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Structural Organization of Glial Cells at the Border between the Neurotransplant and Recipient Brain

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Integration of fetal hippocampal dentate fascia neurotransplants with the neocortical somatosensory region in adult rats was studied by electron microscopy. The growth of nerve fiber through the neurotransplant/brain border formed by the glial cells was studied. The interface zone was organized by various astrocyte subpopulations and ependymocytes forming multilamellar accumulations in some sites of the interface. These conglomerations of the glial cells and their processes did not prevent the growth of axonal and axodendritic bundles; moreover, fibrous astrocyte axons accompanied them. Under conditions of immature nervous tissue transplantation to the focus of mature brain damage, the glial cells created a substrate in the interface permeable for nerve fibers, thus promoting the functional integration of the neurotransplant.

Key Words: *neurotransplant; interface; glial cells; nerve processes; ultrastructure*

Transplantation of immature nervous tissue is used for studies of brain development and morphofunctional plasticity and for the treatment of injuries, stroke, and neurodegenerative diseases. The favorable trophic effects of transplanted cells on damaged brain are determined by secretion of neurotrophins and other bioactive substances. However, in many cases the compensation of the pathological conditions can be attained only after formation of synaptic connections between the neurotransplant and recipient brain neurons. On the other hand, these functional interactions can be impeded by the formation of a glial cicatrix around the neurotransplant preventing the growth of nerve processes. Nerve tissue injury is usually associated with active proliferation of astrocytes, hypertrophy of their axons, and a drastic increase in the number of intermediate gliofilaments. Under these conditions, reactive astrocytes produce factors inhibiting axonal growth and release neurotoxic products causing neuronal death [6,12]. However, experimental studies of the spinal cord recovery after transection have shown positive effects of some glial cell types. Transplantation of specialized olfactory ensheathing cells to the focus of the cortico-spinal injury reduced the glial cicatrix and promoted axonal growth [11].

Here we studied the possibility of neuronal axon penetration through the neurotransplant/brain interface and the contribution of glial cell to this process under conditions of intraneocortical transplantation. The study was carried out on heterotopical neurotransplants of the hippocampal dentate fascia developing in the somatosensory region of the neocortex. The use of the hippocampal dentate fascia as the donor structure was explained by the possibility of identification of the granular neuron axons (mossy fibers) and their giant synaptic terminals at the ultrastructural level without special label by their unique morphological characteristics [1]. One more reason for the choice of this experimental model was that the hippocampus is among the first structures damaged in neurodegenerative diseases. In addition, mossy fibers in temporal epilepsy are subjected to significant reorganization, synaptically interact with atypical targets, and form pathological neuronal chains [14]. We have previously shown on

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similar heterotopical dentate fascia neurotransplants that transplanted neurons formed aberrant synaptic reactions with the recipient brain neurons, with which they normally did not interact [1-3]. It remains unknown how the granular cell axons cross the interface between the neurotransplants and damaged brain. We carried out a detailed electron microscopic analysis of the cellular organization of the neurotransplant/brain interface. The knowledge of specific interactions between the dentate fascia mossy fibers and the glia axons was expected to provide new data essential for the development of methods for improving the regenerative potential of the brain by means of neurotransplants.

MATERIALS AND METHODS

The study was carried out on Wistar rats in accordance with the regulations on humane attitude to animals (GOST R ISO 10993-2–2009, Part 2). All manipulations were carried out under total Nembutal narcosis with local anesthesia. Primordial dentate fascia from 20-day rat fetuses served as donor tissue. The recipients were 7 adult male rats of the same strain. The injury was created in the somatosensory neocortex by sucking off a small volume of tissue with a vacuum pump, after which the donor material (0.3-0.5 mm^3) isolated under the microscope was inserted there. The neurotransplants developed for 9 months, after which they were fixed by perfusion with 2.5% glutaraldehyde through the left heart ventricle. Fragments containing the neurotransplant and the adjacent brain were isolated from the brain, postfixed with 1% OsO_4, and processed by the standard methods for electron microscopy. A fragment with clearly seen interface between the neurotransplant and brain was chosen in semithin sections for ultramicrotomy. Ultrathin sections were examined under an electron microscope. The method of transplantation and processing of the material was described in detail previously [1-3].

