STRUCTURAL ANALYSIS OF REACTIVITY OF THE PLEXIFORM LAYER OF THE CEREBRAL CORTEX TO EXTREMAL FACTORS

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Key Words: cerebral cortex; plexiform layer; dendrites.

The cerebral cortex is a heterogeneous formation consisting of different types of cells with a clearly defined distribution by layers. The plexiform layer differs from the other cortical layers in having virtually no cells, but consisting of apical segments of dendrite of nerve cells in deeper layers, synaptic endings, axons, and glial cells. This layer has attracted the attention of neurologists because most afferents from subcortical nonspecific systems, whose influence is essential for maintaining a definite level of activation of the cerebral cortex, necessary for providing for higher nervous activity, terminate there [9].

The aim of this investigation was to study the response of single layers of the cortex and, in particular, the plexiform layer under the influence of factors widely used in experimental neurology and medicine: general anesthesia and anodal polarization.

General anesthesia was chosen as a general systemic factor, whereas dc polarization is a factor with local action on the cortex [4]. Special attention was paid to quantitative and qualitative analysis of structural changes taking place in the plexiform layer of the cortex: in dendrites, dendritic spines, synaptic endings, and glial cells.

EXPERIMENTAL METHOD

Acute experiments were carried out on 15 albino rats under pentobarbital anesthesia. Control rats were given an injection of pentobarbital in a dose of 40 mg/kg body weight. The tests were conducted on two groups of animals. In the experimental animals of group 1 the action of deep general anesthesia was studied by injecting pentobarbital until definite functional changes appeared, as shown by the electroencephalogram (EEG) and the respiration rate. In the animals of group 2 the left somatosensory cortex was polarized by the anode of a direct current of 50-150 μA for 30-40 min, by means of a flat Ag—AgCl electrode, located on the lower table of the cranial bones. The area of the polarizing electrode was 20 mm^2. The cathode was applied to the animal's ear. The EEG was recorded on an ink-writing instrument, and also recorded simultaneously on magnetic tape, for computer processing. The EEG was derived by epidurally implanted silver ball electrodes, by a monopolar technique from the left and right somatosensory areas of the cortex, and the reference electrode was applied to the nasal bones. Material for morphological analysis was taken from the animals of group 1 at the stage of a sharp decrease in amplitude of the EEG wave and slowing of respiration, and in the animals of group 2 after 30-40 min of continuous polarization of the cerebral cortex. The brain of animals under moderately deep anesthesia with a high-amplitude EEC spectrum served as the control.

Morphological analysis of the somatosensory cortex was carried out by light-optical (methods of Golgi and Nissel) and electron-microscopic methods. Material for electron-microscopic investigation was taken after perfusion through the heart with a solution of fixative consisting of 1.5% glutaraldehyde ("Merck") and 1% formaldehyde ("Serva") in 0.1 M phosphate buffer (pH 7.2-7.4). The material was then treated with 1% osmic acid in the same buffer, dehydrated through a series of alcohols with increasing concentration and in acetone, and then embedded in Araldite. Semithin sections were cut to a thickness of 1 μm of an LKB-IV Ultrotome and stained by Nissel's method for subsequent analysis of the state of the neurons and neuropil of all layers of the cortex in the light microscope. The pyramid was sharpened at the level of layer 1 of the cortex, ultrathin sections were cut to a thickness of 40-80 nm, and these were then analyzed in the JEM-100B electron microscope. Sums of areas of the structures studied (profiles of dendrites, synaptic endings) were measured on the electron micrographs, relative to a strictly constant area

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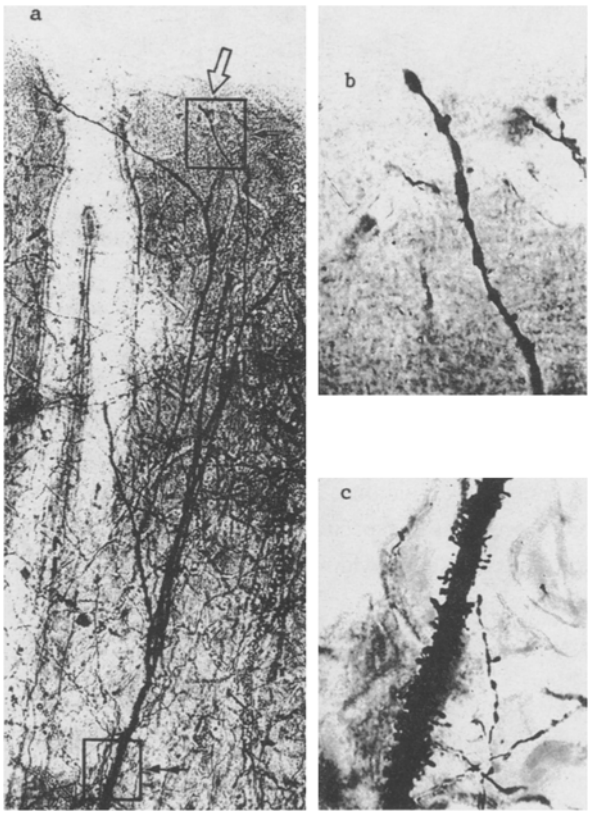

