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METHODS

Intraocular Neurografts as a Model for Studying of Organization of Synaptic Connections in a Denervated Brain Area

Z. N. Zhuravleva[1], S. S. Khutsyan[1,2], and G. I. Zhuravlev[2]

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Intraocular neurografts of the septal region of rats were used as the model of deafferentiated brain area where the lack of adequate innervation is compensated for own interneuronal connections. Septum anlage from the brain of a 17-day fetus served as the donor material. The grafts developing in the anterior eye chamber over 3 months represented well-differentiated samples of the nervous tissue. A comparative morphometric study of the tripartite organization of synapses in the grafts and in the septum *in situ* was conducted. In the grafts, the mean volume and perimeter of synaptic terminals were below the normal. At the same time, postsynaptic densities did not differ from the control. A significant difference was found in the degree of surrounding of presynaptic terminals by astrocytic processes: in the grafts this parameter was higher by 1.8 times. Our results attest to an important role of perisynaptic glia in the formation of functionally active synaptic contacts with unusual neuronal targets.

Key Words: *intraocular neurografts; septum; ultrastructure; synaptic contacts; perisynaptic processes of astrocytes*

Synaptic contacts are the main form of functional interactions in the nervous system of vertebrate animals; they serve for integration of nerve cells into neural networks and provide integrative brain activity. During embryonic development, the number of synaptic contact is excessive. At later terms, during the formation of a mature pattern of neural networks, the contacts formed with inappropriate targets and not receiving the specific signals from the postsynaptic elements and environment are eliminated [5,13]. At the same time, it has been shown that under conditions of neurotransplantation, neurons form synaptic connections with not only appropriate, but also atypical postsynaptic targets and these atypical contacts persist throughout the life of the experimental animal [1,11]. Moreover, in grafts transplanted into ectopic immunoprivileged areas of the body, *e.g.* into the anterior eye chamber and devoid of functional connections with the brain, the neurons actively form synaptic contacts with each other [2,3,15]. Abnormal organization of neuronal networks can also occur in the brain *in situ* after neuronal death resulting from traumatic brain injuries, strokes, and neurodegenerative diseases [10]. It is important to know structural peculiarities of the formed synaptic connections between the neurons under conditions of partial or complete absence of natural relevant afferents.

The participation of astrocytes in the formation, functioning, and elimination of synapses is demonstrated in numerous studies [6,14]. Distal astrocyte

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences; [2]Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow region, Russia. ***Address for correspondence:*** zhuravleva@iteb.ru. Z. N. Zhuravleva

processes surrounding synaptic terminals play a special role in the regulation of synaptic transmission. They perform a homeostatic function by controlling ionic and neurotransmitter balance. Based on these data, it was proposed to include in the composition of synaptic endings not only pre- and postsynaptic components, but also the surrounding perisynaptic astrocytic processes, *i.e*. to consider synapses as three-part structural complexes ("tripartite synapses") [4,14].

Our aim was to compare the three-part organization of synapses in normally functioning and functionally isolated from appropriate brain structures nervous tissue. The main task was to determine the role of perisynaptic astroglia in the maintenance of functional contacts with ectopic targets. For microscopic studies, we used septal area of the brain *in situ* and ectopic septal grafts developing in the anterior eye chamber (deafferentiated tissue).

MATERIALS AND METHODS

The study was performed on Wistar rats in compliance with the requirements for experimental work with animals (GOST R ISO 10993-2-2009). All procedures were performed under Nembutal (40 mg/kg intraperitoneally) or ether anesthesia; for intraocular transplantation, the eye was additionally anesthetized with 2-3 drops of dicaine.

For transplantation into the anterior eye chamber, an anlage of the septum was isolated from the brain of rat embryos on day 17 of gestation under a stereomicroscope according to the atlas of developing rat brain. The fragments of donor tissue was transplanted to male rats (n=3) into the anterior eye chamber with a special Microman pipette through a small incision in the cornea. Three months after surgery, the grafts were isolated from the anterior eye chamber and subjected to histological (Nissl staining) and ultrastructural analysis. For electron microscopy, the grafts were cut into pieces (0.8-1.0 mm^3), fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) and postfixed in 1% OsO_4. After dehydration in alcohols and acetone, the samples were embedded in epon 812, and ultrathin sections were sliced. Specimens of the septal area of healthy brain (n=3) were taken from 3-month-old animals after intracardiac perfusion with the same fixative and similar processing of samples was performed according to the protocol for electron microscopy studies described in detail earlier [15]. Ultrastructural analysis was performed on a JEM 100B electron microscope (Jeol). Micrographs of the neuropil were digitized, saved as computer files, and analyzed using UTHSCSA Image Tool. For morphometric analysis, at least 100 microimages of synaptic endings with distinct structure of functional contacts located on dendritic spines or on dendritic trunks were selected in both experimental groups. Synapses were compared by the following parameters: the length of postsynaptic density (PSD), the area and perimeter of the presynaptic terminal (T), and the length of the astrocyte process surrounding the terminal (A). To calculate the degree of development of the astrocytic environment of the synapse (perisynaptic glia), we introduced coefficient K=A/T.

Significance of differences was evaluated using the Student's t test (Microsoft Excel). The differences were significant at $p \leq 0.05$.

