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Structural Signs of Dynamic State of Synaptic Contacts between Neurotransplant and Brain

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We studied ultrastructure of synaptic connections between long-living dentate fascia transplants and somatosensory area of the neocortex in rats. Giant synaptic terminals of granular neurons upon contact with unusual neuronal targets in the neocortex reproduced their specific constitutive morphological features. At the same time, developing synapses with signs of active structural and metabolic reorganization were revealed. This is indicative of a dynamic state and instability of functional connections between the transplant and brain despite long time after transplantation.

Key Words: *neurotransplants; ultrastructure; giant synapses; dentate fascia; neocortex*

The efficiency of transplantation of neural progenitor cells for restoring the damaged brain largely depends on the formation of functional connections between the transplant and recipient's brain. The transplanted neural progenitors after differentiation form synaptic connections with not only appropriate cell targets, but also to irrelevant neuronal elements [7,15]. Our previous experiments with heterotypic transplantation of the fetal hippocampal dentate gyrus to the rat neocortex have demonstrated that granular neurons of the transplants can form synaptic contacts with neurons of the somatosensory area, although normally these structures neither anatomically, nor functionally interact *in situ*. The process of mutual adaptation of synaptic partners involves neuropeptide co-transmitters and cell adhesion molecules [1,2].

Here we analyzed stability of aberrant synaptic connections between the transplant and the brain in long-living neocortical transplants of the dentate fascia.

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MATERIALS AND METHODS

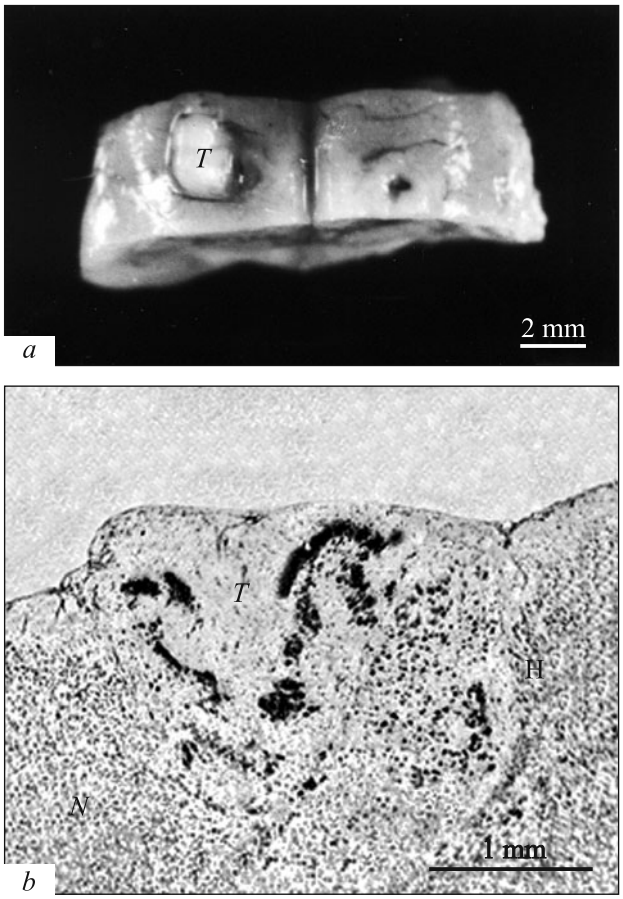
Experiments were carried out on Wistar rats with strict adherence to principles of humane handling of animals. The dentate gyrus anlage from 19-day-old fetuses (0.5 mm^2) was used as donor material. Allotransplantation into the oblique cavity in the somatosensory area of the neocortex was performed in adult males (n=7). All procedures were carried out under Nembutal anesthesia. Ten months after surgery, the transplants were analyzed under an electron microscope. Fixation was carried out by perfusion with glutaraldehyde into the ascending aorta. Brain sections (500 μ) containing the transplant were postfixed with OsO_4, processed routinely, and embedded in epon [1,15]. The area for ultramicroscopy was chosen in semithin sections stained with methylene blue and borax. Contrasted ultrathin sections were studied under a JEM-100B electron microscope. The neocortex adjacent to the transplant was examined in order to detect "chimeric" giant synaptic terminals formed by axons of the dentate fascia granular neurons growing from the transplant with neural elements of the neocortex. Synaptic terminals

were identified by the following specified features: large presynaptic terminals (up to 4-5 μ), numerous intraterminal asymmetric active zones with dendritic spines and batches of symmetric adhesive connections with the trunk of the same dendrite [1,2,15].

