MORPHOLOGY AND PATHOMORPHOLOGY

Development of Neuronal Ganglion Xenografts from Gastropoda in Rat Brain

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Survival of neuronal ganglia from newborn snail (*Helix aspera L.*) in the brain of adult rats was studied. Snail ganglion survived in the brain of warm-blooded animals for 6 months without inducing immune conflict. At early stages (5 days) after transplantation, xenografts increased in size and were several times larger than native ganglia from 10-day-old snails, thereafter (on days 28 and 180) they became smaller still surpassing the sizes of ganglia from snail of the corresponding age. Rapid enlargement of the xenograft was due to cell reactive processes in the ganglion. Deep penetration of large vessels from xenografts to rat brain was observed.

Key Words: *xenogenic neurotransplantation; neuronal ganglia of Gastropoda class; rat brain; reactive processes*

Experiments on animals showed that allogenic (from animals of the same species) immature nervous tissue can survive and stimulate compensatory and recovery processes in the damaged or pathologically changed recipient brain [2,5,7]. Transplantation of fetal allogenic nervous tissue for the treatment of some neurological and neurodegenerative diseases is associated with ethic and technical problems. In light of this, substitution of aborted nervous tissue with xenogenic donor tissue became actual. The search is usually limited to testing donor-recipient pairs within mammals. Under conditions of immunosupression, xenogenic nervous tissue survives in intact and damaged brain and forms neuronal and humoral connections [1,12]. Xenografts are chemically active and can restore impaired behavior in the recipient [11,13]. However, immunosuppressive therapy impairs the immune system of recipients.

A new trend in neurotransplantology is the use of nervous tissue from invertebrates characterized by weak histocompatibility system as a donor material for transplantation to mammalian brain. This xenotransplantation can be successful without immunosupression. Russian scientists first showed that neuronal germ tissue from drosophila transplanted to the brain of laboratory recipient animals survived for 2 months. Being transplanted with fetal allogenic nervous tissue, it stimulated differentiation of neuroblasts, growth of neuronal processes from recipient tissue to the graft, and vascularization of donor and recipient tissues. Moreover, human fetal cells and drosophila embryonic cells can differentiate and form connections with each other and neurons of patients with Parkinson disease [3,8].

The present study is the second one in this field. For the first time neuronal ganglia from Gastropoda molluscs were used as donor tissue for transplantation to mammalian brain. We hypothesized that these grafts will longer survive in rat brain than drosophila grafts, because of significantly longer life-span of mollusc compared to drosophila (4-5 years *vs.* 1 month). Neurotransmitter specificity of individual neurons in va-

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rious Gastropoda ganglia is well studied. Moreover, ion compositions of the media for long-term culturing of dissociated mammalian and Gastropoda neurons are similar [4]. Therefore, the use of nervous tissue from Gastropoda for transplantation to mammalian brain seems to be promising.

We studied the development and state of ganglion xenografts from newborn snail (SGXG) in the brain of adult rats and cellular and vascular interactions between SGXG and recipient brain.

MATERIALS AND METHODS

Adult Wistar rats (n=23) weighing 250-300 g were used as recipients. Neuronal ganglia from 5-day-old snails (*Helix aspera L.*) were used as donor tissue. The ganglia (n=46) were isolated under a microscope and placed into Ringer solution. Some ganglia (n=20) were stained with fluorescent vital dyes, bisbenzimide and fast blue (Sigma), directly before transplantation [10]. Neurotransplantation included the following procedures: nembutal-anesthesized rats (30-40 mg/kg intraperitoneally) were fixed in a stereotaxis, the skin on the head was cut, and bilateral trepanation was performed (AP 2.0-2.5; L 2.0-3.0). Donor tissue in a small volume of nutrient medium was injected bilaterally with a glass needle connected to a syringe. The skin was sutured with surgical silk. All manipulations were performed under aseptic conditions.

