Effect of Emotional Stress Experienced by Female Rats before Pregnancy on Brain Development in Their Offspring

B. Ya. Ryzhavskii, T. V. Sokolova, R. V. Uchakina, Yu. I. Fel'dsherov, Yu. A. Sapozhnikov, E. V. Vasil'eva, and I. R. Eremenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 6, pp. 146-150, June, 2002
Original article submitted March 29, 2002

Twenty-one- and 40-day-old offspring from female rats exposed to emotional stress for 3 weeks and mated with intact males 10 days later were examined. Intact female offspring served as the control. The weight of the brain of 21- and 40-day-old experimental rats varied is a wider range compared to the control. In 40-day-old experimental males, the mean weights of the brain and hemisphere were lower than in controls. In 21-day-old rats, layer V neuronal nuclei were enlarged, while the thickness of the parietal cortex tended to decrease. In 40-day-old animals, morphometric parameters of neurons in the neocortex and hippocampus and RNA concentration in their cytoplasm did not differ from the control. Thirty-day-old experimental rats demonstrated low exploratory activity in the plus-maze test.

Key Words: *brain; development; stress; pregnancy; morphometry*

Stress during pregnancy causes developmental and behavioral changes in the offspring. Prenatally stressed rats are characterized by increased corticotropin content in the amygdala, decreased number of glucocorticoid receptors in the hippocampus, irregular sexual cycles, and changes in anxiety, motor activity, and aggressiveness [1,3,5-9,11,13]. It is possible that the effects of prenatal stress are realized mainly via modulation of functional activity of endocrine glands both in pregnant females and fetuses [5,7]. As was shown earlier, stress during the third trimester changes morphometric parameters of the whole brain, neocortex, and hippocampus, behavioral reactions in the plus-maze test, and histophysiology of the gonads and adrenal cortex [8]. In the present study we examined morphometric and histochemical parameters of the brain, adrenals, and gonads in the offspring of female rats exposed to long-term emotional stress before pregnancy.

Department of Histology, Department of Normal Physiology, Far Eastern State Medical University; Laboratory of Biochemistry, Institute of Maternity and Child Protection, Siberian Division of the Russian Academy of Medical Sciences, Vladivostok

MATERIALS AND METHODS

The study was carried out on 21- and 40-day-old rats from intact females and females subjected to 3-week emotional stress [2]. The stress was reproduced as follows: the animals in cages with glass covers were daily (5 days a week, 6 h per day, from 9.00 to 15.00) exposed to bright light emitted by 3 luminescent lamps located 90-cm above the floor.

Twenty-one-day-old offspring from 3 (n=32) and 4 (n=41) litters of control and experimental females, respectively, and 40-day-old offspring from 4 intact (n=26) and 5 experimental (n=33) litters were studied. Before and during pregnancy, the control and experimental rats and their offspring were kept in the same vivarium. Twenty one- and 40-day-old rats were killed by decapitation.

In 1-month-old rats, the integral exploratory activity was tested in an elevated plus maze. The time and number of acts (sniffing, head dippings, rearings, grooming, movements, and entries into open and closed arms) were considered [12].

Body weight was determined in 1-day-old animals and the brains, hemispheres, adrenal glands and gonads

0007-4888/02/1342-0126$27.00

TABLE 1. Parameters of 21- and 40-Day-Old Offspring from Intact Female Rats and Rats Exposed to Emotional Stress before Pregnancy (*M*±*m*)

Parameter	21-day-old rats				40-day-old rats			
	control	experiment	males	females	control	experiment	males	females
Body weight, g	26.0±1.5	22.3±0.9	23.0±2.3	23.0±2.9	91.7±3.3	82.0±5.4	74±3*	85.0±5.2
Weight of the brain, mg	1284±13	1223±12	1210±49	1167±50	1605±18	1551±20	1511±28*	1538±23.6
Weight of the hemisphere, mg	450.0±7.7	436.0±8.8	434±21	447±20	580.0±9.5	553.0±15.4	549.0±6.6*	561.0±10.6
Weight of the gonad, mg	64.0±2.5	5.9±0.5	48.0±8.8	4.6±0.4*	481.0±19.6	16.7±1.8	399.0±17.5*	19.1±1.8
Weight of the adrenal gland, mg	3.60±0.24	4.00±0.22	3.6±0.6	3.4±0.4	11.4±0.4	11.1±0.5	12.00±0.59	13.0±0.8*
Concentration								
testosterone, nmol/liter					3.6±1.16		2.5±0.89	
estradiol, pmol/liter						108.0±6.2		80.0±7.9*
progesterone, pmol/liter						4.40±1.29		5.80±2.06
StDH activity, arb. units.								
ZG	345±27	358±29	238±39*	332±52	440±24	421±37	415±33	429±28
ZF	441±36	487±22	518±58	412±31	692±48	715±60	740±38	788±33
ZR	590±34	579±29	600±43	512±55	510±35	499±44	641±34*	697±26*
ZG/ZF	0.86±0.09	0.78±0.07	0.54±0.13*	0.83±0.15	0.670±0.048	0.63.00±0.07	0.560±0.038	0.55±0.03
ZG/ZR	0.64±0.07	0.62±0.04	0.44±0.09	0.71±0.14	0.89±0.05	0.9±0.09	0.650±0.043*	0.620±0.036*

