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ULTRASTRUCTURAL CHANGES IN NEOCORTICAL SYNAPSES DURING REHABILITATION OF MICE AFTER LONG-TERM PROTEIN-ENERGY DEFICIENCY

D. I. Medvedev, I. I. Babichenko,
I. Z. Eremina, and A. I. Kravtsova

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Long-term protein-energy deficiency in the early postnatal period of development leads to significant changes in synaptic structure [7, 10, 13, 14]. A few investigations have shown that dietary rehabilitation as a rule does not lead to sufficiently full restoration of the morphological and functional characteristics of the synapses [8, 14]. In previous investigations the writers showed that a combination of dietary rehabilitation with the addition of carnitine to the diet sharply intensifies intracellular metabolism and thereby promotes repair of the ultrastructural changes arising in synapses after exposure of the organism to protein-energy deficiency in the early period of its postnatal development [8]. However, no quantitative electron-microscopic evaluation of changes in neocortical synapses during rehabilitation after protein-energy deficiency has hitherto been undertaken.

It was therefore decided to make a quantitative electron-microscopic study of the ability of dietary rehabilitation alone, and of dietary rehabilitation plus the addition of carnitine to the diet to restore the ultrastructure of synapses disturbed by exposure of mice to protein-energy deficiency.

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TABLE 1. Quantitative Evaluation of Axon Terminals in Neuropil of Layer V of the Neocortex in Mice Aged 70 Days

Experimental conditions	Area of cross section of terminals	Fraction of area filled w/ vesicles	Degree of concen. of vesicles near active zone
Control	3,14±0,013	3,13±0,013	1,21±0,011
Dietary rehabilitation	2,87±0,022*	3,00±0,013*	1,30±0,006*
Dietary rehabilitation + carnitine	3,12±0,020	3,21±0,009*	1,21±0,006

Legend. Here and in Table 2: *p < 0.001 compared with control.

TABLE 2. Quantitative Evaluation of Synapses in Layer V of Neocortex of Mice Aged 70 Days

Experimental conditions	Length of active zone	Width of synaptic cleft, nm	Thickness of postsynaptic condensation, nm	No. of cisterns in spinous apparatus	Degree of change of spinous apparatus
Control	2,83±0,013	21,2±0,24	31,2±0,41	3,46±0,019	1,32±0,010
Dietary rehab.	2,65±0,017*	19,1±0,26*	25,4±0,28*	3,34±0,014*	1,70±0,019*
Dietary rehab. + carnitine	3,39±0,015*	18,8±0,23*	28,4±0,36*	4,97±0,030*	1,20±0,003*

EXPERIMENTAL METHODS

Experiments were carried out on 30 CBA mice. The experimental setup and the method of processing the material for electron-microscopic investigation were described by the writers previously [8]. A method of visual classification of synapses based on a set of features [6] was used for quantitative analysis. Each synapse was assessed relative to nine features, which were expressed in points depending on their degree of representation: from minimal (1 point) to maximal (from 3 to 7 points). The following features were used: 1) the area of cross-section of the terminal; 2) the fraction of the area of cross-section of the terminal which is filled with vesicles (the analog of the number of vesicles in the terminal); 3) the degree of concentration of vesicles around the active zone; 4) the configuration of the active zone (the straight active zone, the active zone with positive curvature, and active zone with negative curvature), classification of Cooke et al. [12]; 5) length of the active zone, 6) number of cisterns in the spinous apparatus; 7) the degree of change of structure of the spinous apparatus; 8) width of the synaptic cleft; 9) thickness of the postsynaptic condensation of the membrane.

The thickness of the postsynaptic condensation and width of the synaptic cleft were measured with a ocular micrometer. For these two features, the significance of differences between the experimental and control groups were determined by Student's test. All other features were evaluated visually. The results were subjected to statistical analysis by the method in [6].

Synaptic contacts located on the spinous processes of dendrites were evaluated.

EXPERIMENTAL RESULTS

The previous quantitative electron-microscopic analysis showed that the mean area of cross section of the terminals, the number of synaptic vesicles in the terminals, the width of the synaptic clefts, and the thickness of the postsynaptic membranes in synapses of the sensomotor neocortex of mice kept from the 10th through the 40th day of life on a low-protein diet, were less than in the synapses of normally nourished animals. An increase also was found in the number of straight active zones and a decrease in the average length of the active zones. At the same time, there was an increase in the degree of change of the structure of the spinous apparatus [5]. Quantitative studies showed that after dietary rehabilitation of the underfed animals the mean area of cross section of the terminals, the number of synaptic vesicles, the average length of the active zones, the width of the synaptic

clefts, and the thickness of the postsynaptic condensations of the membranes remained less than in the control (Tables 1 and 2).

Meanwhile, after dietary rehabilitation an increase was observed in the concentration of synaptic vesicles near the active zone of the synapses (Table 1).

Investigation of the configuration of the active zones of the synapses revealed a decrease in the number of straight active zones after dietary rehabilitation of the underfed animals, on account of an increase in the number of synapses with positive curvature. Changes in the ultrastructure of different components of synapses during normal ontogeny are known to be connected with the functional state of these structures [1, 4]. An increase in the concentration of synaptic vesicles near the active zones and a decrease in the number of straight active zones, which are the least active, can evidently be regarded as a compensatory-adaptive reaction aimed at making synaptic transmission more effective. After dietary rehabilitation sufficiently complete recovery of the ultrastructure of the spinous apparatus likewise does not take place (Table 2).

As the results of the quantitative electron-microscopic analysis showed, dietary rehabilitation for 1 month with the addition of carnitine to the diet led to virtually complete restoration of synaptic ultrastructure (Tables 1 and 2). Parameters such as the number of synaptic vesicles, the length of the active zones, and the number of cisterns in the spinous apparatus also were higher than the control values, and according to data obtained by other workers [1, 9, 11], this can be regarded as enhancement of the functional activity of synaptic transmission. Meanwhile, just as after dietary rehabilitation, the width of the synaptic clefts and the thickness of the postsynaptic condensations of the membranes remained less than in the control. For these ultrastructural parameters to be restored, additional and appropriate physiological stimulation is evidently necessary [2, 3].

Quantitative electron-microscopic analysis of axodendritic synapses on spinous processes of dendrites thus showed that dietary rehabilitation for 1 month does not lead to the complete recovery of their submicroscopic structure. Meanwhile dietary rehabilitation with the addition of carnitine to the diet not only induces complete recovery of synaptic ultrastructure, but also results in hypertrophy of several parameters. The stimulating effect of carnitine is evidently directly linked with prompt activation of intracellular compensatory and adaptive mechanisms.

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