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Microscopic Study of Nervous System Plasticity: Interactions of Sympathetic Nerves with Neurons of Intraocular Hippocampal Transplants

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Functional interactions of sympathetic fibers innervating the iris with the neurons of central origin in intraocular transplants of the rat hippocampus were studied by optic, confocal, and electron microscopy. After formaldehyde fixation, fluorescent dye DiI was applied to the upper cervical ganglion; the dye migrated to the transplant by lateral diffusion via axons. Sympathetic nerves labeled with fluorescent dye grew into the neurotransplants along perivascular membranes of blood vessels. In addition, some fluorescent axons were identified in the transplant parenchyma. Electron microscopy showed large bundles of the peripheral type axons in the vascular adventitia and Schwann-axonal complexes in the transplant neuropil. Autonomic axons formed synaptic contacts with transplanted neurons.

Key Words: *intraocular neurotransplants; hippocampus; autonomic sympathetic nervous system; synaptic contacts*

One of the most important characteristics of the brain is its morphofunctional plasticity, due to which it readily adapts to changes in the tissue microenvironment and in the efficiency of neuronal activity. Restructuring is a constant process running in the brain, and even new neurons emerge. Plastic changes in the receptive fields, afferent-efferent relations, and neuronal ensembles are traced in health and disease. The data on the role of multiple forms of the brain plasticity are analyzed in many specialized reviews [1,9,10]. However, plastic processes in response to contacts of the nervous tissue of the central and peripheral origin received little attention. Mutual replacement of the cells belonging to these two compartments of the nervous system is realized in vertebrates in sites where the cranial/spinal nerves enter the brain/spine or are released from it. Specific intermediate locuses develop in these sites, in which Schwann environment of the peripheral nerves is gradually replaced with oligodendrocytic environment, and *vice versa* [4]. We have shown previously that intraocular neurotransplants can serve as a convenient experimental model for studies of interactions between the peripheral and central compartments of the nervous system, as the transplanted tissue contacts with the iris and is supplied with blood through it [2,3,12]. The iris is innervated with three types of the peripheral nerve fibers: sympathetic adrenergic, parasympathetic cholinergic, and somatic sensitive fibers of the orbital nerve. All types of nerve fibers form vast perivascular networks in the iridal connective tissue stroma and compact plexuses near smooth muscles dilating or contracting the pupil [11]. These data suggest that peripheral nerves from the iris can penetrate into transplanted tissue.

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Here we studied the possibility of growth of the sympathetic nerve fibers from the upper cervical ganglion (UCG) into intraocular neurotransplants and interactions between these nerves and the transplanted hippocampal neurons with microscopic methods.

MATERIALS AND METHODS

The study was carried out on Wistar rats in accordance with regulations for humane handling of experimental animals (GOST R ISO 10993-2-2009). All manipulations were carried out under Nembutal (40 mg/kg intraperitoneally) or ether narcosis. Additional anesthesia with dicain (2-3 droplets) was carried out during transplantation into the anterior chamber of the eye (ACE).

Embryonic hippocampal primordium isolated from the brain of 18-19-day fetuses served as the material for transplantation. The recipients were adult male rats (n=10). Fragments of embryonic tissue (0.8-1.0 mm^3) were inserted into ACE with a special Microman pipette through an incision in the cornea. Transplanted tissue developed in ACE for 3 months. The animals were then separated into 2 groups. In one group (n=5), intraocular transplants together with the iris fixed to it were isolated and processed for histological studies of the neurotransplants organization with Nissl method and for electron microscopy of ultrastructural organization of the transplants. Processing of the material was described in detail previously [3,6,12]. The other group of animals (n=5) was used for evaluating the possibility of intraocular neurotransplant innervation with sympathetic nerves of the autonomic nervous system, penetrating into the transplant from the UCG through the iris. The animals were transcardially perfused with 4% paraformaldehyde in 0.1 M PBS (pH 7.3).

The UCG at the level of cervical vertebrae II-III from postmortem animals were prepared, a small incision in the ganglion was made, through which a crystal of carbocyanine lipophilic dye Dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) was inserted and fixed with a droplet of agar—agar. In fixed tissue the tracer dye Dil incorporated in cytoplasmic membranes and with lateral diffusion migrated, via axons, from the site of its insertion in the antegrade and retrograde directions [5]. The recipient animals were then decapitated, the heads were plunged *in toto* into the same fixative for additional fixation and stored in darkness till the study in a fluorescent microscope. The rat head was an anatomical preparation with UCG (into which carbocyanine tracer was inserted) and a sympathetic pathway with which, via axonal membranes, the dye gradually diffused to innervated targets. This preparation was stored in the fixative for 3 months. The hippocampal intraocular transplant with a fragment of the iris was then resected, sections (40-60 μ) were sliced on a freezing microtome, placed on slides in a droplet of PBS, and examined in a Leica TCS SP fluorescent confocal laser microscope at λ_{ex}=543 nm and λ_{em}=560 nm.

