HETEROGENEITY OF DISTRIBUTION OF FILAMENTOUS-TUBULAR MATERIAL IN REACTIVELY CHANGED SYNAPSES AND NEURITES* OF THE CENTRAL NERVOUS SYSTEM

O. S. Sotnikov and E. M. Zamoskovskii

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Geometrical heterogeneity of neurites has a significant effect on their electrophysiological characteristics [11]. Functional changes associated with enlargement of synaptic swellings along the course of axons have been observed by many workers [2, 4-6]. The development of a varicosity is the typical reaction of neurites to pathology. There are strong grounds for considering that reactive geometrical changes in the structure of synapses and processes of the neuron is accompanied by the appearance of heterogeneity of the substance of the neuroplasm and that this process develops most easily of all in terminal ramifications [10]. Heterogeneity of distribution of filamentous-tubular material is readily observable in synapses on Ranvier nodes [13, 14].

The object of this investigation was to study the nature of cytoplasmic heterogeneity of synaptic swellings and varicosities of neurites in the CNS which develop during nonspecifid reactive structural change connected with disturbance of the homeostasis of the surrounding medium.

EXPERIMENTAL METHOD

There were three series of experiments. In series I (seven frogs) synaptic and nonsynaptic swellings (geometrical heterogeneities) of the neuropil of the spinal cord and olfactory brain, stained with a 0.01% solution of methylene blue in Ringer's fluid, were investigated by means of the GOI 10 dark-field contact objective and an ordinary 40× epiobjective. The meninges and surface layer of the brain and spinal cord tissue were removed. The dye was applied to the surviving preparation in drops. In the experiments of series II (six frogs) synaptic swellings of the anterior horns of the spinal cord were investigated in the electron microscope. The spinal cord surviving *in situ* for 3 h after opening of the vertebral canal was fixed in 1% OsO_4 solution in veronal-acetate buffer, pH 7.4, for 1 h and then processed in the usual way. In the experiments of series III (three frogs and three rats) local swellings of neuroplasm, obtained by mechanical dissociation of the surviving spinal cord by means of a tissue microblender and subsequent differential centrifugation, were investigated with the aid of phase contrast. Microinterferometry of the relative heterogeneity of distribution of dry substance and water in the region of the swellings and constrictions of the neurites was carried out by a striped field technique and a field without stripes with complete image splitting [12, 15]. The main stimulus giving rise to nonspecific swellings in all series of experiments was a disturbance of the homeostasis of the nerve tissue during prolonged survival of the brain and spinal cord under unfavorable conditions connected with the operation and partial disturbance of the blood supply. The irritant effect of the methylene blue was evidently added to this also in the experiments of series I, and the considerable mechanical trauma involved in dissociation of the brain in series III.

*Although "neurite" is synonymous with "axon" in Russian too, a distinction is drawn in Fig. 2 — Translator.

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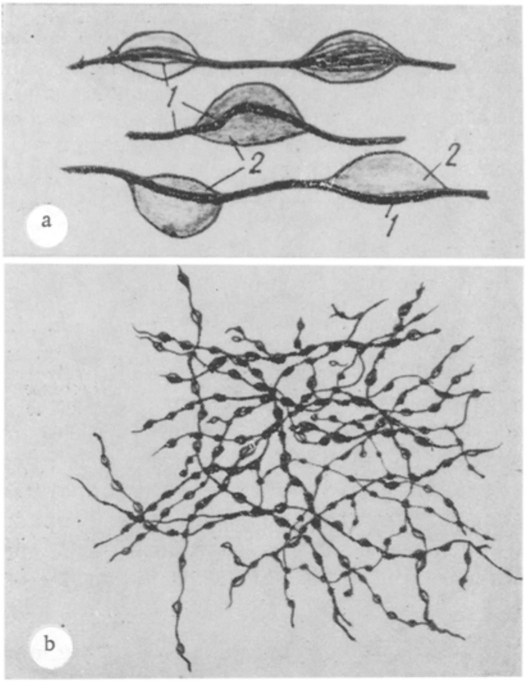

Fig. 1. Swollen synaptic boutons and various swellings of neurites of the frog brain. a) Heterogeneous staining of components of swellings (spinal cord, scheme); b) neuropil of olfactory brain (scheme). 1) Deeply stained axial filamentous band; 2) drop-like swelling of palely stained fraction of neuroplasm. Intravital staining with methylene blue. Dark-field contact GOI 10 objective, ocular 20.

