Degenerative Changes and Cell Death in Long-Living Homo- and Heterotopic Transplants from Embryonic Germ Layers of Rat Neocortex

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Morphological study of allotransplants of rat embryonic neocortex 14-18 months after transplantation into the neocortex, lateral cerebral ventricle, and sciatic nerve of adult animals revealed death of nerve and glial cells in the delayed postoperation period independently on the site of transplantation. After heterotopic transplantation the count of degenerated neurons was 2 times higher that after homotopic transplantation. In heterotopic transplants a considerable number of grafted neurons underwent reversible and irreversible degenerative changes accompanied by their premature aging. Neuronal death is probably determined by insufficiency of trophic influence from afferent structures and target tissues. We hypothesized that antiapoptotic preparations can be used for prevention of transplanted cell death. It was also found that degeneration of neurons was associated with impaired vascularization of transplants and pronounced immune reaction of the recipient in late posttransplantation period. Transplantation of embryonic brain structures can serve as a model system in studies concerning involutive and pathological processes in the central nervous system and in the search for factors improving survival of neurons.

Key Words: *neurotransplantation; neocortex; lateral cerebral ventricle, nerve*

Transplantation of embryonic CNS tissues is widely used in neurobiological experiments concerning the fundamental problems of histogenesis and regeneration of nervous tissues. This procedure serves as a model to study prenatal damage leading to brain pathologies. Apart from experimental damage to developing brain caused by teratogenic factors, irradiation, and hypoxia during prenatal development [2], neurotransplantation allows evaluation of the mechanism of death and histoblastic potencies of neuronal precursors and mature neurons during adaptation to adverse factors. Transplantation of embryonic nervous tissues is used in the studies of histogenesis of transplanted CNS germ tissues after tissue damage in donors and recipients produced by changes in microenvironmental conditions, absence of specific afferent and efferent signals, atypical vascularization, and immune reaction in recipients. The most pronounced changes in transplanted neurons occur in late post-transplantation period. Previous experiments demonstrated degeneration of nerve cells 6-12 months after transplantation [1,6,8, 12]. However, some investigators revealed no changes in long-living neurotransplants [11]. Here we examined long-living allotransplants from embryonic rat neocortex after homo- and heterotopic transplantation. Comparative study of brain cells in delayed periods after transplantation into various tissues of recipients will elucidate the mechanisms of neuronal death and factors affecting the development and viability of cells during late ontogeny.

MATERIALS AND METHODS

Experiments were performed on 45 Wistar rats weighing 200-250 g. Embryonic tissues were routinely

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transplanted into the sciatic nerve, lateral cerebral ventricle, and neocortex of adult animals under ether anesthesia [5,3]. Fragments of the dorsolateral wall of the anterior cerebral vesicle were isolated from Wistar rat embryos (15 days). The animals were kept in a vivarium under standard conditions and sacrificed with ethyl ether 1-2 and 14-18 months after surgery. For a histological study the samples were fixed in Bouin fluid. Paraffin sections (5 μ) were stained with hematoxylin and eosin and by the method of Nissl (toluidine blue). For detection of fragmented DNA in transplanted cells the sections were fixed in an alcohol-formalin mixture and embedded into paraffin (TUNEL method) [9]. The count of neurons per unit area in transplants was determined on a MOP analyzer. The results were analyzed by Student's *t* test. For electron micro-