RESULTS

The neurotransplants were found in all operated animals. They grew in size from the moment of implantation and completely filled the artificially created cavity in the neocortex. The majority of granular neurons in the neurotransplants formed a cell layer, often looking fragmented in histological sections. The neurons in the layer were densely packed and had typical ultrastructural characteristics of granular cells: large nucleus, narrow rim of the cytoplasm, and diffusely distributed cell organelles. In addition, granular neurons were diffusely distributed in some regions of the neurotransplants. Generally, the transplanted tissue (except the granular layer) looked clearer under the light microscope than the adjacent brain, because of lesser density of the nerve and glial cells. No signs of cell destruction were detected. The interface between the neurotransplant and neocortex was clearly seen on the side of the pial vascular network, where large blood vessels grew into the gap between the neurotransplant and the neocortex. The vessels had wide adventitial membranes formed by pericytes, fibrous astrocytes, and numerous interwoven axons with rows of basal membranes between them. The interface zone between the neurotransplant and brain tissues could not be discerned in sites where the recipient and donor tissues were maximally close to each other. The brain and neurotransplant tissues in these sites sometimes could be differentiated only due to strict orderly orientation of the apical dendrites of the recipient neocortical pyramidal neurons. The length of these sites varied in different neurotransplants, reaching about 30% (according to visual estimation).

A significant part of the interface was determined by the higher or lesser concentration of linearly located glial cells. Transplanted tissue by the interface was a typical neuropil consisting of myelinated and unmyelinated axons, dendrites, synaptic terminals, and astrocyte processes. The marginal zone, directly adjacent to the recipient neocortex, was lined with glial cells and their processes (Fig. 1, *a*). The majority of gliocytes were referred to protoplasmic astrocytes with just few gliofilaments. The recipient brain surface damaged during the surgery was also outlined with the astrocytic glia processes in sites of contacts with the neurotransplants, many of these astrocytes containing much more gliofilaments that these cells on the side of transplanted tissue, and were identified as fibrous (Fig. 1, *b*). The adjacent neurotransplant/brain tissues were separated by just fine astrocyte processes in the greater part of the interface. Glial cells characterized by high electron density at the expense of granular filamentous matrix and compact location of the organelles were clearly seen against the background of astrocytes with clear cytoplasm and their processes. These glial cells were elongated, with eccentric nuclei and long cytoplasmic processes stretched along the marginal zone of the neurotransplant (Fig. 1, *a*). The width of these nonbranched processes with smooth contours was comparable to the size of transverse sections through the gliocyte soma. These cells were scattered along the neurotransplant border and formed no layer that could impede the nerve filament growth. The morphology of glial cells of this type resembled unipolar astrocyte precursors of the dentate fascia ventricular germinative zone, described previously; granular neurons migrated after their differentiation in the embryogenesis along these glial cell processes [13]. It seems that analogous precursors of the radial glia, initially present in