Fig. 1. Apical dendrite of pyramidal cell stained by Golgi's method. Deep anesthesia. a) General appearance. Bold arrow indicates surface of cerebral cortex. 100×, b) Fragment of dendrite at level of plexiform layer, indicated in Fig. 1a by a single thin arrow. Note varicose expansions of dendritic trunk. 280×, c) Fragment of proximal part of apical dendrite indicated in Fig. 1a by thin double arrow, with intact spines. 280×.

of part of a brain section, by a semiautomatic method, using a specially devised program (by means of a graphic tablet on an IBM PC computer). Altogether 30 regions, each measuring 18 μm, were studied in each animal. Values, standard deviations, and comparison of samples by Student's test were carried out by a "Statgraphics" program.

EXPERIMENTAL RESULTS

Similar morphological changes, relating mainly to the plexiform layer of the neocortex, were observed in the experimental animals of both groups under the influence of deep anesthesia and polarization.

In sections stained by Golgi's method, disappearance of the spines and appearance of bead-like varicose expansions were observed in distal segments of dendrites of the plexiform layer (Fig. 1b), evidence of swelling of these loci in dendrites of a nerve cell. The diameter of the varicose expansions often exceeded the diameter of the intact dendritic trunk by 5-10 times. In sections stained by Nissel's method this corresponded to the appearance of the "porous" neuropil, and this reaction was observed mainly in the upper third of the plexiform layer.

Examination of the electron micrograph showed that no microtubules were present in the cytoplasm of the varicose region of the dendrites, but the mitochondria remained intact, evidence that these changes were reversible (Fig. 2a). Endocytosis of structures adjacent to it by a dendrite was often seen in the region of a varicose expansion.

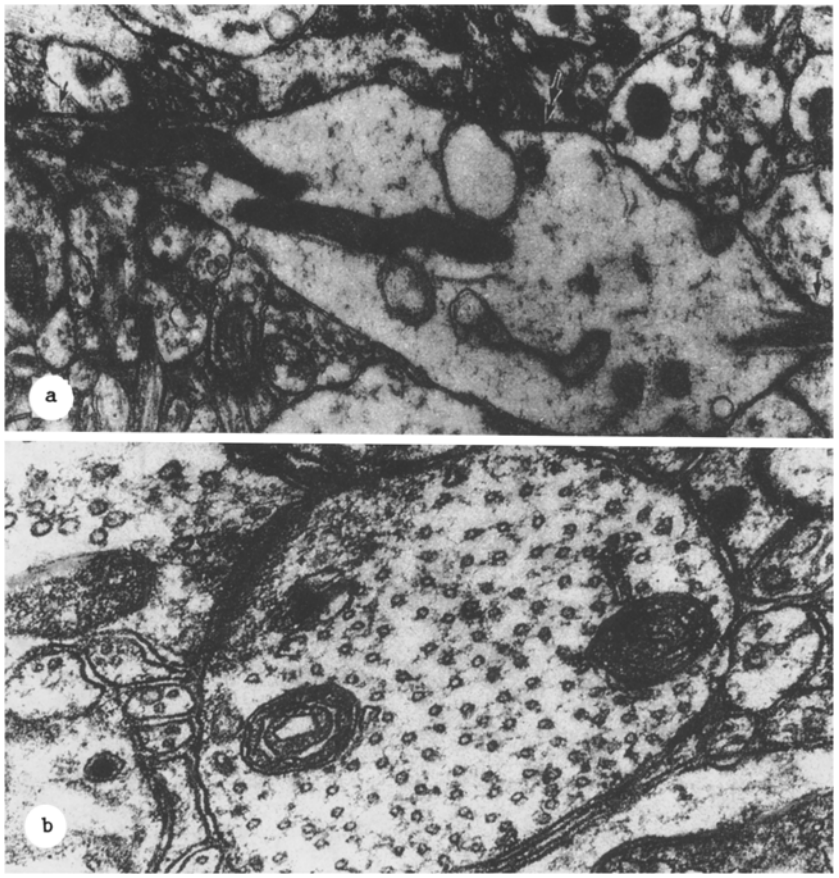

Fig. 2. Micrograph of dendritic fragment. a) Plexiform layer. Varicose expansion. Single arrow indicates intact part of dendritic trunk. Double arrow indicates varicose expansion. Note absence of microtubules. 30,000, b) Layer 3 of cortex. Note well preserved intracellular structures. 60,000×.

