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EFFECT OF COMBINED PROTEIN AND CALORIC DEFICIENCY ON SYNAPTIC ULTRASTRUCTURE IN THE MOLECULAR LAYER OF THE CEREBELLAR CORTEX OF DEVELOPING AND ADULT ANIMALS

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Because of the particular features of its late cytogenesis the cerebellum is more sensitive than any other part of the brain to the action of unfavorable external environmental factors [9-11]. In previous investigations the writers showed that long-term combined protein and caloric deficiency leads to an increase in the number of dark pyriform neurons in developing and adult animals, and also causes ultrastructural changes both in cells and in ultrastructural components of the neuropil of the molecular layer of the cerebellar cortex [5, 6]. Investigation of the structural organization of interneuronal connections under conditions of depressed cellular metabolism is of great interest in connection with the study of compensatory and adaptive reactions reflecting intracellular regeneration [8].

The aim of this investigation was a quantitative electron-microscopic study of disturbances of synaptic ultrastructure in the molecular layer of the cerebellar cortex of young and adult mice, receiving a diet deficient in protein and calories.

EXPERIMENTAL METHOD

CBA mice were used. Protein–caloric deficiency was created by reducing the quantity of nutrients by introducing an extra volume of cellulose into the diet. The content of ingredients in the experimental diet was 50% of that of the control diet. In age group 1, nursing females 10 days after giving birth to their young were switched to a low-protein diet, and from the 22nd through the 40th days, young mice fed themselves on a deficient diet. The animals were killed 1 month after the beginning of starvation. In age group 2, mice aged 2 months received the same synthetic diet for 1 month and were then sacrificed. Under pentobarbital anesthesia the brain was fixed by intravital perfusion through the ascending aorta with a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4, 0.1 M). Sagittal sections of the dermis of the cerebellum were cut, prefixed in 2% OsO_4 solution in the same buffer, stained with uranyl acetate, and embedded in Araldite [5]. The electron-microscopic data were subjected to quantitative analysis by visual classification of synapses in accordance with a number of features [4]. Each synapse was assessed relative to six morphological features, which were rated in points: from minimal (1 point) to maximal (5 points).

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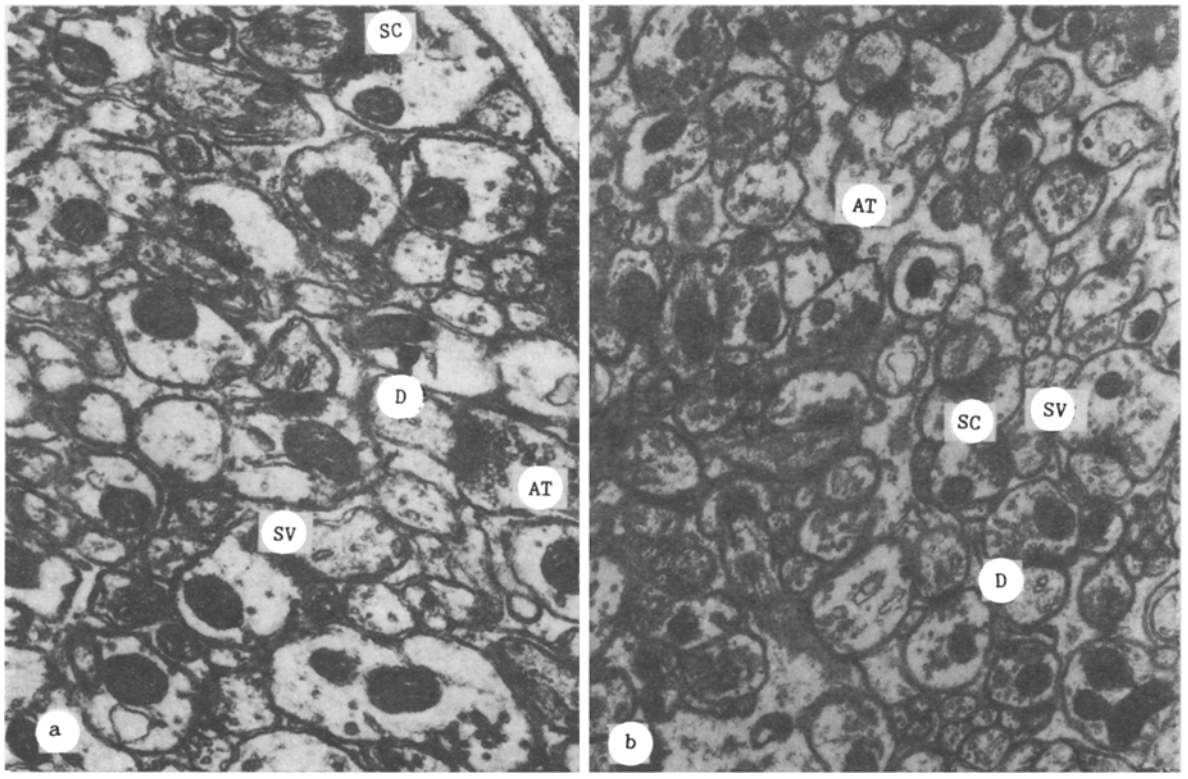

