Reactive hyperemia on the photoplethysmogram corresponded to a rapid decrease in the intensity of reflected light (Fig. 1, II, a, segment 5) followed by recovery to the initial level in the course of 1-3 min. Values of the maximal vasodilator responses obtained by PPG and LDF methods correlated with one another (r = 0.69; p < 0.05; Fig. 2b).

Parameters of vasoconstrictor (induced by noradrenaline) and vasodilator responses (reactive hyperemia), determined by methods of laser doppler fluxometry and photoplethysmography thus correlate with each other. Either of the two methods is adequate for studying cutaneous vascular reactions and can be used independently. A combination of both methods may, however, increase the reliability and informativeness of the investigation.

## LITERATURE CITED

- 1. A. S. Ibragimova, E. V. Érina, I. I. Almazov, et al., Kardiologiya, No. 2, 76 (1986).
- 2. E. V. Oshchepkova and S. E. Ustinova, Kardiologiya, No. 3, 22 (1985).
- 3. D. Bohr and R. Webb, Am. J. Med., 77, 3 (1984).
- 4. M. Mulvany, Blood Vessels, 20, 1 (1983).
- 5. G. E. Nilsson, T. Tenland, and P. A. Oberg, IEEE Trans. Biomed. Eng., 27, 597 (1980).
- 6. V. V. Panfilov, E. V. Oshchepkova (E. V. Oschepkova), G. N. Potapova, and G. G. Arabidze, 12th Scientific Meeting, International Society of Hypertension, Abstracts, Kyoto (1988), N. 0299.

MORPHOMETRY OF GIANT MULTIPOLAR NEURONS OF THE BRAIN-STEM RETICULAR FORMATION OF RATS CARRIED ON BOARD THE BIOSATELLITE "KOSMOS 1667"

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KEY WORDS: gigantocellular nucleus of reticular formation; space flight factors; quantitative analysis of neuron geometry.

Investigation of the brain of animals taking part in space flight is necessary in order to discover the mechanisms of the effect of weightlessness on the CNS. Investigations of the morphology of the nervous system in animals on board biosatellites have been published, but no attempt has been made to study the dendritic ramifications of brain neurons in such animals.

We studied giant multipolar neurons (GMN) of the gigantocellular nucleus of the brainstem reticular formation (GNRF), which is known to be concerned with two brain functions that may be disturbed in flight: motor and vestibular. In our view, GMN are integratively-triggering "premotor cells," triggering motor responses organized at that particular brain level by consolidation into a single functional system of the corresponding number of neurons at different levels of the brain [3, 6]. These cells receive powerful vestibular afferentation [10] although their extensive dendritic territory is such that the most widely different afferent impulses can be integrated [3]. Their afferentation from the somatic [1, 6, 7] and visceral [9] brain systems has in fact been demonstrated.

The aim of this investigation was to study the geometry of the dendrites and bodies of giant multipolar neurons of GNRF of the brain stem in rats kept for 7 days on the biosatellite "Kosmos 1667" during space flight and in control experiments.

## EXPERIMENTAL METHOD

Experiments were carried out on four groups of male Wistar-SPF rats (seven animals in each group): a flight group (F), the animal house control to the flight group (AC1), the ground control experimental group (GCE), and the animal house control to that group (AC2). The animals were decapitated and within 3 min a frontal block of brain tissue 4 mm thick was

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TABLE 1. Morphometric Parameters of GMN of GNRF of Brain Stem of Rats Carried on the Biosatellite "Kosmos 1667" and in Control Experiments

Parameters	Group of animals			
	F	AC-1	GCE	AC-2
$Sel, \mu^2$ $Sel, \mu^2$ $R_{comp}, \mu$ $Ad$ $Ad$ $E_{rs}$ $E_{dt}$ $Ad$ $Ad$ $E_{dt}$	6 (5-8) 1417 (1166-1467) 419 (396-537) 3 (2-4) 15 (11-28) 20,0 (16,0-23,0) 3,2 (1,5-6,0) 0,31 (0,26-0,65) 124 (103-191) 46 (33-68) 0,5 (0,3-1,0)	6 (4-7) 1265 (870-1874) 370 (327-410) 3 (2-4) 18 (13-23) 20,0 (15,0-24,2) 3,5 (2,1-5,3) 0,21 (0,15-0,40) 167 (126-185) 38 (23-55) 0,5 (0,3-1,0)	6 (4—7) 1517 (1419—2043) 381 (376—408) 3 (2—4) 15 (13—23) 23,0 (19,0—25,0) 3,6 (2,3—5,0) 0,35 (0,24—0,51) 173 (124—217) 39 (30—51) 0,6 (0,3—0,7)	7 (5-7) 1301 (934-1569) 343 (330-417) 4 (2-5) 14 (12-18) 22,0 (15,0-24,0) 3,4 (2,3-5,3) 0,31 (0,18-0,45) 152 (123-212) 42 (32-48) 0,7 (0,3-1,0)

Legend. Number of oscillations shown in parentheses.