Rats with SGXG were sacrificed with lethal nembutal dose 5 (n=2), 28 days (n=13), and 6 months (n=8) after surgery. Brain regions with SGXG were fixed in 10% paraformaldehyde on phosphate buffer (pH 7.2-7.4). Frontal serial sections (15-20 μ) were prepared on a freezing microtome and mounted on glass slides coated with 1% gelatine. Sections were stained with thionin and cresyl violet by the method of Nissl. Unstained sections with SGXG labeled by fluorescent dyes before transplantation were examined under a fluorescent microscope. Thereafter, the sections were stained according to Nissl for identification of fluorescing cells.

Cell composition of SGXG and adjacent tissues was examined using an Axioplan-2 light microscope (Zeiss). The lesioned area in brain hemispheres with SGXG (wound canal, lymphocyte infiltrates, reactive gliosis, and SGXG) was estimated using an Image-Pro Plus 3.0 image analysis system. This parameter was measured in 4 hemispheres with fluorescence-labeled SGXG and in 7 hemispheres with unlabeled SGXG 5 and 28 days after transplantation, respectively. Three brain sections with maximum tissue injury from each rat were evaluated.

The dynamics of ganglion growth in the recipient brain was evaluated by similar measurements of the

TABLE 1. Parameters of SGXG and Ganglia Isolated from *Helix aspersa L.* at Different Terms after Hatching ($M\pm m$)

Parameter	Time, days							
	5	10		33		180		
	ganglia for transplantation, n=25	ganglia, n=22	SGXG, n=4	ganglia, n=24	SGXG, n=7	ganglia, n=8	SGXG, n=8
Area, mm^2	0.09±0.01	0.11±0.01	1.57±0.21* (12)	0.14±0.01	0.43±0.04* (21)	0.20±0.03	0.58±0.05* (23)
Diameter, μ							
maximum	366.39±21.74	372.99±16.54	4269.09±469.73*	485.11±19.78	1342.53±77.43*	626.78±41.48	1363.91±74.91*
minimum	273.04±18.99	295.25±16.79	2188.85±265.53*	358.68±21.55	428.38±33.58	406.490±33.017	568.26±47.98

Note. In parentheses: number of SGXG sections, *p<0.0005 compared to ganglia of the corresponding age.

