Effect of Emotional Stress in Pregnant Rats on Brain Development of Their Progeny

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We compared 21- and 40-day old rat pups from rats subjected to emotional stress in trimester III and from intact females. The width of the parietal cortex and the size of layer V neuron cytoplasm and nuclei in the anterior parietal lobe and hippocampus were increased in 21-day-old test pups compared to age-matched controls. In 40-day-old test pups, neurons in layers II and V of the parietal lobe and layer II of the anterior parietal lobe and hippocampal neurons were enlarged compared to the control..

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

Stress stimulation of pregnant rats can induce somatic and behavioral disorders in their progeny [1-3,5,7-12,14]. The prenatally stressed mature rats has higher content of ACTH in the amygdala and increased number of glucocorticoid receptors in the hippocampus [14]. Stress stimulation of pregnant rats disturbs estrous cycle, decreases anxiety level, enhances locomotor activity in female offspring, and increases aggressiveness in male offspring [1], modulates gonadotropic function of the pituitary [9], eliminates sex-related differences in catecholamine content [5], modifies the response to moderate stress in mature offspring [11]. There is evidence that stress-induced changes in functional activity of endocrine glands are an important mechanism underlying the effect of stress on the development of progeny [10,12]. It is noteworthy that not only maternal, but also fetal endocrine glands respond to stress [3,8]. However, morphological changes in the brain of the progeny of stressed rats are little studied, probably, because of the absence of crude structural alterations in these cases. Quantitative analysis reflecting various aspects of brain development can help to these peculiarities. The present study based on morphometric techniques was carried out using a MEKOS complex (Medical Computer Systems).

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MATERIALS AND METHODS

Experiments were carried out on 21- and 40-day-old pups from intact rats (control) and rats subjected to a 5-day emotional stress during trimester III. The stress was modeled as described previously [2]: pregnant rats were daily placed under a ventilated bell glass (floor area of about 300 cm^2) for 1 hour (from 13:00 to 14:00). The area was illuminated with three 100 W lamps placed at the distance of 50 cm from the floor. We examined 21-day-old pups from intact (5 litters, n=32) and stressed females (4 litters, n=23) and 40-day-old progeny of intact (4 litters, n=26) and stressed (5 litters, n=30) females. Before and during pregnancy, the control and test rats and their progeny were maintained under standard vivarium conditions. Integral exploratory activity in an elevated plus-maze was assessed in 1-moth-old rats. The duration and the number of sniffings, hang down movements, rearings, grooming reactions, motor activity, and entries into the open and closed arms were evaluated [13]. The body weight and the weights of the brain, hemisphere, adrenal glands, and gonads were determined. Blood plasma

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TABLE 1. Parameters of 21- and 40-Day-Old Progeny from Rats Subjected to Emotional Stress during Pregnancy (*M*±*m*)

Index	21-day-old				40-day-old			
	control		test		control		test	
	males	females	males	females	males	females	males	females
Body weight, g	31.2±0.6	30.5±0.9	37.2±1.8*	38.1±2.7*	94±2.9	91±2.6	108±4.6*	92±1.9
Brain weight, mg	1329.0±11.2	1279.0±16.8	1352.0±16.2	1338±22*	1635.0±18.5	1579.0±18.0	1618.0±23.5	1497±23*
mg/g body weight		42.80±0.87	42.00±0.95 16.20±0.54*	37.3±1.4*	37.4±1.4*	17.5±0.4	17.50±0.47	15.3±0.6*
Hemisphere weight, mg	470.0±6.4	451.0±8.6	492.0±9.9	480.0±10.1	580±16	544.0±10.7	573.0±14.6	513.0±6.4
Testosterone, nM/liter	—	—	—	—	2.3±0.6	—	2.6±0.8	—
Progesterone, nM/liter	—	—	—	—	—	3.9±0.7	—	3.95±1.20
Weight of gonads, mg	86.0±3.1	8.3±0.4	103.0±6.9*	11.0±1.7	450±57	15.8±1.2	486±29	25.1±1.2*
Diameter of follicle, μ	—	442±28	—	512±35	—	574±22	—	645±36
Diameter of seminiferous tubule, μ	92.0±6.3	—	125.0±5.5*	—	195±12	—	226±11	—
Weight of adrenals, mg	5.4±0.2	5.1±0.4	6.4±0.5	7.6±0.7*	12.0±0.7	11.6±0.6	13.0±0.7	11.4±0.6
3β-HSD activity, arb. units:								
zone glomerulosa	223±21	198±17	281±27	294±44*	301±60	423±48	364±40	451±33
zone fasciculata	366±36	320±34	440±38	479±40*	401±63	452±38	403±27	413±29
zone reticularis	495±32	435±40	548±32	577±50*	364±48	406±31	407±37	417±34
follicular theca	—	410±31	—	526±35*	—	430±31	—	487±30

Note. Here and in Table 2: *significant differences from the control.

testosterone (in males) and progesterone (in females) were determined by enzyme immunoassay in 40-day-old rats. Paraffin sections (7 μ) of brain hemispheres were stained for nucleic acids with gallocyanine, and analyzed morphometrically. The width of the cortex in the anterior parietal lobe (APL) and parietal lobe (PL) was determined using a MOB-15 ocular micrometer. The cross-section areas of the cytoplasm and nuclei of pyramidal neurons in cortical layers II and V and in hippocampal field II were measured using a MEKOS complex and RNA content in the cytoplasm of these cells was determined (25 cells in at least 5 visual fields was examined in each structure). Activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) was measured histochemically [4] on cryostat sections of the adrenals (in different zones of the adrenal cortex), ovaries (theca), and testicles using a MEKOS complex (25 cells in each structure). In the same preparations, the width of the adrenal cortex, diameter of the largest ovarian follicle, and the mean diameter of seminiferous tubules were measured. These indices were analyzed using Statistica software.