Fig. 1. Region of neuropil of molecular layer of cerebellar cortex of experimental mouse aged 40 days (a) and 90 days (b). SC) Synaptic complex; SV) synaptic vesicles; AT) axon terminal; D) dendrite. Magnification: a) 18,000 ×, b) 16,000 ×.

The following parameters were determined: 1) the area of cross-section of an axon terminal; 2) the fraction of the area of an axon terminal filled with vesicles; 3) the concentration of vesicles in the active zone; 4) the length of the active zones; 5) the width of the synaptic clefts; 6) the thickness of postsynaptic condensations of the membranes. In each group consisting of six animals, 300 axodendritic synapses were examined. For each feature, sample values of average point ratings for random tens of synapses were calculated and constituted a frequency distribution of a variable, which obeyed the law of the normal distribution. For each variable (feature) the average of the means and its confidence interval were determined from Strelkov's table [8]. The confidence limits provided a basis for statistical comparison of homonymous values. The significance of differences between the control and experimental parameters, measured by means of an ocular micrometer, was estimated by the standard biometric methods and by Student's test [7].

EXPERIMENTAL RESULTS

The electron-microscopic study showed that the greater part of the neuropil of the molecular layer of the cerebellar cortex of young and adult animals is occupied by small dendrites and axon terminals, forming exodendritic synapses with them. Unmyelinated fibers and processes of glial cells also are located in this region. The axon terminals are filled with round synaptic vesicles (Fig. 1).

Quantitative estimation of axodendritic synapses in the molecular layer of the cerebellar cortex revealed considerable resistance of the adult mice to protein–caloric deficiency. The change in all parameters of the synapses analyzed in young animals were found to be greater in degree than that in adult animals (Tables 1 and 2). For instance, the area of cross section of axon terminals in developing undernourished mice was significantly reduced on the average by 30% compared with the control, whereas estimation of this parameter in adult mice showed a very small and not significant change in the average area of cross section of the axon terminals. In mice developing under conditions of protein–caloric deficiency the number of synaptic vesicles was reduced: on the average by 54% in the young and by 9% in the adult animals. The character of distribution of vesicles in the terminals changed under these circumstances. In

TABLE 1. Axon Terminals in Neuropil of Molecular Layer of Mouse Cerebellar Cortex

Age of mice, days	Experimental conditions	Area of cross section of terminal	Fraction of area occupied by vesicles	Degree of concentration of vesicles near synaptic membrane
		points		
40	Control	3,23 ± 0,018	3,81 ± 0,006	1,92 ± 0,009
	Malnutrition	2,27 ± 0,015*	1,76 ± 0,006*	2,85 ± 0,018*
90	Control	3,26 ± 0,027	2,87 ± 0,006	2,50 ± 0,012
	Malnutrition	3,24 ± 0,012	2,59 ± 0,012†	2,82 ± 0,006 †

Legend. Here and in Table 2: *$p < 0.01$ compared with 40-day control, †$p < 0.01$ compared with 90-day control.