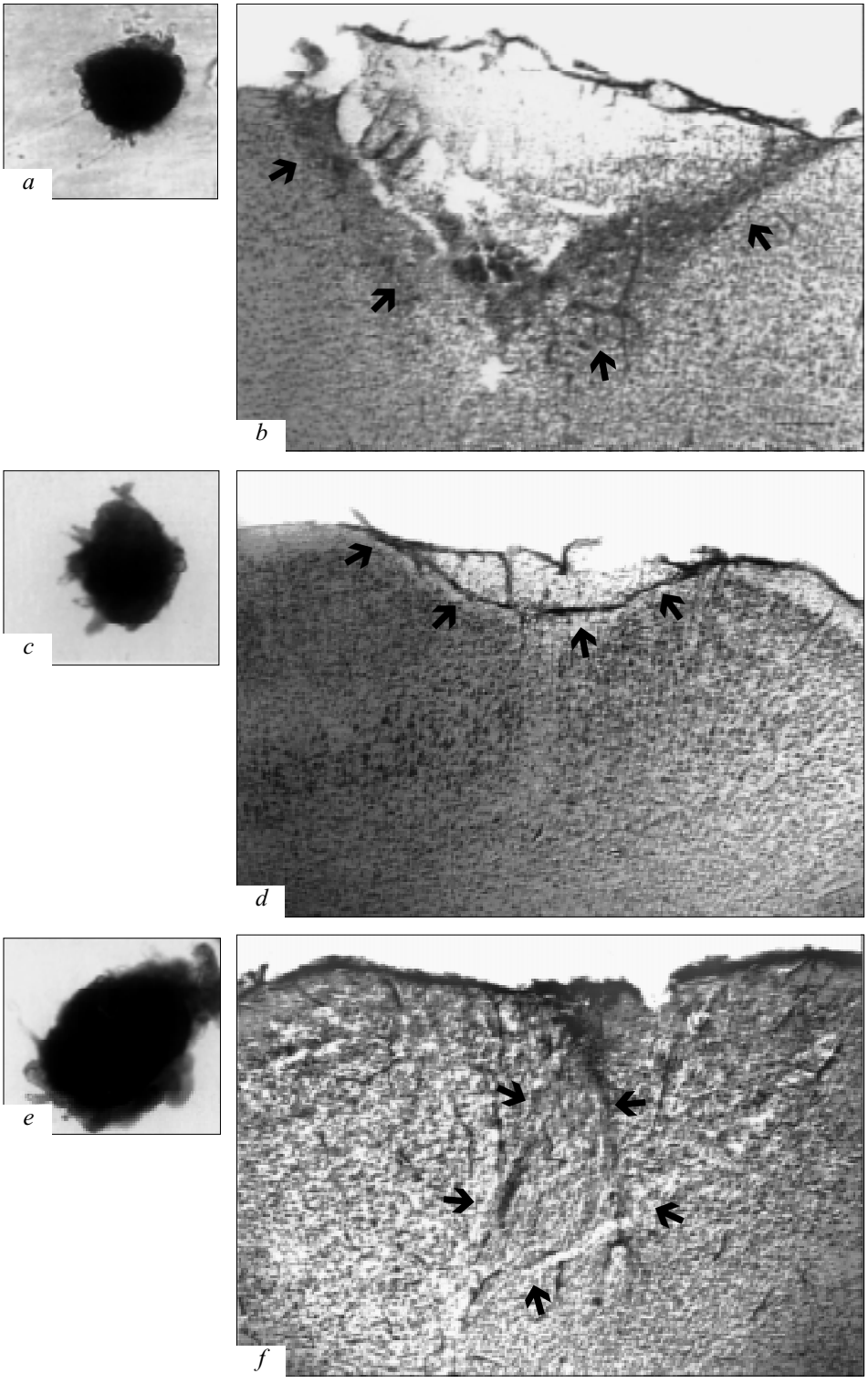

Fig. 1. Pedal ganglia isolated from *Helix aspersa L.* on days 10 (*a*), 33 (*c*), and 180 (*e*) of postnatal ontogeny and snail ganglion xenografts (SGXG) on days 5 (*b*) and 28 (*d*), and 6 months (*f*) after transplantation to rat brain. Arrows indicate SGXG borders. Nissl staining, ×50.

areas and maximum and minimum SGXG diameters (3 sections per SGXG) 5 (n=4), 28 days (n=7), and 6 months (n=8) after transplantation. These parameters were compared with maximum arears and diameters of the native ganglia isolated from 5-, 10-, 33-day-, and 6-month-old snails *Helix aspera L*.

Statistical analysis was performed by Student t test for independent variables using Statistica software.