Note. Here and in Table 2: *p<0.05 compared to the control.

were weighted after sacrifice. Paraffin sections (7-μ) of the parietal cortex of the left hemisphere were stained with gallocyanine for nucleic acids and analyzed morphometrically. RNA concentration in the cytoplasm of neocortical and hippocampal neurons was determined [8]. The right hemisphere and the cerebellum of 40-day-old rats were used for preparing 25-μ cryostat sections, which were stained with sudan black for evaluation of myelinization.

Activity of 3β-ol-steroid dehydrogenase (StDH) [4] in the zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR) of the adrenal cortex was measured on cryostat sections using a MECOS computer complex (Medical Computer Systems) and Morphodensitometry software [8]. In each case, the ratios of StDH activity in ZG to that in ZF (ZG/ZF) and ZR (ZG/ZR) were determined. These parameters reflected the correlation between production of various steroid hormones in the adrenal cortex. Plasma concentrations of testosterone (in males), progesterone, and estradiol (in females) were determined in 40-day-old rats by immunoenzyme assay.

The obtained results were processed on a computer using Statistica software.

RESULTS

No significant differences were found in the body weight of 1-day-old experimental and control rats (5.8±0.2 and 6.0±0.1 g, respectively). However, in the experimental group this parameter varied in a wider range (3.3-7.6 *vs*. 4.5-7.1 g in the control).

The weights of male and female gonads in 21-day-old offspring of stressed rats were lower than in age-matched controls. The males showed low StDH activity in ZG adrenocorticocytes; ZG/ZF, and ZG/ZR ratios also decreased. In 40-day-old male offspring of stressed females the body weight and weight of the testis were significantly lower than in controls. In experimental females the plasma concentration of progesterone increased and that of estradiol decreased. Plasma levels of testosterone in experimental and control males were similar (Table 1). Experimental males and females showed increased StDH activity in ZG of the adrenal cortex and decreased ZG/ZF and ZG/ZR ratios (Table 1). Thus, both 21- and 40-day-old experimental rats showed changes in the synthesis of hormones belonging to different corticosteroid groups and producing different effects. In 40-day-old animals, these changes were associated with higher weight of the adrenals (Table 1). These results agree with the data on changes in the hypothalamic-pituitary-adrenal system in the offspring of rats exposed to stress during pregnancy [8,10,13].

No significant differences in the brain weight were noted in experimental 21-day-old males and females (Table 1). However, this parameter in experimental animals varied in a wider range than in the control group: 920-1410 and 100-1470 mg in males and females, respectively, compared to 1200-1410 and 1110-1320 mg in control males and females, respectively. Thus, experimental group included animals with extremely low brain weight and high brain weight surpassing the maximum value of the control group. In

TABLE 2. Morphometric Parameters of Brain in 21- and 40-Day-Old Rats from Mothers Exposed to Emotional Stress before Pregnancy (*M±m*)

Parameter	21-day-old rats		40-day-old rats	
	control	experiment	control	experiment
Thickness of the cortex, μ	851±52	758±35	1113±13	1100±17
Area of neuronal cytoplasm, μ^2				
layer V	108.0±4.4	109.0±8.8	108.0±5.4	121±6
layer II	60.0±5.4	64.0±4.1	70.0±3.2	70.0±2.8
hippocampus	76.0±4.4	82.0±5.4	104.0±6.6	107.0±7.1
Area of neuronal nuclei, μ^2				
layer V	89.0±2.6	103±3*	121.0±4.2	120.0±4.1
layer II	68.0±4.1	68.0±2.1	95.0±3.4	90.0±3.9
hippocampus	88.0±2.6	103.0±9.3	126.0±4.4	128.0±4.6
RNA concentration in neuronal cytoplasm, arb. units.				
layer V	223.0±8.3	233.0±11.2	223.0±8.4	222.0±7.7
layer II	296±14	288.0±12.3	197.0±9.5	210.0±5.6
hippocampus	267.0±23.4	268.0±23.3	224.0±6.1	218.0±8.6

40-day-old experimental males, the weight of the brain was significantly decreased (Table 1). Similarly to 21-day-old rats, variability of this parameter was higher in the experimental group: the differences between the minimum and maximum values were 380 and 400 mg in females and males, respectively, compared to 230 and 260 mg in control females and males, respectively. It should be noted that in 40-day-old experimental animals the minimum values were lower and the maximum values were higher than in the control rats. Experimental animals showed higher brain weight variability in the same litter and between litters. This suggests that the consequences of stress could not be adequately characterized by differences between means.

