Structural Basis of Changes in Deformation of Synaptic Contacts of the Sensorimotor and Cerebellar Cortex in Health and Acute Ischemia

V. V. Semchenko, S. S. Stepanov, and E. D. Sergeeva

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Paramembranous specialized formations of the synaptic cytoskeleton - dense projections and postsynaptic condensation of axospinous synapses of the molecular layer of white rat sensorimotor and cerebellar cortex - in health and acute total ischemia are studied by selective contrast staining with phosphotungstic acid. A direct relationship is revealed between the pattern and degree of deformation of the contact plane, on the one hand, and the postsynaptic condensation and ratio of the volumes of pre- and postsynaptic accumulations of paramembranous filaments, on the other.

Key Words: *neocortex; cerebellum; interneuronal synapse; ischemia*

Synaptic deformation is regarded as the basis of morphological characterization of synapse shape [3]. Experimental findings attest to a relationship between the pattern of deformation of the plane of a synaptic contact and its functional state [2,3,7]. The factors determining the type (positive or negative) and degree of deformation include various external and internal factors (state of the synaptic cytoskeleton, type of transmitter metabolism, size of the synapse, complexity of arrangement of the contact, its localization on the neuron, and its appurtenance to a particular brain compartment) [2,3,5,6]. Discrepancies in the data, however, leave unsolved the problem of the basic structural mechanisms of synapse formation in health and disease.

In this study we assessed the effect of the spatial organization of the system of paramembranous filamentous formations (dense projections, postsynaptic condensation) on the degree of changes in the deformation of the synaptic contact plane in health and ischemia.

Histology Department, Omsk Medical Institute. (Presented by L. V. Poluektov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The objects of this study were the axospinous synapses of the molecular layer of the sensorimotor cortex (SMC) and the cerebellar cortex, which differ appreciably in biochemical composition and in the spatial arrangement of postsynaptic condensation [4]. Experiments were carried out with 6 male white rats weighing 190 to 210 g under ether narcosis. Acute total ischemia of the brain was induced by 10-min clamping of the cardiac vascular bundle [1]. The brain was fixed by perfusion of a mixture of 1% glutaraldehyde, 4% paraformaldehyde, and 5% sucrose in phosphate buffer (pH 7.4) through the left ventricle of the heart for 15 min, and then postfixed for 2 h in the same solution at 4°C. Oriented pyramidal fragments of SMC and cerebellar cortex were contrast-stained in ethyl solution of phosphotungstic acid as described elsewhere [7]. The fragments were embedded in an epon-araldite mixture. Ultrathin slices were prepared in a tangential plane at the level of the molecular layer of the SMC and cerebellar cortex. The slices were examined under an EVM 100AK electron microscope; 30 random visual fields of the neuropil were photographed at the standard 15,000

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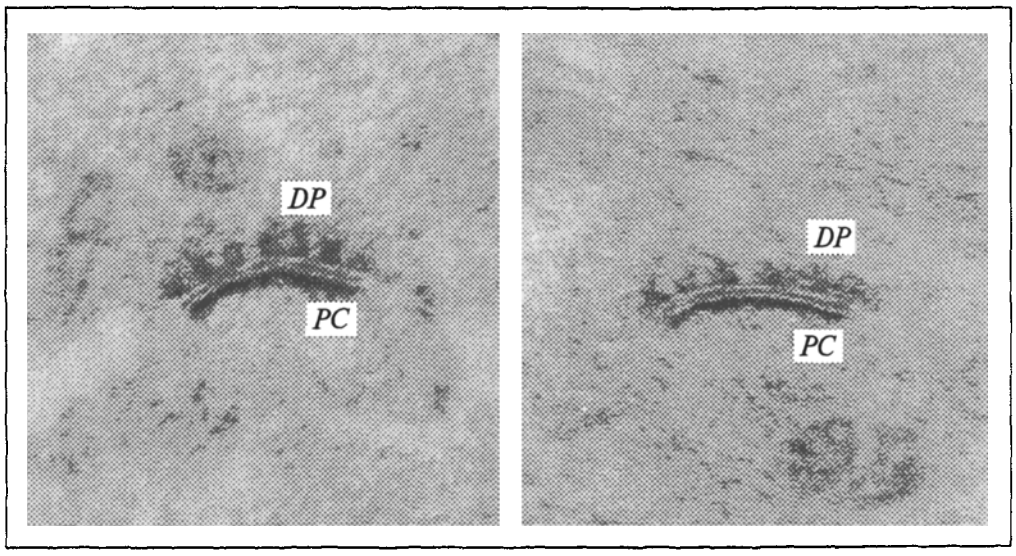

Fig. 1. Paramembranous neurofilamentous formations of synapses of molecular layer of SMC (*a*) and cerebellar cortex (*b*) of a control rat. Contrast staining with phosphotungstic acid. ×60,000. *DP*: dense projections; *PC*: postsynaptic condensations.

magnification for each block. The number of flat and positively and negatively deformed contacts, the length of synaptic profiles, and the size of dense projections and postsynaptic condensation were counted on photographs at a final magnification of 30,000; in addition, the degree of deformation of the synaptic contact plane was assessed. The synaptic curvature was estimated as the $1/R$ ratio using the formula: $R=a/2+b^2/8a$, where R is the radius of the circumference drawn as a continuation of the synaptic profile, b is the line connecting the ends of the synaptic profile, and a is the perpendicular from the synaptic membrane to b [7]. The numerical density of all parameters was estimated per 100 μ^2 of neuropil section plane. Digital material was statistically processed using Student's t test.