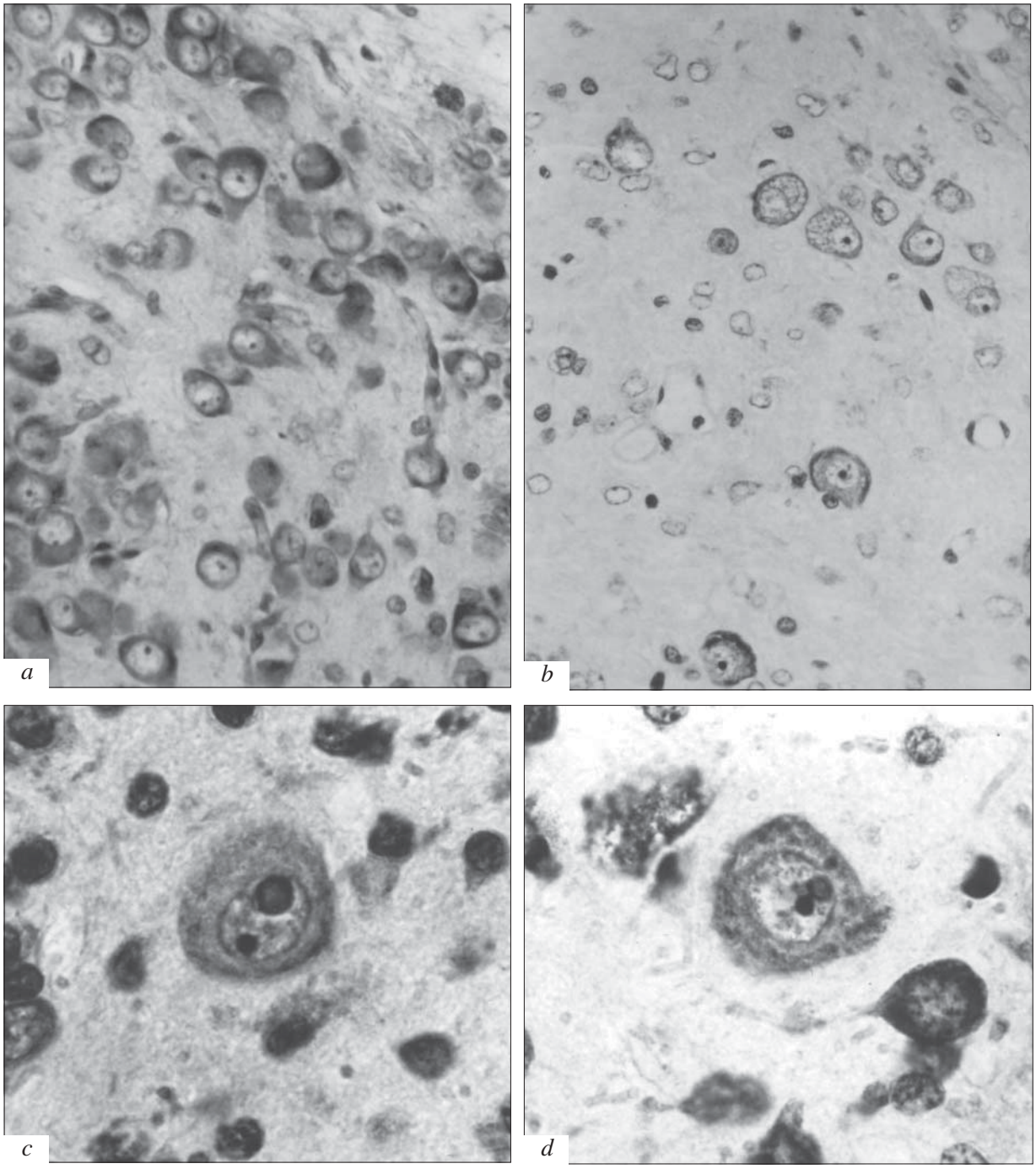

Fig. 1. Fragments of transplants from embryonic germs of the neocortex (E15) in the lateral cerebral ventricle of adult rats: 1 (*a*) and 15 months after transplantation (*b*); intranuclear inclusions in neurons of transplants 15 months after surgery (*c*, *d*). Staining with toluidine blue by the method of Nissl. ×400 (*a*, *b*), ×900 (*c*, *d*).

scopy the nerves with transplants were fixed in 2.5% glutaraldehyde and 2% OsO_4 and dehydrated in alcohols and propylene oxide 8 months after surgery. The sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-100B microscope.

RESULTS

The structure of long-living transplants from embryonic rat neocortex grafted into the sciatic nerve, lateral cerebral ventricle, and neocortex of adult animals was studied 14-18 and 1-2 months after surgery. Our previous studies showed that 1-2 months after surgery the transplants contain a considerable number of nerve and glial cells [3,5]. Transplanted cells were characterized by high degree of maturity (Fig. 1, *a*). Homo- and heterotopic transplants had a well-developed neuropil and many blood vessels. A specific feature of transplants developing in nerves is the formation of a glial multilayer consisting of ependymal cells [3].

The density of neurons in transplants developing in the neocortex of adult rats markedly decreased 14-18 months after surgery (Fig. 2). The number of nerve cells per unit area of the transplant decreased by 36%. Transplanted cells did not undergo massive degeneration. Dystrophic neurons were practically absent. However, the volume of long-living transplants decreased at this stage and large pyramidal cells were absent (these cells were preserved in transplants 1-2 months after surgery).

