Ultrastructural Reorganizations of Synaptic Contacts in an Organotypic Culture of the Brain Cortex in the Presence of Ethanol

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The effect of various ethanol concentrations (0.5 and 1%) on the ultrastructure of interneuronal contacts is studied in an organotypic culture of the brain cortex from newborn rats. It is shown that ethanol in the culture medium causes geometric complications in the synaptic contacts. Morphometric analysis of synapses reveals an increase of the area and perimeter of axon terminals and of the length of the active zone of the contact, as well as a decrease of the coefficient determined by the ratio of the number of synaptic vesicles to the length of the active zone of the contact.

Key words: *ethanol; nerve tissue culture; ultrastructure; morphometry; synapses*

Intellectual impairment in children is one of the main manifestations of fetal alcohol syndrome; in models using experimental animals this syndrome is expressed in a variety of behavioral disturbances [6,15]. Neuromorphological changes induced by ethanol include a broad range of synaptic involvement [1,5,7,8,11,12]. The definitive differentiation of the cortex is accomplished postnatally and the first 10 days after birth correspond to the period of rapid brain growth in rats, when myelinization, synaptogenesis, and the formation of neurotransmitter systems proceed very vigorously [15]. The effect of different ethanol concentrations on the state of interneuronal contacts was assessed in the present investigation using the model of an organotypic culture of the brain cortex from newborn rats.

MATERIALS AND METHODS

Explants of the sensorimotor cortex of newborn rats were cultured by the method of "flying" slides in rotating test tubes [3]. Ethanol was added to the experimental cultures upon explantation and during the entire period of growth *in vitro* (20 days). The alcohol concentration was 0.5 and 1% in the culture medium. The tissue was routinely processed for electron microscopy on the 20th day. The preparations were examined in JEM-100B и JEM-100CX (Joel) electron microscopes. A semiautomatic MOP-Videoplan (Reichert) image analyzer was used to study the synaptic contacts. Measurements were carried out on negatives obtained under a magnification of 19,000.

The indexes estimated morphometrically were as follows: 1) the area of the presynaptic terminal; 2) the perimeter of the presynaptic terminal; 3) the length of the active zone of the contact (AZC); 4) the total area of mitochondrial sections and their number in the axon terminal; 5) the number of synaptic vesicles in AZC; 6) the calculated ratio of the number of such synaptic vesicles to the length of the corresponding postsynaptic thickening. The percentage of perforated, convergent, and divergent synapses as well as the ratios of synapses with AZC of different curvature (straight, concave, and convex) were taken into consideration. The results were processed statistically by Student's *t* test.

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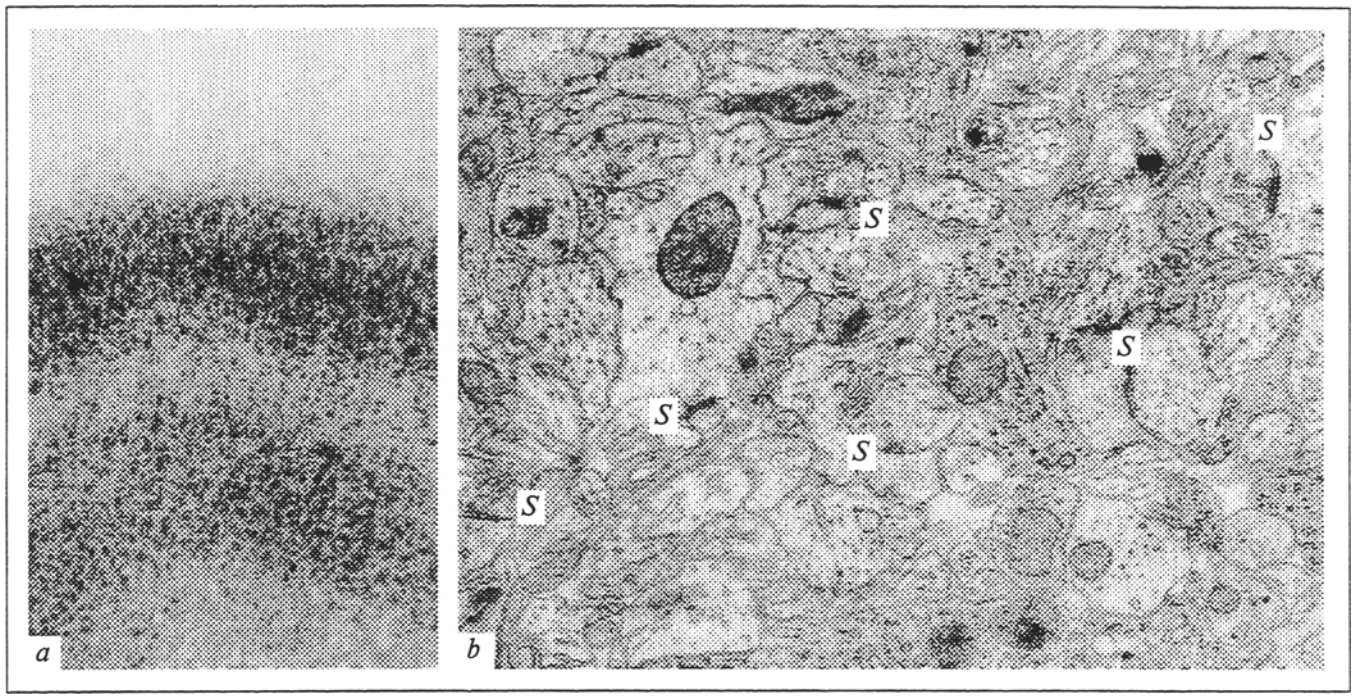