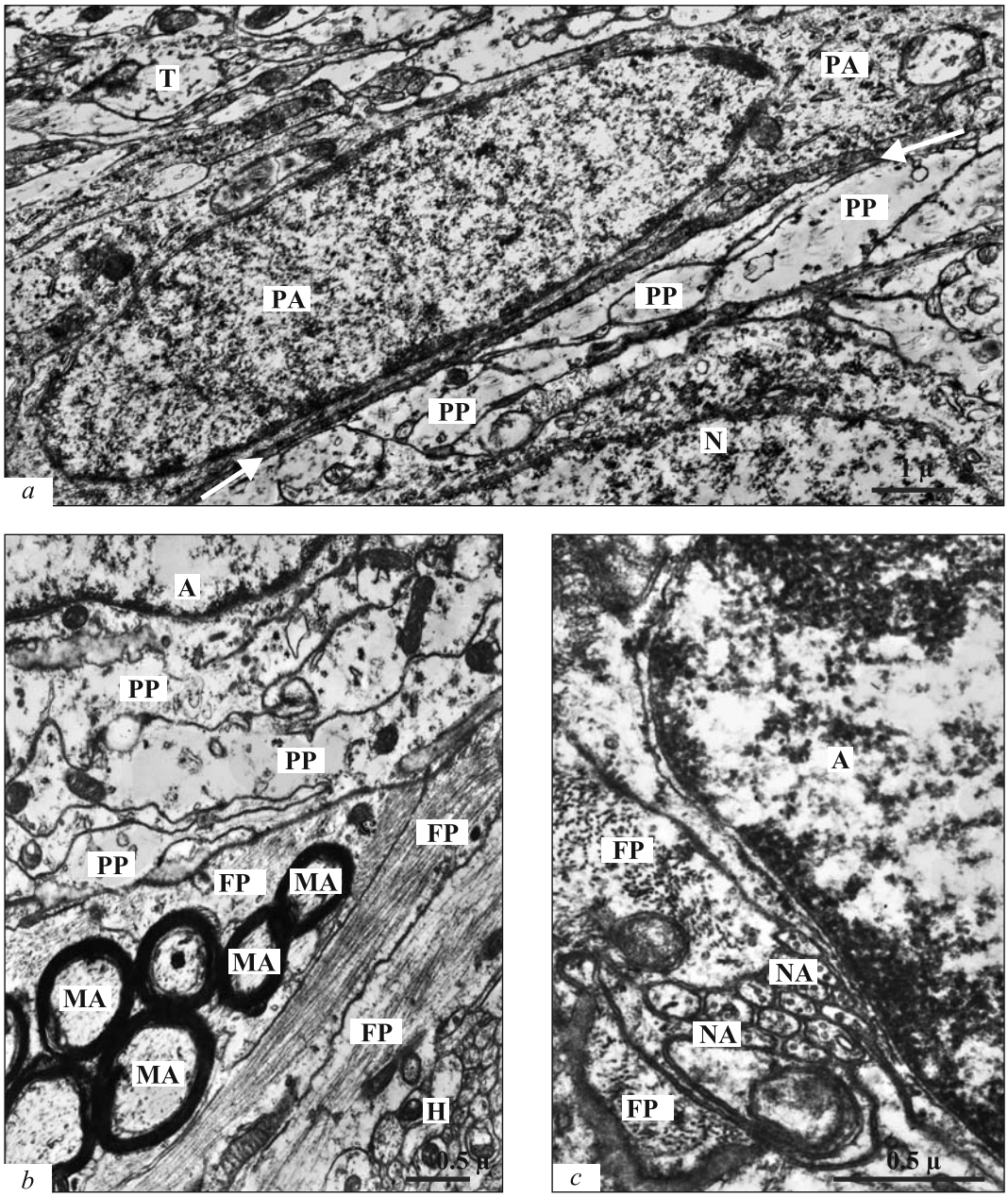

Fig. 1. Astrocyte subpopulation at the interface (arrows) between dentate fascia neurotransplant and recipient neocortex. *a*) Protoplasmic astrocyte processes and presumably progenitor astrocytic cell lining the neurotransplant and brain surfaces; *b*) bundle of myelinated axons, crossing the astrogliosis region in the interface; *c*) bundle of fine unmyelinated axons in the interface, consisting of accumulation of astrocytes and their processes. Here and in Fig. 2: NT: neurotransplant; N: neocortex; PA: progenitor astrocyte; A: astrocyte; PP: protoplasmic astrocyte processes; FP: fibrous astrocyte processes; MA: myelinated axons; NA: unmyelinated axons.

the donor tissue fragment, remained undifferentiated after transplantation and contributed to the damage repair. Previously we had noted that some signs of juvenility were retained in the neurotransplants under conditions of common high maturity of the nerve and glia elements. For example, they contained immature oligodendrocyte forms, axons with loose myelin membranes, synaptic contacts during the development stage [2,3]. Moreover, radial glial cells were present in the dentate fascia throughout life, and neurogenesis from one's own neural precursors was in progress [4,7].

