Association of Brain Weight Value with Morphometric and Histochemical Properties of Cortical Neurons and Higher Nervous Activity Parameters in Rats in Prepubertal Period

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We compared thickness of the neocortex, morphometric and histochemical characteristics of neurons in frontoparietal and parietal lobes and hippocampal field I in animals from great litters with lower brain weight (group 1) and from small (artificially reduced) litters with higher brain weight (group 2). It was found that the thickness of the neocortex in the frontoparietal and parietal lobes does not differ in the compared groups, while the size of neuronal cytoplasm in layer II of the frontoparietal and parietal lobes and in layer V of the frontoparietal lobe in group 2 animals was lower than in group 1. Nuclei of cortical neurons in layer II of the parietal and frontoparietal lobes and in frontoparietal lobe layer V in group 2 animals were smaller than in group 1. Neuronal nucleoli in group 2 animals were also smaller than in group 1 rats. RNA concentration in neuronal cytoplasm in the hippocampus and neocortex of group 2 rats was higher than in group 1 animals. NADPH-dehydrogenase activity in neurons of parietal lobe (layer II) and hippocampus in group 2 rats was lower than in group 1 animals, NADH-dehydrogenase activity was lower in parietal lobe layer II neurons. Group 2 rats demonstrated increased number of hanging down, sniffing, movements, entries into open and closed arms, and lower immobility time in the elevated plus-maze test.

Key Words: *brain weight; development; neurons; morphometry; histochemistry*

The relationship between brain weight and its functional properties was studied for many years and still attracts much attention [2,5,8-12]. Many studies are focused on comparison of the dynamics of organ growth during the early stages of ontogeny with mental capacity indices at later stages. These studies were conducted as a part of brain development analysis in both mature and premature newborns with significant body weight deficit and low brain weight. Specific role of brain size and growth rate during the early ontogeny in determination of its functions was established [5,8-12]. In light of this it is interesting to compare macroscopic parameters of the brain (weight, size) to morphometric parameters of the cortex and neurons. We previously showed that the difference in brain weight between newborns rats was associated with differences in cortex thickness, density of cortical neurons, and size of neuronal nuclei; it should be noted that higher brain weight correlated with morphometric indices indicative of high level of brain development [5,6]. At the same time, the corresponding data for other stages of ontogeny are absent.

Here we studied these relationships on prepubertal rats; brain development is virtually completed by this time.

Various approaches can be used to obtain animals of this age with different brain weights. For instance, in prereproductive period brain weight value can be determined by different litter size, which negatively

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correlates with body weight in growing animals, which in turn positively correlates with brain weight [1,5].

MATERIALS AND METHODS

We used offspring of 4.5-5.5-month-old intact rats maintained simultaneously in the same vivarium with free access to water and food. The animals were divided into 2 groups. Group 1 comprised animals from great litters (n=11-13), group 2 consisted of animals from small litters where the number of rat pups was reduced artificially. To this end, 4-5 pups were eliminated from 6 medium-sized litters after attaining the age of 1 day (n=9-10). The total number of pups in groups 1 and 2 was 24 (2 litters) and 30 (6 litters), respectively. Each litter was kept with the dam in a standard cage (length 59 cm, width 35 cm, height 30 cm). At the age of 30 days (by the end of preweaning period), all animals were tested in an elevated plus maze (EPM). "Elementary" behavioral acts (sniffing, hanging down, time spent in open and closed arms, rearings, grooming acts, movements, and immobility time) were recorded using special software [7]. Each rat was tested for 3 min. At the age of 14, 21, 30 and 40 days, the rats were weighed on electronic scale. At the age of 40 days, the pups from the compared groups were decapitated and the weight of the brain, brain hemispheres, gonads, and adrenal glands was determined. The left hemisphere was fixed in Carnoy fluid. Then sections through the frontoparietal lobe (RPL) and parietal lobe (PL) were cut strictly perpendicular to the longitudinal axis and the hemisphere surface and embedded into paraffin. RPL and PL sections (thickness 7 μ) were stained for nucleic acids with gallocyanin according to Einarson. Thickness of the cerebral cortex and RPL and PL layer I was determined using a MOB-15 ocular micrometer at ×3.7. Cross-section area of nuclei, nucleoli, and cytoplasm of pyramid neurons in necocortex layers II and V of RPL and PL and in hippocampus layer I and RNA concentration in neuronal cytoplasm were measured on gallocyanin-stained sections using a Mekos microphotometric apparatus. In all studied areas, 25 cells in at least 5 fields of view were measured. The right hemisphere PL was used for preparing 20-μ cryostat sections immediately after decapitation. They were placed onto glass coverslips and NADH-D and NADPH-D reactions were conducted in strictly standard conditions. Taking into account an association between endocrine glands activity and brain development [5] we also studied some parameters of gonads and adrenal glands. The left adrenal gland was used for preparing 20-μ cryostat sections passing through the central part of the organ. Staining for 3β-hydroxysteroiddehydrogenase (3β-HSD) was carried out as described elsewhere [4]. NADH-D, NADPH-D and HSD activities were estimated by optical density (λ=530 nm) of the reaction products (25 cells in each localization for each case) using Mekos apparatus. Serum concentrations of estradiol (in females) and testosterone (in males) were measured. Statistical analysis was performed using Statistica 6.0 software.

