maternal stress modifies development of the offspring is unknown. However, serotonin is believed to play a role in early brain development [see, for example, (16, 17, 38)] and an increase in fetal brain 5-HT synthesis may be involved as a mediator in at least some effects of prenatal stress. Stress increases brain levels of tryptophan and 5-HT by a mechanism which involves, at least in part, an elevated plasma free tryptophan level (11,12). Some effects of stress in adult rodents appear to be mediated through an increase in brain tryptophan uptake, for example, stress-induced analgesia can be blocked by a procedure which prevents the increase in brain tryptophan (10).

We therefore questioned whether an increase in maternal plasma free tryptophan could also be involved in the mechanisms by which the effects of maternal stress are transmitted to the fetus. If the stress-induced elevation in maternal plasma free tryptophan increases brain tryptophan uptake in the fetus, as it does in the adult (12), then brain 5-HT synthesis would be expected to increase since tryptophan loading has been shown to enhance fetal brain 5-HT synthesis during the last few days of pregnancy (9).

The present study was designed to test whether an increased plasma free tryptophan level induced by maternal stress during the third week of pregnancy is capable of increasing fetal brain tryptophan uptake and 5-HT synthesis. Since changes in food intake could influence the amount of available tryptophan, a stress procedure was selected that did not affect food or water intake but was still capable of increasing plasma corticosterone level at least 2-fold even on the final day of treatment. The stress procedure that we used (once daily saline injections combined with crowding) was selected based on an earlier preliminary study with pregnant rats (D.A.V.P., unpublished data) which showed that this combination of an intermittent stress (injections) with a chronic stress (crowding) reliably elevated plasma corticosterone 2- to 3-fold when measured 15 minutes after the saline injection on the 14th day without affecting food and water intake. Crowding or once daily saline injections alone were less effective in elevating plasma corticosterone.

METHOD

Animal Procedures

The rats were maintained throughout the experiment in temperature- and humidity-controlled rooms under a reverse light cycle (12 hr light, 12 hr dark). Groups of 4 female (220–230 g) and 1 male (320–340 g) Sprague-Dawley rats (Charles River, Montreal) were left together for a single 4-hour period each day. At the end of each period the sperm-positive females were assigned to either control or stress groups. The sperm-positive day was defined as gestation day (GD) 1. The pregnant rats were kept in groups of 2 in standard 20×40 cm polycarbonate cages until gestation day (GD) 16 when the stress treatments commenced. The stress procedure consisted of crowding combined with once daily saline injections, as previously described (29). Rats in the stress group were kept in crowded conditions, 5 rats in each standard cage, and were taken once each day to an adjacent laboratory where each received a 0.1 ml subcutaneous saline injection. The control rats were kept in groups of two in identical cages in a separate quiet room.

For the first experiment 8 control and 8 stress females were killed by decapitation on each of GD's 18, 19, 20 and 21 at the time normally scheduled for the injections. Six fetuses were quickly removed from each rat and a maternal blood sample collected in a heparin-containing tube. The fetal brains were removed, weighed, and frozen in liquid nitrogen in individual plastic capsules. The blood samples were centrifuged at low speed to obtain 1–2 ml plasma samples.

For the second experiment a total of 30 females were mated, 20 for the control group and 10 for the stress group. After the final injections on day 21 the females were transferred to individual breeding cages. Within 12 hours of birth the litters were weighed, culled to 10 pups/litter with as close as possible to equal numbers of males and females, and fostered. Two litter groups were obtained, the control group consisting of 8 litters fostered between control dams, and a prenatal stress group of 8 stress litters transferred to control dams. The remaining litters were born on days when no second litter was available for fostering and were discarded. One male and 1 female pup from each litter were killed on each of postnatal days 0 (day of birth), 2, 5 and 10. The brains were quickly removed and dissected on an ice-cooled glass plate into cortex, cerebellum, pons-medulla, corpus striatum, hippocampus, hypothalamus and a remainder. The brain parts were weighed and frozen in liquid nitrogen in individual plastic capsules. Tissues from both experiments were stored at -80°C until assayed. For this study only cerebral cortex was used; the remaining tissues were stored for other investigations. Cerebral cortex was selected for two reasons. Firstly, in a previous study offspring of pregnant rats given daily saline injections throughout pregnancy showed significant increases in 5-HT and 5-HIAA levels in cerebral cortex at 16 days of age whereas most other regions studied showed no change (25). Secondly, previous data on the effects of the same stress procedure on offspring brain 5-HT receptor binding were obtained using cerebral cortex (28,29).