RESULTS

Twenty-one-day-old progeny of stressed rats had higher indices of body weight, gonads, and adrenal glands compared to control rats. The with of the adrenal cortex, the mean diameter of seminiferous tubules, the diameter of the largest ovarian follicle (insignificantly) also increased in this group compared to the age-matched control. Activity of 3β-HSD in the theca of ovarian follicles and all zones of the adrenal cortex was higher than in controls (significant differences were observed only in females, Table 1). The body weight of 40-day-old test males significantly surpassed the control value, while in females no difference in this index was observed between the experimental and control groups. The differences in 3β-HSD activity in the adrenal cortex, weight of testicles, diameter of seminiferous tubules disappeared; plasma concentrations of testosterone in males of both groups were practically the same. The weights of the ovaries in the progeny of stressed rats surpassed the control values, the diameter of the largest follicle and 3β-HSD activity in

TABLE 2. Morphometrical Indices of the Brain in 21- and 40-Day-Old Progeny from Rats Subjected to Emotional Stress during Pregnancy (*M*±*m*)

Index		21-day-old		40-day-old	
		control	test	control	test
Cortex width, μ	APL	1311±35	1369±45	1588±37	1563.0±21.3
	PL	895.0±27.2	980.0±11.6*	1043.0±20.7	1041.0±19.2
Neuronal cytoplasm area, μ^2					
layer V	APL	105.0±6.5	124.0±4.8*	136.0±6.5	139.0±6.3
	PL	112.0±4.7	92.0±4.4*	115.0±3.8	115.0±4.6
layer II	APL	62.0±2.8	67.0±2.6	71±3	70.0±2.2
	PL	57.0±1.5	63.0±2.4	68.0±2.4	62.0±1.7
hippocamp		65.0±2.6	81.0±2.8*	88.0±3.5	110.0±5.7*
Neuronal nucleus area, μ^2					
layer V	APL	103.0±4.2	110.0±3.5	111.0±3.4	109.0±3.2
	PL	89.0±2.6	103±3*	87.0±2.3	93.0±1.9*
layer II	APL	86.0±3.8	80.0±2.5	78.0±1.6	109.0±3.2*
	PL	68.0±4.1	68.0±2.1	65.0±1.6	93.0±1.9*
hippocampus		83.0±2.8	100.0±3.6*	85.0±3.3	102.0±2.1*
RNA concentration in cytoplasm, arb. units					
layer V	APL	241.0±7.8	234.0±9.3	229.0±7.6	240.0±6.3
	PL	243.0±7.3	233.0±11.2	214.0±10.2	209.0±7.2
layer II	APL	204.0±9.1	234.0±11.5*	234.0±8.0	241.0±8.8
	PL	296±14	288.0±12.3	197.0±9.5	210.0±5.6
hippocampus		275.0±17.5	260.0±12.2	235.0±11.9	213±10

thecocytes also increased ($p>0.05$). Progesterone concentration did not differ in both groups (Table 1). The absence of the parallelism in deviations of morphologic indices and progesterone level seems can be explained by the fact that corpora luteum, the main source of progesterone in the body, are formed only after sexual maturation.

The absolute brain weight in 21-day-old test rats surpassed the control value (difference was significant only for females), but the relative brain weight decreased in both male and female test rats (Table 1). There was no sex-related difference in brain morphometric indices in both the control and test groups, therefore the data obtained on males and females were pooled (Table 2). The width of PL cortex and the size of cytoplasm in layer V of the APL cortex and hippocampal neurons increased in 21-day-old test rats. Hippocampal neurons had also enlarged nuclei. The size of the cytoplasm of layer V neurons in the PL cortex decreased, while the size of their nuclei increased. However, the size of neurons did not change, and nucleus/cytoplasm ratio increased. Most studied parameters reflecting somatic development and the growth of endocrine glands in 40-day-old test rats did not significantly differ from the control (Table 1); however the absolute (females) and relative (males and females) brain weights in these animals were below the control values, neurons in layers II and V of the PL cortex and layer II of APL cortex had enlarged nuclei, and hippocampal neurons had enlarged nuclei and cytoplasm (Table 2).

Behavioral reactions of 30-day-old control and test rats in an elevated plus maze significantly differed: prenatally stressed rats exhibited higher horizontal activity (by 2.35 times), the number of rearings and sniffins surpassed the control values by 58% and 25.9%. These differences attested to enhanced motor and exploratory activity of test rats at the end of the suckling period.

The peculiarities of 21-day-old test rats indicate that the revealed differences of their brain are essential parts of other differences, which include the signs of more rapid somatic and sexual development. The peculiarities of brain morphology attest to accelerated development and enhanced functional activity of cortical neurons in prenatally stressed rats. It is noteworthy that deviations in indices characterizing neurons were different in functionally different cortical zones and layers. Some differences in morphometric parameters of cortical neurons persisted in 40-day-old rats, whereas many differences in somatic development observed in 21-day-old rats disappeared at this term (Table 2). As a whole, the revealed differences in the brain of progeny of stressed rats can be considered as an element of morphological equivalent of previously described functional features of the brain in these animals. We believe that our data on the dynamics of body weight, development and histophysiology of the adrenals and gonads will help to elucidate the mechanisms underlying changes in the brain of prenatally stressed animals.

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