Morphogenesis of Asymmetry of Rat Brain Nuclei under Normal Conditions and during Exposure to Microgravitation

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Histological and morphometrical analysis of the brain and peripheral analyzers revealed proliferative migration abnormalities of brain development and neuron differentiation in mammals during a space flight, which can limit space exploration.

Key Words: *brain; asymmetry; microgravitation; porencephalia*

During long-term space flights experimental animals adapt to microgravitation, but adaptive changes in adults differ from adaptation of embryos, fetuses, and larvae. Experiments on clawed frog (*Xenopus laevis*) larvae carried out under conditions of microgravitation (Biokosmos sputnik) showed considerable changes in cell differentiation and delayed development of the main analyzer systems (visual, auditory, and olfactory). However, weightlessness did not influence asymmetry of the development of amphibian nervous system [2].

The present study was carried out within the framework of embryological experiment (NIH-R1) on the Shuttle spaceship devoted to studies of morphogenesis of laboratory rats (*Rattus rattus*). In this paper we describe the effect of 11-day microgravitation on the development of the brain and its nuclei.

MATERIALS AND METHODS

Embryonal development of asymmetry of rat brain nuclei was studied on 20-day embryos, newborn rats, a group of synchronous control to the flight, and a group of space flight effect on maternal organism. The animals in space and on the Earth were kept and fed similarly.

During space flight, 10 laboratory rats were housed on the middle floor of Shuttle spaceship from day 9 to day 20 of pregnancy. Rat pups born 2 days after landing were experimental group. The first rat pup from each dam was taken. The study was carried out in cooperation with the Institute of Biomedical Problems.

The rats were decapitated under isofluran narcosis. The material was treated by routine histological methods and stained with hematoxylin and eosin and after Mallori. The volume and surface of the upper and lower colliculi, lateral and median habenular nuclei, and the caudate nucleus-shell complex on the left and right brain were measured on microprojections of the rat heads using Wacom Computer Systems GmbH devices. The areas and perimeters of cerebral structures were evaluated using Canvas 5.0.2 software (Deneba Systems Inc.). Borders and location of the nuclei were determined from the atlas of rat brain [9].

Asymmetry coefficient was estimated by absolute values using the formula:

$$\frac{R-L}{R+L} \times 100\%,$$

where R and L are the size of right- and left-brain structures, respectively [8].

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The results were statistically processed using Wilcoxon's test for paired data.

RESULTS

Embryos were exposed to microgravitation during the placentation and initial organogenesis, the periods most sensitive to damaging factors. Embryofetal and fetal periods of prenatal development passed over the flight [1].

Histological analysis of experimental embryos detected foci of neuronal degeneration characterized by the absence or decreased number of neurons and the presence of few glial cells and individual capillaries (Fig. 1). These foci were detected in the hippocampus, neocortex, dorsolateral nucleus of the thalamus, caudate nucleus, hypothalamus, and spinal cord.

Foci of neuronal degeneration were detected only in the experimental group (70% cases), which indicates a pathogenetic effect of microgravitation on the development of mammalian brain. This cast some doubt on the possibility of normal organogenesis in mammals under conditions of microgravitation and necessitates further long-term orbital experiments. Cavitation and stratification of the white matter indicate the development of porencephalia in experimental animals.

Presumably the detected abnormalities of the embryonal nervous system development are characteristic of space flights. It should be emphasized that the term "porencephalia" is used with certain amendments, because the detected defects differ from the common classification of this pathology of the nervous system.

Studies of the effects of these abnormalities on the development of brain structures and their asymmetry are of particular interest. We selected 5 structures: upper and lower colliculi, medial and lateral habenular nuclei, and the caudate nucleus-shell complex. These structures were chosen because they lie in different compartments of the brain, which allowed us to evaluate the effects of various factors on these compartments, and because they are the components of different functional systems of the brain: the upper colliculi are the components of the visual system and the lower of the auditory system, habenular nuclei are related to the limbic system of the brain, the caudate nucleus and the shell are also related to the limbic system and are the components of the nigrostratal and extrapyramidal systems [4,6]. In addition, these large structures are easily detected, which is particularly important for stereological analysis.