As calculations showed, the mean ratio of the sum of areas of the varicose expansions of the dendrites to the total constant area of section in the plexiform layer of the cortex was 14.1 ± 1.3% (n = 150; Fig. 3a). In the experimental animals (deep anesthesia) this index was about twice as high, namely 31.4 ± 2.1% (n = 150; Fig. 3b).

Synaptic endings, astroglia, and myelin sheaths of the axons, incidentally, showed no significant changes. For example, the difference in the mean ratio of the sum of the areas of synapses in the plexiform layer to the total area of section, between the control and experiment was not statistically significant, but amounted to 13.7 ± 1.2% (n = 150) and 14.2 ± 1.9, respectively (n = 150).

In the lower layers of this same part of the sensomotor cortex no clear ultrastructural changes were found. The structure of the proximal regions of the apical dendrites also remained unchanged, as could be seen in photographs taken from preparations impregnated by Golgi's method (Fig. 1c) and also on electron micrographs (Fig. 2b). It is also clear from these photomicrographs that the spines and cytoplasmic components of the proximal regions of the dendrites remained intact. The essential feature is that the structural changes in the dendrites of the plexiform layer of the cortex described above were reversible.

Thus the distal regions of the dendrites of the plexiform cortex are the most reactive and variable parts of the nerve cell, as shown by the appearance of varicose expansions on the dendritic trunk under the influence of various extremal factors on the brain. It is probable that the appearance of these varicosities on the dendrites, together with other changes in them (loss of microtubules, disappearance of spines) may lie at the basis of the functional "blocking" of these loci of the nerve cell. Since it was shown in [5] that the threshold of excitability in the region of a varicose expansion of an axon may be 50 times higher than in the corresponding region of an intact axon of a nerve cell.

