Effects of Infant Rat Fostering in Artificially Formed Litters on the Development of Brain, Adrenals and Gonads

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Morphometric and histochemical properties of neurons in the frontoparietal and parietal lobes and CA1 hippocampal area, thickness of the neocortex, histochemical and morphometric properties of adrenals and gonads, and concentrations of sex hormones were compared in 40-day rats fostered in artificially formed (experimental group) and natural (control) litters. Animals of the experimental group had lower body and brain weight, thickness of the neocortex in the parietal lobe, sizes of nuclei and cytoplasm of layer 2 and layer 5 neurons of the frontoparietal and parietal lobes and in the hippocampus, lower NADPH-dehydrogenase activity in the hippocampus, and lower NADH- dehydrogenase activity in layer 2 neurons of the parietal lobe in comparison with control rats. RNA concentration in neuronal cytoplasm in neocortex and hippocampus was higher in rats from experimental group, than in animals from the control group. Higher estradiol concentration, higher activity of 3β-hydroxysteroiddehydrogenase in thecal cells of ovarian follicles were found in females from experimental group; decreased testicle weight, reduced diameter of seminiferous tubules, reduced activity of 3β-hydroxysteroiddehydrogenase in Leydig's cells, and trend forward lowering of testosterone concentration were found in males from experimental group.

Key Words: *development; combined litters; brain; gonads; morphometry*

Conditions of early postnatal period, nutrient supply during this period, and stressfulness of environment can substantially affect the parameters of higher nervous activity and concentration of some hormones in the future periods [4-6,11]. Changes in mother status induced by long-term emotional stress and administration of glucocorticoid hormone derivatives to dams or newborn pups are also responsible for similar consequences [7-9]. Fostering in "alien" environment without mother can significantly affect living conditions at early stages. Fostering of young rats in alien litters was established to affect functional activity of the adrenals and predisposition to hereditary hypertension [2,3,10].

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The objective of this study was to investigate the effects of fostering of rat pups in alien litters on functional parameters of their brain, gonads, and adrenals.

MATERIALS AND METHODS

We studied the offspring of 4.5-5.5-month intact mongrel albino rats kept simultaneously in the same vivarium with food and water *ad libitum*. The animals were divided into two groups. The experimental group consisted of 4 artificially formed litters (n=47) formed 1 day after birth from litters of 9-13 pups, from which 2-6 pups were removed and 4-6 "adopted" pups were added; the control group consisted of 2 natural litters of 11 and 13 pups. Each litter was kept with mother in a standard cage.

At the age of 40 days, the pups were weighted, then animals were decapitated, the brain and the right hemisphere, the gonads, and the adrenals were weighed.

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The left hemispheres were fixed in Carnoy's fluid and then sectioned in frontoparietal lobe (FPL) and parietal lobe (PL) strictly perpendicular to the longitudinal axis and top surface and were embedded into paraffin. The sections of FPL and PL (7 μ) were stained with gallocyanine to detect nucleic acids [5]. The thickness of FPL and PL cortex was measured using eyepiece micrometer MOV-15. Cross-section area of nucleoli, nuclei, and cytoplasm of pyramidal neurons of layers 2 and 4 of FPL and PL and CA1 hippocampal area, as well as RNA concentration in neuronal cytoplasm were determined by computer morphometry on MEKOS apparatus on the gallocyanine-stained preparations (λ=530 nm). Twenty-five cells in at least 5 fields of view were measured in the studied brain areas of each animal. The number of neurons per field of view was calculated at objective magnification 40 in 5 standard fields of view in neocortex layers 2 and 5. Immediately after decapitation, 20-μ cryostat sections were prepared from PL of the right hemisphere. They were used for the reaction for NADH- and NADPH-dehydrogenases (NADH-d, NADPH-d). The left adrenals, testicles, and ovaries were used to prepare 20-μ cryostat sections for the reaction for 3β-hydroxysteroiddehydrogenase (HSDG). NADH-d, NADPH-d, and HSDG activities were evaluated by optical density (λ=530 nm) of the reaction products (25 cells of each localization for each case) using a MEKOS apparatus. The same preparations were used for measuring the thickness of the adrenal cortex, diameters of convoluted seminiferous tubules, and of the largest vesicular ovarian follicles using eyepiece micrometer MOV-15. Serum concentrations of estradiol (in females) and testosterone (in males) were measured by ELISA.

The results were processed statistically using Statistica 6.0 software.