Biochemical Assays

The fetal whole brains and neonatal cerebral cortices were assayed for tryptophan, tyrosine, 5-HT and 5-HIAA using HPLC with electrochemical detection by a method similar to that described by Wagner *et al.* (34). The HPLC system comprised an LKB dual piston solvent delivery system, manual injector with 20 μl loop, a 10 μm C18 column, LKB model 2143 electrochemical detector and Fisher model 2220 computing integrator-printer. The mobile phase consisted of 20% (v/v) methanol containing 2.75 mM octane sulfonic acid, 0.1 mM EDTA, 0.1 mM NaCl and 0.25 mM triethylamine adjusted to pH 3.30. Briefly, the tissue parts were homogenised in 5 volumes of ice-cold 0.2 M perchloric acid containing 0.26 mM EGTA. The homogenates were centrifuged at $25{,}000 \times g$ for 10 minutes at 4°C and the sample injected through a 0.45 μm nylon membrane filter.

Two 0.1 ml aliquots of each plasma sample were reserved for the assay of plasma whole tryptophan and the remainder subjected to filtration through Amicon Centriflo CF 50 filters to prepare a protein free filtrate for the assay of unbound (free) tryptophan (2). Duplicate samples of both whole plasma and the ultrafiltrate were assayed for tryptophan and tyrosine in the same HPLC equipment by a method similar to that described by Marshall, Kennedy and Eccleston (21). Whole plasma was diluted 20-fold with water before injection while the ultrafiltrate was injected undiluted. The mobile phase consisted of 15% (v/v) methanol containing 75 mM NaH_2PO_4, 0.1 M Na citrate, 0.75 mM octane sulfonic acid and 0.1 mM NaCl adjusted to pH 4.00. Since tyrosine is unbound in plasma, the measured concentrations of tyrosine in the plasma and ultrafiltrate could be used to determine the recovery of free tryptophan in the ultrafiltrate (2).

RESULTS

Offspring body and brain weights were not significantly affected by maternal stress. Measurements taken on GD's 18, 19, 20 and 21, at birth, and on postnatal days 2, 5 and 10 showed all body and brain weights in the stress group to be within the control ranges. There was also no significant stress-related change in the time of birth (control 22.5 ± 0.2 days, stress 22.5 ± 0.3 days).

The effects of stress during the period of GD's 16–21 on maternal plasma tryptophan and tyrosine levels are summarised in Table 1. A two-way ANOVA (treatment × GD) showed a significant treatment effect for plasma free tryptophan, $F(1,40) = 9.29$, $p<0.005$, with no change in either total tryptophan, $F(1,40) = 1.25$, $p>0.05$, or tyrosine, $F(1,40) = 0.92$, $p>0.05$. As expected from experiments with nonpregnant adult rats (6) stress also increased maternal brain 5-HIAA but not 5-HT (data not presented).

TABLE 1

EFFECT OF STRESS ON PLASMA TRYPTOPHAN AND TYROSINE LEVELS IN PREGNANT RATS

		Tryptophan (μg/g)		Tyrosine (μg/g)
		Total	Free	Total
GD 18	Control	33.0 ± 2.7	2.19 ± 0.17	8.58 ± 0.11
	Stress	29.2 ± 1.2	2.75 ± 0.16*	9.03 ± 0.43
		(88)	(126)	(105)
GD 19	Control	29.0 ± 2.9	2.44 ± 0.18	7.31 ± 0.55
	Stress	29.2 ± 3.2	3.17 ± 0.44	6.87 ± 0.34
		(101)	(130)	(94)
GD 20	Control	27.0 ± 2.5	2.50 ± 0.29	7.52 ± 0.61
	Stress	27.3 ± 1.4	4.40 ± 0.68*	9.24 ± 1.24
		(101)	(176)	(123)
GD 21	Control	29.9 ± 3.0	1.70 ± 0.74	3.98 ± 0.74
	Stress	23.6 ± 5.4	2.51 ± 0.59	4.05 ± 0.66
		(79)	(148)	(102)

