Effect of Experimental Litter Reduction in Female Rats on Parameters of Brain and Endocrine Gland Development in the Progeny

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 3, pp. 349-353, March, 2007
Original article submitted May 29, 2006

We studied the relationship between parameters of brain development, elevated plus-maze behavior, and the status of the endocrine glands in the progeny of 4.5-5- and 8-9-month-old females after litter reduction by removal of one uterine tube. The progeny of young experimental females differed from the progeny of control animals by brain weight (at the age of 1 day), morphometrical characteristics of the cortex and its neurons, activity of 3β-hydroxysteroid dehydrogenase in the adrenal cortex (at the age of 1 and 40 days), and behavioral reactions in the elevated plus-maze (at the age of 30 days). The differences in these parameters between the progeny from old females with experimentally reduced litter size and control females were significantly less pronounced.

Key Words: *brain; litter size; endocrine glands*

The relationship between the pre- and postnatal development of the brain and conditions of fetal development was demonstrated in many papers [1,3-6,8-12]. This process depends on the maternal endocrine status and reproductive potential. It was found that one-day-old progeny of females with the reproductive potentials below the average and with small litters had larger brain, while morphometrical characteristics of the cortex indicating advanced development of the organ, which was in line with advanced development of the brain in the progeny of old females in comparison with the progeny of young ones [4,6].

We studied the possibility of modulating brain development by experimental reduction of the number of fetuses in the litter.

MATERIALS AND METHODS

The progeny of 4.5-5-month-old (young females, 8 litters) and 8-9-month-old (old females, 2 litters) rats, in which the right uterine tubes were removed under ether narcosis 30-35 days before mating, was studied. One-day-old and 40-day-old progeny of intact females of the same age (6 litters from young and 3 from old females) served as the controls. All females were mated with 4-5-month-old males. Rats of all groups were kept in the same vivarium.

On day 1 of life, rat pups of each litter were weighed, counted, after which 1-3 males and 1-3 females (depending on the size of the litter) with body weight mean for the litter were decapitated (a total of 52 pups). The animals were sacrificed during the morning hours.

The brain and the right hemisphere were weighed on electron scales. The left hemisphere was fixed in Carnoy fluid, after which sites containing the anterior parietal lobe (APL) and parietal lobe proper (PLP) were cut out strictly perpendicularly to the hemispheric surface and the log. The material was embedded in paraffin, 7-μ sections were sliced and stained for nucleic acid detection by gallocyanin after Einarson. The nuclear section areas in APL and PLP cortical layers II and V and CAI

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0007-4888/07/1433-0376

hippocampal field were measured on a Mekos programmed device (25 measurements per zone in each case) and the concentrations of nucleic acids in the nuclei were evaluated. The numbers of neurons per standard visual field were counted in APL cortical layers II and V. Tests for the key steroidogenesis enzyme (3β-hydroxysteroid dehydrogenase HSDH) were carried out on 20-μ cryostat sections of the adrenals and gonads [2]. Enzyme activity was measured on a Mekos device in monochromatic light at λ=530 nm (in 25 cells of each zone for every case). The thickness of the adrenal cortex was measured by an ocular micrometer in the same preparations.

Animals left in each litter were tested in an elevated plus-maze at the age of 30 days. The duration of the "elementary" components of behavior (sniffing, peeping down, time spent in open and closed arms, rearing, grooming, movements) was registered using computer software. Each rat was tested for 3 min [7]. The remaining animals were decapitated in the prepubertal age (40 days) and morphological studies were carried out as described previously. The data were analyzed using Statistica 6.0 software.