Neuronal density in heterotopic transplants14-18 months after surgery decreased by 60-70% (compared to that 1-2 months postoperation, Fig. 1, *a*, *b*, Fig. 2). Neurons underwent reversible and irreversible degenerative changes. Reversible changes included acute swelling, *i.e.* enlargement and deformations of neurons, chromatolysis, and clarification of the cytoplasm. Shrunken nerve cells and neurons with the vacuolized cytoplasm were often seen (Fig. 1, *b*). Intranuclear incorporations were found in some neurons (Fig. 1, *c*, *d*). Long-living heterotopic transplants contained irreversibly changed cells with lysed nuclei, ghost cells, and cells undergoing neuronophagy.

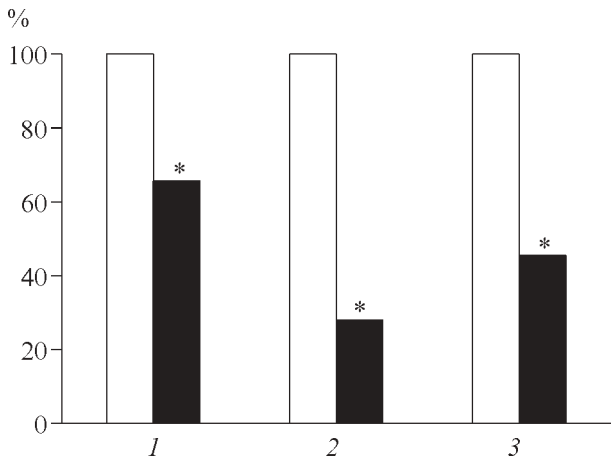

Fig. 2. Decrease in the number of neurons per unit area of neurotransplants in the delayed period after transplantation in the neocortex (*1*), lateral cerebral ventricle (*2*), and sciatic nerve of adult animals (*3*). Light bars: control level of neuronal density 1-2 months after transplantation (100%). Dark bars: 14-18 months after transplantation. Ordinate: number of neurons per unit area of the transplant. *$p<0.05$ compared to the control.

Study of the ultrastructure of neurons in heterotopic transplants developing in the sciatic nerve for 8 months revealed changes typical of aging brain cells [4]. The cytoplasm of many cells was depleted in organelles. Cells with agglomerates of lipofuscin granules were often seen (Fig. 3, *a*, *b*). Some neurons had intranuclear incorporations. The cells were characterized by deep invaginations of the nuclear membranes (Fig. 3, *d*). The number of synaptic contacts in the neuropil decreased. Edema and degeneration of astrocytic pedicles near blood vessels were revealed. The cytoplasm of glial cells contained lipofuscin granules (Fig. 3, *c*). Blood vessels were enlarged, and their number increased. Basal membranes of capillaries were thickened. Neurons were often localized near blood vessels, which is characteristic of aging brain [4]. Premature aging of neurons in heterotopic transplants is consistent with previous data obtained in experiments on transplants developing in the anterior eye chamber [12]. L. C. Doering *et al.* reported that changes in neuron cytoskeleton in long-living transplants are similar to those observed during Alzheimer's disease [8]. The observed signs of cell aging in transplants suggest that some of them undergo apoptosis. The study of fragmented DNA showed that long-living transplants developing in the nerve contained apoptotic glial cells.

The development of long-living heterotopic transplants is accompanied by a pronounced immune reaction of recipients. It was observed during transplantation into the lateral cerebral ventricle. A considerable number of mononuclear cells migrated from the white matter of recipients into the neurotransplant. The ependyma disappeared at the site of contact between transplanted tissues and ventricle wall. Migrating cells occupied a large area of the transplant and caused death of neurons and glial cells. When the transplant neighbored the vascular network in recipients, lymphocytes migrated from the vascular plexus into the transplant. Focal degeneration of tissues transplanted into the sciatic nerve was revealed in the delayed period. We observed death of nerve, glial, and blood vessel cells. Calcified deposits similar to psammoma bodies were formed in these regions [1]. They probably contained amyloid deposits. Similar amyloid deposits and their calcification are found in the posterior horns of the