RESULTS

Body weight of 40-day-old rat pups from artificial litters (both males and females) was significantly lower than in control. In parallel, significant decrease in absolute weight of the brain, adrenals, ovaries, and particularly testicles was observed in the experimental group. The diameter of vesicular ovarian follicles and seminiferous tubules and the thickness of the adrenal cortex also decreased. Activity of HSDG, the key enzyme of steroidogenesis, in adrenocytes of all adrenal cortex zones was similar in all groups, while in thecal cells of ovarian follicles it was significantly higher and in testicular Leydig cells it was lower than in the control. Estradiol concentration increased in females and trended to a decrease in males (Table 1). Higher estradiol concentration and higher HSDG activity in thecal cells in ovarian follicles in prepubertal females of the experimental group with lower body weight are in line with the data that the development of secondary sexual characteristics in girls with microsomic and micromesosomic types anticipates that in mesomacrosomic and macrosomic girls [1].

Morphometry of the cortex showed that its thickness in FPL was similar in all groups, while PL thickness was significantly lower in the experimental group compared to the control. Moreover, numerical density of layer 2 neurons in FTL neocortex in experimental males was significantly lower than in controls. In experimental animals, the size of neuronal cytoplasm, nuclei, and nucleoli in the studies areas of the neocortex and hippocampus was lower and RNA concentration in the cytoplasm of these cells was significantly higher that in the control (Table 2). Reduced NADH-d activity in PL layer 2 neurons and reduced NADPH-d activity in hippocampal neurons were revealed in the animals from experimental litters (Table 1).

In artificially formed litters we compared all studied parameters between "kin" and "adopted" pups (Tables 3 and 4). "Kin" and "adopted" pups were virtually similar in the studied gravimetric parameters as well as in morphometric characteristics of adrenals and gonads, and estradiol and testosterone blood concentrations. In "adopted" pups, the thickness of the cortex in FTL was higher and neuronal density PL layer 2 was lower than in "kin" animals. Layer 2 neurons in FPL were characterized by smaller nucleoli, while in PL they had larger nucleus and cytoplasm; PL layer 5 neurons were characterized by larger nuclei. In "adopted" animals, NADH-d and NADPH-d activities in neurons of all studied areas significantly surpassed those in "kin" animals (Table 3), which attests to more intense processes of mitochondrial and extramitochondrial oxidation in brain neurons in "adopted" pups.

Comparison of these data led us to the following conclusions: first, fostering conditions in artificially formed litters differed from that in control litters for both "adopted" and "kin" pups giving rise to a number of intergroup differences between rat pups from naturally and artificially formed litters; second: similar fostering conditions for "kin" and "adopted" pups within artificially formed litters gave rise for lower number of differences in parameters of brain, gonad, and adrenal development, although the number of morphometric and metabolic characteristics of the cortex and its neurons differed significantly. In general, our findings suggest that changes in fostering environment at early stages of postnatal ontogeny can substantially affect parameters of physical development, histophysiology of the gonads and adrenal glands, cortex development, and state of neurons in the neocortex and hippocampus.

TABLE 1. Parameters of the Development of the Brain, Gonads, and Adrenals in Male and Female Rats from Control and Experimental Groups (*M*±*m*)

Parameter			Control group			Experimental group		
			total (*n*=24)	males (*n*=13)	females (*n*=11)	total (*n*=47)	males (*n*=26)	females (*n*=21)
Weight	body, g		81±2	82±3	78±3	63±4*	67±5*	59±5*
	brain, mg		1481±11	1503±16	1454±11	1434±14*	1457±19	1405±21
	hemisphere, mg		547±9	555±10	535±15	523±9	536±10	507±14
	adrenal, mg		10.4±0.4*	10.0±0.6	11.0±0.7	9.0±0.4	9.0±0.5	9.0±0.8*
	testicle, mg		462±13			282±31*		
	ovary, mg		19.0±1.5			15.0±0.8*		
NADPH-d activity, arb. units								
	layer 2		0.416±0.014	0.406±0.024	0.426±0.017	0.393±0.016	0.394±0.025	0.392±0.019
	layer 5		0.396±0.010	0.387±0.022	0.405±0.006	0.387±0.017	0.384±0.025	0.392±0.022
	hippocampus		0.436±0.018	0.398±0.029	0.472±0.016	0.388±0.010*	0.386±0.015	0.391±0.014*
NADH-d activity, arb. units								
	layer 2		0.445±0.012	0.454±0.017	0.437±0.018	0.404±0.011*	0.416±0.016	0.390±0.015
	layer 5		0.389±0.011	0.406±0.015	0.375±0.017	0.409±0.019	0.395±0.023	0.428±0.033
	hippocampus		0.421±0.015	0.440±0.021	0.404±0.022	0.405±0.018	0.380±0.015	0.433±0.034
HSDG activity, arb. units								
	adrenals	zona glomerulosa	0.537±0.036	0.535±0.051	0.539±0.053	0.543±0.020	0.549±0.028	0.537±0.031
		zona fasciculata	0.551±0.029	0.560±0.046	0.544±0.038	0.5560±0.0212	0.573±0.026	0.534±0.035
		zona reticularis	0.518±0.028	0.504±0.042	0.531±0.041	0.574±0.018	0.569±0.020	0.582±0.032
	testicles	Leydig cells		0.603±0.027			0.491±0.018*	
	ovaries	follicle thecal cells			0.558±0.009			0.622±0.025*
Thickness of adrenal cortex, μ			793±11	815±21	773±9	725±12*	736±16*	711±17*
Diameter of the largest follicle, μ					456±20			404±12*
Diameter of seminiferous tubules, μ				245±9			207±9*	
Blood concentration								
	estradiol, pg/ml				34.4±4.5			57.4±5.3*
	testosterone, nmol/liter			3.17±0.60			2.15±0.30	