RESULTS

One-day-old progeny of young females in which one uterine tube was removed, was characterized by a significantly larger brain, hemisphere, thickness of the APL cortex and layer I, size of neurons in PLP layer II, APL and PLP layer V, and hippocampus (Table 1). Hence, a complex of signs indicated advanced development of the brain in experimental rat pups. One-day-old progeny of old intact females differed from one-day-old progeny of intact young females by signs indicating advanced development of the brain (Table 1), which confirms the previous data [4,6]. Removal of the uterine tube in old rats led to a decrease in the number of pups in the litter, but did not change

TABLE 1. Morphometric Characteristics of the Brain and Endocrine Glands in 1-Day-Old Pups (*M*±*m*)

Parameter		Progeny of young females		Progeny of old females	
		intact	tubectomied	intact	tubectomied
Number of litters		6	8	3	2
Number of pups in litter		10.6±0.3	4.8±0.4*	8.2±1.5	6.5±0.3
Body weight, g		5.60±0.08	6.40±0.16*	5.4±0.2	7.10±0.13^{+}
Brain weight, mg		232±4	261.0±6.9*	256.0±11.7*	275.0±8.6
Hemispheric weight, mg		76.0±1.4	87.0±2.5*	88.0±4.5*	91.0±2.6
Thickness, μ					
layer I	APL	57.0±2.2	65.0±2.6*	58.0±7.2	62.0±4.6
	PLP	57.0±1.7	64.0±4.5	44.0±4.4*	57.0±2.6^{+}
cortex	APL	541.0±7.9	591.0±10.9*	543.0±19.1*	604.00±20.05^{+}
	PLP	520±8.8	534±20.7	481±18.3	527±14.7
Nucleus section area, μ^2					
layer II	APL	30.7±1.2	32.5±1.2	37.6±1.7*	34.4±1.5
	PLP	25.2±0.8	28.4±1.2*	34.3±0.7*	32.9±1.4
layer V	APL	35.8±0.7	43.0±1.5*	48.0±1.3*	42.2±0.8
	PLP	32.5±0.6	35.30±1.04*	42.1±0.7*	40.9±2.1
hippocampus		40.4±1.6	45.6±1.6*	50.3±1.5*	52.7±1.3
Thickness of adrenal cortex, μ		356.0±11.1	387.0±20.1	355.0±17.8	390.0±12.3
HSDH activity, arb. units					
zona glomerulosa		0.296±0.010	0.394±0.030*	0.421±0.040*	0.451±0.030^{+}
zona fasciculata		0.328±0.010	0.425±0.030*	0.436±0.050*	0.433±0.050^{+}
zona reticularis		0.402±0.010	0.474±0.030*	0.410±0.040	0.468±0.050
zona glomerulosa/zona reticularis		0.738±0.030	0.851±0.050	1.19±0.05*	0.978±0.050*
Leydig cells		0.480±0.020	0.441±0.030	0.579±0.040*	0.562±0.020*

Note. $p<0.05$ compared to progeny of *young intact females, $^{+}$old intact females.

TABLE 2. Morphometric Characteristics of the Brain and Endocrine Glands of 40-Day-Old Rats and Their Behavioral Parameters in an Elevated Plus-Maze (*M*±*m*)