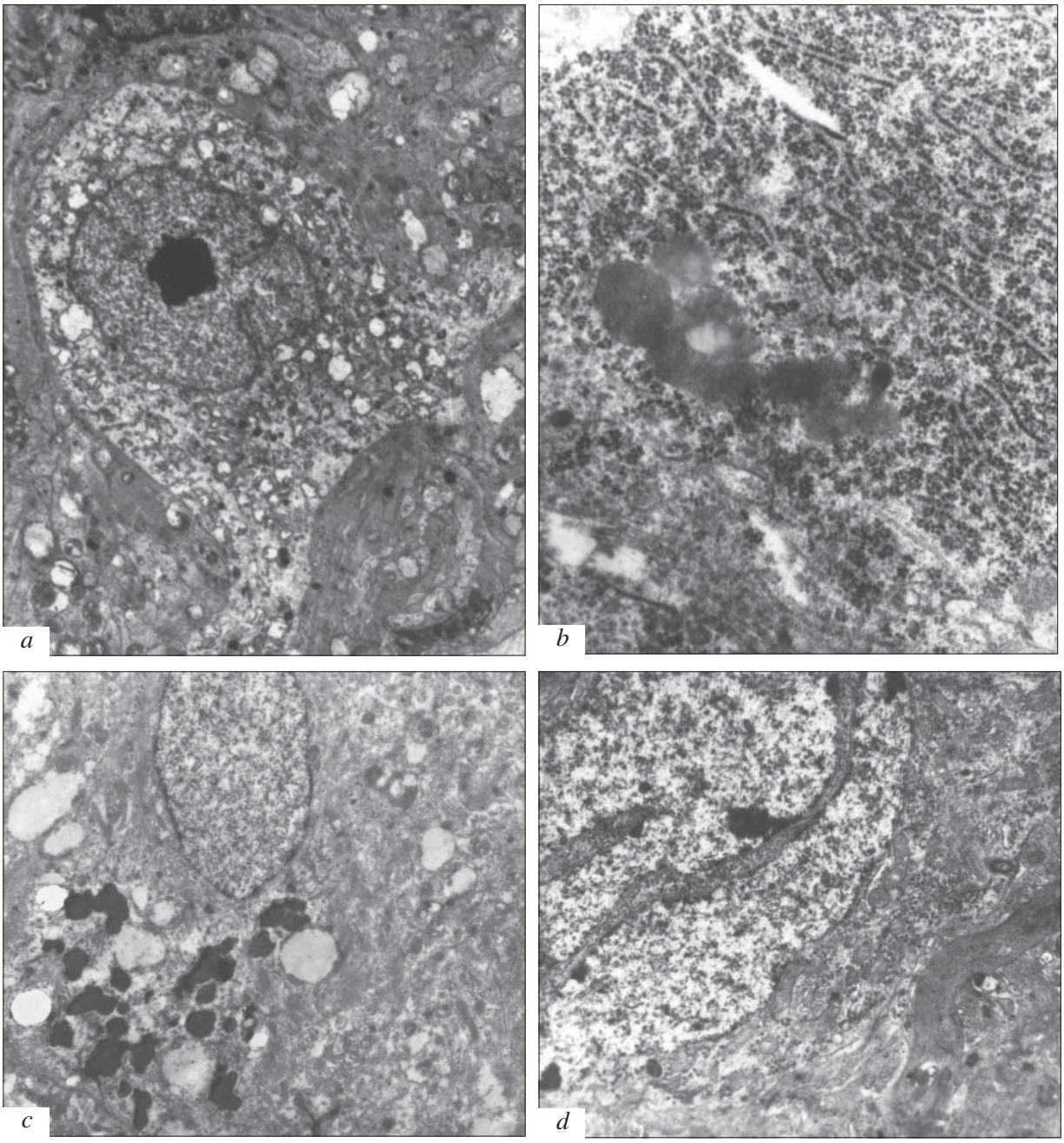

Fig. 3. Ultrastructure of neurotransplants from embryonic neocortex 8 months after transplantation in the peripheral nerve of adult rats. Nerve cell with the cytoplasm depleted in organoids (*a*, ×5000); lipofuscin in the nerve cell cytoplasm (*b*, ×10,000); lipofuscin in the glial cell cytoplasm (*c*, ×7000); deep invaginations of nuclei in the neuronal membrane (*d*, ×7000).

spinal cord during aging and in some pathologies. The presence of lymphocytes, monocytes, and plasma cells in the perivascular space reflects activation of the immune response in the delayed period after transplantation into the nerve. Multinuclear giant macrophages were often seen at the site of transplantation in the nerve, which was characteristic of chronic inflammation.

Our results show that some neurons died 14-18 months after surgery independently on the site of transplantation. Neuronal death was most pronounced in heterotopic transplants. Low trophic influence from afferent structures and target tissues can contribute to neuronal death. This problem can be solved by transplantation of embryonic layers from various brain structures [7,10,13]. Antiapoptotic preparations hold promise for the prevention of transplanted cell death. Degeneration of neurons is probably related to the impairment of vascularization in transplants (*e.g.*, peripheral nerve) and pronounced immune reaction of recipients in the delayed post-transplantation period.

Transplantation of embryonic brain structures can serve as a model to study involutive and pathological processes in the central nervous system and search factors increasing survival of neurons.

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