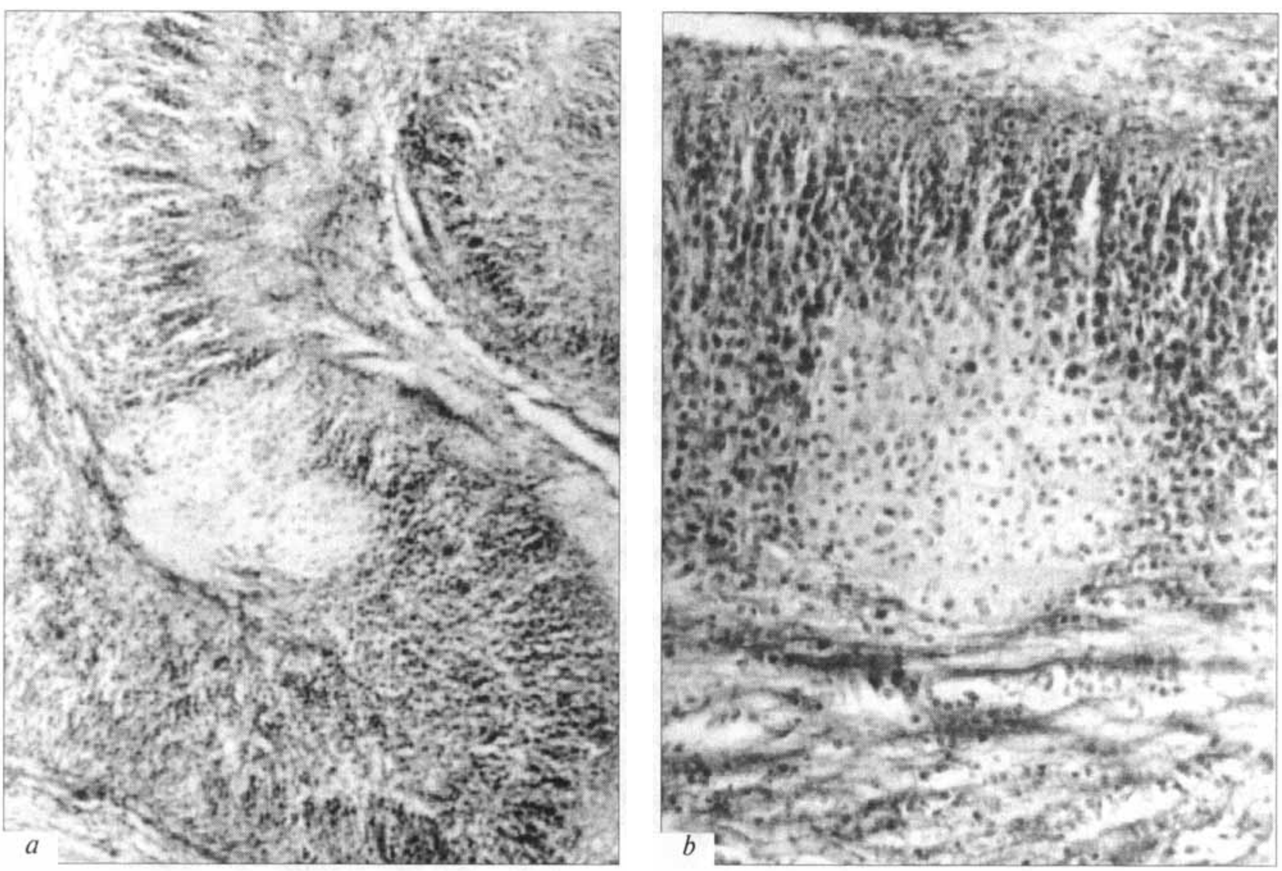

Fig. 1. Foci of neuronal degeneration in the hippocampus (*a*) and cerebral cortex (*b*) of a newborn rat prenatally exposed to microgravitation. Staining after Mallori, ×220 (*a*), ×440 (*b*).

