EFFECT OF MALNUTRITION ON NEOCORTICAL SYNAPSES

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Changes in the conditions of functions of the brain during individual development are known to be closely interconnected with changes in the structure and time of formation of synapses. It has been shown that synaptic ultrastructure in the CNS exhibits considerable plasticity and is largely dependent on the influence of various external environmental factors [3]. Malnutrition, with deficiency of protein and sources of energy, may be used as an experimental model of changes in the morphological and functional state of the brain and, in particular, of its synaptic apparatus [5, 7]. In earlier studies of the effect of malnutrition on neocortical ultrastructure of developing and adult mice it was shown that malnutrition gives rise to significant structural changes both in neurons themselves and in the neuropil surrounding them. However, the most marked changes are found in the structure of synapses located on the dendritic spines [7]. Data in the literature have been obtained principally by the study of only single parameters reflecting the morphological and functional state of synapses under the influence of malnutrition [11-13]. There has been virtually no attempt to study the action of malnutrition on neocortical synaptic ultrastructure in adult animals.

For the reasons given above, it was decided to undertake a quantitative electron-microscopic study of the action of malnutrition on synaptic ultrastructure in young and adult mice.

EXPERIMENTAL METHODS

Experiments were carried out on 24 CBA mice (12 young and 12 adult animals, each age group divided into six experimental and six control animals). The experimental mice received a diet containing 5% casein and the control mice a diet containing 10% casein for 30 days [4]. The young animals were kept on the diet from the 10th to the 40th day of life, the adult mice from the 60th to the 90th day. The animals were then killed and the sensomotor cortex removed. The technique of processing the material for electron microscopy was described previously [7]. The method of visual classificaiton of synapses based on a series of features [6] was used for quantitative analysis. The morphological and functional state of each synapse was estimated on the basis of nine features which, depending on the degree to which they were represented, were expressed in points, ranging from minimal (one point) to maximal (3-7 points). The features were as follows: 1) area of cross section of the terminal; 2) fraction of the area of cross section of the terminal occupied by vesicles (the analog of the number of vesicles in the terminal); 3) the degree of concentration of vesicles near the active zone; 4) the configuration of the active zone (straight, or with positive or negative curvature); the classification is taken from Cooke [10]; 5) the length of the active zone; 6) the number of cisterns in the spinous apparatus; 8) the width of the synaptic cleft; 9) the thickness of the postsynaptic condensation of the membranes. When item 4 was evaluated, frequency analysis was used, i.e., the frequency with which straight active zones and zones with positive and negative curvature (expressed as percentages of the total number of active zones studied for that particular group of animals) was investigated. The thickness of the postsynaptic condensation and the width of the synaptic space