Parameter			Progeny of young females		Progeny of old females	
			intact	tubectomied	intact	tubectomied
Weight, mg	brain		1549±15	1580±18	1486±11*	1576±20^{+}
	hemisphere		536±7	549±16	506±9*	550±8^{+}
Thickness, μ	layer I	APL	147.0±6.6	190±24	168.6±8.8	152.0±8.1
		PLP	133.0±3.8	137.0±6.8	149±4	146.8±4.9
	cortex	APL	1571±30	1657±102	1481.0±44.8	1521.0±56.5
		PLP	1294.0±22.5	1446.0±67.8	1206.0±25.7*	1279.5±41.8
Number of neurons in visual field, APL	layer II		21.0±0.9	23.4±0.7*	24.5±1.2	23.8±1.0
	layer V		12.0±0.6	14.6±1.1	16±1^{x}	13.4±0.5^{+}
Area, μ^2	nucleoli	APL, layer II	2.70±0.08	3.1±0.1*	3.30±0.12*	3.40±0.14
		PLP, layer II	2.50±0.06	3.2±0.2*	3.1±0.1*	3.20±0.08
		APL, layer V	2.60±0.06	3.0±0.2	3.00±0.04*	3.20±0.07
		PLP, layer V	2.50±0.05	2.80±0.14	2.90±0.12*	3.10±0.06
	hippocampus		2.70±0.06	3.3±0.2*	3.30±0.05*^{x}	3.60±0.08^{+}
Area, μ^2	nucleus	APL, layer II	53.0±1.6	60.6±3.7*	69.1±2.4*	72.1±2.2
		PLP, layer II	52.5±1.3	56.9±1.7	63.6±1.9*^{x}	68.5±1.8^{+}
		APL, layer V	49.8±1.6	52.6±2.7	60.22±0.80*	63.73±1.40
		PLP, layer V	46.38±1.00	50.33±2.40	60.05±20*	62.19±0.80
	hippocampus		45.4±1.1	61.7±2.1*	77.7±1.8*	75.8±1.7
Cytoplasm area, μ^2	APL, layer II	PLP, layer II	43.5±1.1	44.1±1.7	52.1±1.8*	50.80±1.07
		APL, layer V	41.0±1.4	41.8±2.6	49.4±2.6*	46.7±1.4
		PLP, layer V	36.4±1.0	38.2±2.4	46.3±2.5*	42.6±0.6
	hippocampus		43.2±1.0	44.5±2.6	58.8±4.2	50.6±1.3
RNA concentration in neuron cytoplasm, arb. units		APL, layer II	0.277±0.010	0.199±0.008*	0.274±0.040	0.367±0.030
		PLP, layer II	0.267±0.010	0.220±0.020	0.302±0.040	0.308±0.050
		APL, layer V	0.276±0.012	0.219±0.030	0.336±0.040	0.345±0.030
		PLP, layer V	0.269±0.011	0.190±0.030	0.251±0.040	0.272±0.030
	hippocampus		0.309±0.014	0.256±0.010*	0.417±0.050	0.440±0.040*
Time in EPM, sec	peeping down		2.6±0.7	5.6±3.4	7.9±1.4*	5.5±2.1
	rearing		8.2±1.1	5.3±2.0	3.5±0.6*	9.7±2.6
	grooming		22±3	14.3±3.7	9.2±3.3*	19.8±4.3
	sniffing		146±4	160.8±3.9*	164.9±4.0*	151.1±4.7
	movements		68.6±5.6	52.1±12.9	75.6±7.1	58.3±7.2
	in open arms		13.7±2.8	18±9	43.6±6.8*	32.6±9.4
	in closed arms		164.0±2.8	159.1±9.0	133.7±6.8*	139.3±10.0
Number of episodes in EPM	peeping down		1.3±0.3	2±1.4	6.1±1.1*	4.6±1.3
	rearing		5.0±0.5	3.8±1.0	3.4±0.5*$^{+}$	7±1^{+}
	grooming		4.4±0.5	5.0±0.6	2.8±0.4*^{x}	5.4±0.7^{+}
	sniffing		6.0±0.6	6.6±0.7	3.2±0.2*^{x}	6.1±0.6^{+}
	movements		7.4±0.6	6.6±0.5	11.1±1.1*	9.4±1.4
	excursions into open arms		1.5±0.2	2.2±1.0	2.7±0.5*	2.4±0.5
	excursions into closed arms		2.3±0.2	3±1	3.3±0.5	3.1±0.5
Thickness of adrenal cortex, μ			725±14	763±18	619±18*^{x}	704±13^{+}
HSDH activity, arb. units	adrenal cortex					
	zona glomerulosa		0.429±0.024	0.243±0.036*	0.526±0.026^{x}	0.511±0.015
	zona facsiculata		0.478±0.029	0.282±0.037*	0.586±0.018^{x}	0.528±0.022
	zona reticularis		0.531±0.027	0.316±0.034*	0.606±0.016^{x}	0.604±0.013
	Leydig cells		0.397±0.030	0.264±0.006*	0.568±0.042*^{x}	0.457±0.027*$^{+}$
	follicular thecocytes		0.438±0.038	0.237±0.016*	0.585±0.054*	0.532±0.025*$^{o+}$