Morphometric parameters of the brain cortex showed no significant sexual differences in the experimental and control groups and, therefore, the data were united. Lower thickness of the parietal cortex was noted in 21-day-old experimental rats, however, this difference was insignificant. Neurons of layer V parietal cortex and their nuclei were enlarged in 21-day-old experimental animals. In 40-day-old experimental and control animals, the sizes of neurons, their cytoplasm, and nuclei in layers II and V of the parietal cortex and hippocampus were similar. RNA concentration in these cells did not differ significantly (Table 2). The intensity of sudan black staining was lower in experimental animals in the white matter of both hemispheres (344±18 vs. 386±22 arb. units in the control) and cerebellum (469±20 vs. 683±28 arb. units in the control, $p<0.05$). This can be interpreted as a decreased rate of myelinazation in the offspring of stressed females.

Plus-maze experiments revealed significant intergroup differences: the number of rearings and sniffings in the experimental group was 2.9-fold and by 15.3% lower than in control. These differences were equally pronounced in males and females and attested to decreased exploratory activity in experimental rats by the end of suckling period. These changes were opposite to those observed in the offspring of rats exposed to stress during the third trimester of pregnancy [8]. This discrepancy was associated with different changes in morphometric parameters of the neocortex and hippocampus, which were numerous and highly pronounced in the prenatally stressed rats and were practically absent in the offspring of rats stressed before pregnancy.

In the whole, the obtained data suggest that long-term emotional stress before pregnancy causes changes in the offspring manifested in changes in the morphological and functional parameters of the brain, and histophysiology of the gonads and adrenal cortex. The increased variability of the brain weight and the appearance of animals with significant deviations from the mean value deserve special attention. These findings suggest that changes in the offspring of animals exposed to stress during [8] and before pregnancy are not identical. At the same time, in both cases, stress-induced consequences were stable and persisted at the end of suckling period and in the prepuberty. These changes involved important parameters of the brain, gonads, and adrenal cortex.

REFERENCES

1. A. S. Batuev, E. P. Vinogradova, and O. N. Polyakova, *Zh. Vyssh. Nervn. Deyat.*, **46**, No. 3, 558-563 (1996).
2. E. V. Emel'yanov, *Ecological and Morphological Studies of Early Mammalian Ontogenesis* [in Russian], Moscow (1984), pp. 5-39.
3. I. N. Zaitshenko, F. I. Proimina, and F. I. Ordyan, *Zh. Vyssh. Nervn. Deyat.*, **49**, No. 1, 106-112 (1999).
4. Z. Loida, R. Gossrau, and T. Shibler, *Enzyme Histochemistry* [in Russian], Moscow (1982).
5. M. S. Mitskevich, *Hormonal Regulation in Animal Ontogenesis* [in Russian], Moscow (1978).
6. N. D. Nosenko, *Fiziol. Zh.*, **82** ,No. 4, 46-52 (1996).
7. B. Ya. Ryzhavskii, *Zh. Vyssh. Nervn. Deyat.*, **50**, 1046-1054 (2000).
8. B. Ya. Ryzhavskii, T. V. Sokolova, Yu. I. Fel'dsherov, *et al., Byull. Eksp. Biol. Med.*, **132**, No. 8, 149-152 (2001).
9. L. V. Tarasenko, P. V. Sinitsin, and A. V. Reznikov, *Fiziol. Zh.*, **82**, No. 4, 39-45 (1996).
10. J. C. Day, M. Koehi, V. Deroche, *et al., J. Neurosci.*, **18**, No. 5, 1886-1892 (1998).
11. R. Diaz, K. Fuxe, and S. O. Orgen, *Neuroscience*, **81**, No. 1, 129-140 (1997).
12. S. Pellow, P. Chopin, and S. E. File, *J. Neurosci. Methods*, **14**, No. 3, 149-167 (1985).
13. M. Weinsctock, *Neurosci. Biobehav. Rev.*, **21**, No. 1, 1-10 (1997).