# PARAMEMBRANOUS MICROFIBRILLAR STRUCTURES OF RAT CEREBRAL CORTICAL SYNAPSES DURING SENSITIZATION BY BRAIN ANTIGENS

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The immunologic reactivity of an organism largely determines the character of morphological changes in the brain when affected by various pathological processes [4, 7, 8]. This is evidence of the need to distinguish immune mechanisms of damage and repair of its components in the pathogenesis of diseases of the nervous system. The state of interneuronal junctions, responsible for the integrative function of the brain as a whole is particularly important in this respect. High antigenic activity of brain synapses has now been established [10, 11]. However, detailed studies of highly specialized structures of interneuronal junctions and of the synaptoarchitectonics of the brain during sensitization with brain antigens have not been reported. The aim of this investigation was to study the state of specialized paramembranous microfibrillar structures of axodendritic synapses in the molecular layer of the sensomotor cortex during sensitization by brain antigens.

## EXPERIMENTAL METHOD

Experiments were carried out on 10 male Krushinskii-Molodkina rats weighing 190-210 g. The animals were sensitized with a 20% saline extract of homologous brain in a dose of 0.5 mg protein/kg body weight every 3rd-4th day, 15 times (45 days of the experiment). Antigens of brain extract (ABE) were prepared by the method in [2, 9]. The sensitization control consisted of determination of the antibody content to ABE by the method in [13] and also by Hoigne's microprecipitation reaction in the modification [3]. Test and control sera were exhausted with dry plasma or with dry liver powder [1]. Parallel controls for specificity of the immunologic tests also were set up. The brain of animals in whose peripheral blood antibodies to ABE were found in diagnostic titers was used for the morphologic investigation. The brain of animals anesthetized with ether was fixed for 15 min by perfusion through the left ventricle with a mixture of 1% glutaraldehyde solution and 4% paraformaldehyde solution in 1M phosphate buffer (pH 7.4) with sucrose (5%), under a pressure of 110 mm Hg. After removal the brain was postfixed in a solution of the same composition for 2 h at 4°C. Oriented fragments of sensomotor cortex (areas $FP^a$ and FPP) [5] were washed in 0.1M phosphate buffer and dehydrated in ethanol of increasing concentration. At the 100° ethanol stage the material was stained in a 5% solution of phosphotungstic acid (PTA) for 3 h and embedded in Epon-Araldite.

Ultrathin sections were cut on a UMTP-4 ultramicrotome in the tangential plane at the level of the molecular layer of the neocortex. Randomly chosen areas of neuropil were photographed under standard magnification of 15,000 on an ÉVM-100 LM electron microscope. Stereologic analysis of the material was carried out on electron micrographs with a final magnification of 30,000. The density of PTA-positive interneuronal junctions with a clearly defined line within the synaptic cleft per 100 $\mu^2$ of neuropil was calculated. Depending on the organization of the paramembranous material, the synapses were subdivided into symmetrical

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TABLE 1. Content of PTA-Positive Junctions in Neuropil of Molecular Layer of Rat Cerebral Cortex on Sensitization with ABE

| Character of procedure | Density of junctions per 100 μ² | | |
|---|---|---|---|
| | Total | asymmetrical | symmetrical |
| 15 injections of ABE (5) | 8,15±0,55* | 7,63±0,44* | 0,52±0,17* |
| Control (5) | 17,69±0,41 | 14,54±0,63 | 3,15±0,21 |

Legend. *) Significant differences compared with control ($p < 0.01$). Number of animals shown in parentheses.

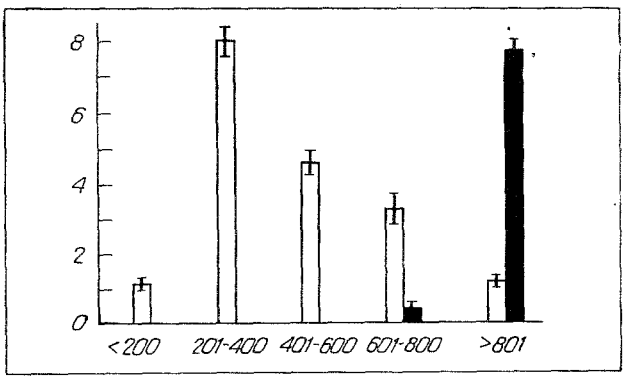


Fig. 1. Density of PTA-positive synapses differing in the length of their active zone of contact in layer I of the rat cerebral cortex on sensitization by ABE. Abscissa, length of active zone of contact (in nm); ordinate, number of synapses per 100 μ² of neuropil. Unshaded column – control, black column – sensitization with ABE.

