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The Role of Cell Fusion in Physiological and Reparative Regeneration of the Cerebral Cortex

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The prefrontal (cognitive) cerebral cortex of rats was studied by morphological and physiological methods 56 days after stroke induced by photothrombosis. The cognitive capacity impaired after the intervention was completely restored by this time. The count of fused cells (dikaryons) increased significantly in experimental and sham-operated (control) animals in comparison with the early period (7 days) after surgery. Normalization of the dikaryon and mononuclear cell structure was observed after 56 days. Presumably, cell fusion promotes their morphological restoration and regeneration of the lost functional capacity. Fusion is regarded as a manifestation of physiological and reparative regeneration of the cortex.

Key Words: *cell fusion; physiological and reparative regeneration of CNS; stroke*

Today the majority of scientists regard proliferation and differentiation of neural stem cells into neurons as the main and even the only mechanism of the nervous system regeneration, including regeneration of its highest compartments [8,9]. However, this opinion is often disputed. Some authors suggest that the methodological errors [3,4,7] in studies of neural stem cell differentiation could distort the results. Since the problem is very intricate and needs sophisticated experiments for its solution, a weighed and, presumably, not ungrounded opinion was expressed: neuronal regeneration by means of differentiation of neural stem cells has been persuasively proven only for intermediate neurons of the olfactory bulb and dentate gyrus of the hippocampus [5,6]. The mechanisms of regeneration of the rest compartments of the brain remain unknown. We started research aimed at clearing out the role of cell fusion in the CNS in health and disease [2].

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This paper presents the results of morphological studies of the cell fusion phenomenon and its correlation with the functional changes after ischemic stroke.

MATERIALS AND METHODS

The study was carried out on outbred male rats (200-230 g; 2 months). Bilateral focal ischemic infarction of rat cerebral prefrontal cortex (fields Fr1 and Fr2) [10] was induced by the photothrombosis method [11].

The animals were narcotized with chloralhydrate (300 mg/kg intraperitoneally). Bengal rose (Sigma), a photosensitized dye, was injected into the jugular vein (3% solution, 40 mg/kg). Animal's head was fixed in the stereotaxis and the periosteum was removed after a longitudinal incision of the skin. The light guide (light beam output diameter 3 mm) was fixed at a distance of 1 mm from the skull surface. Exposure to cold light (λ=560 nm) was carried out for 20 min on each side. Sham-operated animals (control) were subjected to the

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same procedures except injection of bengal rose dye.

The CNS function in rats was evaluated by the latency (sec) of conditioned passive avoidance response (CPAR). The reflex was trained as described previously [1] in the Biotest RK-5201 shuttle chamber. The reflex was considered trained if its latency was 300 sec; animals with shorter latency were not taken into the experiment.

All animals were tested for CPAR reproduction 7 and 56 days after surgery. Experimental animals lost CPAR 7 days after bilateral photochemical thrombosis, while controls retained the reflex trained before the intervention.

After 56 days, when the cognitive function (CPAR performance) was restored in the experimental animals, the rats of both groups were perfused (under chloralhydrate narcosis) with 2.5% glutaraldehyde solution in phosphate buffer with sucrose. The prefrontal zones were embedded in epoxy resin by the standard method for electron microscopy. Semithin sections were examined and measurements were carried out in an Olympus microscope fitted with Cell-F image analysis software. Ultrathin sections were examined in a Leo 912AB microscope (Omega).

Cells with 2 nuclei in the cytoplasm (dikaryons) were considered fused. Quantitative intensity of the fusion process was evaluated by the mean area of a section containing one dikaryon. Statistical significance of differences was evaluated by the Wilcoxon test.

RESULTS

The prefrontal zone of experimental animals was examined on day 56 after ischemic stroke. Microscopic examination of exposed zones of the cortex showed normal cortical tissue. The brain structure of control animals was the same. The incidence of dikaryons in experimental and control animals 56 days after the stroke was higher than after 7 days (if the zones beyond the penumbra were compared in the 7-day preparations).

The results are summed up in Table 1. The data indicate that cell fusion process is more intense closer to the necrotic zone. This process takes place in cells subjected to chromatolysis, which lost many ultrastructures (Fig. 1). This result is in line with our initial hypothesis according to which cell fusion is a form of nervous system regeneration. Fusion develops in the zone with the greatest (but still compatible with life) alterative changes. This corresponds to the classical scheme of the development of the reparative regeneration process. Though the fused cells are dystrophic, the direction of their subsequent changes is not progressive alteration, but regeneration. This is proven by the fact that fused cells do not die; their number increases by the later period of the study. Fusion of two damaged cells into one can be a reparative event, if the injuries are qualitatively different and the fusion has a complementarity effect. Both cells could die without fusion, but their fusion retains at least one, and, which is remarkable, more potent cell with two genomes. The morphology of dikaryons was completely restored 56 days after the stroke induced by photothrombosis (Fig. 2). Hence, morphological regeneration at the cellular level corresponded to recovery of the functional capacity at the organism level (Table 2).