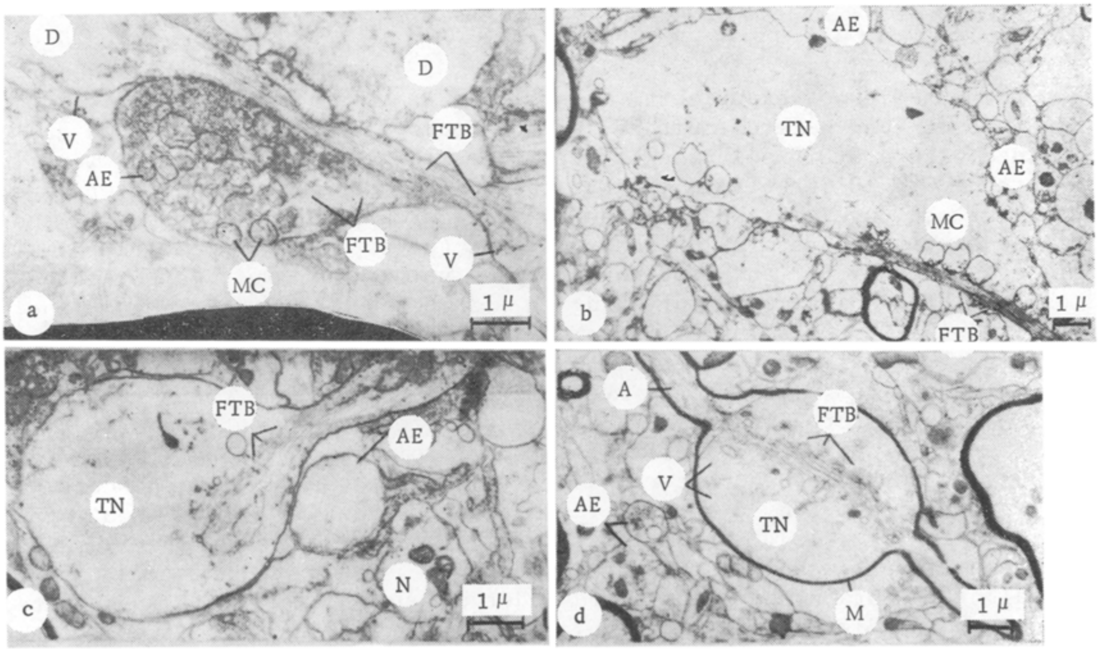

Fig. 2. Heterogeneity of distribution of filamentous-tubular material in reactively changed synaptic boutons and neurites in the CNS: a) decrease in concentration of filamentous-tubular material in region of varicose swellings located near axodendritic synapse. 7800 ×; b) compact bundle of filaments and neurotubules in region of postsynaptic swelling. 4200 ×; c) reduced compactness of filamentous-tubular bundle in region of swelling. 7800 ×; d) filamentous-tubular band in varicose expansion of myelinated axon; 5600 ×. A) Axon; D) dendrite; N) neurite; M) myelin sheath; MC) mitochondria; V) varicose expansion; FTB) filamentous-tubular band; TN) translucent fraction of neuroplasm; AE) axon ending.