Groups of 6 pregnant rats were killed by decapitation on gestation days (GD's) 18, 19, 20, and 21. Blood samples were collected in heparinised tubes and plasma obtained by centrifugation. Total tryptophan and tyrosine were assayed in whole plasma and free tryptophan in ultrafiltrates. Results are mean ± s.e.m. The figures in parentheses are the percentages of control values. Two-way ANOVA's showed a significant treatment effect for free tryptophan ($p<0.005$) but not for total tryptophan or tyrosine ($p>0.05$). Student t-test: *$p<0.05$.

TABLE 2

EFFECT OF MATERNAL STRESS ON FETAL BRAIN LEVELS OF TRYPTOPHAN, 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA)

		Tryptophan (μg/g)	5-HT (ng/g)	5-HIAA (ng/g)
GD 18	Control	5.84 ± 0.14	72.8 ± 3.1	66.3 ± 6.3
	Stress	5.79 ± 0.30	73.0 ± 5.2	55.6 ± 5.8
		(99)	(100)	(84)
GD 19	Control	5.58 ± 0.63	80.5 ± 6.6	83.5 ± 7.2
	Stress	8.34 ± 0.70*	109.4 ± 13.0	98.0 ± 6.4
		(149)	(136)	(117)
GD 20	Control	6.68 ± 0.53	86.1 ± 3.2	87.6 ± 3.4
	Stress	8.17 ± 0.51*	122.9 ± 8.0‡	107.9 ± 5.9†
		(122)	(143)	(123)
GD 21	Control	5.23 ± 0.17	92.6 ± 13.8	94.4 ± 7.1
	Stress	5.63 ± 0.28	94.8 ± 15.4	124.2 ± 10.5*
		(108)	(102)	(132)

Groups of 8 pregnant rats were killed on gestation days (GD's) 18, 19, 20, and 21. The fetuses were quickly removed and the brains stored at −80 until assayed for tryptophan, 5-HT and 5-HIAA. The results are mean ± s.e.m. for groups of 8 fetuses each from a different female. Two-way ANOVA's showed significant treatment effects for tryptophan ($p<0.001$), 5-HT ($p<0.05$) and 5-HIAA ($p<0.01$). Student t-test: *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

The stress-induced increase in maternal plasma-free tryptophan was paralleled by increases in fetal brain tryptophan and 5-hydroxyindoles (Table 2). A two-way ANOVA showed a significant treatment effect for tryptophan, $F(1,56)=12.86$, $p<0.001$; 5-HT, $F(1,56)=6.17$, $p<0.05$; and 5-HIAA, $F(1,56)=7.90$, $p<0.01$. Tryptophan and 5-HIAA but not 5-HT also showed a significant treatment × age interaction [tryptophan, $F(3,56)=3.82$, $p<0.05$; 5-HT, $F(3,56)=1.84$, $p>0.05$; 5-HIAA, $F(3,56)=3.26$, $p<0.05$]. When the data were further tested with two-tailed Student t-tests tryptophan was found to be significantly increased on GD's 19 ($t=2.93$, $p<0.02$) and 20 ($t=2.23$, $p<0.05$), 5-HIAA was increased on GD's 20 ($t=2.98$, $p<0.01$) and 21 ($t=2.35$, $p<0.05$), while 5-HT was increased on GD 20 only ($t=4.27$, $p<0.001$).