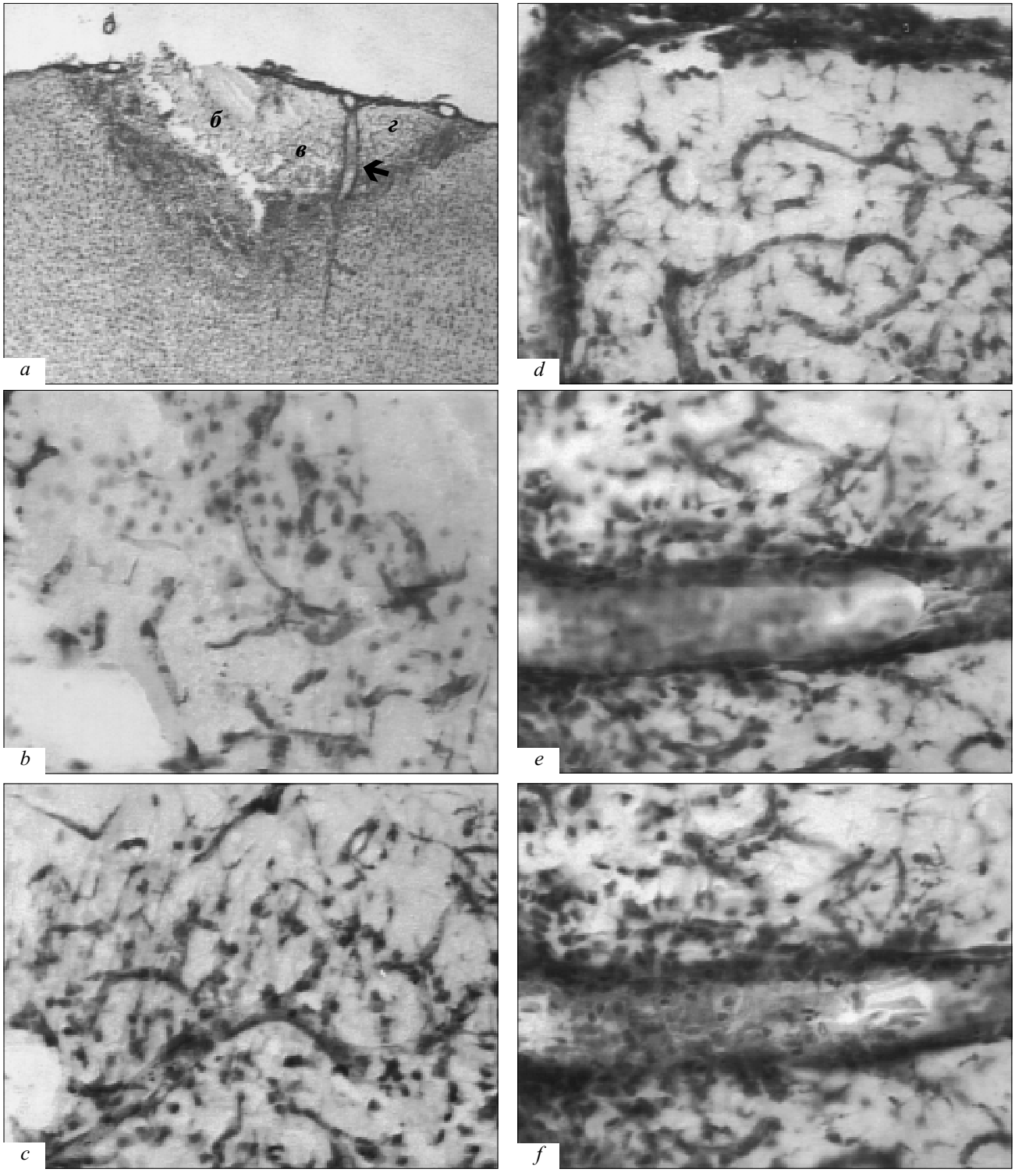

Fig. 2. Rat neocortex with snail ganglion (procerebrum) xenograft (SGXG) on day 5 after transplantation. Nissl staining, ×50 (*a*), ×400 (*b-f*). *a*) general view, *b-f*) SGXG fragments. The following components can be distinguished: round neuronal nuclei (*b*), hypertrophic elongated cell elements (*c*), growth of small vessels (*d*) near a large vessel penetrating into rat brain (indicated by arrow on fragment *a*), vessel wall (*e*) and blood cells in the vessel (*f*).

RESULTS

No SGXG rejection was observed 5 (Fig. 1, *b*; Fig. 2, *a*), 28 days (Fig. 1, *d*), and 6 months (Fig. 1, *f*) after transplantation and no reactive processes in the recipient brain were found. SGXG with multipolar neurons were found in 5 of 8 rats 6 months after transplantation (Fig. 1, *f*). SGXG were incorporated