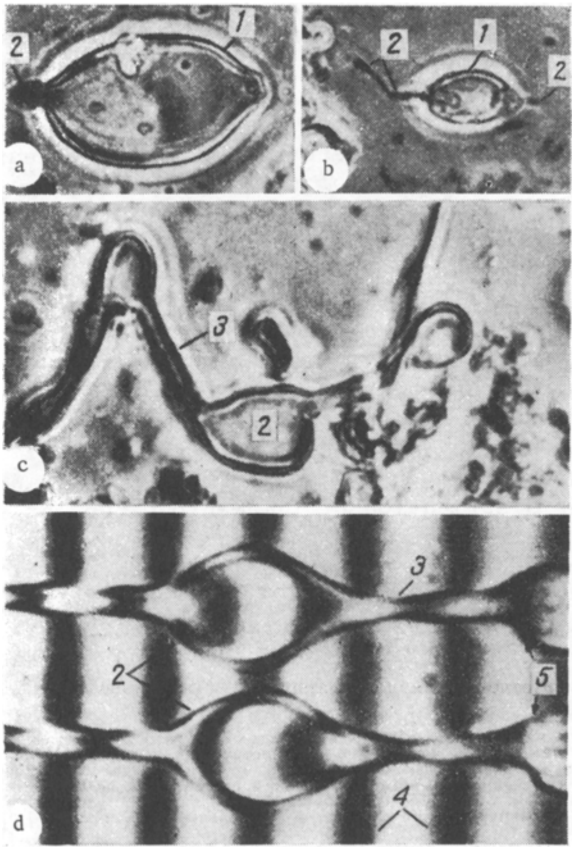

Fig. 3. Single and grouped varicose swellings of myelinated fibers isolated from frog and rat brain after fragmentation and centrifugation. a, b) Isolated varicosities of thick and thin frog fibers; c) fragment of varicose fiber of rat brain; d) interferometry of varicose swelling of frog in striped field. 1) Varicosity; 2) fragment of filamentous band; 3) constriction between varicosities; 4) interference bands; 5) split image. a-c) Phase contrast, 400 ×; d) 630 ×.

EXPERIMENTAL RESULTS

In the course of intravital observation on the surviving frog's brain and spinal cord (series I) swollen synaptic boutons and swellings of presynaptic branches were detected simultaneously with methylene blue staining after 3-10 min (Fig. 1), and they were virtually similar in all respects. To begin with they appeared in the zone corresponding to the gray matter. In different parts of the CNS varicose deformations were uniform in structure (Fig. 1a), but their structure could be made out most clearly in the region of the olfactory brain (Fig. 1b). The distribution of the dye was irregular. Not all the neuroplasm of the swellings, but only very thin and uniform bands about 1 μ in diameter, stained a deep blue. All were studied with a large number of palely stained bouton-like structures, whose diameter gradually increased and could be 5-7 times greater than the diameter of the blue band. These swellings were close to drop-like (spherical) in shape. Often they were localized along one side of a blue axial filament (Fig. 1a). According to all their features these geometrical heterogeneities must be classed in type 4 [7]. Considering that there are no nucleic acids in the cytoplasm of nerve fibers and that most of the stain was bound with proteins [9], heterogeneity of its distribution in the boutons can be interpreted as heterogeneity of protein localization. The deeply stained central band-like structure of the "en passant" synapses and varicosities was probably a complex of condensed fibrillary proteins, and the palely stained part of the bouton was the hydrated fraction of the neuroplasm. This hypothesis could be tested by electron microscopy.

On electron-microscopic investigation (experiments of series II) both presynpatic and postsynaptic portions of the synapses appeared reactively changed (Fig. 2). Marked heterogeneity of the neuroplasm and the formation of considerable drop-like swellings (boutons) were common to both. The concentration of filamentous-tubular material in the region of the varicose swellings of the neurites was sharply reduced compared with that in the region of the constricted areas (Fig. 2a). The densely packed bundle of neurotubules and neurofilaments, entering into the zone of the bouton, often preserved its compactness (Fig. 2b). In these cases it resembled the dense filamentous-tubular band in boutons stained with methylene blue. The strongly transparent neuroplasm surrounding it was evidently hydrated. This picture corresponds completely to the results of intravital observations on the reaction of the processes in the frog's brain in the previous series. Sometimes the compactness of the filamentous-tubular bundle on entering the bouton diminished to a greater or lesser degree (Fig. 2c), although evidently the material of the bundle in the region of the swelling was never uniformly distributed. On electron micrographs the diameters of the swellings could be as much as 6-9 times greater than the diameters of the constrictions. A direct relationship was found between the size of the swelling and the degree of heterogeneity of the neuroplasm in it. Active synaptic zones of neurilemma (heterogeneities of the membrane) were distributed both in the region of the swellings and in the region of the constrictions (Fig. 2a, b), and consequently they were not directly related to the geometrical heterogeneity of the neurites and heterogeneity of the neuroplasm. As a result of the accumulation of relatively unchanged organoids in the synaptic boutons, which preserved a considerable degree of electron density, it was nevertheless possible to distinguish an isolated area occupied only by an accumulation of filamentous-tubular material (Fig. 2a). Varicose swellings of uniform type were observed not only in the terminal portions, but also along the course of the preterminal neurites (Fig. 2d). A translucent fraction of neuroplasm, containing vesicles of different caliber and granular and floccular masses, was observed in these swellings along the sides of the filamentous axial band. The results of the electron-microscopic investigations confirmed the view that heterogeneity of distribution of filamentous-tubular material takes place in the region of the reactively changed terminal structures, just as in other parts of the altered nervous processes. In the region of the swellings the total concentration of fibrillary organoids was much lower than in the zone of the constrictions, and they had a tendency to aggregate. The mechanism of reactive structural change is evidently connected with separation of the colloid of the neuroplasm into layers: the hydrated fraction and the comparatively more compact, aggregated filamentous-tubular component [10]. The concentration of protein in the region of the local swellings and constricted parts of the neurites could be compared by intravital microinterferometry.