Note. Here and in Table 2: *$p<0.05$ in comparison with corresponding control.

TABLE 2. Morphometric and Histochemical Parameters of Brain Development in Males and Females from Control and Experimental Groups (*M*±*m*)

Paremeter	Control group			Experimental group		
	total (*n*=24)	males (*n*=13)	females (*n*=11)	total (*n*=47)	males *n*=26)	females (*n*=21)
Cortex thickness, μ						
FPL	1586±12	1595±19	1575±16	1574±15	1582±22	1565±20
PL	1360±17	1355±24	1367±24	1209±12*	1214±17*	1203±18*
Neuron number in the field of view						
FTL layer 2	20.00±0.68	21.3±1.0	18.50±0.58	18.80±0.23	18.6±0.3*	19.0±0.4
layer 5	6.80±0.16	6.70±0.25	6.90±0.21	6.8±0.1	6.9±0.2	6.8±0.1
PL layer 2	20.80±0.53	21.20±0.79	20.20±0.71	19.8±0.2	19.8±0.3	19.9±0.4
layer 5	7.10±0.23	7.40±0.38	6.80±0.19	6.9±0.1	6.9±0.1	6.9±0.1
FPL layer 2						
cross-section area of neuronal nucleoli, μ^2	2.47±0.11	2.53±0.17	2.40±0.16	1.99±0.03*	1.97±0.04*	2.01±0.05*
cross-section area of neuronal nuclei, μ^2	61.36±1.34	60.5±1.9	62.5±1.8	52.28±0.66*	51.89±0.93*	52.7±0.9*
cross-section area of neuronal cytoplasm, μ^2	45.83±1.22	45.8±1.6	45.8±1.9	38.33±0.94*	37.7±1.1*	39.07±1.60*
RNA concentration in neuronal cytoplasm, arb. units	0.259±0.013	0.277±0.020	0.237±0.015	0.349±0.018*	0.348±0.023*	0.351±0.029*
FPL layer 5						
cross-section area of neuronal nucleoli, μ^2	4.77±0.11	4.90±0.17	4.63±0.14	4.5±0.1	4.66±0.08	4.3±0.2
cross-section area of neuronal nuclei, μ^2	105±2	105.5±3.4	104.4±3.6	96.53±1.23*	96.9±1.5*	95.9±2.1*
cross-section area of neuronal cytoplasm, μ^2	88.7±2.4	88.6±3.2	88.8±3.9	80.5±2.5*	80.9±3.2	79.9±3.9
RNA concentration in neuronal cytoplasm, arb. units	0.312±0.017	0.309±0.024	0.316±0.024	0.400±0.018*	0.394±0.022*	0.408±0.031*
PL layer 2						
cross-section area of neuronal nucleoli, μ^2	2.56±0.17	2.76±0.23	2.32±0.25	1.96±0.03*	1.93±0.03*	2.00±0.06
cross-section area of neuronal nuclei, μ^2	60.0±1.5	58.9±1.7	61.5±2.8	50.02±0.63*	49.1±0.8*	51.07±0.92*
cross-section area of neuronal cytoplasm, μ^2	45.3±1.3	46.8±1.8	43.4±1.6	37.17±0.95*	37.3±1.3*	37.01±1.40*
RNA concentration in neuronal cytoplasm, arb. units	0.243±0.015	0.241±0.023	0.245±0.018	0.338±0.015*	0.325±0.022*	0.353±0.022*
PL layer 5						
cross-section area of neuronal nucleoli, μ^2	4.35±0.08	4.50±0.11	4.19±0.10	4.48±0.08	4.45±0.09	4.52±0.15
cross-section area of neuronal nuclei, μ^2	95.0±2.4	93.2±3.7	97.1±2.9	91.50±1.04	91.5±1.4	91.5±1.5
cross-section area of neuronal cytoplasm, μ^2	82.0±1.9	82.1±2.3	81.9±3.3	75.3±2.8*	73.9±3.6	77.01±4.45
RNA concentration in neuronal cytoplasm, arb. units	0.307±0.017	0.311±0.020	0.302±0.031	0.386±0.018*	0.377±0.024*	0.396±0.029*
Hippocampus						
cross-section area of neuronal nucleoli, μ^2	2.94±0.20	2.80±0.23	3.0±0.3	2.26±0.04*	2.21±0.05*	2.31±0.07
cross-section area of neuronal nuclei, μ^2	73.8±1.9	72.4±3.1	75.4±1.9	63.7±1.4*	64.30±1.01*	63.03±2.90*
cross-section area of neuronal cytoplasm, μ^2	50.50±2.06	51.2±3.1	49.7±2.7	39.7±0.7*	39.2±0.9*	40.4±1.03*
RNA concentration in neuronal cytoplasm, arb. units	0.255±0.019	0.252±0.021	0.259±0.034	0.373±0.020*	0.370±0.024*	0.377±0.034*