The number of fusions on day 56 is significantly higher in comparison with day 7 not only after stroke, but even after sham operation (Table 1). This fact seemingly contradicts the initial hypothesis according

TABLE 1. Mean Section Area (S_{mean}) Containing One Dikaryon during the Early and Late Periods after Stroke

Parameters	Group I		Group II	
	day 7 after stroke; cognitive capacity is lost		day 56 after stroke; cognitive capacity is restored	
	experiment	control	experiment	control
Number of animals	5	3	5	5
Number of blocks examined	20	23	26	22
S_{mean} per dikaryon for the entire cortical zone, μ^2	982,872	937,749	81 389*	113,920+
S_{mean} per dikaryon in the peri-infarction zone (penumbra), μ^2	146,971*			

Note. $p<0.01$ compared to: *other cortical zones in experiment and control 7 days after stroke, +incidence of fusions in the experiment and control 7 days after stroke.

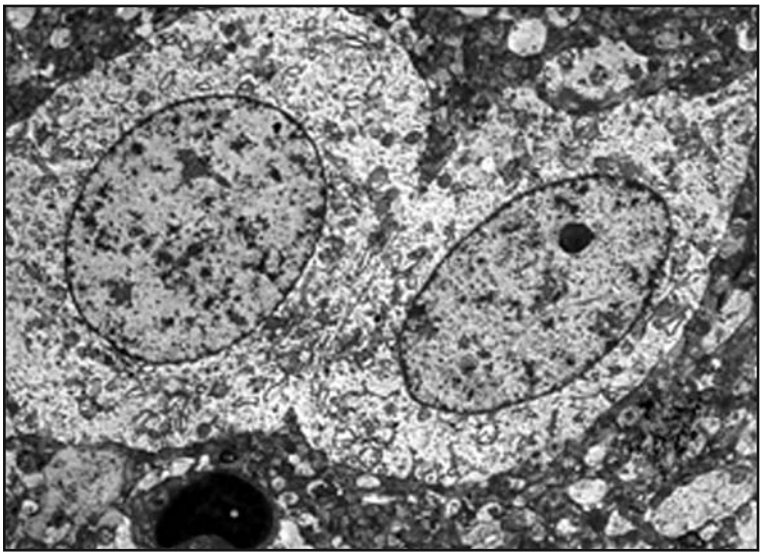

Fig. 1. Neuron dikaryon in the penumbra. Loss of significant number of cytoplasmic structures led to a reduction of electron density of the cytoplasm, it is significantly more clear than the adjacent neuropil and even than the nuclei. In terms of optic microscopy this corresponds to chromatolysis, ×15,000.

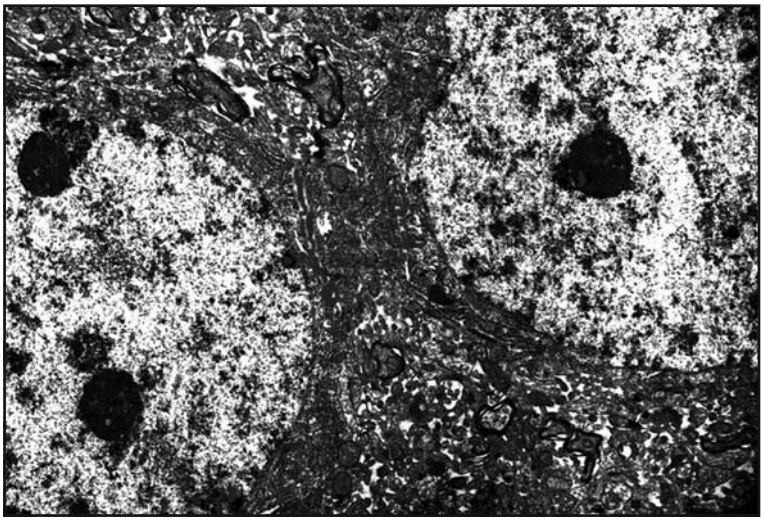

Fig. 2. Neuron dikaryon 56 days after stroke. Cytoplasm with numerous ultrastructures, its electron density does not differ from that of the neuropil. The nuclei are obviously more clear, ×17,000.