Significant concentration of the glia elements was noted in the interface, where gaps between the donor tissue fragment and recipient brain formed initially after transplantation. Entanglement of gliocytes and their processes promoted close anatomical contacts between these tissues. Subpopulations of protoplasmic and fibrous astrocytes were clearly differentiated by

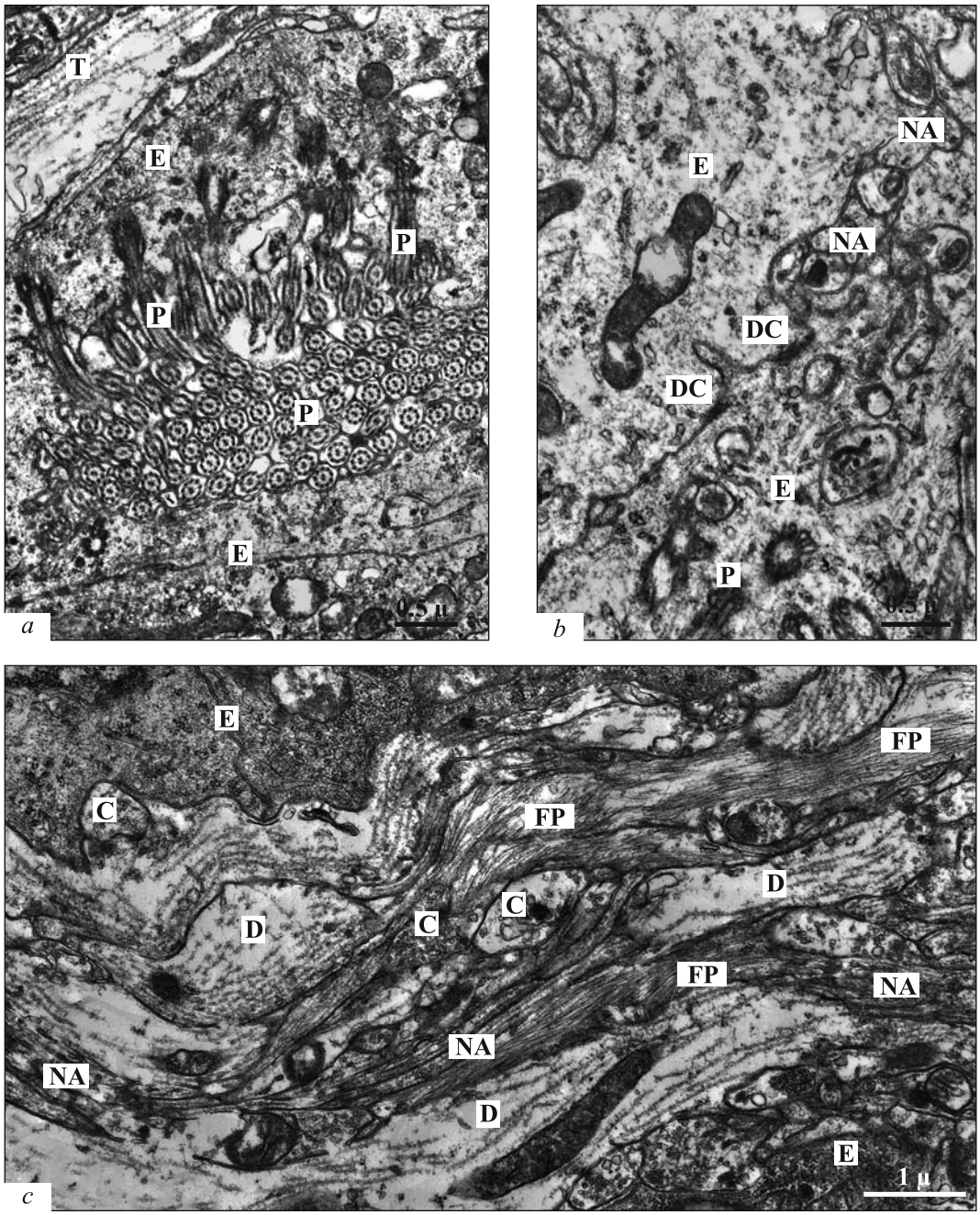

Fig. 2. Ependymal cells in neurotransplant/brain interface. *a*) Clia filling cell–cell space between ependymocytes; *b*) unmyelinated axons penetrating in narrow fissures between closely connected ependymocytes; *c*) potent axodendritic bundle accompanied by fibrous astrocyte processes, perforating the ependymocyte layer in the interface. E: ependymocytes; C: cilia; D: dendrites. DC: desmosome-like contacts.