RESULTS

Visual and light-microscopic analysis revealed transplants in all operated animals. In the majority of cases, they filled the whole cavity space and sometimes were rising above the surface of the recipient brain. The areas occupied by the transplanted tissue and neocortex with clear-cut interface were seen in histological sections (Fig. 1, *a*, *b*). Prevalent cell elements consisted of typical for dentate fascia granular neurons, most of them participated in cell layer formation. They reproduced their cytological properties: a large nucleus and a thin cytoplasm ring with diffusely distributed small groups of tigroid substance (Fig. 1, *c*). There were no significant degenerative changes in the transplanted tissue. Synaptic terminals forming functional contacts with neocortex cell elements were searched in areas with clear-cut interface between the transplant and the brain. Giant (up to 4-6 μ in diameter) synaptic terminals of axons growing from the transplant could be easily recognized among relatively small (0.5-0.8 μ) synaptic profiles of the neocortex. Aberrant terminals were often grouped in clusters and formed two types of cell contacts typical of normal mossy fiber system: synaptically active zones and desmosome-like adhesive connections. Adhesive contacts were clearly seen in areas where axonal buds contacted with dendrite surface. Synaptic active zones were observed mainly on dendrite spines invaginated into terminals and very rarely on membranes of dendrites themselves. The terminals was filled with synaptic vesicles concentrated in the area of active zones (Fig. 2, *a*, *c*; Fig. 3). In general, giant terminal forming functional contacts with foreign neuronal targets in recipient's neocortex reproduced their constitutive ultrastructural properties.

However, detailed investigation of ectopic terminals revealed signs of ongoing development or degradation of individual sub-synaptic organelles in some of them. So, groups of growth vesicles, tubular structures and polymorph vacuoles were present in some terminals. Moreover, large vesicles (80 nm in diameter) with electron-dense content and spicular surrounding were found in them. They looked like small gear wheels in ultrathin sections (Fig. 2, *a*, *b*). Vesicles with spicu-



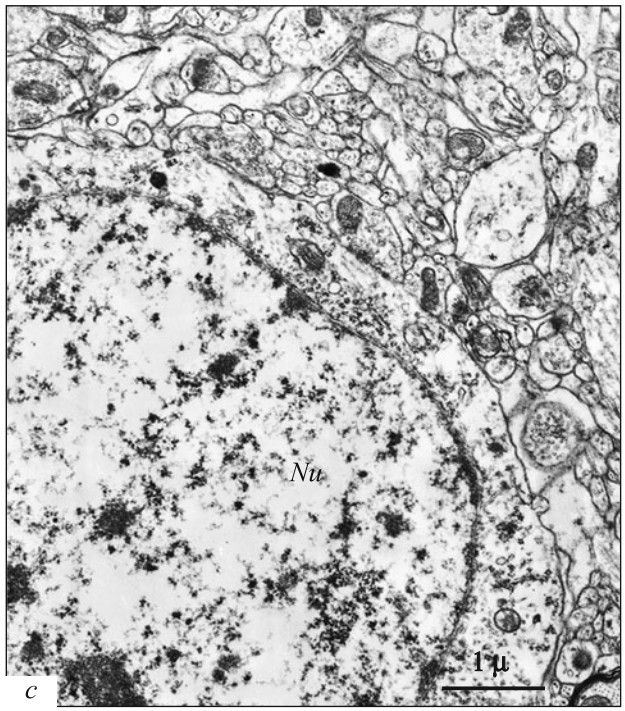


Fig. 1. Dentate fascia transplants developing in the somatosensory area of rat neocortex during 10 months. *a*) Fragment of the recipient's brain with neurotransplant, *b*) histological section of the transplant (Nissl staining), *c*) ultrastructure of a granular neuron and a neuropil in the transplant. *T*: transplant, *N*: recipient neocortex, *Nu*: nucleus of granular neuron.