Fig. 1. Organotypic control culture on the 20th day. *a*) cytoarchitectonics of a sensorimotor cortex explant. Nissl staining, ×80. *b*) fragment of neuropil, ×10,000. *S*: synapse.

RESULTS

The cytoarchitectonics of the cortical layers becomes quite distinct toward the 20th day of development *in vitro* (Fig. 1). The density of distribution of processes rises in the neuropil and the ultrastructure of synapses corresponds to the main types of synaptic contacts [2]. Axodendritic synapses of asymmetric type are mostly represented in explants as well as a small number of axospinal synapses with a developed spiny apparatus (Fig. 2, *b*). The synapses have a weakly pronounced postsynaptic thickening, while large vesicles preserved from the growth period are found along with small synaptic vesicles in the presynaptic terminals.

The ultrastructure of interneuronal contacts in the experimental groups does not differ significantly from the control and no gross destructive changes are found. Just severely jagged profiles of presynaptic terminals are noted in test cultures as well as a decrease of the number of synaptic vesicles, especially in explants with 1% ethanol (Fig. 3, *b*). In the presence of 0.5% ethanol individual terminals filled with synaptic vesicles are found in parallel with presynaptic profiles containing a small number of vesicles. A complication of synaptic geometry is revealed in explants with ethanol, manifested as a high percentage of perforated synapses containing several AZC as well as a slight increase of the number of convergent and divergent synapses (Figs. 2, *a* and 3, *b*). In cultures with 0.5% ethanol a 7.3% increase is found in the number of concave synapses (functionally active) and a slight decrease of straight profiles (reserve synapses) in comparison with the control.

A number of reliable changes and synaptic reorganizations are established by morphometric analysis of a large number of synapses (Table 1).

TABLE 1. Main Parameters of Synaptic Contacts in Different Experimental Groups

Index	Control (n=363)	0.5% ethanol (n=261)	1% ethanol (n=379)
Area of presynaptic terminal, m^2	0.510±0.014	0.541±0.017**	0.598±0.016*,**
Perimeter of presynaptic terminal, m	2.878±0.045	2.954±0.049**	3.079±0.043*,**
Length of AZC, m	0.375±0.007	0.395±0.009**	0.432±0.008*,**
Total area of mitochondrial section, m^2	0.021±0.002	0.026±0.004*,**	0.018±0.002**
Ratio of number of synaptic vesicles to AZC length	9.928±0.197	9.304±0.234**	7.938±0.225*,**

Note. *signifies differences from the control group, **between experimental groups (p<0.05); n – the number of contacts.