to which fusion is a form of regeneration and is more manifest in sites of reparative regeneration. In fact, this is really so. The incidence of fusions is higher in experimental samples, though the difference is statistically negligible. But, on the other hand, minor difference in the incidence of fusions in experiment and control 56 days after stroke is presumably the most interesting experimental finding. There was no reparative regeneration in the control, but physiological regeneration, a permanent process in any living object, did take place in these samples. Moreover, it was not just age-associated physiological regeneration (the rats

TABLE 2. Motor Activity and CPAR Latency in Rats with Bilateral Prefrontal Cortical Ischemia and Sham-Operated Rats

Period of study	Experiment		Control	
	motor activity	CPAR latency	motor activity	CPAR latency
Before photothrombosis	207.4	300	186.6	300
Day 7 after operation	140.4	41.6*+x	150.6	300
Day 56 after operation	132	261.2	148.4	270

Note. Means for groups (n=5) are presented. p<0.05 compared to: *level before intervention, +level on day 56 postoperation, xsham-operated animals.

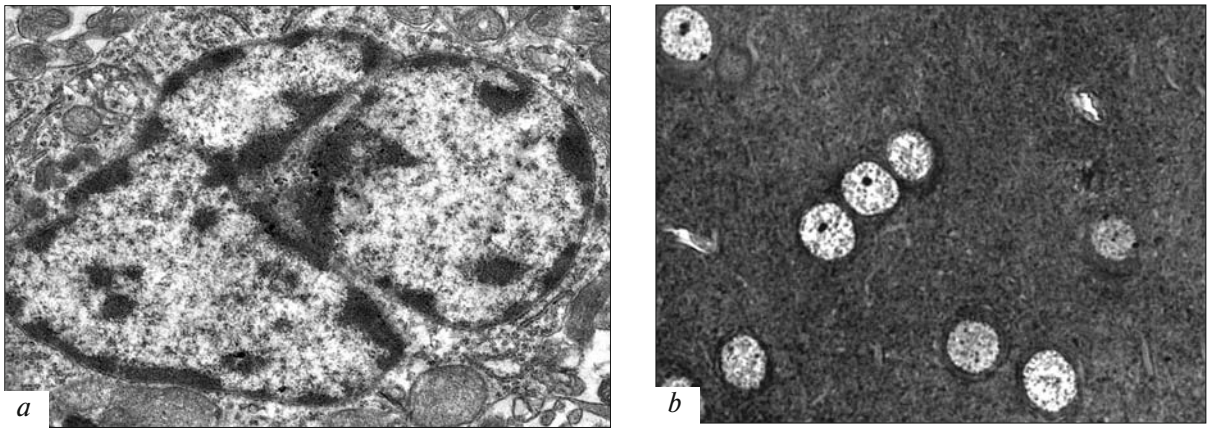

Fig. 3. Variants of cell fusion in the cognitive cortex. *a*) oligodendrocyte dikaryon in the cortex 56 days after stroke, ×18,000; *b*) neuron trikaryon (in the center) in a control rat 56 days after sham operation, ×600.

grew 2-fold older over the time of the experiment), but intense physiological regeneration under conditions of forced functional loading. The animals were trained before sham operation and then were tested for reproduction of acquired habits.

A significant increase in dikaryon count in the control indicates that physiological regeneration is also realized through fusion. This finding supports the conclusion on the regenerative role of fusion in the context of the detected fact: similarity of mechanisms of physiological and reparative regeneration. Similarity of the mechanisms, certainly, does not cancel the regularity according to which the intensity of reparative regeneration is often many-fold more intense than physiological one. But then, why the increase in the number of fusions 56 days after experimental stroke (after physiological and reparative regeneration) is just slight and statistically negligible in comparison with the control, in which only physiological regeneration took place? This important problem will be the object of our further studies. Now we explain this result by small area of the penumbra in comparison with the entire examined cortical zone, because of which the local increase in the dikaryon count in the penumbra is leveled in the summary estimation of the large cortical area, in which the penumbra is indiscernible. Fusion of a neuron with an oligodendrocyte was reported at least for the penumbra [2]. It was found that oligodendrocytes also can fuse (Fig. 3, *a*). The stimuli to fusion can be so strong or the location of the cells so favorable, that trikaryons formed by neurons were detected, though rarely, in experimental and control animals (Fig. 3, *b*). Experiment showed that cell fusion is not a rare event in the cerebral cortex, not confined to neurons exclusively. The results best of all conform to interpretation of cell fusion as a mechanism of physiological and reparative regeneration of the CNS.

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