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Morphometric and Statistical Analysis of Macro- and Microscopic Characteristics of the Brain and Their Relationships with Adrenocortical Histophysiology in Newborn Rats

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A direct relationship between the weights of the brain and hemisphere and parameters characterizing the development of the cortex and its neurons in intact newborn rats was revealed. Parameters of brain development in newborn rats correlate with 3β-hydroxysteroid dehydrogenase activity in the adrenal zona glomerulosa.

Key Words: *brain; newborns; morphometry; adrenal cortex*

Brain development during the embryonic and early postnatal ontogeny includes changes in its weight, thickness of the hemispheric cortex, and numerous morphometric characteristics of these cells [5]. Functional interpretation of differences in the organ weight is different [5,8]. We found no data on the relationship between brain weight and many morphometrical characteristics of the cortex and neurons. Study of this aspect, which became our purpose, can lead to more precise and detailed evaluation of differences in brain weight as a characteristic of its development during the early ontogeny. One more purpose was evaluation of the relationships between the level of brain development in newborn rats and activity of their adrenal cortex (injection of corticosteroids to pregnant animals modifies brain development in their progeny [3,5, 6,9-11]). The effects of hormones produced by maternal adrenal glands and developing fetus at concentrations not surpassing the normal (which is highly variable) on the brain are little studied [1]. We previously detected a relationship between some parameters of brain development in 21-day fetuses and newborn rats and activity of 3β-hydroxysteroid dehydrogenase (HSDH) in the adrenal cortex and ovaries of their mothers [7]. Fetal glands also produce corticosteroids during gestation period [1,3,4], and therefore we studied the relationship between their activities and fetal brain development.

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MATERIALS AND METHODS

One-day-old progeny of 4-5-month-old rats (6 litters, n=55) were studied. All rat pups were weighed and 1-3 males and 1-3 females with the weight mean for the litter were selected from each litter (a total of 24 pups). The animals were decapitated, the brain and right hemisphere were weighed using an electronic balance. Paraffin sections (7 μ) from the left hemispheric anteroparietal (APL) and parietal lobe (PL) were sliced strictly perpendicularly to the hemispheric surface and log and stained with gallocyanin after Einarson. The thickness of APL and

PL was measured using a MOV-15 ocular micrometer. The number of neurons in cortical layer V in these zones per standard visual field was counted. Section areas of neuronal nuclei in layers of II and V of APL and PL cortex and in the hippocampal CA1 field were measured on a Mecos (medical computer systems) device (20 nuclei per structure for each case) and the nucleoli were counted in the measured nuclei. Reaction for the key enzyme of steroidogenesis (HSDH) was carried out [2] on cryostat sections (20 μ) of the adrenals. Enzyme activity was measured on a Mecos device using monochromatic light (λ=530 nm) in 25 cells of each zone for each case. The thickness of the adrenal cortex was measured in the same preparations using an ocular micrometer.

The results were processed using Statistica software.

RESULTS

The mean weight of newborn rat pups was 5.70±0.08 g, mean weight of the brain 238.0±4.7 mg, which is in line with previous data [5]. These parameters were in strong correlation (r=0.76). The weight of the brain correlated with HSDH activity and the ratio of HSDH activities in the zona glomerulosa and zona reticularis (r=0.73 and r=0.82, respectively). These data are in line with previous data on correlations between the weight of the brain in 21-day rat fetuses and HSDH activity in maternal adrenal cortex and with the data on the stimulatory effect of deoxycorticosterone acetate (drug with the characteristics of mineralocorticoids produced by the zona glomerulosa) on the brain [7]. The thickness of the cortex in APL and PL was 561.0±14.7 and 546.0±10.5 μ, respectively. Thus, there was a trend to local differences in the parameter. The largest nuclei were found in hippocampal neurons (39.70±3.39 μ^2) and smallest in layer II of PL (25.0±1.8 μ^2). The mean number of the nucleoli was virtually the same (Table 1).

TABLE 1. Morphometricl Characteristics of Cerebrocortical Neurons in Newborn Rats (M±m)

Parameter	Nucleus size, μ^2	Number of nucleoli per nucleus
APL, layer II	32.3±2.2	1.300±0.046
APL, layer V	34.8±1.3	1.300±0.053
PL, layer II	25.0±1.8	1.200±0.053
PL, layer V	33.60±1.27	1.340±0.076
Hippocampus, CA1	39.70±3.39	1.30±0.07

Analysis of the morphometrical parameters reflecting the development of the cortex and its neurons, brain weight, and HSDH activity in the adrenal cortex revealed significant correlations between these parameters (Table 2). A direct significant or close to significant relationship was detected between brain weight and parameters characterizing

TABLE 2. Correlations between Morphometric Parameters of Cerebrocortical Neurons, Brain Weight, and HSDH Activity in the Adrenal Cortex of Newborn Rats