(discrete dense projections not present, functionally immature) and asymmetrical (dense projections formed; functionally mature synapses) [6, 12]. Asymmetrical junctions were divided into groups depending on the height of the dense projections (DP); A (> 61 nm), B (51-60 nm), and C (< 50 nm) [6, 12]. The length of the synapses was determined with the aid of a test grid with a step of 3 mm. Depending on the length of the active zone, junctions were divided into very small (< 200 nm), small (201-400 nm), average (401-600 nm), large (601-800 nm), and very large (> 801 nm). The numerical results were subjected to statistical analysis by Student's t test.

## EXPERIMENTAL RESULTS

Small and average asymmetrical synapses with well developed dense projections, belonging to type A, predominated in the molecular layer of the sensomotor cortex. The system of subsynaptic units (SSU), material of the synaptic cleft, and the postsynaptic condensation (PSC) of these synapses had clear outlines. PTA-positive material of the pre- and postsynaptic cytoplasm was connected structurally with SSU.

On sensitization with ABE the clarity of outlines of SSU was reduced in some synapses and structural connection of SSU with the surrounding synaptoplasm was disturbed. The degree of destruction of DP was much greater, whereas that of PSC was less. The destructive changes varied in severity from a very small reduction of clarity of the outlines of SSU and of the height of DP to their fragmentation, detachment of DP from the presynaptic membrane, and complete disappearance of DP and SSU. The relative content of synapses with low DP of type C was increased by 4%.

Evidence of a marked reduction in the content of interneuronal junctions on sensitization with ABE was given by a decrease in the total density of synapses by 53.9% (Table 1). The almost complete absence of symmetrical junctions reflects their transformation into more mature

forms and the insufficient intensity of formation of new connections. SSU was hypertrophied in some asymmetrical junctions without any destructive changes. Large and very large junctions predominated (Fig. 1), with the almost total absence of smaller synapses. Parallel with hypertrophy of SSU, its three-dimensional organization was more complex. On the predominantly disk-shaped form it was transformed into circular, horseshoe-shaped, or completely broken up into fragments with a diameter of 300-400 nm. Hypertrophy of SSU is compensatory in character and allows integrative activity of the neocortex even after death of many interneuronal junctions. Changes of the same kind have been described in the cerebral cortex in hypoxia [6].

The synaptic pool of adult intact animals kept under uniform conditions is known to exhibit marked stability over a long period of time [12, 14]. This is on account of a constantly operating process of replacement of disintegrated synapses by new ones [15]. On sensitization by ABE the formation of new synapses and their differentiation are blocked. This leads to compensatory structural hypertrophy of the residual synapses and to the rapid exhaustion and death of some of them, as shown by a marked reduction in the total number of junctions and of immature forms of synapses. The process of fragmentation of the rest of the hypertrophied junctions is intensified as a compensatory mechanism. Moreover, sensitization with ABE is itself a powerful factor in synapse destruction. Under these circumstances, the synaptic membrane is not the only immunologic target, but the cytoskeleton of the synapse and, in particular, its paramembranous specialized formations (DP and PSC), also serve this purpose. This is evidently because of increased permeability of the blood-brain barrier and of the nerve cell membranes, intensification of phagocytosis and pinocytosis and, as a result of this, increased access of immunocompetent macromolecules to the cytoskeleton of the synapse.

Thus sensitization to ABE significantly changes the paramembranous microfibrillar structures of interneuronal junctions and the synaptoarchitectonics of the cerebral cortex, and this plays an important role in the changes in integrative activity of the brain in various pathological processes associated with an autoimmune mechanism of nerve tissue damage.

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