TABLE 3. Parameters of Brain, Gonad, and Adrenal Development in "Kin" and "Adopted" Rats from Experimental Group ($M \pm m$)

Parameter		"Kin" rats			"Adopted" rats		
		total (n=29)	males (n=17)	females (n=12)	total (n=18)	males (n=9)	females (n=9)
Weight	body, g	62±5	67±6	55±7	65±5	66±8	64±8
	brain, mg	1418±20	1442±25	1383±31	1459±19	1485±25	1434±26
	hemisphere, mg	513±12	531±15	487±16	540±11	547±9	533±21
	adrenal, mg	9.0±0.6	9.2±0.7	8.4±1.0	10.0±0.7	9.4±0.8	10.4±1.2
	testicle, mg		283±39			281±51	
	ovary, mg			15±1			14.4±1.3
NADPH-d activity, arb. units							
	layer 2	0.330±0.013	0.317±0.017	0.348±0.020	0.483±0.016*	0.508±0.020*	0.450±0.021*
	layer 5	0.323±0.013	0.312±0.015	0.339±0.023	0.479±0.020*	0.491±0.033*	0.462±0.018*
	hippocampus	0.356±0.012	0.348±0.015	0.368±0.200	0.434±0.011*	0.443±0.018*	0.422±0.008*
NADH-d activity, arb. units							
	layer 2	0.365±0.013	0.372±0.018	0.355±0.020	0.457±0.010*	0.481±0.010*	0.430±0.014*
	layer 5	0.353±0.016	0.351±0.024	0.357±0.020	0.479±0.031*	0.461±0.035*	0.498±0.053*
	hippocampus	0.343±0.011	0.339±0.013	0.348±0.019	0.474±0.028*	0.435±0.019*	0.509±0.050*
HSDG activity, arb. units							
	adrenals zona glomerulosa	0.566±0.033	0.549±0.043	0.590±0.057	0.519±0.022	0.548±0.037	0.489±0.022
	zona fasciculata	0.565±0.034	0.560±0.042	0.573±0.062	0.546±0.024	0.592±0.024	0.500±0.037
	zona reticularis	0.551±0.017	0.550±0.017	0.552±0.036	0.604±0.033	0.597±0.043	0.611±0.054
	testicles						
	Leydig cells		0.481±0.030			0.504±0.016	
	ovaries						
	follicle thecal cells		0.624±0.042				0.620±0.033
Thickness of adrenal cortex, μ		713±16	726±18	691±31	738±17	751±30	726±19
Diameter of the largest follicle, μ				420±17			389±15
Diameter of seminiferous tubule, μ			210±12			203±12	
Blood concentration							
	estradiol, pg/ml			55.7±7.0			59.6±8.4
	testosterone, nmol/liter		2.32±0.43			1.84±0.28	

Note. Here and in Table 4: *p<0.05 in comparison with corresponding parameter in "kin" rats.