TABLE 2. Synapses in Neuropil of Molecular Layer of Mouse Cerebellar Cortex

Age of mice, days	Experimental conditions	Length of active zone, points	Width of synaptic cleft, nm	Thickness of postsynaptic condensation of membrane, nm
40	Control	2,70 ± 0,015	20,6 ± 0,15	32,5 ± 0,26
	Malnutrition	2,46 ± 0,018*	10,1 ± 0,17*	22,1 ± 0,24*
90	Control	2,75 ± 0,012	18,8 ± 0,28	34,7 ± 0,25
	Malnutrition	3,25 ± 0,009 †	17,6 ± 0,24 †	31,8 ± 0,24

the undernourished animals, both young and adult, they were concentrated in the region of active zones of synapses. However, the degree of disturbance of this parameter varied, and was 33 and 11% respectively. The area occupied by active zones was reduced in the young undernourished mice on the average by 9%, whereas in the adult undernourished animals it was increased on the average by 18%.

The averaged values of the width of the synaptic clefts in developing mice kept on a low protein diet were reduced by half, those of adult undernourished mice by 7%. Estimation of the thickness of the postsynaptic condensations of the membranes revealed a statistically significant decrease of 32% in young animals and of 8% in adult animals.

The results are thus evidence of a greater effect of protein–caloric deficiency on the ultrastructure of axodendritic synapses in the neuropil of the molecular layer of the cerebellar cortex in young animals than in adults.

Changes in synaptic ultrastructure during normal ontogeny have been linked by some workers with differences in the function of these structures [1, 2]. Our own data showed that the concentration of synaptic vesicles in the active zone of synapses in undernourished animals is accompanied by narrowing of the width of the synaptic cleft and a decrease in thickness of the condensations of postsynaptic membranes; these changes are evidently the structural expression of a reduction in the quantity of receptor protein in postsynaptic structures, leading to depression of synaptic function. Under these circumstances the concentration of synaptic vesicles in the active zones of synapses can be regarded as the expression of compensatory and adaptive reactions aimed at making synaptic transmission more efficient.

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EFFECT OF ALCOHOLIC INTOXICATION OF FEMALE RATS BEFORE PREGNANCY ON NEURONAL ULTRASTRUCTURE OF THE SENSOMOTOR CORTEX IN THE PROGENY

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Many clinical studies have shown that developmental disturbances characteristic of the fetal alcohol syndrome may sometimes arise in the offspring of mothers with alcoholism. The role of maternal alcoholic intoxication before pregnancy in the pathology of development of the fetus and offspring has received little study, and available data are contradictory. There is evidence that the average body weight of newborn infants of mothers taking alcohol before pregnancy is less than in the case of nondrinking women [9, 10]. According to other researchers, alcohol consumption before pregnancy (100 g alcohol per week) reduces the risk of the newborn infants being underweight [14]. In the case of alcohol abuse before pregnancy, in 30% of cases various abnormalities were found in the newborn infants; correlation has been found between the level of alcohol consumption and lowering of the mental development of children [11]. It has been shown experimentally that prolonged alcohol consumption by female rats before pregnancy leads to early embryonic mortality in some animals, to reduction of the body weight of the offspring at the age of 3 weeks, a tendency toward increased excitability of their CNS at the age of 1 month, inhibition of motor activity in the open field test, and some

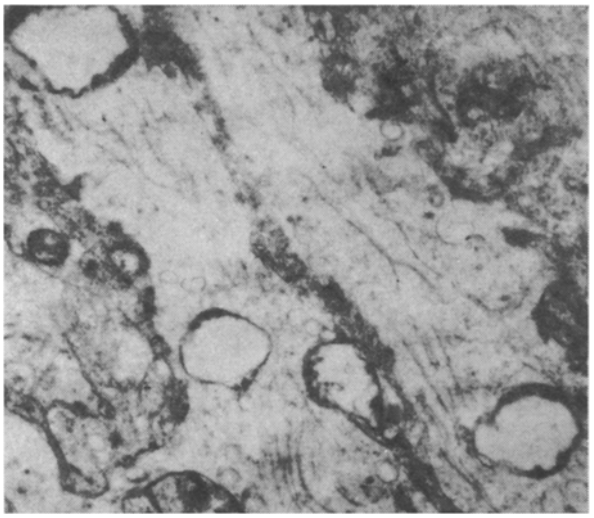

Fig. 1. Changes in ultrastructure of mitochondria in dendrites of sensomotor cortex of experimental rat aged 14 days (5800 ×).

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