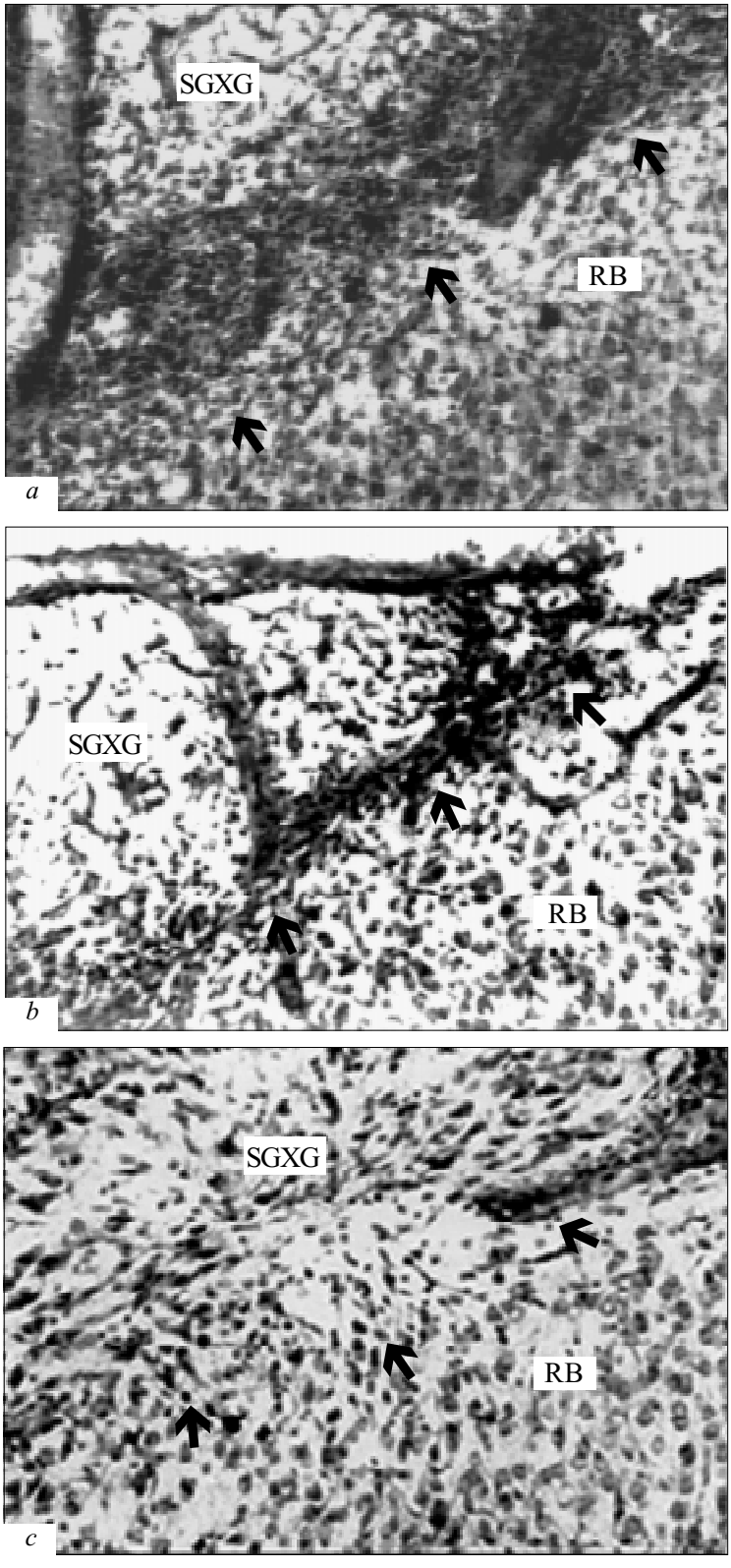

Fig. 3. Types of borders between snail ganglion xenografts (SGXG) and rat brain (RB). Nissl staining, ×200. *a*) 5 days after transplantation: wide sheath consisting of small cells (glia and lymphocytes) is seen on SGXG-RB border; *b*) 28 days after transplantation SGXG is surrounded by a thin membrane typical of ganglia; reactive processes are not pronounced. Scar-free zone between SGXG and RB 28 days after transplantation. Arrows indicate SGXG borders.

into the neocortex forming a single whole with the brain surface. In contrast to SGXG, xenografts of embryonic chick forebrain were rejected 5 days after transplantation and caused graft-versus host reaction leading to degradation of the recipient brain tissue [6].