TABLE 4. Morphometric and Histochemical Parameters of Brain Development in "Kin" and "Adopted" Rats from Experimental Group (*M*±*m*)

Parameter	"Kin" rats			"Adopted" rats		
	total (*n*=29)	males (*n*=17)	females (*n*=12)	total (*n*=18)	males (*n*=9)	females (*n*=9)
Cortex thickness, μ						
FPL	1530±16	1537±22	1519±23	1646±20*	1667±32*	1626±25*
PL	1167±16	1167±23	1167±21	1176±21	1172±26	1180±35
Neuron number per field of view						
FPL layer 2	19.0±0.3	18.8±0.4	19.2±0.6	18.4±0.3	18.0±0.4	18.7±0.5
layer 5	6.8±0.1	6.8±0.2	6.8±0.2	6.9±0.1	7.0±0.3	6.8±0.1
PL layer 2	20.3±0.3	20.2±0.4	20.5±0.4	19.0±0.3	18.9±0.3*	19.0±0.7
layer 5	6.9±0.1	7.1±0.1	6.8±0.2	6.9±0.1	6.7±0.3	7.1±0.2
FPL layer 2						
cross-section area of neuronal nucleoli, μ^2	2.03±0.04	2.02±0.05	2.05±0.08	1.92±0.03*	1.87±0.05*	1.97±0.05
cross-section area of neuronal nuclei, μ^2	52.32±0.95	51.45±1.23	53.56±1.48	52.22±0.87	52.73±1.42	51.71±1.08
cross-section area of neuronal cytoplasm, μ^2	37.9±1.3	37.15±1.48	38.99±2.46	39.00±1.26	38.81±1.67	39.18±1.98
RNA concentration in neuronal cytoplasm, arb. units	0.344±0.024	0.347±0.029	0.339±0.046	0.358±0.027	0.349±0.042	0.367±0.037
FPL layer 5						
cross-section area of neuronal nucleoli, μ^2	4.57±0.09	4.7±0.1	4.40±0.17	4.37±0.22	4.58±0.15	4.15±0.41
cross-section area of neuronal nuclei, μ^2	96.55±1.64	97.92±1.67	94.61±3.21	96.5±1.8	95.22±2.86	97.78±2.53
cross-section area of neuronal cytoplasm, μ^2	78.44±3.34	80.3±4.4	75.8±5.2	83.8±3.6	82.08±4.46	85.53±6.00
RNA concentration in neuronal cytoplasm, arb. units	0.405±0.027	0.407±0.031	0.403±0.047	0.392±0.024	0.369±0.028	0.416±0.039
PL layer 2						
cross-section area of neuronal nucleoli, μ^2	1.97±0.03	1.98±0.04	1.95±0.05	1.96±0.07	1.84±0.05*	2.07±0.12
cross-section area of neuronal nuclei, μ^2	48.88±0.86	47.44±0.50	50.92±1.43	51.86±0.73*	52.45±1.00*	51.26±1.10
cross-section area of neuronal cytoplasm, μ^2	35.6±1.0	35.8±1.5	35.45±1.34	39.62±1.79*	40.1±2.4	39.08±2.80
RNA concentration in neuronal cytoplasm, arb. units	0.331±0.021	0.314±0.031	0.356±0.028	0.348±0.022	0.347±0.024	0.349±0.037
PL layer 5						
cross-section area of neuronal nucleoli, μ^2	4.38±0.06	4.35±0.07	4.42±0.10	4.65±0.19	4.63±0.21	4.66±0.33
cross-section area of neuronal nuclei, μ^2	89.51±1.18	89.61±1.56	89.37±1.88	94.75±1.72*	95.12±2.59	94.37±2.43
cross-section area of neuronal cytoplasm, μ^2	73.24±1.62	74.2±2.1	71.89±2.53	78.7±6.8	73.57±9.93	83.83±9.67
RNA concentration in neuronal cytoplasm, arb. units	0.381±0.023	0.363±0.028	0.407±0.040	0.393±0.032	0.404±0.047	0.382±0.045
Hippocampus						
cross-section area of neuronal nucleoli, μ^2	2.26±0.03	2.22±0.04	2.31±0.06	2.250±0.091	2.21±0.10	2.30±0.15
cross-section area of neuronal nuclei, μ^2	62.77±2.09	64.04±0.90	60.98±4.93	65.36±1.47	64.95±2.35	65.7±1.9
cross-section area of neuronal cytoplasm, μ^2	38.97±0.73	38.57±1.04	39.55±1.01	40.95±1.32	40.42±1.82	41.47±2.01
RNA concentration in neuronal cytoplasm, arb. units	0.380±0.024	0.381±0.028	0.380±0.044	0.361±0.037	0.350±0.049	0.372±0.058

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