The investigation demonstrated the marked protective action of HBO relative to morphological preservation of cortical pyramidal neurons in the zone of developing ischemia. Definite dependence of the effect on dose was observed in only one case, namely 24 h after a late session. The inverse character of this dependence may perhaps be connected with the spastic effect of a dose of 2 atm on the cerebral arterioles and capillaries, which we found in the course of an electron-microscopic investIgation [4]. The action of HBO on transcription demonstrates a somewhat complex dependence on the conditions of HBO. Unlike an early session, the effect of late treatment is normalizing only for nucleolar transcription, and activity of extranucleolar chromatin is inhibited by both doses.

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MULTIVARIATE DATA ANALYSIS TO STUDY THE EFFECT OF SPACE FLIGHT FACTORS ON NEURONAL STRUCTURE IN THE RAT BRAIN

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KEY WORDS: factor analysis; plastic changes; dendrites

The study of the morphological basis of compensatory processes taking place in the nervous system during adaptation to weightlessness, an important role in the development of which is played by the visual system, has necessitated the study of the state of the visual cortical neurons in the brain of rats exposed to the influence of space flight factors (SFF). Considering the complexity and diversity of interneuronal interactions in the neocortex, it seems problematical that all neurons (even of the same class) would give an identical morphological response to SFF. It is perfectly possible that a quite sizeable group of neurons does not participate in the formation of compensatory processes and does not undergo morphological changes. Consequently the problem arises of how to distinguish from the whole population of experimental neurons, groups of cells that possess a characteristic set of features which is not found in control neurons. The parametric and nonparametric statistical methods (the Kruskal–Wallis test, anovar, the t test) used traditionally [3, 5] in studies of plastic changes in dendrites, utilize a priori information on the subdivision of the test population into groups and cannot help to solve the present problem.

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In the present study we examined the possibility of using some methods of multivariate statistical analysis to determine the presence and character of changes in the dendrite system of neurons experimentally, with particular reference to pyramidal cells in the visual cortex of rats taking part in manned space flights.

EXPERIMENTAL METHOD

The experimental material consisted of preparations of the visual cortex of five adult rats, on board the "Kosmos-1887" manned spacecraft, and of 10 control animals, stained by Golgi's method. Drawings (magnification 400) of pyramidal neurons of layer III were obtained under an "Ortholux" microscope: 14 cells belonging to control animals (CN) and 15 taking part in space flight (FN). Altogether 30 parameters characterizing the structure of the dendritic system of the neurons were measured by means of an "ASM" semiautomatic image analysis system ("Leitz," Germany). Parameters describing the orientation of the dendrites [2] and parameters of length and degree of branching were measured separately for the system of the apical and the system of the basal dendrites.

The data were analyzed by PDP-11/03 (OZU 64K) computer, using our own programs realizing the methods of multivariate statistical analysis examined previously. To carry out certain operations with matrices we used subprograms from the standard "Scientific Subroutine Package." To find structurally different groups of neurons we used the *o*-technique of factor analysis, adopting the method of chief factors and orthogonal rotation [1]. To determine the character of the morphological changes under the influence of SFF, canonical analysis was carried out [1,5]. The significance of discriminant functions thus revealed was verified by Bartlett's test ($\alpha = 0.01$), and the significance of differences between groups of cells was determined by Hotteling's T^2 statistic ($\alpha = 0.05$) [4]

EXPERIMENTAL RESULTS

We postulated that the morphological uniqueness of each neuron is due both to the specific integrative function which it performs and to a set of causes not directly connected with this activity (trophic functions, topology of surrounding structures). It can be postulated that the structure of the dendritic system of each neuron is the sum of morphological complexes, each of which is necessary for the performance of one of the biological functions of the cells. Some of these complexes are evidently represented in many neurons, although to a different degree, while others are unique and reflect individual differences in functioning of the cell. Plastic changes taking place with neurons under experimental conditions can be regarded and revealed as a change in value of the contributions of different complexes to the structure of the dendritic tree, and the appearance of new complexes and disappearance of some previously present.

Of all the methods of data analysis known to us, that described above seems to be in closest agreement, in our view, with the model of the *o*-technique of factor analysis. This method is based on the principle of representation of variation of test objects as the result of realization of a limited number of general principles of structure (factors). Each object, represented by a vector of values of measured parameters, is regarded as a linear function of general factors. The coefficients of this function (factor loadings) determine the contribution of each factor to the structure of the object. An advantage of this method is that it presupposes a procedure of rejection of individual distinguishing features. Thus the factor analysis model fully corresponds to our hypotheses for formation of the structure of the dendritic system of neurons and does not require a priori information on the statistical properties of the processes under investigation.

Application of the algorithm of *o*-factor analysis to the general correlation matrix enables all neurons to be represented by points in a space of general factors. In order to find plastic changes in neurons taking place experimentally, it is necessary to distinguish from the general factors those whose loadings differ in cells belonging to control and experimental animals. The position of the neurons in the space of these factors will be determined by exposure to the experimental conditions. It is evident that we do not obtain full information on the action of the experimental conditions on neuronal structure. The picture may be incomplete because of the limitation of the initial set of parameters, and also because of an unsuccessful rotation procedure. Regarding the latter it must be emphasized that some existing principles may not be found in the loadings of the neurons, but the algorithm does not enable the investigator to add his own considerations to the phenomena present in the material.