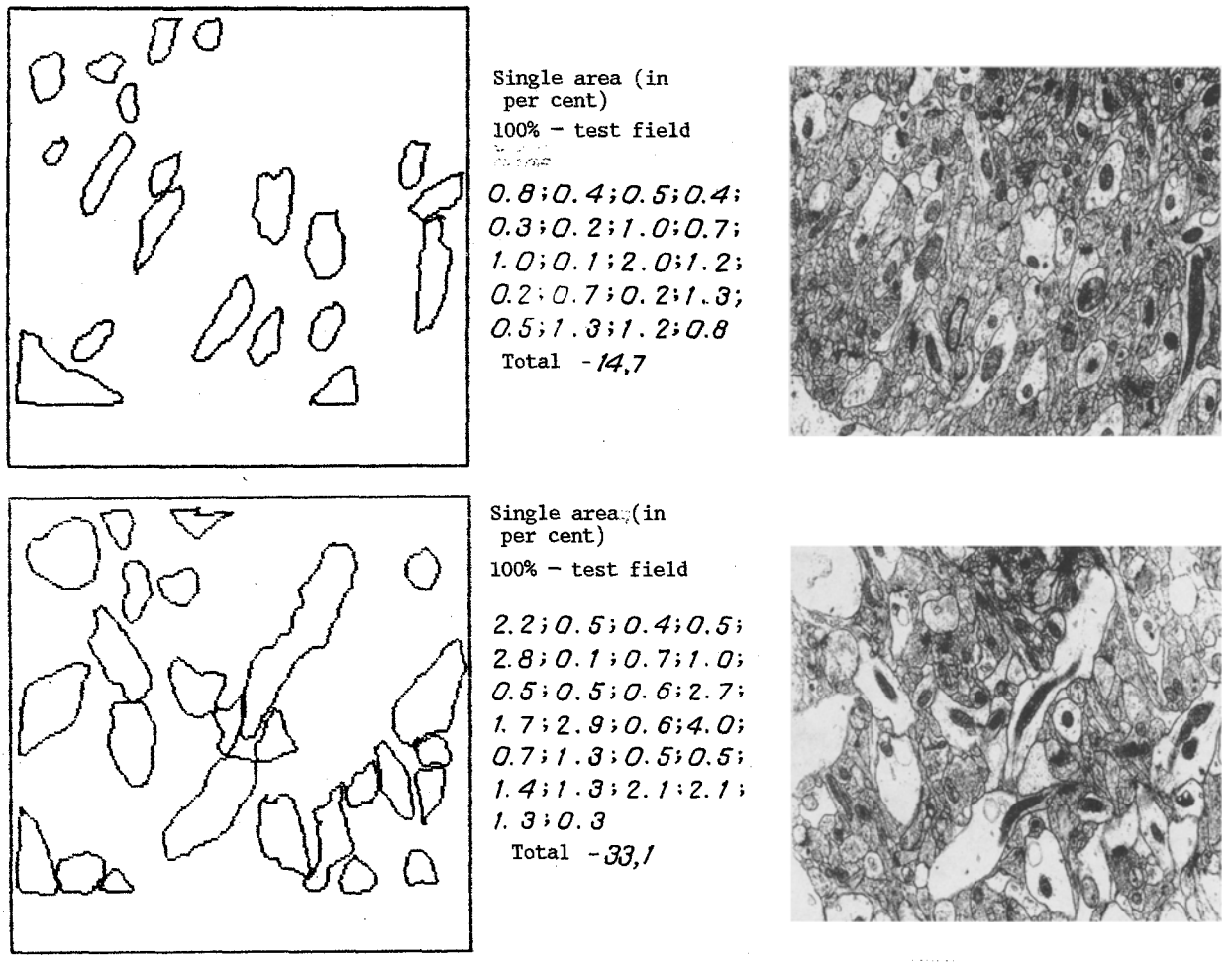

Fig. 3. Structural analysis of dendritic profiles in normal and experimental animals. a) On right: electron photomicrograph of standard region of fixed area of plexiform layer of sensomotor cortex, on left: mask corresponding to electron micrograph with areas of dendritic varicose expansions identified and with results of calculation of their area. Control. 9000×; b) deep anesthesia. Remainder of legend as to Fig. 3a. 9000×.

Most nonspecific afferents running into the cortex are known to terminate on dendrites in the plexiform layer, and mainly in its upper third [6], where, according to our data, the most important morphological changes were found in the dendrites. An increase in the degree of swelling of the distal regions of the dendrites of the plexiform layer at the stage of deep anesthesia may be the cause of blocking of incoming impulsation from the nonspecific activating system of the brain, which leads to a fall of the level of activation of the neocortex and, correspondingly, to a decrease in amplitude of the EEG waves.

It can be concluded from these results that distal regions of apical dendrites of the plexiform layer can play an important role in the realization of signals entering the cortex from the activating subcortical centers. The reversibility of changes in geometry of the dendrites and their cytoplasm may provide the structural basis for functional states of the brain such as the level of consciousness, sleep, and limiting inhibition.

It follows from the results of this investigation that the nerve cell is a heterogeneous unit which can be divided into different loci, not only on the basis of structural and functional characteristics [1], but also on the basis of differences of reactivity to extremal factors. The greater sensitivity of dendrites to the action of factors of this kind have been reported in several investigations [2, 3, 7, 8]. It can be concluded from our data that the plexiform layer is specially sensitive to the action of extremal factors, and that it is mainly the dendrites of this layer that undergo structural modification, whereas other structures of the neuropil remain virtually unchanged.

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CHANGES IN SURFACTANT ACTIVITY AND ULTRASTRUCTURE OF THE AIR–BLOOD BARRIER IN EXPERIMENTAL ALCOHOL POISONING

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In alcoholism, ethanol acts on the system of local and general defense [2], but there is no information in the literature on the state of the lung surfactant (LS) system in alcohol poisoning, although changes in the surface activity of that system play an important role in the pathogenesis of various diseases of the bronchopulmonary system [1, 3].

The aim of this investigation was accordingly to study changes in the surface-active properties of LS and in the ultrastructure of the air–blood barrier (ABB) in experimental alcohol poisoning.

EXPERIMENTAL METHOD

The lungs of 26 Wistar albino rats of both sexes, weighing from 290 to 350 g, in which acute and chronic poisoning with ethyl alcohol was induced, were subjected to physicochemical [determination of maximal and minimal surface tension (ST_{min}) of the surface-active fraction of lung extracts from the animals on Wilhelmy scales], biochemical (determination of total lipids and phospholipids in LS followed by thin-layer chromatography of the latter), and electron-microscopic investigation. Acute poisoning was produced by intragastric administration of 50% ethyl alcohol, in a dose of 7.5 ml pure ethanol/kg body weight, divided into fractions and given at 5-min intervals. Group 1 consisted of six such animals. Chronic ethanol poisoning was produced by giving 50% ethyl alcohol by gastric tube in a dose of 4 ml/kg body weight in a single dose at 24-hourly intervals. All the animals were killed by decapitation under thiopental anesthesia 1.5 months (group 2 – five observations), 3 months (group 3 – five), 6 months (group 4 – three), and 12 months (group five – seven) after the beginning of the experiment. The lungs of five healthy animals were used as the control.

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