Effect of the Age of Pregnant Females on Brain Development in the Offspring

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We examined offspring of 9-10 and 3.5-4 month-old female rats. Female offspring (14, 21, 28, 35, and 40 days) of old rats had higher body weight than offspring of young animals. No intergroup differences were revealed in the body weight of male offspring. At the age of 40 days the offspring of old females differed from the offspring of young rats by higher absolute weight of the brain (females), lower size of ganglionic neurons in the parietal lobe (males and females), and lower blood testosterone concentration (males). Thirty-day-old offspring of old rats exhibited higher locomotor activity and lower degree of anxiety compared to the offspring of young animals.

Key Words: *brain; offspring; maternal age; hormones*

The "maternal factor" determines embryogenesis of the offspring. The endocrine status of pregnant females depends on a variety of factors, including age [1,3,4,8,9]. In humans pregnancy can occur in age interval exceeding 30 years. It should be emphasized that the concentration of estrogens, gonadotropins, and corticosteroids and histophysiological characteristics of the ovaries and adrenal cortex are characterized by age-related changes [6,7]. Changes in the concentration of hormones during pregnancy also depend on age [1]. Age-related peculiarities of the endocrine status in female rats aging 9-10 and 3.5-4 months determine the differences between 1-day-old rat pups. The offspring of old females differs in a higher absolute and relative weight of the brain, greater thickness of the cortex, and lower density of neurons. These signs reflect accelerated brain development [5]. Here we studied the dependence of brain development in the 40-day-old offspring on the age of female rats.

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MATERIALS AND METHODS

We examined offspring of female rats aging 9-10 (old animals) or 3.5-4 months (young animals) and 4-5-month-old males. Group 1 and 2 animals produced 33 and 19 pups, respectively. Adult rats and offspring were kept in a vivarium and received food and water *ad libitum*. Body weight was measured at the age of 14, 21, 28, 35, and 40 days. Behavioral activity of 30-day-old animals was studied in the elevated plus-maze (EPM) test for 3 min. The duration and rate of overhanging and vertical rearing postures, grooming, nuzzling, ambulations, and entries into open and closed arms were recorded on a computer using special software. The animals were decapitated on day 40 of life. Enzyme immunoassay was used to measure the concentration of blood testosterone and estradiol in males and females, respectively. The left hemisphere was fixed with Carnoy fluid. The anteroparietal and parietal lobes were prepared and embedded in paraffin. Paraffin sections perpendicular to the long axis of the left hemisphere and its surface (7 μ) were stained with gallocyanin for nucleic acids (method of Einarson).

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NADH diaphorase activity reflecting the intensity of oxidative processes in mitochondria was measured on cryostat sections of the anteroparietal lobe in the right hemisphere [2]. The width of the cortex and molecular layer was measured using a MOB-15 ocular micrometer. The size of neurons, nuclei, and cytoplasm, RNA concentration in the cytoplasm of layer V cortical neurons in the anteroparietal and parietal lobe, and NADH diaphorase activity were measured on a MECOS device. We examined 25 neurons in each area of the

TABLE 1. Effect of Age of Female Rats on the Development of Offspring

Index	Offspring of old females		Offspring of young females	
	males	females	males	females
Body weight, g				
14-day-old	21.0±1.3	25.0±1.1*+	22.0±1.6	20±2
21-day-old	36.0±2.8	38.0±1.5*	36.0±1.8	30.0±2.4+
28-day-old	56.0±3.7	58.0±1.6*	51.0±2.6	42±3+
35-day-old	82.0±6.4	84.0±2.6*	78.0±2.8	65±4+
40-day-old	96.0±7.8	101±4*	98.0±2.9	86.0±2.2+
Concentration				
testosterone, nmol/liter	1.76±0.34*		5.4±1.5	
estradiol, pg/ml		7.50±1.67		10.2±2.1
Weight, mg				
testes	366±63		409±31	
ovaries		12.1±1.2*		8.8±0.4
brain absolute, mg	1557±39	1552.0±17.1*	1561.0±12.7	1483.0±24.6
relative, mg/g	17.2±1.3	15.6±0.5*	16.1±0.58	17.2±0.3
cerebellum, mg	197.0±8.8	195±5	195.0±4.1	187±9
Width of cortex, μ				
parietal lobe	1141±26	1124±31	1107±22	1129±45
anteroparietal lobe	1711±25	1657±37	1626±46	1613±57
Width of cortical layer I, μ				
parietal lobt	147±6	145±6	142±8	153±11
anteroparietal lobe	146±7	145±5	149±8	146±12
Section area, μ^2				
neurons in the parietal lobe	161±9*	160±5*	189±6	199±13
nuclei	92±5	88±6	95±5	94±7
cytoplasm	69±7*	71±5*	94±5	105±12
neurons in the anteroparietal lobe	240±9	245±19	237±13	221±16
nuclei	120±7	124±7	119±8	110±8
cytoplasm	120±9	123±14	117±8	111±8
NADH diaphorase activity, arb. units	462±65	440±43	478±68	453±52
Time, sec				
overhanging	6.3±1.8*	4.6±1.3	0.38±0.20	1.6±1.0
rearing postures	8.9±1.4	7.3±1.7	9.1±2.0	9.7±3.8
grooming	7.9±1.7	8.4±2.0	10.6±2.0	14.9±4.4
nuzzling	87.0±2.9	83.0±3.3	79.0±3.1	81.0±5.7
ambulations	17.0±2.1*	13.3±1.3	10.2±1.2	14.0±2.5
open arms	19.0±3.7*	14.7±2.5	3.7±1.0	9.8±3.7
closed arms	79.0±3.6*	84.3±2.6	96±1	89.4±2.8

Note. $p<0.05$: *compared to offspring of young females; +compared to males.

cortex. The results were analyzed using Statistica 5.0 software.