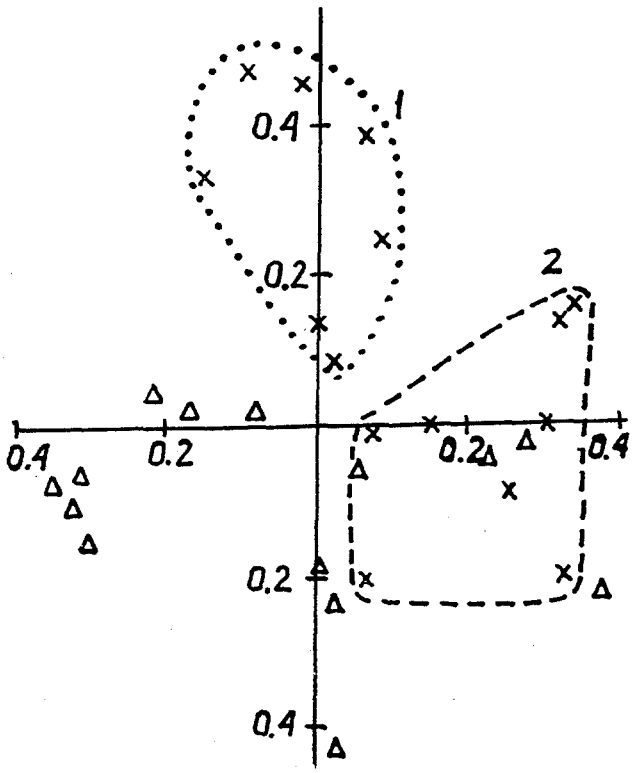

Fig. 1. Arrangement of neurons in factorial space: a) triangles – neurons of control animals, b) (crosses) – experiment. 1) First flight group, 2) second flight group.

In the present investigation, during *o*-factor analysis 14 meaningful factors were distinguished, making grouping of all 29 test neurons possible. By orthogonal rotation, two factors were distinguished, which can be interpreted as the result of exposure to SFF. Loadings of the neurons to these factors enabled two groups of FN to be distinguished and, correspondingly, two directions of morphological variation (Fig. 1). One of them, leading to the formation of FN1, is the formation of a new morphological complex. The other direction, connected with isolation of FN2, can be explained by a change in the contribution of the normally existing complex to the structure of the dendritic system in some FN. It is possible, however, that under normal conditions also, neurons of the type studied can be subdivided into two groups. In that case it can be postulated that isolation of FN1 is connected with the acquisition by a neuron of one of these groups of a new morphological complex, whereas the structure of the dendritic system of the neuron of the other group does not change significantly. It became evident that a more profound classification of the pyramidal cells of layer III of the intact visual cortex, previously regarded as a homogeneous group, must be carried out.

We considered it unnecessary to assess the connection between the original parameters and the isolated factors within the scope of the model of *o*-factor analysis, in view of the heterogeneity of the test neuron population revealed. It was also essential to prove that the position of the factor axes discovered not only enables the character of grouping of the neurons to be discovered, but also corresponds accurately to the directions of the morphological changes taking place during flight, which in our view is impossible. In our case the task of describing the directions of the changes discovered can be solved by other methods, for division of the neurons by types of their response to SFF was found.

Within the scope of the canonical analysis which we used for this purpose, discriminant functions were calculated for "CN–FN1" and "CN–FN2" pairs. Analysis of the signs of the coefficients of these functions and contributions of the parameters to their intergroup dispersion enables the direction and degree of changes in

individual parameters during exposure to SFF to be determined. For example, high values of contributions of parameters describing orientation of dendrites of the apical system, obtained for FN1 cells are evidence of more significant changes in this dendritic system compared with changes in the basal system (small values of contributions of parameters describing orientation of dendrites of the basal system).

By analyzing the character of changes in the whole set of parameters, we could determine the functional importance of structural reorganization of the dendritic system of neurons during exposure to SFF, but this task lies outside the scope of the present publication.

The study of the response of visual cortical neurons to SFF can be regarded as a special case of the frequently arising task of studying the reaction of biological objects to exposure to environmental-factors of complex nature. Our suggested approach, using methods of multivariate data analysis, may prove to be of substantial help when problems of this kind are being solved, especially if variation of the conditions of exposure is difficult or, as is often the case when studying human biology, impossible.

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IS TRANSFERRIN THE NEUROTROPHIC FACTOR CONTROLLING THE COMPOSITION OF SKELETAL MUSCLE MYOSINS?

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It is generally accepted that skeletal muscle phenotypes are under neurotrophic control [7]. It is suggested that this control is realized by motoneurons by two mechanisms: through hypothetical trophic factors of peptide nature synthesized in the perikarya of nerve cells and transported to muscle by axonal transport systems, and it may also be determined by the character of spike activity [6, 13]. What are these trophic factors that are transported intraaxonally to muscle? We have as yet no unambiguous answer to this question. It has been shown that certain organoids, enzymes, and high-molecular-weight compounds can be transported by the axonal current to target cells [11]. In 1976 [15] a protein, which was called sciatin, was isolated from nerve tissue, and according to some of its features, it could possibly claim the role of one such neurotrophic factor. It was soon discovered that this protein is none other than transferrin, bound with Fe [14]. Exogenous administration of transferrin and even of trivalent ferric ions could prevent the development of certain denervation disturbances, such as: a decrease in the area of cross section of the muscle fibers (MF), an increase in expression of proteins of acetylcholine receptors, a change in the

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