Note. EPM: elevated plus-maze. $p<0.05$ compared to progeny of *young intact females, ^{o}young tubectomied females, $^{+}$old intact females, ^{x}old tubectomied females.

their brain weight and virtually none of the studied morphometrical characteristics (Table 1). Hence, a decrease in the number of pups in the litter had different effects on brain development in one-day-old progeny of females of different age. Presumably, the mechanisms promoting advanced development of the brain in the progeny are activated in intact old females, as a result of which experimental reduction of the number of pups in the litter cannot appreciably stimulate this process.

The morphometrical parameters of the brain of 40-day-old progeny of young tubectomied rats differed from those in the progeny of intact females of the same age: they had larger nucleoli, nuclei, higher RNA concentration in the neuron cytoplasm (Table 2). The differences between 40-day-old progeny of old tubectomied rats and age-matched controls were less pronounced than between the progeny of experimental and control young females. Hence, the most pronounced delayed effects were observed in the progeny of young tubectomied female rats. Brain weight in 40-day-old progeny of young tubectomied rats and of old females in both groups was not greater than in pups of intact young females. A different situation was observed in 1-day-old pups (Tables 1, 2). Hence, higher rates of brain growth during the prenatal period of ontogeny do not cause similar processes later.

Testing in an elevated plus-maze showed that the behavior of 30-day-old pups from the litters of tubectomied young rats differed from that of control pups. They spent more time for sniffing ($p<0.05$) and stayed longer in open arms, they more often explored the open and closed arms (Table 2). The behavioral reactions of the progeny of old intact females differed significantly from those of young females (Table 2), which confirms previous data on higher exploratory activity of the progeny of old females [4,6]. The 30-day-old progeny of tubectomied old rats exhibited a greater number of rearing, grooming, and sniffing episodes ($p<0.05$). They spent more time for peeping down, rearing, grooming, and less time for movements and in the open arms (Table 2).

These differences in the parameters of brain development in newborn animals, progeny of tubectomied females, can be regarded as a result of better provision of the fetuses by nutrients due to lesser number of fetuses in the litter. This hypothesis is supported by, for example, a positive relationship between brain and body weights of the newborns and a negative relationship between body weight and number of fetuses in the litter [4], which confirm our findings (Table 1). The differences from brain development parameters in the control group could be due to changes in the endocrine characteristics in the mother—placenta—fetus system determined by a lower summary weight of the fetal endocrine organs, lower summary weight of the placentas in the presence of a lower number of fetuses. The study of the endocrine glands to a certain measure confirmed the differences in the endocrine status of the progeny of operated females. The thickness of the adrenal cortical matter in 1-day-old progeny of tubectomied young females was virtually the same as in the control group, while activity of HSDH was higher in all adrenocortical zones of experimental rats. On the other hand, HSDH activity in the adrenals of 1-day-old progeny of old rats virtually did not differ from that in the control groups, though was significantly higher than in pups of intact young females. In 40-day-old progeny of young tubectomied rats enzyme activity in all adrenocortical zones, Leydig cells, and follicular thecocytes was significantly lower than in the control. The cortex in the progeny of old experimental females had greater thickness, while HSDH activity in their adrenals did not differ from that in the control group, though a trend to its reduction could be traced. The decrease in HSDH activity in the testicular Leydig cells in the progeny of old tubectomied females was statistically significant (Table 2).

Hence, the data indicate the possibility of purposeful modulation of the important characteristics of brain development during the prenatal period of ontogeny by modifying the number of fetuses in the litter. Interestingly that this approach can be practiced for obtaining animals with advanced development of the brain at the early stages of ontogeny, which will open new vistas for the analysis of various aspects of this organ development and factors regulating it. Our results confirm the relationship between brain development in the progeny and number of newborns in the litter and maternal age. Presumably, this, to a certain measure, is due to different patterns of changes in the endocrine glands of young and old rats, in which the number of fetuses in the litter was experimentally reduced.

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