Parameter		Brain weight	HSDH activity in adrenals			
			zona glomerulosa	bundle zone	reticular zone	glomerular/ reticular zones
APL		0.85*	0.6*	-0.1	-0.69*	0.77*
PL		0.52	0.28	-0.64*	-0.59*	0.59*
APL, layer II	nucleus size, μ^2	0.63*	0.62*	-0.37	-0.37	0.66*
	number of nucleoli per nucleus	0.16	0.51	-0.23	0.06	0.4
APL, layer V	nucleus size, μ^2	0.49	0.22	0.06	-0.34	0.23
	number of nucleoli per nucleus	0.66*	0.6*	0.44	0.04	0.34
PL, layer II	nucleus size, μ^2	0.59*	0.36	0.39	-0.02	0.22
	number of nucleoli per nucleus	0.78*	0.39	0.19	-0.43	0.44
PL, layer V	nucleus size, μ^2	0.46	0.32	-0.05	-0.15	0.32
	number of nucleoli per nucleus	0.66*	0.6*	0.54	0.05	0.33
Hippocampus, CA1	nucleus size, μ^2	0.52	0.55	0.33	0.1	0.35
	number of nucleoli per nucleus	0.33	0.62*	0.64*	0.37	0.16

Note. *Correlations are reliable.

TABLE 3. Morphometric Characteristics of Cerebral Cortex, Activity of Adrenocortical 3β-Hydroxysteroid Dehydrogenase in Newborn Rats Differing by Brain Weight

Parameter	Rats with "small" brain	Rats with "large" brain
Body weight	5.70±0.11	6.2±0.2*
Abs. weight of brain	220.0±2.5	262.0±3.6*
hemisphere, mg	72.0±1.8	84.6±2.0*
Rel. weight of brain, mg/g	43.20±0.47	42.4±0.9
Cortex thickness, μ		
APL	533±13	611±8*
PL	531.0±9.8	572±18*
Number of neurons in visual field of layer V		
APL	26.3±1.3	23.5±1.4
PL	23.2±1.8	22.0±2.1
Size of nucleus, μ^2		
APL, layer II	28.6±1.9	38.8±3.2*
APL, layer V	33.20±1.27	37.5±2.5
PL, layer II	22.2±1.4	29.80±3.36
PL, layer V	32.0±1.7	36.60±0.73*
Hippocampus	34.6±4.1	48.70±2.18
Number of nucleoli in nucleus		
APL, layer II	1.250±1.038	1.30±0.14
APL, layer V	1.270±0.038	1.48±0.10
PL, layer II	1.110±0.028	1.38±0.09*
PL, layer V	1.240±0.018	1.53±0.19
Hippocampus	1.240±0.077	1.40±0.14
HSDH activity in adrenal, arb. units		
zona glomerulosa	0.236±0.018	0.320±0.013*
bundle zone	0.318±0.026	0.338±0.018
reticular zone	0.402±0.032	0.381±0.020
glomerular/reticular zones	0.589±0.026	0.8600±0.0058*
Thickness of adrenal cortex, μ	314±27	370±10

Note. *$p<0.05$ *vs.* "small" brain.

the size of the APL and PL cortex, neuronal nuclei in these regions of the cortex and in the hippocampus, and number of nucleoli per nucleus. These relationships are not *a priori* obvious, but we consider that their presence renders greater significance to such a parameter as brain weight in studies of the early stages of its organogenesis.

Analysis of correlations also showed direct significant or close to significant relationships between HSDH activity in the zona glomerulosa and ratio of enzyme activity in the zona glomerulosa to that in the zona reticularis and the studied morphometrical parameters of cerebral cortex development. This supplements our data on correlations between gland activity and parameters of brain development.

At the next stage of data processing all animals were divided into 2 groups depending on brain weight (animals with "large" and "small" brain, Table 3). The groups differed also by the thickness of the cortex, size of neuronal nuclei, and HSDH activity in the adrenals. The density of neuronal distribution in the cortex, decreasing with its development, was somewhat lower in APL and PL of rat pups with "large" brain (Table 3) than in those with "small" brain ($p>0.05$). The thickness of the cortex in APL and PL differed significantly in animals with large brain and virtually did not differ in animals with small brain. Presumably, greater differences in the thickness of the APL and PL cortex also indicates more advanced development of the brain in this group.

The thickness of the adrenal cortical matter was virtually the same. On the other hand, activity of HSDH in the zona glomerulosa of rats with large brain was higher and in the zona reticularis lower than in rats with small brain. The ratio of enzyme activities in these zones was 0.860±0.058 and 0.589±0.026 in animals with large and small brain, respectively (Table 3). These data suggest that the ratio of blood concentrations of steroids produced in different zones of the adrenal cortex differed in rats of the two groups. Presumably, higher concentrations of mineralocorticoids produced by the zona glomerulosa is, due to their anabolic characteristics [4], a factor determining advanced development of the brain in animals with large brain. Their effects can be realized as early as during the prenatal period due to appearance of appropriate receptors in the organ cells [4,8].

Hence, normally the weights of the brain and hemispheres, morphometrical characteristics of the cortex and its neurons are in direct relationship during the neonatal period, this indicating a relationship between changes in the macroscopic parameters of the organ development and the development of its major components, these processes correlating with HSDH activity in the adrenal cortex of these animals.

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