RESULTS

Body weight in the offspring of young females was characterized by sexual dimorphism. In all periods of life, males had higher body weight compared to females. In 14-day-old offspring of old rats, body weight of males significantly exceeded that of females. Female offspring of old rats had higher body weight compared to the offspring of young animals. No differences were revealed in body weight of male offspring (Table 1). Body weight gain was pronounced in female offspring of old rats, which probably determined the absence of sexual dimorphism in animals of this group (Table 1).

Significant intergroup differences were revealed in the absolute weight of the brain in 40-day-old females (offspring of old rats had higher brain weight compared to offspring of young animals). These differences were absent in males. We found no intergroup differences in the weight of the cerebellum in males and females (Table 1). The width of the cortex and molecular layer in the parietal and anteroparietal love did not differ in animals of different groups. In the offspring of old rats the size of pyramidal neurons in the ganglionic layer of the parietal lobe was lower than in the offspring of young animals. Significant intergroup differences were found in the cytoplasm area in males and females (36 and 48%, respectively). RNA concentration in the cytoplasm of neurons did not differ in male and female offspring of old (283±24 and 275±49 arb. units, respectively) and young rats (246±5 and 255±14 arb. units). Table 1 shows age-related changes in NADPH diaphorase activity and RNA concentration in the cytoplasm of brain cells in male and female offspring of old (282±14 and 252±54 arb. units, respectively) and young rats (295±39 and 275±12 arb. units).

EPM behavior significantly differed in offspring of young and old rats. These differences primarily concerned the time of elementary behavioral reactions. The offspring of old rats was characterized by longer time of overhanging, rearing postures, nuzzling, and stay in open arms. However, the time of grooming reactions and the time spent in closed arms was shorter in this group. The observed differences were statistically significant in males (Table 1). Similar differences were revealed in the number of elementary reactions over a certain time interval. These data indicate that the offspring of old rats is characterized by higher locomotor activity and lower degree of anxiety. Higher nervous activity underwent more significant variations than morphological indexes of the brain. Therefore, higher nervous activity of animals requires further detailed investigations.

Blood estradiol concentration did not differ in females of different groups. The weight of the ovaries in the offspring of old rats was much higher than in the offspring of young animals (Table 1). Blood testosterone concentration in male offspring of old rats was lower than in the offspring of young animals. The weight of the testes tended to decrease in the offspring of old females. Differences in higher nervous activity of animals with various testosterone concentrations in the blood are of considerable interest. Published data show that testosterone concentration in pupils trained at schools for talented children is lower than in pupils of normal schools [10].

Our results show that on the first day of life the offspring of 9-10-month-old females had morphological signs of accelerated brain development (compared to the offspring of 3.5-4-month-old animals) [5]. These intergroup differences were not observed in the 40-day-old offspring. It should be emphasized that on day 40 of life the absolute weight of the brain in female offspring of 9-10-month-old rats was higher than in the offspring of 3.5-4-month-old animals. The degree of intergroup differences in newborn rat pups progressively decreases in the follow-up period. After birth these animals are not exposed to the influence of "maternal factors" determined by age-related histophysiological characteristics of reproductive and endocrine organs. However, the offspring of young and old rats differs in the dynamics of body weight, development of the reproductive system, morphological indexes of the brain, and higher nervous activity.

REFERENCES

1. S. V. Kuznetsova, R. V. Uchakina, N. A. Belomytsina, and N. V. Orzhekhovskaya, *Fiziol. Chel.*, **21**, No. 6, 85-91 (1995).
2. Z. Loida, R. Gossrau, and T. Shibler, *Histochemistry of Enzymes* [in Russian], Moscow (1982).
3. A. G. Reznikov, *Sex Hormones and Brain Differentiation* [in Russian], Kiev (1982).
4. V. B. Rozen, *Bases of Endocrinology* [in Russian], Moscow (1994).
5. B. Ya. Ryzhavskii and S. I. Biryukova, *Fiziol. Zh.*, **80**, No. 1, 119-122 (1995).
6. B. Ya. Ryzhavskii and G. B. Koval'skii, *Aging. Adaptation. Reversibility* [in Russian], Vladivostok (1992).
7. V. P. Smetnik and L. G. Tumilovich, *Nonoperative Gynecology* [in Russian], Moscow (2001).
8. J. P. de Bruin, M. Dorland, H. W. Bruinse, *et al.*, *Early Hum. Dev.*, **51**, No. 1, 39-46 (1998).
9. H. H. Kay, I. M. Bird, C. L. Coe, *et al.*, *J. Soc. Gynecol. Investig.*, **7**, No. 5, 269-278 (2000).
10. D. Ostatnikova, M. Dohnanyiova, A. Mataseje, *et al.*, *J. Bratisl. Lek. Listy*, **101**, No. 8, 470-473 (2000).