Attempts to distinguish uncontracted single synaptic swellings for this purpose by differential centrifugation were unsuccesful. However, more resistant single and grouped varicose swellings of nervous processes covered with myelin were consistently distinguished in the nuclear fraction of the supernatant (experiments of series III). At the sides of these swellings, in the region of the discontinuities, projections could be seen in the phase-contrast microscope (Fig. 3a). However, these were not projections of liquid neuroplasm, but the remains of a dense band (Fig. 3b), with fibrillary structure. This evidently was the same filamentous-tubular band as that observed in synaptic boutons on electron micrographs.

It was shown by interferometry (Fig. 3c) that the path difference is about equal at different points along the axis of the swellings and constrictions. The path difference is directly proportional to the concentration of dry mass and the thickness of the test structure; for that reason, knowing the thickness of the object and the path difference, it is easy to calculate the concentration of dry mass. We were interested in the relative value of the concentration of dry substances in the region of a varicosity compared with that in the constrictions. Since these structures possess axial symmetry, their thickness means their diameter. With an equal path difference at different points along the axis of the varicosity and constriction, the concentration of dry substance will be inversely proportional to the diameter of the fiber at these points. In the region of the swellings it was found to be 5-8 times less, i.e., just as could be postulated on the basis of the results of the previous series of experiments, in this case also there was considerable hydration of the neuroplasm, by contrast with the constricted regions. These direct measurements, demonstrating significant liquefaction of the neuroplasm, suggest that the swellings along the course of the processes may be formed through the action of the force of surface tension, in the same way as a drop. It also follows from the measurements described above that the quantity of dry substance at different points along the axis of the swellings and constrictions is the same. Since most of the dry substance of the neuroplasm consists of filamentous-tubular proteins, it can be asserted that in a certain region along the length of the reactively changed process there is a definite constant quantity of the fibrillary component of the neuroplasm. This was in fact what was observed in the previous series of experiments, in the form of compact filamentous-tubular aggregates, running without any particular changes from the constricted portions of the neurite into the expansions (Fig. 1a; Fig. 2b, d). The appearance of marked local swellings of synapses and neurites has been observed under the electron microscope in hypoxia, during intensive electrical stimulation, exposure to nonspecific factors, and in certain diseases [1, 3, 5, 6], and for that reason we regard these changes as a relatively nonspecific response. The experimental results indicate that during nonspecific reactive structural changes in the synapses and nervous processes marked local heterogeneity of the distribution of filamentous-tubular material and considerable hydration of the neuroplasm are observed in the region of the swellings (geometric heterogeneities) along the course of the process, which are independent of the localization of the synaptic specializations on its membrane. In other words, the external geometric and internal cytoplasmic heterogeneity are evidently not directly connected with the "planar heterogeneity of the neurilemma" [8]. In the writers' view reactive structural changes can be explained by conformational changes in the proteins of the neuroplasm, and this is manifested as an increase in the adhesive properties of the filamentous-tubular and other structures and the consequent segregation of the more hydrated fraction of the cytoplasm, which under the influence of the force of surface tension tends to form drop-like swellings along the course of the nervous process.

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