Morphometric analysis showed that destructive processes in rat brain with SGXG were less extensive

(1.817±0.311 mm^2 on day 5 and 0.711±0.069 mm^2 on day 28, $p<0.0005$ and $p<0.0001$, respectively) than after xenotransplantation of chick embryonic nervous tissue (4.857±0.272 mm^2 on day 5 after transplantation) [6].

At early terms after transplantation, the ganglia enlarged in the recipient brain; then they decreased in size but were larger than native ganglia of the corresponding age (Table 1, Fig. 1).

Morphometric analysis showed that initial (within 5 days) dramatic enlargement of SGXG in the brain of warm-blooded recipients resulted from intensive reactive processes in the ganglia and along their borders with the brain (Fig. 2). Neurons with round nuclei could be revealed in SGXG (Fig. 2, *b*). Neurons were surrounded by numerous proliferating cells (round or elongated, Fig. 2, *c*) identified as connective tissue cells, lymphocytes, and glia [9,14]. Some areas contained numerous small vessels (Fig. 2, *d*) consisting of proliferating cells. Large vessels resembling mollusc lacunae growing from SGXG surface penetrated through the graft and recipient tissue (Fig. 2, *a, e, f*; Fig. 3, *a*) and sometimes reached the corpus callosum. The vessels contained densely packed large cells with nuclei (Fig. 2, *f*). SGXG were separated from the recipient brain tissue by a scar of consisting of glial and lymphocyte cells. However, reactive processes in SGXG area did not involve surrounding tissue of the recipient brain (Fig. 2, *a*, Fig. 3, *a*).

Reactive processes on the SGXG border became less intensive 28 days after transplantation, the size of the grafts decreased (Table 1; Fig. 1, *d*). SGXG were surrounded by a membrane typical of ganglia, glial elements accumulated along the graft borders did not infiltrate the recipient brain (Fig. 3, *b*). In 2 animals scar-free zones were observed along SGXG-brain borders (Fig. 3, *c*), which attested to partial integration of the snail ganglia with the recipient rat brain.

Fluorescence microscopy of the brain sections with SGXG labeled with fluorescent dyes revealed bright blue fluorescence in numerous small cells and vessel membrane. Analysis of Nissl-stained sections revealed fluorescent endothelial cells in growing vessels, glial cell nuclei and macrophages. Glial cells were seen along the track of transplantational needle and migrated to about 500 μ away from the track. It was concluded that labeling of membrane-coated ganglia with fluorescent dyes might be not reasonable, because the dye poorly penetrated through the membrane. Higher percent of graft rejection in case of fluorescent dye-labeled ganglia compared to unstained ones cannot also be excluded, in these cases fluorescent cells can originate both from SGXG and rat brain (recipient glia and macrophages can phagocytize the label from destroyed SGXG cells).

Thus, neuronal mollusc ganglia transplanted into the rat brain cause no immune conflict during 6 months after surgery and probably longer. As soon as on post-transplantation day 5, vessels from SGXG penetrated into the recipient brain providing its humoral integration. The recipient brain presents a specific environment for SGXG promoting intensive proliferation of ganglion cell at the early terms after transplantation. The subsequent decrease in SGXG size can be due to inhibition of reactive processes in the ganglia due to their adaptation to recipient brain tissue. SGXG nutrition is provided via vessels growing from the graft into the recipient brain. Neurons in SGXG were preserved 6 months after transplantation.

We assume that, apart from scientific purposes, the use of neuronal ganglion xenografts from invertebrates with weak immune system can be applied in veterinary and clinical practice. However, before clinical application, it is important to study functional activity of these grafts in the mammalian brain and the absence of unfavorable effects on recipient behavior. Previously, we reported encouraging results on the effect of SGXG on conditioned behavior in rats attesting to functional activity of SGXG [15].

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