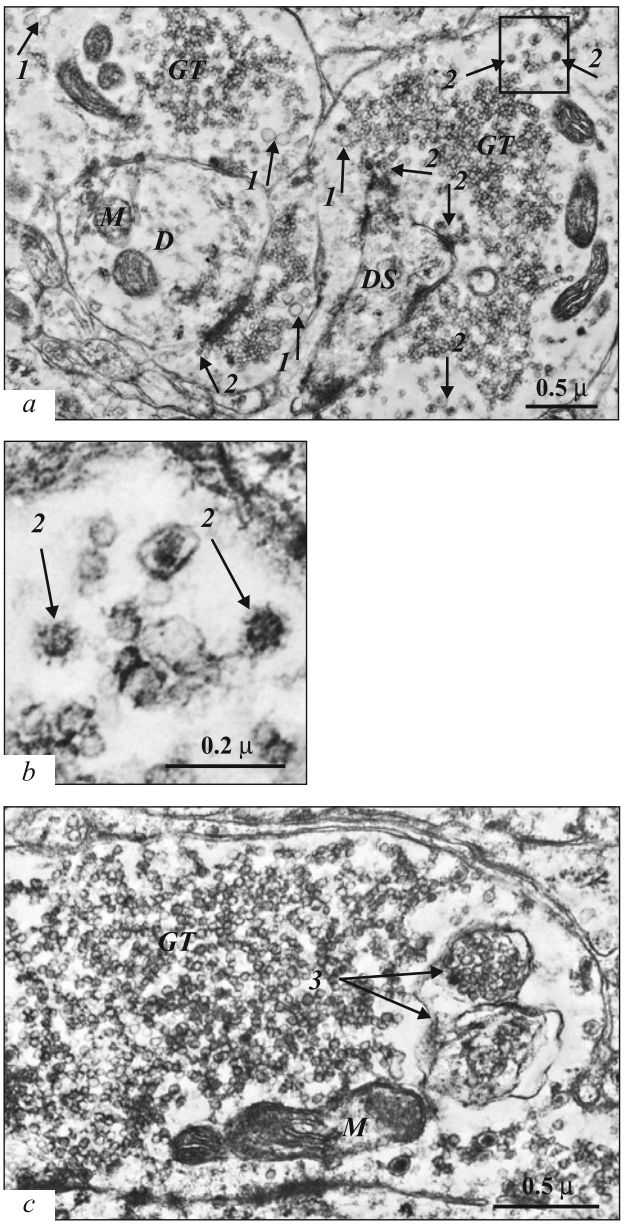


Fig. 2. Ultrastructural signs of ongoing development (*a, b*) and degradation (*c*) of organelles in presynaptic regions of ectopic giant synapses of granular neurons. *b)* Area in fragment *a* marked with a rectangular box is shown at high magnification. *1*) Growth vesicles; *2*) large vesicles with spicular denticular surface; *3*) autophagosomes. Here and in Fig. 3: *GT*: giant terminal profiles filled with synaptic vesicles form synaptic active zones; *M:* mitochondria; *D*: dendrite of a recipient neocortical neuron; *DS*: dendritic spine invaginated into a terminal.

lar surrounding were arranged singly or were part of small conglomerates together with smoothly outlined membrane structures. Such heterovesicular aggregates were found near the active zones as well as on the fringes of terminals. Immunochemical studies, carried out by other authors, have established that subcellular organelles in the form of vesicles with needle-like denticulate surface by themselves or along with other vesicles are macromolecular packages of presynaptic proteins, which are transported from pericaryons and stored for new active zone construction. Each transport package is a unitary element and contains a set of proteins, needed for active zone organization, including cytomatrix molecules, exocytosis system and voltage-dependent calcium channels [9,12,13]. At the same time, some ectopic giant terminals contained autophagosomes with sequestrated synaptic vesicles and organelle remnants (Fig. 2, *c*). Occasionally terminal profiles also contained lysosome-like dense bodies. Normally, damaged organelles and waste proteins are permanently destructed in cells for the maintenance of structural and chemical homeostasis. This process is activated by stress, inflammation, and neurodegenerative diseases and at initial stages plays a protective role [3,8]. Under conditions of transplantation, the appearance of destructed organelles can reflect adaptation of mossy fibers to foreign microenvironment of mature recipient's brain.

Postsynaptic elements of "chimeric" giant synapses were represented by dendrites and their spines in the recipient neocortex. Most of them had a cytoplasm rich in organelles. Numerous ribosomes and polysomes, organelles, responsible for metabolite synthesis, were seen; mitochondria, endosomes, tubular and polymorph vacuoles were also present (Fig. 2, *a*; Fig. 3, *a*). Similar to presynaptic terminals, lysosome-like bodies were rare. Enhanced local protein synthesis in postsynaptic compartments upon synaptogenesis and potentiation was described not once; it has been established that newly synthesized proteins undergo post-translational modification and control receptor traffic and synaptic transduction [5,6,10]. Structural signs of metabolic reorganization in our material were not exclusively confined to synaptic apparatus, they were also revealed in areas of dendritic trunks, where terminals of growing mossy fibers formed desmosome-like adhesive contacts. Unusual agglomerations of cell adhesion molecules in the perimembrane zones of the neocortical dendrites indicate that their synthesis was induced by mossy fibers. Agglomerations in dendrites were arranged strictly symmetrically to aggregations in the presynaptic terminal (Fig. 3, *b*). The neighboring dendroplasm always contained cisterns of the endoplasmic reticulum opening their channels towards the desmosome-like complexes and partially covered by ribosomes. Some subsurface cisternas resembled the spine apparatus, they were smoothly outlined and located in sites of dendritic spine branching. On the presynaptic side, mitochondria were concentrated near the adhesive connection, which attests to energy dependence and coordination of these transmembrane communications between the presynaptic and postsynaptic compartments. Due to formation of