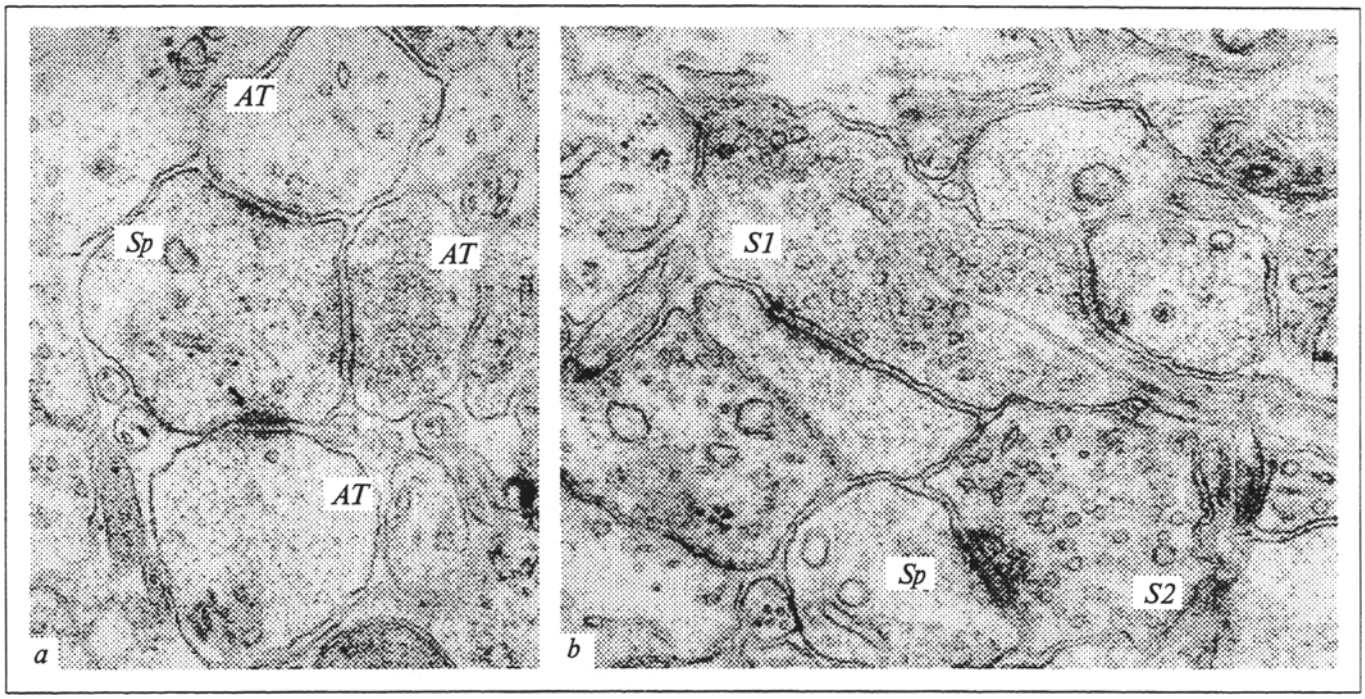

Fig. 2. Ultrastructure of complicated synaptic contacts. ×19,000. *a*) junction of convergent type; axon terminals contain a differing number of synaptic vesicles (0.5% ethanol). *AT*: axon terminal; *Sp*: spine. *b*) *S1*: divergent synapse, *S2*: axospinal synapse (control).

AZC length correlates positively both with the perimeter (r=0.4) and with the section area of the axon terminal (r=0.4) in control cultures only. An increase of the mean area and perimeter of the presynaptic terminals as well as an elongation of the postsynaptic thickening are observed in cultures with 0.5% ethanol, but the reliability of differences is insignificant. The presence of 1% ethanol results in a 17% increase of the area of presynaptic terminals (p<0.05) as compared to control cultures and a 10.5% increase (p<0.05) as compared to the second experimental group. The enlargement of the presynaptic perimeter is 7% (p<0.05) as compared to the control and 4.2% (p<0.05) in comparison with the series containing 0.5% ethanol. The extent of AZC is 15.2% greater (p<0.05) as compared to the control and 9.4% greater (p<0.05) in relation to the preceding group.

The number of synaptic vesicles making contact with AZC does not differ in any of the culture groups, while the ratio of vesicle number to unit of AZC length declines in both experimental groups. A 6.3% decrease of this coefficient (p<0.05) is noted in cultures with 0.5% ethanol and a 20.1% decrease (p<0.05) in explants with 1% ethanol in comparison with the control. As the ethanol concentration rises, the correlation between the number of synaptic vesicles in contact with AZC and its extent diminishes. The value of the correlation coefficient is r=0.61 in the control, while in the group with 0.5% ethanol it is r=0.55 and in the presence of 1% ethanol r=0.4.

The percentage of presynaptic terminals with mitochondria is practically the same in all groups and comprises 25-27%. The number of mitochondria per terminal is the same as well. On the other hand, the total area occupied by mitochondria in cultures with 0.5% ethanol is 34.8% (p<0.05) greater than in the control and 32.4% (p<0.05) higher than that in the series with 1% ethanol. The relative share of the area of a presynaptic terminal occupied by mitochondria is also higher in cultures with 0.5% ethanol.