excised at the level of the root of the seventh cranial nerve, and impregnated by Golgi's method. The neurons were drawn by means of a drawing apparatus under an "ortholux" microscope (15 neurons for F, 10 for ACl, 9 for GCE, and 8 for AC2). By means of an ASM system (Leitz, West Germany) and PDP-11 computer, the number of dendrites (d), the area of the cell body ( $S_{Cb}$ ), the greatest radius of the dendritic field ( $R_{Comp}$ ), the degree of ramification of the dendrite ( $A_{C}$ ) and of the whole cell ( $A_{C}$ ), the relative length of the dendrites ( $E_{CS} = R_{CS} =$ 

 $\frac{R_{comp}}{\sqrt{Scb/\pi}}$ ), the relative density of the lengths of the dendrites in the medium (Nd), the mean number of foci of maximal branching on the dendrite (XFMB) and in the whole dendritic territory  $(F_{
m dt})$ , the volume of the total dendritic territory  $(V_{
m dt})$ , and the total length of the dendrites in it  $(I_{dt})$  were calculated (by Leontovich's method, 1978). The orientation of the dendrites relative to six formations of the brain stem was analyzed by a special method: to the central gray matter (C), the sensory vestibular nuclei (V), the sensory trigeminal nuclei (T), the superior olive (0), the pyramidal tract (P), and the midline of the brain (K). For the more complete discovery of all branches of the dendrites, reconstruction of a neuron was carried out from neighboring sections and the reconstructed drawing was analyzed, and its exact location noted on the projection of the section. A transparent test grid with 15° sectors was placed on the drawing of the neuron so that its center coincided with the center of the cell body, and the zero radius was arranged parallel to the midline of the brain. In each sector the total length of the dendrites was measured and expressed as a percentage of the total length of the dendrites of the whole cell, and on the basis of these percentages, polarograms of the neurons were constructed (relative to the median for each group of neurons). The significance of the differences was calculated by the Kolmogorov-Smirnov test and the results are given as both median and quartiles (upper and lower).

#### EXPERIMENTAL RESULTS

A comparative study of GMN of GNRF of rats carried on the biosatellite "Kosmos 1667" in ACl, GCE, and AC2 revealed no significant differences with respect to the above-mentioned 11 parameters (Table 1), including the total length of the dendrites in the whole dendritic territory of the neuron. However, a tendency was observed for parameters such as the area of the cell body, the greatest radius of the dendritic field, and the volume of the whole dendritic territory, to increase in the F and GCE groups compared with the ACl and AC2 groups.

The orientation of GMN dendrites compared to a circular structure (Fig. 1) shows a definite decrease in total dendrite length in sectors in the direction of the vestibular sensory nuclei, and an increase in sectors along the midline for group F compared to groups AC-1, GCE, and AC-2. We must also note a tendency towards a decrease in the length of dendrites oriented in the direction of the central gray matter for group F compared with the 3 remaining groups.

Although in the present investigations no significant differences were found in the four groups of animals studied with respect to the 11 basic morphometric parameters characterizing each class of CNS neurons [3], the tendency toward an increase in the area of the cell body, the maximal radius of the dendritic field, and the volume of the whole dendritic territory in rats of the F and GCE groups suggests that although these changes were minimal, they were regular and were connected, not with weightlessness, but with excitation factors common to both experimental groups (stress, overloading, and so on).

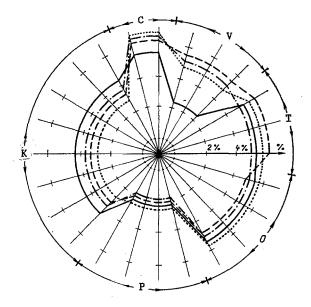


Fig. 1. Changes in orientation of dendrites of GMN of GNRF under conditions of weightlessness and of control experiments. C) Central gray matter, V) vestibulary sensory nuclei, T) trigeminal sensory nuclei, O) nucleus of superior olive, P) pyramidal tract, K) midline of brain Continuous line denotes P, dotted line AC-1, broken line GCE, and dots and dashes AC2.

Significant "reorganization" of the dendritic mass of GMN in the rats of group P only, including changes in their construction (reduction of the total length of the dendrites running toward the vestibular nuclei and an increase in the length of those running toward the midline of the brain) indicates that these changes can be associated with weightlessness. Such complex "reorganization" of the dendrites of the same cell under the influence of procedures of any kind has not been described previously. However, investigations have shown atrophy of dendrites as a result of loss of their principal sensory inputs [2, 8]. The decrease in size of certain dendrites of GMN, which was described, may reflect a deficiency of vestibular information under flight conditions and its leading importance for these neurons. GMN of GNRF are evidently highly specialized in relation to vestibulomotor responses, and this may account for the disturbance of these responses under flight conditions. The increase in mass of the dendrites running toward the midline of the brain can be explained by the fact that a deficiency of one afferent input leads to compensatory enhancement of afferentation of the cell from another [11, 12].

