Morphology of the Cerebellum in the Progeny of Female Rats Exposed to Long-Term Emotional Stress before Pregnancy

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The structure of the cerebellum was studied in 40-day-old progeny of female rats exposed to 3-week emotional stress and mated with intact males after 10 days and in controls (progeny of intact females). The cerebellum in the experimental group was smaller, the nucleus and cytoplasm of Purkinje cells were smaller, the concentration of RNA in their cytoplasm and increased, the percentage of dark Purkinje cells increased, and the concentration of lipids in the molecular cortical layer and white matter decreased.

Key Words: *cerebellum; progeny; stress; histochemistry; morphometry*

Prenatal stress, including emotional stress, has a negative impact on the higher nervous activity (HNA) of the progeny [4]. The progeny of females exposed to chronic emotional stress during pregnancy differs from the controls by morphometric characteristics of the neocortical and hippocampal neurons and histophysiology of the adrenal cortex at the age of 21 and 40 days [6]. Long-term emotional stress before pregnancy leads to the appearance of the progeny differing from the control by the weight of the brain and cerebral hemispheres, morphometrical characteristics of the neocortex, and HNA [5]. The cerebellum is usually not referred to brain compartments playing the key role in reactions to emotional stress; on the other hand, cerebellar cells are characterized by high density of glucocorticoid receptors [11]. Injection of dexamethasone to pregnant rats promoted apoptosis of cerebellar granular cells in their 7-day-old progeny induced by exposure to mercury compounds and colchicine *in vitro*. Changes in mitochondrial functions and marked inhibition of catalase were detected [10]. Unfavorable conditions during embryogenesis lead to changes in the cerebellar morphology and characteristics of cerebellar cells after birth [13]. We found no data on the effect of emotional stress in females on the postnatal development of the cerebellum in their progeny. We investigated the morphology of the cerebellum in the progeny of females exposed to stress before pregnancy.

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MATERIALS AND METHODS

The study was carried out on 40-day-old progeny of intact female rats (control) and experimental females exposed to stress for 3 weeks. Experimental animals were housed in cages with glass cellar illuminated with 3 luminescent lamps at a distance of 90 cm from the cage floor (5 days for week). The duration of illumination was 6 h (9.00-15.00). Forty-day-old progeny of intact females (4 litters, n=26) and females exposed to stress (5 litters, n=33) was examined. The control and experimental females before and during pregnancy and their progeny were kept simultaneously in the same vivarium. All rats were sacrificed by decapitation; body weight and weights of the brain and cerebellum were determined. The cerebellum of two males and two females from each litter was taken for histological study. Frontal paraffin sections of the cerebellum (7 μ, left hemisphere), were stained with hallocyanine for nucleic acids and morphometrical analysis

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of the lateral zone of the hemisphere (neocerebellum) was carried out [2]. The thickness of the cerebellar cortex and layers was measured using a MOB-15 ocular micrometer, the apices of 5 gyri were measured in each case. RNA concentration in the cytoplasm of Purkinje cells was measured using a MEKOS programmed complex (Medical Computer Systems) and Morphodensitometry software. Twenty-five neurons in 8-12 visual fields were studied in each case. The mean RNA content in the cytoplasm was calculated from the area of the cytoplasm section and RNA concentration. The number of Purkinje cells per standard length (1000 μ) of the ganglionar layer was determined. These measurements were carried out in the cortical sulci (400-600 μ to the depth of the sulcus from the surface of the ganglionar layer) in 5-7 gyri. The section area of neuronal nuclei in the granular layer of the cortex was estimated on the basis of 25 measurements per case and the number of neurons in a standard visual field was determined. Cryostat sections (25 μ) were prepared from the right cerebellar hemisphere and stained with Sudan black for evaluation of myelination. The reaction intensity was evaluated on a MEKOS complex.

The results were computer-processed using Statistica software.

RESULTS

Maternal stress had different impact on some parameters in male and female progeny. The weight of the brain and cerebral hemispheres virtually did not differ from the control in experimental female pups, but decreased in male pups. The weight of the cerebellum decreased in both males and females (by 14.4 and 7%, respectively). The percentage of cerebellar weight from the weight of the whole brain decreased, *i. e.* the changes in the cerebellum were more pronounced than in the whole brain (Table 1). Sudan black staining showed a considerable decrease in the content of phospholipids, an important component of the myelin membranes [8,12], in the white matter of the cerebellum

TABLE 1. Effect of Long-Term Emotional Stress Experienced by Female Rats before Pregnancy on the Cerebellum of Their Progeny