RESULTS

Visual analysis showed no signs of inflammation in ACE 3 months after transplantation in all recipients; round or elongated cellular formations, up to 4-5 mm

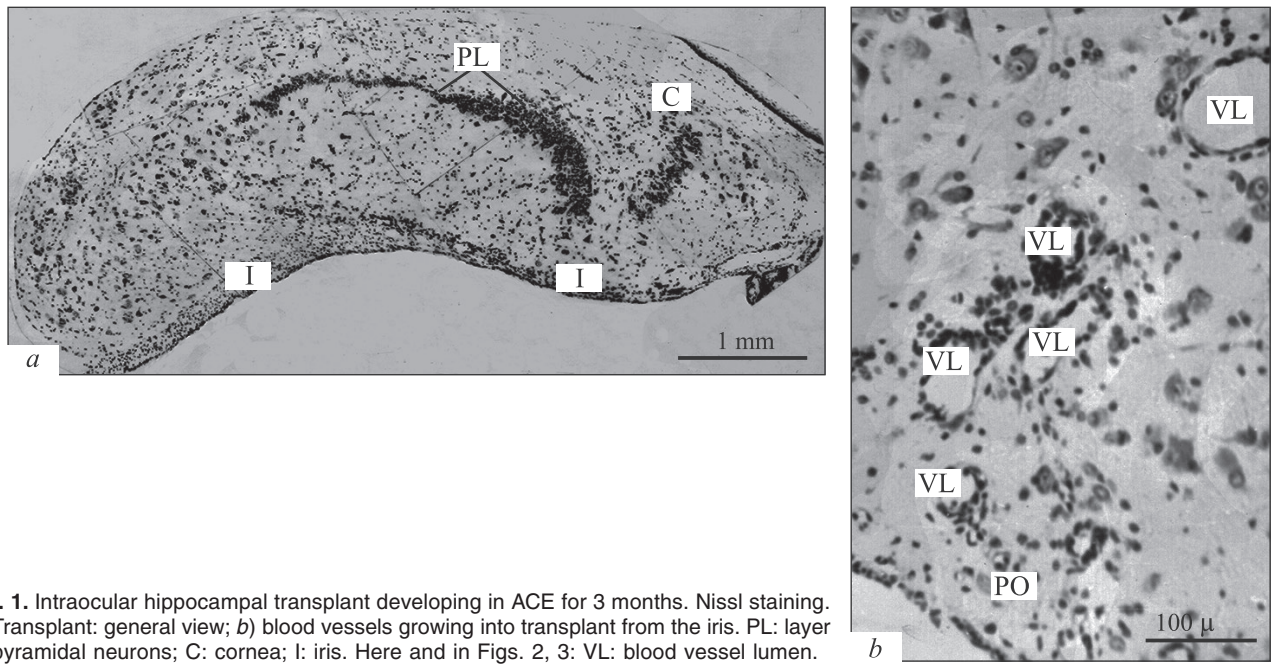

Fig. 1. Intraocular hippocampal transplant developing in ACE for 3 months. Nissl staining. *a*) Transplant: general view; *b*) blood vessels growing into transplant from the iris. PL: layer of pyramidal neurons; C: cornea; I: iris. Here and in Figs. 2, 3: VL: blood vessel lumen.