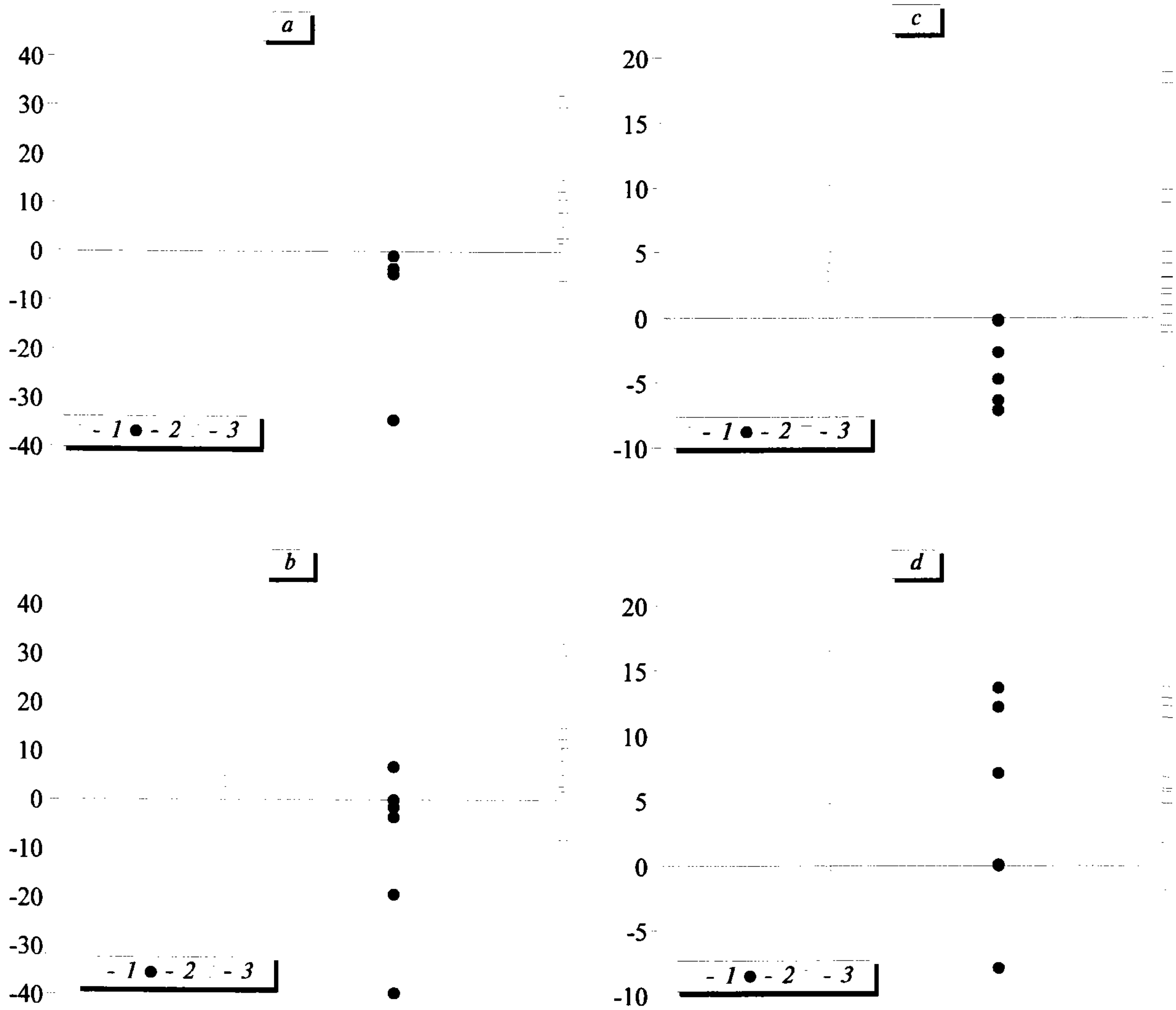

Fig. 2. Asymmetry of the volume of lower (*a*) and upper (*b*) colliculi and surface of the medial (*c*) and lateral (*d*) habenular nuclei. Ordinate: asymmetry coefficient. *1*) 20-day rat embryos; *2*) newborn rats (synchronous control); *3*) newborn rats prenatally exposed to microgravitation.

Analysis of the lower colliculi in 20-day rat embryos showed predominance of this nucleus on the right. In experimental group, the volume and surface of the lower colliculi were also larger on the right, while in the control group they predominated on the left (Fig. 2, *a*). No deviations from the norm or synchronous control were detected in the auditory analyzer system of rat pups prenatally exposed to microgravitation [3], except two cases of retarded development, changes in the asymmetry in the lower colliculi are related to more fine changes in brain structures in this group. The extent and consequences of these changes can hardly be evaluated now.

Normally, the left-brain upper colliculi predominate in newborn rats. In rats developed under conditions of microgravitation they were significantly larger then right-brain structures (as in 20-day embryos, Fig. 2, *b*). Presumably, this is associated with impaired development of the visual system in these animals. The retina of the experimental animals (space flight group) was thinner, which was easily seen even at the qualitative level of analysis. A special study showed that the decrease in the retina thickness in experimental animals was due shortening of retinal bipolar cell processes. Physiologically, such changes in retinal structure are normal adaptive reaction, but in long-term space flights they can affect visual function [3].

Structural asymmetries of the habenular nuclei have been known for a long time, starting from pilot investigations of Braitenberg, Kemali, and Morgan [7, 9] performed on amphibians. Our experiments showed asymmetry of the diencephalic habenular nucleus in rats, which can be modified during embryogenesis. In two control groups, the median habenular nucleus in newborn rats significantly predominated on the left side, while in the space flight group it predominated on the right (Fig. 2, *c*). It is most probably due to certain underdevelopment of the median habenular nucleus in the space flight group, which is in line with the data on the 21% decrease in these structures after prenatal exposure to microgravitation. The same right-side asymmetry was detected in 20-day embryos. Significant right-side asymmetry of the lateral habenular nucleus was detected in 20-day control embryos and in the experimental group (Fig. 2, *d*), but not in the control groups of newborn rats. It is most probably due to underdevelopment of this nucleus in the flight group.

No asymmetry of the caudate nucleus-shell complex was detected in all groups.

Hence, qualitative and quantitative changes in brain structure were detected in rat embryos developed under conditions of microgravitation. Changes in the brain morphogenesis under conditions of microgravitation do not lead to animal death. Histological and morphometric analysis of the brain and peripheral analyzers in animals developed during space flight demonstrated proliferative migration abnormalities of brain development and neuron differentiation in mammals exposed to microgravitation, which can limit exploration of the space by man.

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