The increased fetal brain tryptophan and 5-hydroxyindole levels in the prenatal stress group were maintained into the neonatal period (Table 3). When the data were analysed by three-way ANOVA (treatment × age × sex) prenatal stress was found to have increased all three indoles in cerebral cortex [tryptophan, $F(1,112)=47.71$, $p<0.001$; 5-HT, $F(1,112)=48.12$, $p<0.001$; 5-HIAA, $F(1,112)=9.36$, $p<0.005$]. The levels of tryptophan, 5-HT and 5-HIAA also showed a significant age dependence [tryptophan, $F(3,112)=2.96$, $p<0.05$; 5-HT, $F(3,112)=3.11$, $p<0.05$; 5-HIAA, $F(3,112)=15.16$, $p<0.001$]. There was a significant treatment × age interaction for tryptophan, $F(3,112)=2.85$, $p<0.05$, and 5-HT, $F(3,112)=5.80$, $p<0.001$, but not 5-HIAA, $F(3,112)=1.45$, $p>0.05$. When the data for each age were analysed separately by two-way ANOVA (treatment × sex) tryptophan was found to be increased at birth, $F(1,28)=15.55$, $p<0.001$, and on postnatal days 2, $F(1,28)=12.29$, $p<0.001$; 5, $F(1,28)=20.67$, $p<0.001$; and 10, $F(1,28)=5.34$, $p<0.05$. 5-HT levels were increased at birth, $F(1,28)=26.99$, $p<0.001$, and on postnatal days 2, $F(1,28)=8.56$, $p<0.01$; 5, $F(1,28)=6.31$, $p<0.05$; and 10, $F(1,28)=8.89$, $p<0.01$, while 5-HIAA levels were increased on days 2, $F(1,28)=8.62$, $p<0.01$; and 10, $F(1,28)=8.03$, $p<0.05$, but not at birth, $F(1,28)=0.01$, $p>0.05$, or on postnatal day 5, $F(1,28)=1.32$, $p>0.05$.

DISCUSSION

Recent evidence suggests that serotonin plays a role in brain development and may be involved in the mechanisms by which functional links are established between serotonergic neurons and other brain cells during the perinatal period. For example, Whitaker-Azmitia and Azmitia (38) concluded that development of the serotonergic neuron is dependent on a signal which regulates factors originating within the neuron itself and factors in the target tissue and proposed that the signal is 5-HT itself. In addition, Lauder *et al.* (18) have reported that 5-HT axons appear to have a close relationship with some proliferating cells during the period from late gestation and extending into the postnatal period and they suggest that 5-HT neurons may influence the development of less differentiated cells that they contact. Important evidence has come from in vitro studies, usually involving addition of 5-HT or a 5-HT agonist to cultures of fetal or neonatal brain tissue. For example, the ability of a monoamine oxidase inhibitor to reduce growth of fetal raphe cells cocultured with fetal hippocampal cells appeared to be related to the increase in 5-HT (38), while addition of a 5-HT agonist (8-OH-DPAT) to a fetal hypothalamic cell culture resulted in an increase in the number of cells taking up and storing 5-HT (3). In vivo studies have provided supporting evidence. For example, depletion of fetal brain 5-HT with p-chlorophenylalanine was associated with developmental changes in 5-HT terminal fields but not in other brain regions (16,18). It is of interest that the p-chlorophenylalanine-induced depletion of

TABLE 3

EFFECT OF MATERNAL STRESS ON TRYPTOPHAN, 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) LEVELS IN OFFSPRING CEREBRAL CORTEX

Age	Sex	Group	Tryptophan (μg/g)	5-HT (ng/g)	5-HIAA (ng/g)
Birth	Male	Control	4.30 ± 1.36	143 ± 39	137 ± 39
		Stress	7.92 ± 1.60 (184)	289 ± 53* (202)	130 ± 26 (95)
	Female	Control	4.80 ± 0.56	128 ± 16	205 ± 47
		Stress	10.07 ± 0.60‡ (210)	507 ± 75‡ (396)	205 ± 24 (100)
Day 2	Male	Control	4.94 ± 0.39	172 ± 31	257 ± 27
		Stress	7.74 ± 0.61† (157)	300 ± 42* (174)	340 ± 31* (132)
	Female	Control	6.80 ± 0.81	198 ± 22	308 ± 26
		Stress	9.37 ± 1.08 (138)	243 ± 17 (123)	383 ± 23* (124)
Day 5	Male	Control	4.38 ± 0.54	237 ± 42	228 ± 20
		Stress	6.54 ± 0.57* (149)	398 ± 35* (168)	276 ± 34 (121)
	Female	Control	4.55 ± 0.49	244 ± 45	224 ± 34
		Stress	7.13 ± 0.48† (157)	286 ± 39 (117)	264 ± 56 (118)
Day 10	Male	Control	5.72 ± 0.62	258 ± 8	224 ± 31
		Stress	6.66 ± 0.42 (116)	314 ± 19* (122)	306 ± 24 (137)
	Female	Control	5.58 ± 0.46	280 ± 15	229 ± 29
		Stress	7.18 ± 0.66 (129)	358 ± 37 (128)	305 ± 27 (133)