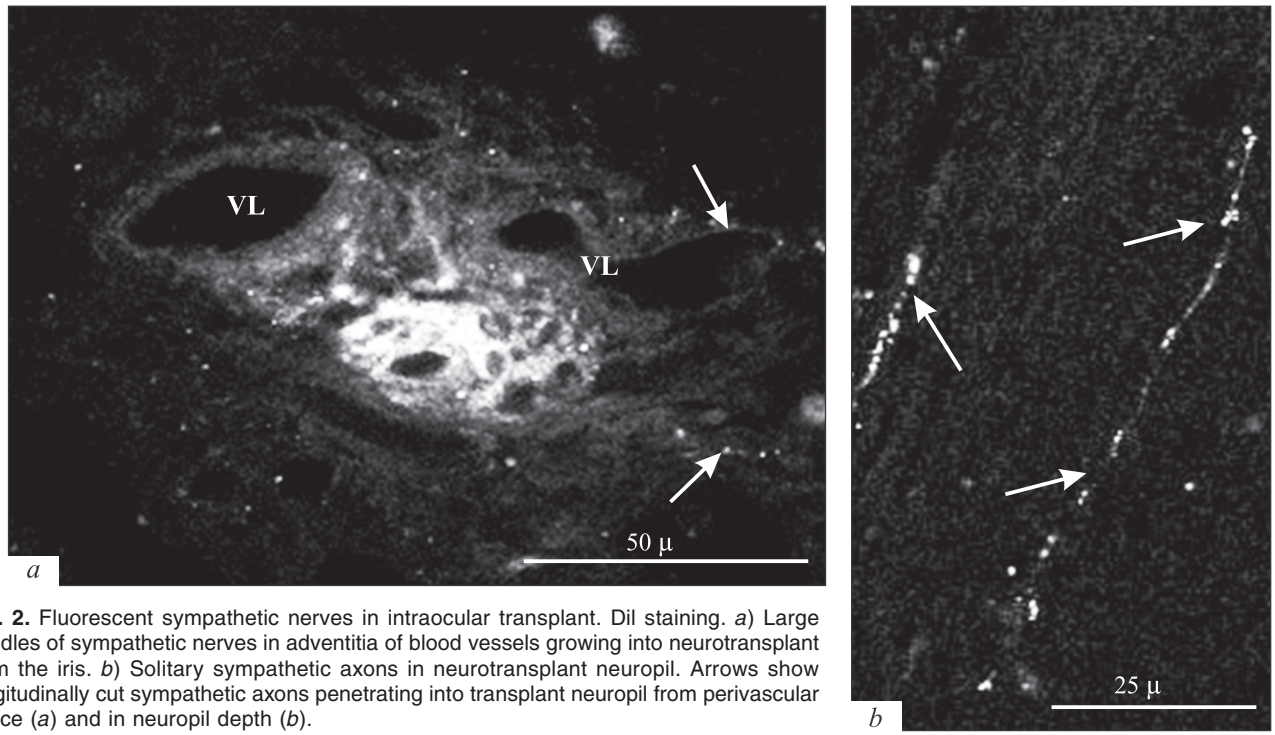

Fig. 2. Fluorescent sympathetic nerves in intraocular transplant. DiI staining. *a*) Large bundles of sympathetic nerves in adventitia of blood vessels growing into neurotransplant from the iris. *b*) Solitary sympathetic axons in neurotransplant neuropil. Arrows show longitudinally cut sympathetic axons penetrating into transplant neuropil from perivascular space (*a*) and in neuropil depth (*b*).

long, were seen through the cornea. In histological sections they were located between the cornea and iris and to a certain measure resembled the cytoarchitecture of the hippocampus *in situ* (Fig. 1, *a*). The cells in them were presented mainly with the pyramidal neurons, with apical dendrites directed towards the iris. Large blood vessels penetrated deep into the transplant and then ramified to form smaller branches (Fig. 1, *b*). We showed in a previous study that in the transplant parts close to the iris and containing a lesser number of neurons the blood vessels had wide perivascular walls, compact basal membranes, and perforated endothelium. As the vessels grew into parts with higher density of neurons, they became thinner and acquired morphologic signs typical of the cerebral capillaries with blood-brain barrier [2].

Fluorescent study of intraocular neurotransplants from rats with DiI tracer inserted into UCG showed that the tracer, migrating via axonal membranes for 3 months, reached the transplanted tissue. This was proven with orange-red fluorescence of wide perivascular spaces of blood vessels near the iris — the peripheral nerves grew into the transplant via these vessels. A series of microphotographs taken during