When the results are compared with qualitative data reported previously [3,4], the state of the intracellular organelles in cultures with 0.5% ethanol is found to be typical for active metabolic processes and the changes observed in synapses are of a functional nature. Various abnormalities of neuropil ultrastructure are found in the presence of 1% ethanol, ranging from a slight lightening of the dendroplasm and a decrease of the number of microtubules and neurofilaments to the appearance of concentric membrane structures of different types (Fig. 3, *a*). It is believed that these changes may hinder the formation of the interneuronal network, whereas the synaptic hypertrophy accompanying AZC elongation is aimed at maintaining neuronal function in the presence of ethanol. A decrease of the number of synapses under different effects of ethanol was established previously [9,10,12-14]. Ex-

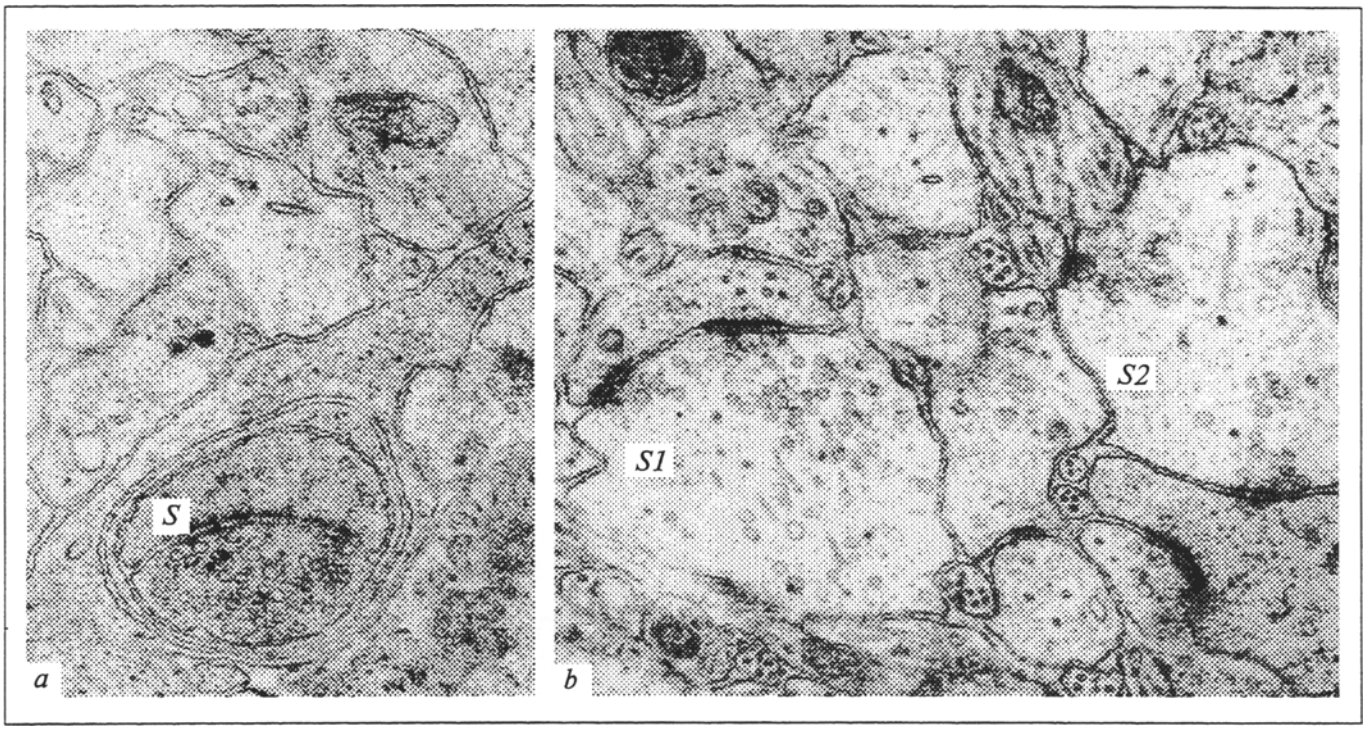

Fig. 3. Interneuronal contacts in a culture with 1% ethanol. ×19,000. *a*) insulation of synapse by glial process. *S*: synapse. *b*) axon terminals contain a small number of synaptic vesicles located close to AZC. *S1*: perforated synapse, *S2*: divergent synapse.

posure to ethanol vapors in the postnatal period resulted in a decrease of the number of synapses in the neocortex on the 56th day. Interneuronal junctions were of larger extent and contained a greater number of synaptic vesicles, but these changes were not found by the 76th day [12]. A more significant decrease in synapse density was noted in the case of prenatal exposure to ethanol [10].

Thus, the action of ethanol during the period of active brain growth results in structural changes of synapses which may give rise to functional disturbances of brain activity in fetal alcohol syndrome.

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