RESULTS

Group 2 rat pups had higher body weight at the age of 14, 21, 30 and 40 days (Table 1). The difference in body weight could be partially explained by better supply with mother's milk in small litters. At the age of 40 days, the weight of the brain and hemisphere in group 2 pups was also higher than in group 1 animals. The intergroup difference in brain weight (6.2%) was less pronounced than the difference in body weight (31.25%), which indicates that brain growth rate depends on body weight gain, but does not "passively follow" it. The weight of the adrenal glands in group 2 animals was also higher than in group 1 animals. At the same time, the weight of the gonads, blood concentrations of testosterone and estradiol, and HSD activity in the adrenal glands did not significantly differ between the groups (Table 1).

Brain morphometry showed that the thickness of the neocortex and its layer I in RPL and PL did not differ between the groups. Taking into account higher weight of the brain and hemisphere in group 2 animals, we can hypothesize that the total volume of the cortex in these animals is higher than in group 1 animals. The size of neuronal cytoplasm in RPL and PL layer II and in RPL layer V in group 2 animals was lower than in group 1. Neuronal nucleoli in the cortex of group 2 animals were smaller than in group 1 rats. Thus, the studied components of neurons in various cortical areas in group 2 animals were smaller than in group 1 rats (Table 2).

RNA concentration in the neuronal cytoplasm in the hippocampus and neocortex of group 2 rats was substantially higher than in group 1 animals (Table 2). NADPH-D activity associated predominantly with extramitochondrial oxidation processes and synthesis of various substances in the cell [3] was lower in neocortical (layer II) and hippocampal neurons of group 2 rats compared to group 1 animals. NADH-D activity reflecting the intensity of mitochondrial oxidative processes [3] was lower in layer II neurons of group 2 animals. Intergroup differences in brain morphology were associated with differences in rat behavior in EPM at the age of 30 days. Thus, group 2 rats demonstrated more acts of hanging down, sniffing, movements, exits into open and closed arms; on the contrary, immobility time was by more than 2-fold shorter (Table 1).