The increased excitability of deafferented brain structures is well known from physiology [13]. Possibly some phenomena of motion sickness and weightlessness (vomiting, changes in cardiac rhythm) may depend on increased excitability of the GMN described, in view of the vestibular afferentation deficit. In fact, the location of the cells which we tested corresponds to the location of the vomiting and cardiovascular centers [14]. Compensation of these disorders during subsequent flight was perhaps due to a decrease in excitability of GMN in view of their compensatory afferentation from other fiber systems, located near the midline.

The character of the change in dendrite geometry of GMN under flight conditions (reduction of the mass of some dendrites with an increase in that of others, while the total length of the dendrites of the whole neuron is preserved) suggests that in this case there is no dysfunctional atrophy or "growth" of the dendrites, but a phenomenon of retraction of some dendrites because of loss of their own synaptic connections [5] and the apparent "displacement" of the cell cytoplasm into other dendrites.

From the theoretical point of view it is important that the "reorganization" and structural changes of the dendrites were observed in adult animals; such phenomena have been described previously in an adult rabbit on neurons of the amygdala in response to its partial deafferentation [4]. In the few studies which have revealed changes in orientation or lengthening of dendrites of CNS cells in response to various forms of intervention [2, 15], young animals were used and they were exposed to the much longer action of the various factors (weeks or months). Our own data indicate the high plasticity of brain neurons in adult mammals, though manifested for a shorter period of time. This suggests that structural changes found in neurons carried on space flights are normalized after returning to earth.

### LITERATURE CITED .

1. P. V. Belichenko, Structural and Functional Principles of Brain Organization [in Russian], No. 7, Moscow (1978), p. 17.

- 2. N. G. Gladkovich, E. A. Lushchekina, T. A. Leontovich, and K. V. Shuleikina, Neirofiziologiya, 17, No. 4, 522 (1985).
- 3. T. A. Leontovich, Neuronal Organization of Subcortical Formations of the Forebrain [in Russian], Moscow (1978), p. 384.
- 4. T. A. Leontovich, N. G. Gladkovich, E. A. Lushchekina, and V. L. Chernova, Mechanisms of Brain Plasticity [in Russian], Vol. 2, Makhachkala (1982), p. 3.
- 5. O. S. Sotnikov, Dynamics of Structures of the Living Neuron [in Russian], Leningrad (1985), p. 160.
- 6. K. V. Shuleikina, The Genesis of Systems [in Russian], Moscow (1980), p. 123.
- 7. M. J. Cronin and M. A. Baker, Brain Res., 128, 461 (1977).
- 8. R. Harris and T. Woolsey, Brain Res., 161, 143 (1979).
- 9. A. D. Miller and V. J. Wilson, Brain Res., 270, 154 (1983).
- 10. B. W. Peterson, M. Filion, L. P. Felpel, and C. Abzug, Exp. Brain Res., 22, 335 (1975).
- 11. G. Raisman, Brain Res., 14, 25 (1969).
- 12. J. P. Rauschecker, Hum. Neurobiol., 3, 109 (1984).
- 13. D. K. Sharpless, Annu. Rev. Physiol., <u>26</u>, 357 (1964).
- 14. J. M. Siegel, Brain Res. Rev., 1, 69 ( $\overline{1979}$ ).
- 15. F. A. Valverde, Exp. Brain Res., 5, 274 (1968).

# STRUCTURAL MOBILITY OF LIVING MYELINATED NERVE FIBERS EXPOSED TO PROTEOLYTIC ENZYMES

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KEY WORDS: glio-neuronal relations; mobility of the neurolemmocyte; demyelinization; myelin sheaths.

The structure of living myelinated nerve fibers exhibits considerable mobility [3, 6, 9]. All rapid morphological reconstructions have a significant effect on functional properties of the conductor and are involved in the development of the early stages of pathological demyelinizing processes [1, 4, 5, 8]. The attention of research workers is currently concentrated on the study of the role of proteolytic processes in the development of early structural changes in myelinated fibers and, in particular, in Ranvier nodes [2, 7, 12]. The view on "elimination," retraction of myelin at the nodes, which develops under the influence of proteolysis, separating the neurolemmocyte and axon in the early stages of demyelinizing processes [11, 13] and in certain functional changes [2, 5, 11], is under discussion. The mobility of other structures of the myelinated fiber under the influence of proteolytic enzymes has not been studied. This process must be analyzed first of all on living objects.

The aim of the investigation was to study the character of morphological changes in all structural components of the living myelinated fiber, developing under the influence of proteolytic enzymes, and to clarify the mechanisms of its reactive transformation.

### EXPERIMENTAL METHOD

Experiments were carried out on single surviving myelinated fibers of Rana temporaria, isolated by mechanical dissection (on the method of isolation, see [3]). The fibers were placed in a microchamber with a continuous-flow system. The nodes, clefts, and perikaryon of the neurolemmocyte were studied. The zone of observation was not less than two nodes away from the zone of injury to the fiber. For proteolysis, a 0.2% solution of pronase E in Ringer's solution was used; this is the usual method for dissociation of neurons and glia in experimental physiology [4, 10]. Observations were made with the ordinary and phase-contrast

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