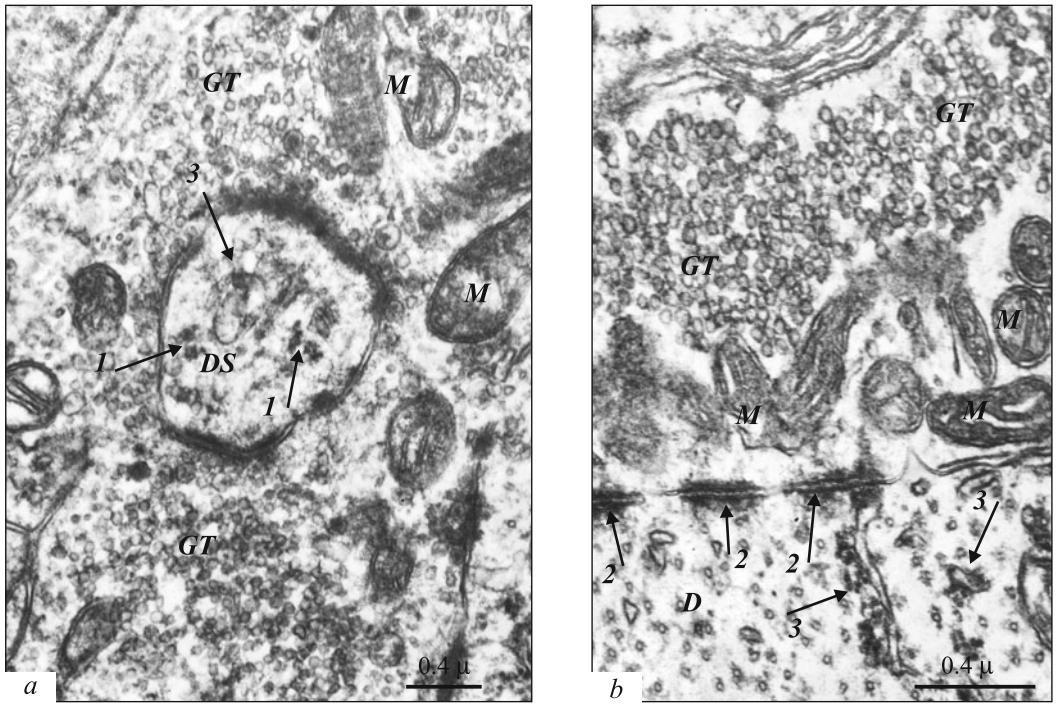


Fig. 3. Ultrastructural signs of structural reorganization in postsynaptic compartments of ectopic giant synapses. *a*) In a dendritic spine; *b)* in a large dendrite. *1*) Groups of polysomes; *2*) symmetric adhesive connections between synaptic terminal and dendritic surface, *3*) cisterns of endoplasmic reticulum.

symmetric desmosome-like connections, the ectopic synaptic terminals acquired an important specific ultrastructural feature of giant synapses *in situ – puncta adhaerentia* contacts. Our previous comparative quantitative analysis indicates that the number and length of desmosome-like complexes in ectopic terminals is sufficiently higher than in control ones [2]. It is also known that adhesive contacts to dendrites are differentiated during ontogeny earlier than active zones, being their precursors [4].

Thus, electron microscopy findings suggest that in case of integration of transplanted neurons with recipient's brain cells many functional contacts possess signs of ongoing development or structural and chemical modeling. It is of particular importance that organization-reorganization events in aberrant connections persist long after transplantation. This is probably due to exceptional plasticity of the brain structure, selected for transplantation: granular neurons of the dentate gyrus are being generated and incorporated into the neuronal networks of adult brain throughout life [11]. Moreover, mossy fibers possess high capacity for the formation of ectopic collaterals, which can be the basis for pathological neuronal networks upon temporal lobe epilepsy [14]. The degree of stability of aberrant synaptic connections upon transplantation of other brain areas has not been investigated. The data on dynamic and non-stable nature of functional connections between the dentate gyrus transplants and neocortex, obtained in this study, should be taken into account in therapeutic use of neurotransplantation.

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