RESULTS

Functionally mature axospinous synapses predominate in the molecular layer of the SMC and cerebellar cortex of control white rats. Contrast staining in ethyl solution of phosphotungstic acid permits clear imaging of the profiles of synaptic contacts represented on transverse sections by dense projections and postsynaptic condensation (Fig. 1). The total numerical density of functionally mature synapses in the cerebellar cortex of control rats is somewhat higher than in the SMC. Flat and positively deformed contacts predominate in both formations. The number of negatively deformed contacts is much lower in the cerebellar cortex (1.23%, $p<0.05$) than in the SMC (19.58%, $p<0.05$). The mean length of synaptic profiles in the cerebellar cortex is 408±46 nm and 348±17 nm in the SMC ($p>0.05$). The mean height of dense projections in the cerebellar cortex is 54±5 nm, that in the SMC 51±5 nm ($p>0.05$); the thickness of postsynaptic condensation is 33±4 nm in the cerebellar cortex and much higher, 55±4 nm, in the SMC ($p<0.05$). The postsynaptic condensations of the cerebellar cortex synapses are flattened and have no conjugated structures of any type, whereas in SMC synapses they possess short, thickened processes of feltlike material positively reacting to phosphotungstic acid, these processes growing inside the spine. The degree of deforma-

Table 1. Numerical Density of Axospinous Synapses with Various Contact Deformation in the Molecular Layer of the SMC and Cerebellar Cortex of White Rats in Health and Ischemia ($M\pm m$)

Type of synaptic contacts	Number of synapses per 100 μ^2 of neuropil			
	cerebellar cortex		SMC	
	control	ischemia	control	ischemia
Flat	14.4±1.4	13.7±2.0	8.4±0.6**	7.2±0.7**
Positively deformed	9.7±0.3	10.8±1.3	6.8±0.2**	2.8±0.2*,**
Negatively deformed	0.3±0.1	—	3.7±0.1**	7.2±0.5*,**
Total:	24.4±1.3	24.5±2.1	18.9±0.6**	17.2±1.6**

Note. Asterisk shows $p<0.05$ *vs.* the control, two asterisks $p<0.05$ *vs.* the cerebellar cortex.

tion of the contact plane in the cerebellar cortex is +0.088±0.005, which does not appreciably differ from that in positively deformed SMC contacts: +0.092±0.006. The degree of deformation of negatively deformed SMC synapses was -0.077±0.004.

At the end of acute ischemia the total numerical density of synapses in the cerebellar cortex and SMC (Table 1), the mean length of synaptic profiles (378±33 and 346±18 nm, respectively), the height of dense projections (52±4 and 51±4 nm), and the thickness of postsynaptic condensations (34±3 and 57±4 nm) were the same as in the control. However, the number of synapses with different types and degrees of deformation of the contact plane varied within a wide range. In the SMC the content of positively deformed contacts dropped to 16.28% ($p<0.05$) while that of negatively deformed increased to 41.86% ($p<0.05$), with the numerical density of flat contacts unchanged. The content of positively deformed synapses in the cerebellar cortex was 44.08%, which was not reliably different from the control (Table 1). The degree of contact deformation was increased 45.46% ($p<0.01$) to +0.128±0.006 in the cerebellar cortex and remained the same in the SMC.

Hence, the axospinous synapses of the molecular layer of the SMC and cerebellar cortex, differing upon contrast staining in ethyl solution of phosphotungstic acid in the thickness and contours of postsynaptic condensation, are characterized by different types of deformation of contacts in health and react differently to acute total ischemia. In the cerebellar cortex the degree of positive deformation of the synaptic contacts is increased after 10 min of ischemia, although the type of deformation is the same. In the SMC positively deformed contacts are transformed into negatively deformed ones. This is proved by the reduced numerical density of positively deformed contacts in the presence of an increased number of negatively deformed contacts and an unchanged number of flat contacts. A correlation between the shape of a synaptic contact and the organization of its postsynaptic condensation is clearly seen. In SMC synapses with thick, uneven postsynaptic condensations the degree of contact plane deformation varied within a wider range, from positive to negative, whereas in the cerebellar cortex synapses with thin, smooth postsynaptic condensations it varied only within the range of positive deformation. This means that the cycle of synaptic functioning of axospinous synapses of the SMC includes phases of flat → positively → negatively deformed → flat contact, and that of the cerebellar cortex synapses: flat → positively deformed → flat contact.

Evidently, the pattern of changes of synaptic deformation in health and total ischemia depends on the ratio of the volume occupied by the filaments of dense projections and postsynaptic condensations. In the SMC the volume of postsynaptic condensation surpasses the volume of dense projections, whereas in the cerebellar cortex the opposite situation is observed. That is why the functional and pathological conformational changes in the filaments of dense projections and postsynaptic condensations associated with changes of their volume cause opposite deformations of the synaptic contact plane in the synapses of the SMC and cerebellar cortex.

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