Rat pups were killed either within 6 hours of birth (day 1) or at 2, 5 or 10 days of age. At each age 1 male and 1 female pup were used from each of 8 control and 8 prenatal stress litters. The brains were quickly removed, dissected and stored at −80 until assayed for tryptophan, 5-HT and 5-HIAA by HPLC with electrochemical detection. Three-way ANOVA's showed significant treatment effects for tryptophan ($p<0.001$), 5-HT ($p<0.001$) and 5-HIAA ($p<0.005$). Student t-test: *$p<0.05$; †$p<0.01$; ‡$p<0.001$.

fetal brain 5-HT delayed the timecourse of neuronal genesis in 5-HT projection areas, whereas the stress of vehicle injections, which from our studies could be expected to increase fetal brain 5-HT synthesis, had the opposite effect (16,18). These data suggest that any treatment that affects fetal brain levels of serotonin, including stress, has the potential of influencing brain development, perhaps irreversibly.

Footshock stress in adult rats has been reported to increase plasma free tryptophan concentration, brain tryptophan uptake and brain 5-HIAA with little effect on 5-HT [see discussion in (6)]. Unlike other amino acids tryptophan in plasma is highly protein bound (20) and changes in the proportion of unbound tryptophan can drastically alter the amount that is transported into the brain (33) to become available for synthesis of serotonin. Uptake of unbound plasma tryptophan into brain is also influenced by competition with other neutral amino acids for a common uptake system (24). In the present study maternal stress significantly increased plasma free tryptophan in the pregnant rats without affecting either the total tryptophan or the level of tyrosine, one of the competing amino acids. It is not clear whether stress first elevates plasma free tryptophan which then leads to increased brain 5-HT turnover, or whether stress releases brain 5-HT which then triggers an increased systemic availability of tryptophan (6). Regardless of which mechanism operates the net effect is to increase plasma free tryptophan level and thereby to increase the amount of tryptophan available to the fetal brains.

The stress-induced increase in maternal plasma free tryptophan was accompanied by increases in fetal brain tryptophan, 5-HT and 5-HIAA, consistent with a report that tryptophan loading in pregnant rats elevated both fetal brain hydroxyindoles (9). The tryptophan and 5-hydroxyindole levels in the cerebral cortices of the prenatally stressed rats remained high until postnatal day 10, the latest age studied. However, an earlier study provides evidence that the early postnatal increases in offspring brain 5-hydroxyindole levels are transient; in the offspring of rats stressed throughout pregnancy both 5-HT and 5-HIAA levels in cerebral cortex were elevated at 16 days of age but not at 23 or 60 days (25).

In the rat, raphe neurons show evidence of 5-HT synthesis as early as embryonic day 13 and begin to receive synaptic contacts about 1 week later (19,35). By that time 5-HT fiber ingrowth into the neocortex has begun (35) and growth of 5-HT dendrites is rapid up until the end of the first postnatal week (19). Maternal stress was found to elevate offspring brain 5-hydroxyindoles throughout most of this period and might therefore interfere with synapse formation. Our previous report of prenatal stress-induced changes in brain 5-HT receptor densities on postnatal day 40 with little effect at 16 or 23 days (26) may possibly be explained in terms of stress-induced changes in synaptic connections between 5-HT neurons and adjacent neurons. Brain 5-HT_2 receptor numbers were increased in all regions studied, whereas the numbers of 5-HT_1 sites were either increased, decreased, or unchanged depending on the brain region (26). Although we did not differentiate between subtypes of the 5-HT_1 receptor in our earlier study further evidence suggests that the 5-HT_{1a} subtypes may have been involved. Thus, we reported that maternal stress reduced the intensity of the 5-HT behavioral syndrome in the adult offspring (29) and several components of this syndrome are now believed to originate through an interaction with the 5-HT_{1a} subgroup of receptors (32). Since at least some of the 5-HT_{1a} and 5-HT_2 receptors are located postsynaptically (7,8) there is a possibility that the development of neurons receiving a serotonergic input may have been affected by prenatal stress exposure.