TABLE 1. Effect of Litter Size on Behavioral Parameters in 40-Day-Old Rats

Index			Group 1	Group 2
Body weight, g		14 days	20.3±0.2	25.5±1.1*
		21 days	31.6±0.5	43.0±1.8*
		30 days	54.1±0.9	66.4±3.1*
		40 days	80±2	104.0±0.5*
Weight of the brain, mg		hemisphere, mg	547±95	74±10*
		adrenal gland, mg	10.4±0.4	13.90±0.65*
		testis, mg	462±13	485±79
		ovary, mg	19.0±1.5	19.2±1.3
Blood concentration of		estradiol, pg/ml	34.36±4.51	24.81±5.12
		testosterone, nmol/liter	3.17±0.57	6.77±2.55
HSD activity, arb. units	adrenal gland	zona glomerulosa	0.537±0.036	0.562±0.016
		zona fasciculata	0.551±0.029	0.562±0.020
		zona reticularis	0.518±0.028	0.583±0.015
Duration, sec	hanging down		2.70±0.44	5.14±0.75
	rearings		8.00±1.12	7.50±1.05
	grooming		14.60±2.24	13.40±1.66
	sniffing		136.0±4.8	154.00±2.28*
	movements		70.9±5.2	87.60±5.28
		open arm movements	25.4±7.0	35.6±4.7
		closed arm movements	153.0±7.1	142.6±4.7
	immobility		29.8±4.3	12.50±1.65*
Number of	hangings down		2.70±0.43	5.40±0.72*
	rearings		7.30±0.91	8.40±0.88
	acts of grooming		7.10±0.57	7.1±0.5
	acts of sniffing		5.70±0.43	7.0±0.4*
	movements		9.50±0.75	13.40±1.02*
	exits into open arms		1.90±0.31	2.80±0.35*
	entrances into closed arms		2.40±0.26	3.44±0.37*

Note. *$p<0.05$ compared to group 1.

Thus, higher brain weight in pups from reduced litters was associated with higher hemisphere weight and apparently with higher neocortex weight. Moreover, morphometric and histochemical peculiarities of cortical neurons in these pups reflect important time points for their development and functional state. The revealed macro- and microscopic differences in brain structures seemed to be a factor determining behavioral differences between the groups in EPM.

Our findings suggest that relations between brain growth on one hand and important parameters of its neurons on the other exist in the course of postnatal ontogeny and brain development. The pattern of intergroup differences in morphometric parameters reflecting brain development in 40-day-old rats differs from that observed in 1-day-old animals [6], which is indicative of changes in the character of association between brain size and morphometric and histochemical properties of the neocortex and various cortical neurons occurring during ontogeny. These regularities are of interest for morphological investigation of the brain and for evaluation of correlations between various morphological and functional characteristics of brain development.

TABLE 2. Effect of Litter Size on Parameters of Brain, Gonad, and Adrenal Gland Development in 40-Day-Old Rats

Index			Group 1	Group 2
Thickness, μ		RPL, layer I	173±4	165±3
		cortex	1797±20	1787±18
		PL, layer I	156±3	150±3
		cortex	1422±21	1478±31
Section area, μ^2	Cytoplasm	RPL, layer II	45.3±1.2	39±1.4*
		RPL, layer V	88.7±2.4	78.4±1.7*
		PL, layer II	45.3±1.3	38.5±1.2*
		PL, layer V	82±1.9	75.6±2.6
		hippocampus	50.5±2.1	46.8±2.2
	Nuclei	RPL, layer II	61.4±1.3	56±0.6*
		RPL, layer V	105±2	95.3±2.3*
		PL, layer II	60±1.5	55.5±1.5*
		PL, layer V	95±2.4	92.7±2.1
		hippocampus	73.8±1.9	70.6±1.4
	Nucleoli	RPL, layer II	2.7±0.11	2±0.04*
		RPL, layer V	4.8±0.11	4.1±0.13*
		PL, layer II	2.6±0.17	2±0.03*
		PL, layer V	4.4±0.08	4±0.1*
		hippocampus	2.9±0.2	2.5±0.07
RNA concentration, arb. units		RPL, layer II	0.259±0.013	0.299±0.014*
		RPL, layer V	0.312±0.017	0.359±0.014*
		PL, layer II	0.243±0.015	0.300±0.019*
		PL, layer V	0.307±0.017	0.369±0.019*
		hippocampus	0.255±0.019	0.346±0.0178*
NADPH-D activity, arb. units		Layer II	0.416±0.014	0.363±0.018*
		Layer V	0.396±0.01	0.376±0.021
		hippocampus	0.436±0.018	0.384±0.019*
NADH-D activity		layer II	0.445±0.012	0.386±0.017*
		layer V	0.389±0.011	0.377±0.020
		hippocampus	0.421±0.015	0.428±0.013

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