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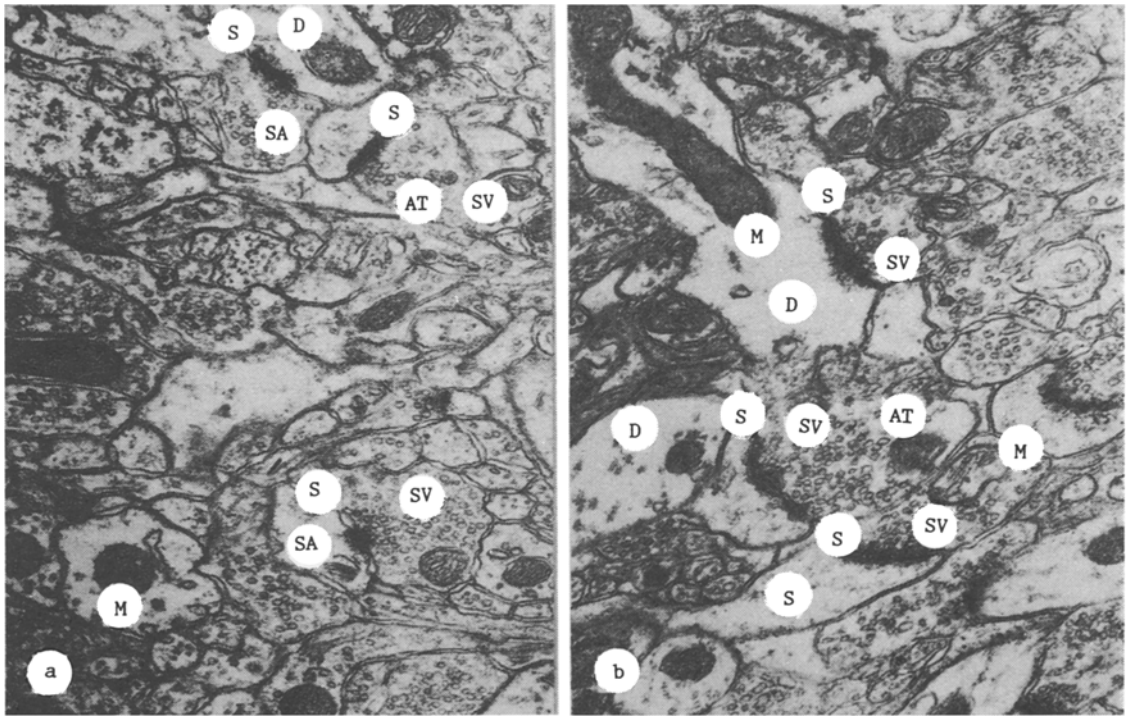

Fig. 1. Neocortical neuropil of undernourished mice aged 40 days (a: 30,000×) and adult (b: 34,000×). S) Synapse, SA) spinous apparatus, SV) synaptic vesicles, AT) axon terminal, D) dendrite, M) mitochondria.

TABLE L. Quantitative Evaluation of Axon Terminals in Neuropil in Layer V of the Mouse Neocortex

Age of mice, days	Expt. conditions	Area of cross section of terminals	Fraction of area occupied by vesicles	Degree of concn. of vesicles in active zone
40	Control	3,14±0,022	3,13±0,008	1,61±0,017
	Malnutrition	2,86±0,010*	2,88±0,008*	1,57±0,023
90	Control	3,09±0,017	3,49±0,007	1,20±0,007
	Malnutrition	3,10±0,016	3,34±0,015**	1,19±0,012

Legend. Here and in Table 2: *p < 0.001 compared with control aged 40 days, **p < 0.001 compared with control aged 90 days.

were measured by means of an ocular micrometer. The significance of differences between the experimental and control groups for these two features was determined by Student's test.

All other features were evaluated visually. The statistical analysis of the data followed the method in [6]. By contrast with most authors of publications on this subject, however, we studied only those synapses that are located on dendritic spines, i.e., synapses of the same morphological and functional type.

EXPERIMENTAL RESULTS

The electron-microscopic study of the neuropil in layer V of the neocortex of young undernourished mice showed that the ultrastructure of the axon terminals is homogeneous. Some have the ultrastructure characteristic of the control animals. Others have translucent axoplasm, containing a few synaptic vesicles. In synapses formed by such terminals the width of the synaptic clefts and thickness of the postsynaptic condensations of the membranes are less than in the control. The spinous apparatus is substantially altered. Its cisterns

TABLE 2. Quantitative Evaluation of Synapses in Neuropil of Layer V of the Mouse Neocortex

Age of mice, days	Exptl. conditions	Length of active zone	Width of synaptic cleft, nm	Thickness of postsynaptic condensation, nm	Number of cisterns in spinous apparatus	Degree of change of spinous apparatus
40	Control	2,59±0,012	22,4±0,20	31,6±0,36	3,62±0,028	1,36±0,006
	Malnutrition	2,50±0,020*	16,1±0,18*	24,5±0,32*	3,31±0,017*	2,13±0,004*
90	Control	2,71±0,014	20,4±0,12	31,3±0,19	3,28±0,022	1,23±0,008
	Malnutrition	2,80±0,004**	17,6±0,07**	28,2±0,15**	3,07±0,017**	1,49±0,016**

are dilated and irregular in shape, and the laminae of electron-dense material are difficult to distinguish (Fig. 1a).