Brain 5-HT is known to be involved in many behaviors and it is tempting to speculate that the prenatal stress-induced changes in brain 5-HT that we have reported in this study and elsewhere (25–29) may be closely related to the behavioral changes. However, we have no clear evidence that changes in any behavior, other than the 5-HT behavioral syndrome, may be mediated by serotonergic neurons.

In summary, this study therefore provides additional evidence that maternal stress affects brain development and suggests that at least part of the mechanism involves a stress-induced elevation of maternal plasma free tryptophan.

ACKNOWLEDGEMENTS

We thank Peter Borodchak for expert technical assistance and the Ontario Mental Health Foundation for financial support.

REFERENCES

1. Allen, T. O.; Haggert, B. N. Group housing of pregnant mice reduces copulatory receptivity of female progeny. Physiol. Behav. 19:61–68; 1977.
2. Bourgoin, S.; Faivre-Bauman, A.; Benda, P.; Glowinski, J.; Hamon, M. Plasma tryptophan and 5-HT metabolism in the CNS of the newborn rat. J. Neurochem. 23:319–327; 1974.
3. De Vitry, F.; Hamon, M.; Catelon, J.; Dubois, M.; Thibault, J. Serotonin initiates and amplifies its own synthesis during mouse central nervous system development. Proc. Natl. Acad. Sci. USA 83:8629–8633; 1986.
4. Dorner, G.; Gotz, F.; Docke, W. D. Prevention of demasculinization and feminization of the brain of prenatally stressed rats by perinatal androgen treatment. Exp. Clin. Endocrinol. 81:88–90; 1983.
5. Dunlap, J. L.; Zadina, J. E.; Gougis, G. Prenatal stress interacts with prepuberal social isolation to reduce male copulatory behavior. Physiol. Behav. 21:873–875; 1978.
6. Dunn, A. J. Changes in plasma and brain tryptophan and brain serotonin and 5-hydroxyindoleacetic acid after footshock stress. Life Sci. 42:1847–1853; 1988.
7. Fischette, C. T.; Nock, B.; Renner, K. Effects of 5,7-dihydroxytryptamine on serotonin$_1$ and serotonin$_2$ receptors throughout the rat central nervous system using quantitative autoradiography. Brain Res. 421:263–279; 1987.
8. Goodwin, G. M.; De Souza, R. J.; Green, A. R.; Heal, D. J. The pharmacology of the behavioural and hypothermic responses of rats to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT). Psychopharmacology (Berlin) 91:506–511; 1987.
9. Howd, R. A.; Nelson, M. F.; Lytle, L. D. L-Tryptophan and rat fetal brain serotonin. Life Sci. 17:803–812; 1975.
10. Kelly, S. J.; Franklin, K. B. J. Evidence that stress augments morphine analgesia by increasing brain tryptophan. Neurosci. Lett. 44:305–310; 1984.
11. Kennett, G. A.; Joseph, M. H. The functional importance of increased brain tryptophan in the serotonergic response to restraint stress. Neuropharmacology 20:39–43; 1981.
12. Knott, P. J.; Curzon, G. Free tryptophan in plasma and brain tryptophan metabolism. Nature 239:452–453; 1972.
13. Kinsley, C. H.; Bridges, R. S. Prenatal stress and maternal behavior in intact virgin rats: response latencies are decreased in males and increased in females. Horm. Behav. 22:76–89; 1988.
14. Kinsley, C.; Svare, B. Prenatal stress reduces intermale aggression in mice. Physiol. Behav. 36:783–786; 1986.
15. Kinsley, C.; Svare, B. Prenatal stress alters maternal aggression in mice. Physiol. Behav. 42:7–14; 1988.
16. Lauder, J. M.; Krebs, H. Serotonin as a differentiation signal in early neurogenesis. Dev. Neurosci. 1:15–30; 1978.
17. Lauder, J. M.; Wallace, J. A.; Krebs, H. Roles for serotonin in neuroembryogenesis. Adv. Exp. Med. Biol. 133:477–506; 1981.