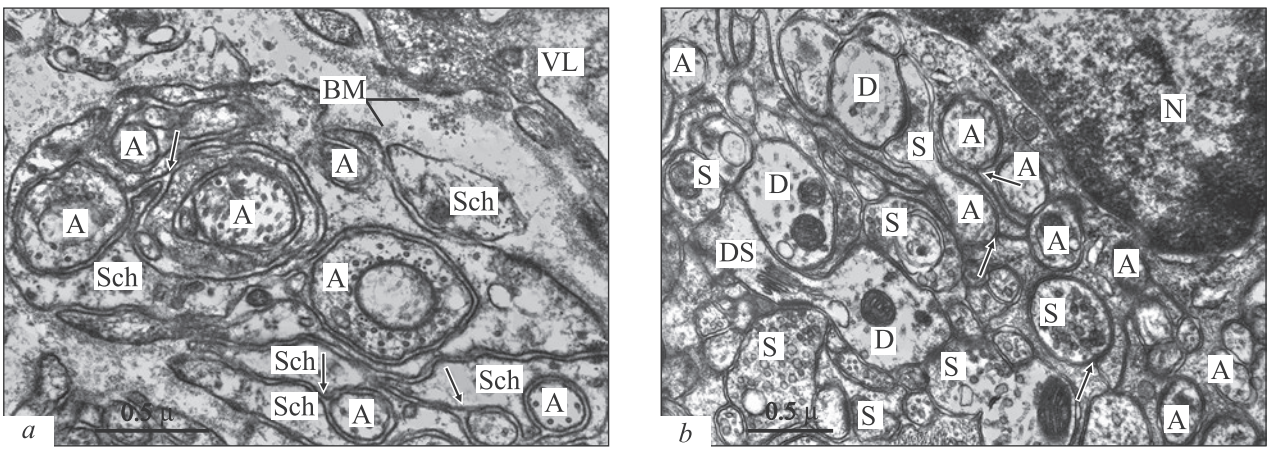

Fig. 3. Electron microscopic image of autonomic nervous system nerves in intraocular hippocampal transplant. *a*) Axon bundle in perivascular space of a peripheral blood vessel; each axon located in a bed from Schwann cell processes. *b*) Schwann-axonal complex in transplant neuropil; some peripheral axons form synaptic contacts with dendrites and dendritic spines of hippocampal neurons. N: Schwann cell nucleus; A: axon; BM: basal membrane; D: dendrite; DS: dendritic spine; S: synaptic terminal, Sch: Schwann cell processes; arrow shows mesaxon.

scanning in a confocal laser microscope showed the lumens of vessels of various sizes and intense fluorescence in their adventitia (Fig. 2, *a*). On the other hand, perivascular spaces of some blood vessels did not fluoresce at all or exhibited just slightly colored basal fluorescence with bright dots, presumably belonging to crossed sympathetic axons innervating the vascular wall muscles. The absence of fluorescence in part of perivascular spaces could be attributed to the fact that the iris was innervated with sympathetic nerves from UCG and with postganglionic fibers from other ganglia, which were not labeled in our experiments. Importantly, no stained nerve fibers projecting from the iris into neurotransplants were detected outside perivascular membranes. However, some longitudinally cut fragments of fine fluorescent axons were seen (rarely) in the transplant parenchyma close to the blood vessels growing into the transplant (Fig. 2, *b*). This fact indicated that some of peripheral nerve fibers were penetrated from perivascular membranes into the adjacent neuronal tissue, but their further destiny could be traced only at the electron microscopic level.

Ultrastructural study of neurotransplants showed large bundles of growing peripheral nerves. In addition to myelinated nerve processes, presumably belonging to somatic sensitive fibers (not studied within the framework of our research) and large groups of nonmyelinated axons of peripheral type were seen. The number of fibers forming a bundle varied from 4-5 to 20-30; the diameter of axial axonal cylinders was 0.15-0.50 μ. Each axon (sometimes 2-3 axons together) was located inside a groove formed with Schwann cell cytoplasmic processes. Mesaxons, typical of the peripheral nervous system nerves, formed in sites where the processes closed the grooves. The entire bundle was enveloped in the basal membrane and loose fibrous connective tissue of perivascular space (Fig. 3, *a*).

Schwann-axonal complexes were also found in the transplant parenchyma, as a rule, not far from capillaries. These complexes were not enveloped in basal membrane and completely integrated in the neuropil (Fig. 3, *b*). Some axons, remaining inside the glia groove, contained synaptic vesicles and, released from the glia membrane, formed synaptic contacts with the adjacent dendritic processes. In order to form functional bonds, axons sometimes created dilatations filled with synaptic vesicles (Fig. 3, *b*). In some cases the peripheral neurons contacted with dendritic spines, containing the spine system, characteristic of the hippocampal synapses in the brain *in situ*. These findings indicated that autonomic nerves, innervating the intraocular transplants, were subjected to plastic reorganization and formed synaptic interactions with the CNS neurons when getting into a different tissue microenvironment (hippocampal transplant). The ultrastructure of these contacts was characteristic of the CNS synapses. According to some reports, the UCG catecholaminergic autonomic neurons sometimes replaced their mediator nature with cholinergic one under the effect of the target [8]. In addition, growth of adrenergic sympathetic axons into the hippocampus increased with the death of cholinergic innervation [7], while hippocampal transplants developing in ACE outside the brain were devoid of their natural cholinergic outlet from the septal region. This fact seemed to promote their innervation with autonomic nerves of the autonomic nervous system.

Hence, microscopic study has shown that UCG sympathetic nerves innervated the intraocular hippocampal transplants. They grew via the perivascular spaces from the iris of the eye, penetrated into neuropil regions of the neurotransplants, and formed synaptic contacts with dendrites and dendritic spines of transplanted neurons. These data indicated the possibility of synaptic neuron reprogramming and high morphofunctional plasticity of the nervous system in general.

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