The ultrastructure of the axon terminals of the undernourished adult animals showed less marked changes than in mice kept on a low protein diet from the 10th to the 30th day after birth. Some decrease in the number of synaptic vesicles is observed in some axon terminals in this region. Unlike in the control, lysosomes and large, irregularly shaped vesicles are found in individual terminals. Meanwhile in many synapses the width of the synaptic clefts and the thickness of the postsynaptic membrane concentrations are less in the undernourished adult mice than in the control. Significant changes in ultrastructure of the spinous apparatus are observed in these synapses (Fig. 1b).

The quantitative electron-microscopic analysis showed that the average area of cross section of the terminals occupied by vesicles, the width of the synaptic clefts, and the thickness of the postsynaptic concentration of membranes are significantly reduced in the undernourished young and adult animals (Tables 1 and 2). Since, according to data in the literature, only those synapses which possess a well marked postsynaptic condensation and a synaptic cleft of sufficient width are capable of functioning activity [13, 14], it can be tentatively suggested that restricting the diet of young and adult animals leads to depression of the functional activity of a high proportion of axodendritic synapses, located in the sensomotor cortex.

Meanwhile, in young undernourished mice we found a signficant decrease in the mean area of cross section of the terminals, whereas in adult undernourished animals the mean area of cross section of the terminals was not significantly changed (Table 1). This fact is evidently linked with delayed growth of axon terminals in the period of early postnatal development [8].

Investigation of the relative percentages of straight active zones and zones with positive and negative curvature in both young and adult undernourished animals revealed an increase in the number of straight active zones. Moreover, in the young undernourished animals the average length of the active zones was reduced, whereas in the adult undernourished mice the average length of the active zones was increased compared with that of the control animals of the same age (Table 2). We know from the literature that the information capacity of synapses is directly proportional to the area of the active zone and its curvature. Straight active zones are in reserve and their functional capacity is low [1, 2, 9]. Consequently, the decrease in the length of the active zones and the increase in the number of straight active zones, observed in mice exposed to malnutrition, may evidently be responsible for the reduced information capacity of these synapses. Meanwhile the increase in the mean length of the active zones of the synapses discovered in undernourished adult animals may be interpreted as a compensatory and adaptive response of the adult brain to malnutrition.

No statistically significant change in the degree of concentration of synaptic vesicles was observed in the active zones of undernourished young or adult mice. Just as in the control, they had a tendency toward uniformity of their distribution (Table 1). Meanwhile, in undernourished animals of both groups the changes in the structure of the spinous apparatus were intensified and the number of its cisterns reduced (Table 2). Disturbance of the structure of the spinous apparatus in malnutrition may reduce both the possibility of formation of new connections and also the possibility of potential neuronal interactions [15].

The results of this quantitative electron-microscopic investigation thus demonstrate the influence of malnutrition on the basic structural elements of synapses, and that this influence is stronger in young than in adult animals. Meanwhile, malnutrition in young and adult mice produces significant changes in ultrastructure of synapses located on dendritic spines, and these changes may lead to depression of functional activity of the CNS.

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ULTRASTRUCTURE OF THE CEREBRAL CORTEX AND HIPPOCAMPUS IN RATS IN THE EARLY PERIOD AFTER RESUSCITATION FROM TOTAL ISCHEMIA

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The study of changes in the brain at the subcellular level is a leading approach to the elucidation of the mechanisms of development of brain damage in anoxia, knowledge of which is essential for the development of prevention and treatment of postresuscitation encephalopathy [6].

EXPERIMENTAL METHODS

The frontal cortex and area CA_1 of the hippocampus of 12 noninbred male rats weighing 180-200 g were studied. Systemic circulatory arrest was produced in seven animals for 10 min by retrosternal compression of the vascular bundle [4]; five rats remained intact. The brain of animals with complete visible restoration of their neurologic status on the 4th day after clinical death was investigated. Material was fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.6) and then treated with OsO_4, dehydrated in alcohols

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