the content of gliofilaments (Fig. 1, *b*). Astrogliosis, the typical reaction of glial cells to CNS injury, was usually attributed to its inhibitory effect on the regeneratory processes [6]. In our material, bundles of nerve axons perforated the interface even in the most compactly organized astroglia sites, where gliocytes looked hypertrophic and their neighboring processes were fixed by solid contacts. Transit axonal bundles were surrounded by fibrous astrocyte processes, the orientation of filaments in them corresponding to the direction of axon growth. Nerve bundles of 10-15 myelinated axons and fibrous astroglia processes resembled the neocortical axonal fascicles (Fig. 1, *b*). Groups of fine unmyelinated axons were morphologically identical to mossy fibers – dentate fascia granular neuron axons (Fig. 1, *c*). These observations indicated that astrocytosis at the interface between the dentate fascia neurotransplants and the neocortex was not the

typical "glia cicatrix" and did not prevent the establishment of reciprocal functional interactions between them. The formation of similar permeable astrocytic complexes in the damaged spine after transplantation of the glia precursors was described previously [8,9].

The neurotransplant/brain interface consisted in some sites of ependymal cells. Their presence was explained by the presence of ependymocyte precursors in tissue specimens used for transplantation. In a previous study on intraocular neurotransplants of the hippocampus we showed that ependymal cells actively proliferated in their marginal zone facing the anterior chamber of the eye [2]. Ependymocytes were also clearly identified on the surface of the dentate fascia intraneocortical neurotransplants. They looked much darker than the adjacent astrocytes at the expense of their numerous organelles and filamentous matrix in the cytoplasm; separate microvilli were detected in their free surface. The cilia, an important morphological criterion for ependymocyte identification, were so numerous that they completely filled the cell–cell spaces in some places. The profiles of crossed cilia and their basal bodies were also seen in the cytoplasm (Fig. 2, *a*). So active ciliogenesis was characteristic of ependymal cells during ontogenesis [5], while abundant cilia under conditions of transplantation seemed to be a sign of their incomplete differentiation. Accumulation of immature ependymocytes at the neurotransplant interface formed solid cell layers, in which the adjacent cells were fixed by cytoplasmic processes and desmosome-like contacts. Despite so solid cell construction, nerve fibers crossing the ependymal interface were often found inside it (Fig. 2, *b*). Small groups of thin unmyelinated axons passed through narrow fissures between the cells, while myelinated axons penetrated through wider cell–cell spaces. Ruptures emerged in some sites of the ependymal interface site, and potent axodendritic bundles, accompanied by the glial cell filamentous processes, penetrated through these ruptures. Axons and dendrites formed synaptic contacts inside these transit bundles (Fig. 2, *c*). In transverse sections, they looked like neuropil islets in the depth of the interface. In the early postnatal brain astrocyte processes served as "direction guides" for growing blood vessels [10]. One more function of astrocytes – organization of transitory nerve pathways between the neurotransplant and brain – was observed under conditions of transplantation. This function of reactive astrocytes was confirmed by published data on their chemical markers common with the radial glia [11,12].

Ultrastructural analysis of the dentate fascia neurotransplants showed that, despite their long life and heterotopic position in the brain, no morphological signs of inflammation or destruction were detected in the adjacent area. Areas of complete anatomical union of the neurotransplant and the neocortical somatosensory area were seen in the interface between these tissues; there were also sites separated by solitary glial cells or layers thereof. Ultrastructural characteristics of the interface gliocytes indicated their phenotypical and functional heterogeneity. Use of the primordial dentate fascia, adjacent to the surface of the anterior cerebral vesicles of the embryonic brain, for transplantation made it possible to trace the fate of ventricular zone cells. Cells differentiated from ependymal glioblasts were involved in organization of the glial environment of the neurotransplants. It somewhat limited, but did not completely prevent the functional integration of the neurotransplants with the brain. Importantly, some gliocytes in the interface had morphological signs of incomplete maturity. Fibrous gliocyte axons, directing the axodendritic projections through the interface, resembled the radial glia processes by topography and presence of intermediate filaments. The interface was organized by gliocyte precursors from transplanted tissue and by astrocytes from damaged mature brain. The demarcation zone was not a physical barrier for nerve filaments growing between the neurotransplant and brain even in sites of active astro- and ependymogliosis. These data suggested a new view on the phenotypical plasticity of astrocyte-like gliocytes and their role in the creation of a permeable tissue substrate for transition of axonal and dendritic processes.

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