Parameter	Experiment		Control	
	males	females	males	females
Body weight, g	74.00±2.98*	85.0±5.2	92.00±3.28	82.0±5.4
Brain weight, mg	1511±28*	1538±23	1605±18	1551±20
Cerebellum weight, mg	167.0±1.9*	172.0±3.9*	195.0±3.6	185.0±4.5
Cerebellum/brain weight, %	11.20±0.17*	11.00±0.16*	12.00±0.17	12.20±0.25
Thickness (μ) of				
cerebellar cortex	372±26	377±21	379±30	357±48
molecular layer	163±18	180±10	168±19	156±9
granular and ganglionar layer	210±13	197±12	219±31	200±15
Cell number				
Purkinje/1000 μ	26.0±0.9*	25.0±1.2*	17.7±0.6	19.2±0.3
of these, dark cells of granular layer	6.3±0.9*	5.7±0.6*	2.4±0.6	2.7±0.6
per visual field	5.7±0.1*	5.5±0.2*	6.9±0.1	7.0±0.2
Section area, μ^2				
Purkinje cells	194.0±6.8*	184±9*	241.0±5.3	212.0±8.7
cytoplasm	110.0±5.0*	97.0±6.8*	132.0±4.1	131.0±6.4
nucleus	84.0±2.6*	87.0±4.1	109.0±4.4	81.0±3.4
Section area of granular layer cells, μ^2	20.0±1.2	21.7±1.1	19.6±1.5	21.0±0.9
RNA concentration in cytoplasm of Purkinje cells, arb. units	0.411±0.026*	0.373±0.015*	0.308±0.021	0.31±0.09
RNA content per cytoplasm section, arb. units	79.6±5.3	68.7±4.4	74.4±5.8	66.0±4.2
Lipid concentration, arb. units				
molecular cortical layer	345±22*	378±22*	515±20	514±28
white matter	472±29*	511±27*	696±36	664±46

Note. *The differences from the control are significant.

and in the molecular layer of cerebellar cortex (consists mainly of nerve fibers) in the progeny of stressed females (Table 1). This probably attests to decelerated myelination. In rats most intensive myelination occurs during the first month of postnatal ontogeny [3,8,12]. Presumably, delayed myelination is responsible for decreased weight of the cerebellum in experimental rats.

The thickness of the cerebellar cortex and its layers in the control and experimental animals considerably differed on the surfaces of different gyri and, even more so, in sulci, at different depth thereof. However, the mean values measured on the gyrus surfaces virtually did not differ for the groups. The number of Purkinje cells per standard length of the ganglious layer of the cerebellar cortex was significantly higher in experimental rats in comparison with controls. Presumably, this depended on the lesser volume of fibers lying between these neurons, which was due to their slower myelination. There were 24.2% dark cells in experimental males and 22.8% in females *vs.* 13.5 and 14% in controls, respectively, while the absolute number of dark cells per standard length of the ganglious layer differed more than 2-fold between the groups (Table 1). Morphometric analysis of Purkinje cells showed that the area of these cells (their cytoplasm) in experimental males and females decreased. The size of nuclei in these cells decreased significantly in males, but little differed from the control in females. RNA concentration in the cytoplasm of Purkinje cells significantly increased in the progeny of stressed females (in both males and females). The content of RNA in the total section area of the cytoplasm did not differ significantly between the groups (Table 1), which indicates opposite direction of changes in RNA concentration and size of the cytoplasm in experimental rats in comparison with controls. The number of cells per standard section area of the granular cerebellar layer was significantly lower in experimental males and females compared to controls. The size of nuclei in these cells little differed from the control. Since the nuclei occupy the greater part of granular cells, predominant cells in this layer, we could expect that the sizes of cells also little differed. Therefore, the lower number of granular layer cells in visual field in experimental rat cerebellum can be considered to be due to a greater relative volume of the neuropil and glyocytes and/or more intensive neuronal death in this layer during the prenatal or postnatal ontogeny. However, both these hypotheses are to be verified.

Hence, the cerebellar cortical layers containing both associative and efferent neurons in the progeny of females exposed to emotional stress differ from those in the controls. These differences were accompanied by delayed myelination in the molecular layer of the cerebellar cortex and white matter. The cerebellum is a brain compartment undergoing essential changes in the progeny of animals exposed to chronic emotional stress. The mechanisms underlying the formation of these differences are related to abnormal histophysiology of the adrenal cortex in animals exposed to chronic emotional stress [1] and with previously detected specific features in histophysiology of the adrenal cortex in the progeny of stressed females, manifesting, among other things, in higher activity of 3β-ol-steroid dehydrogenase [5]. The effects of corticosteroids on the formation of neurons and myelination of nerve fibers (deceleration of myelination under conditions of increased concentrations of these hormones) were discussed previously [4]. The study of the cerebellum in animals, whose embryogenesis was associated with maternal stress can be useful in the analysis of HNA deviations in animals exposed to stress or glucocorticoids during prenatal or neonatal period and for explaining changes in their motor activity [9,14], because motor coordination and programming is the most important function of the cerebellum [7].

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