18. Lauder, J. M.; Wallace, J. A.; Krebs, H.; Petrusz, P.; McCarthy, K. In vivo and in vitro development of serotonergic neurons. Brain Res. Bull. 9:605–625; 1982.
19. Lidov, H. G. W.; Molliver, M. E. Immunohistochemical study of the development of serotonergic neurons in the rat CNS. Brain Res. Bull. 9:559–604; 1982.
20. McMenamy, R. H.; Oncley, J. L. The specific binding of L-tryptophan to serum albumin. J. Biol. Chem. 233:1436–1447; 1958.
21. Marshall, E. F.; Kennedy, W. N.; Eccleston, D. Whole blood serotonin and plasma tryptophan using high-pressure liquid chromatography with electrochemical detection. Biochem. Med. Metab. Biol. 37:81–86; 1987.
22. Meisel, R. L.; Dohanich, G. P.; Ward, I. L. Effects of prenatal stress on avoidance acquisition, open field performance and lordotic behavior in male rats. Physiol. Behav. 22:527–530; 1979.
23. Moore, C. L.; Power, K. L. Prenatal stress affects mother-infant interaction in Norway rats. Dev. Psychobiol. 19:235–245; 1986.
24. Perez-Cruet, J.; Chase, T. N.; Murphy, D. L. Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans. Nature 248:693–695; 1974.
25. Peters, D. A. V. Prenatal stress: Effects on brain biogenic amines and plasma corticosterone levels. Pharmacol. Biochem. Behav. 17:721–726; 1982.
26. Peters, D. A. V. Prenatal stress: Effect on development of rat brain serotonergic neurons. Pharmacol. Biochem. Behav. 24:1377–1382; 1986.
27. Peters, D. A. V. Prenatal stress increases the behavioural response to serotonin agonists and alters open field behaviour in the rat. Pharmacol. Biochem. Behav. 25:873–877; 1986.
28. 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. 30:669–673; 1988.
29. Peters, D. A. V. Effects of maternal stress during different gestational periods on the serotonergic system in adult rat offspring. Pharmacol. Biochem. Behav. 31:839–843; 1988.
30. Politch, J. A.; Herrenkohl, L. R. Prenatal stress reduces maternal aggression by mice offspring. Physiol. Behav. 23:415–418; 1979.
31. Power, K. L.; Moore, C. L. Prenatal stress eliminates differential maternal attention to male offspring in the rat. Physiol. Behav. 38:667–671; 1986.
32. Smith, L. M.; Peroutka, S. Differential effects of 5-hydroxytryptamine$_{1a}$ selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. 24:1513–1519; 1986.
33. Tagliamonte, A.; Biggio, G.; Vargiu, L.; Gessa, G. L. Free tryptophan in serum controls brain tryptophan level and serotonin synthesis. Life Sci. 12:277–287; 1973.
34. Wagner, J.; Vitali, P.; Palfreyman, M. G.; Zraika, M.; Huot, S. Simultaneous determination of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, dopamine, 4-hydroxy-3-methoxyphenylalanine, norepinephrine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin and 5-hydroxyindoleacetic acid in rat cerebrospinal fluid and brain by high-performance liquid chromatography with electrochemical detection. J. Neurochem. 38:1241–1254; 1982.
35. Wallace, J. A.; Lauder, J. M. Development of the serotonergic system in the rat embryo: An immunocytochemical study. Brain Res. Bull. 10:459–479; 1983.
36. Ward, I. L. Exogenous androgen activates female behavior in non-copulating, prenatally stressed rats. J. Comp. Physiol. Psychol. 91:465–471; 1977.
37. Ward, I. L. Prenatal stress feminises and demasculinises the behaviour of males. Science 175:82–84; 1972.
38. Whitaker-Azmitia, P. M.; Azmitia, E. C. Autoregulation of fetal serotonergic neural development: Role of high affinity serotonin receptors. Neurosci. Lett. 67:307–312; 1986.