RESULTS

The allografts of the septal area of the brain developing in the anterior eye chamber over 3 months represented isolated tissue formations consisting of nerve and glial cells and located between the iris and the cornea. Their surface was lined with a thin layer of glial cells that extended to the iris epithelium. The cytoarchitectonics and neuroglial ratio in the grafts did not visually differ from the control septal area of the brain; no destructive changes in the grafts were revealed (Fig. 1). Numerous blood vessels grew from the iris into the grafts. We have previously shown that the peripheral nerves grew into the grafts along the perivascular spaces and form few synaptic contacts with CNS neurons [2].

At the ultrastructural level, the transplanted tissue was well differentiated. The neurons contained distinct nuclei with diffuse chromatin and clear-cut nucleoli; the cytoplasm contained all typical organelles (Fig. 2). The neuropil sites of the grafts with profiles of dendrites, axons, glial processes, and numerous synaptic endings also showed signs of high differentiation. An important sign of nervous tissue maturity was well-developed tightly packed lamellae of myelinated axons. The quantitative proportions of synaptic complexes

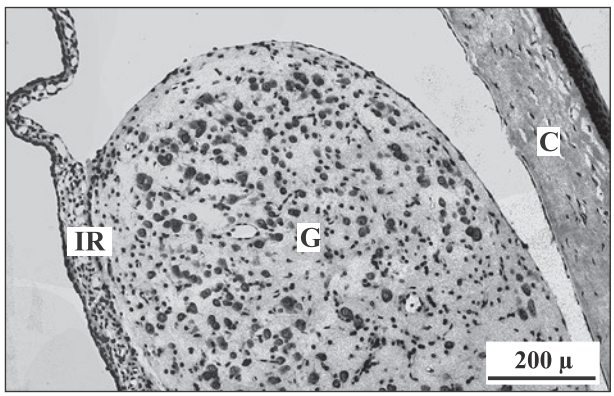

Fig. 1. Histological section through the intraocular graft of the septal region of the brain. Nissl staining. G: graft, C: cornea, IR: iris.

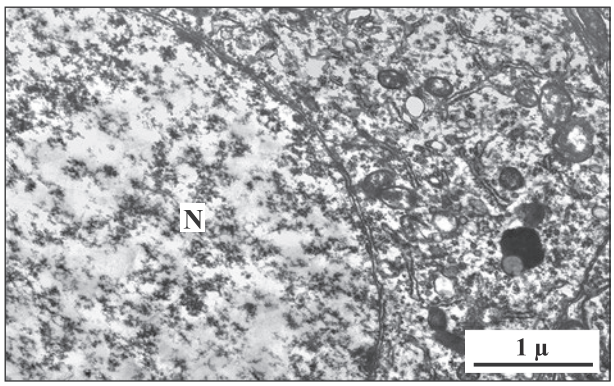

Fig. 2. Ultrastructure of a neuron in an intraocular septum graft. N: nucleus of the neuron.

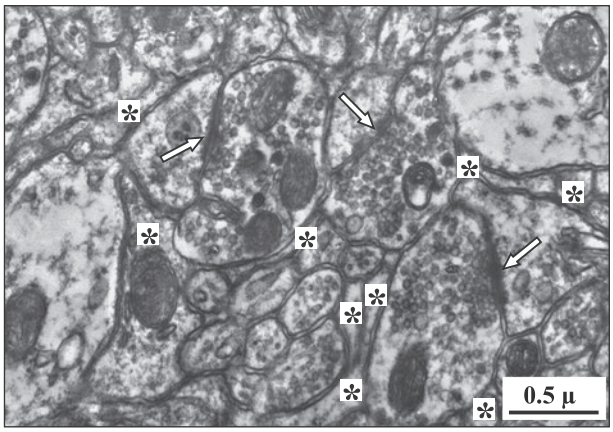

Fig. 3. Neuropil in intraocular septal graft. Arrows show synaptic contacts with the heads of dendritic spines, asterisks show perisynaptic processes of astrocytes.

and other elements of the neuropil visually did not differ from those in the brain *in situ*. Approximately one-third of synaptic contacts were formed on dendritic spines and the rest were localized on dendrite trunks. In synaptic active zones, clusters of synaptic vesicles from the presynaptic side and pronounced electron dense spots near the postsynaptic membrane were typically seen (Fig. 3). Practically all synaptic endings structurally contacted with terminal processes of astrocytes and formed three-part complexes. The perisynaptic glial processes had irregular shape and surrounded synaptic profiles by different extents. The distances between the opposing synaptic and astrocyte membranes also differed.

During electron microscopy of the septal region of the brain that served as the control in this study, special attention was paid to the neuropil zones. The septum receives afferents from the brain stem structures and hypothalamus and also contains a local system of connections formed by its own cholinergic and glutamatergic neurons [7]. Routine microscopy does no allow identifying the origin of different axonal terminals; therefore, randomly selected zones were used for the morphometry of synapses. The number of axospinic contacts in the control septal region was higher than in the grafts (36.0±4.5% *vs.* 22.5±4.0%; $p \leq 0.025$). In addition, perforated active zones were observed by 2 times more frequently in the control than in the grafts, and the presynaptic zones were more densely filled with vesicles.

A comparative morphometric study of synaptic endings in both control samples and grafts took into account their three-part structure. Postsynaptic densities (PSDs) are functionally important structural components of synapses, because they are the site of concentration of receptor molecules and protein signaling complexes and their size correlates with the efficiency of synaptic transmission [9]. The results showed that the length of PSD in individual synapses in both groups widely varied from 0.1-0.2 to 0.8-1.0 μ. Higher values were observed in axodendritic synapses of the neurografts. At the same time, the mean size of PSD in the two groups did not differ significantly (Table 1). This shows that neurons have a certain internal mechanism that regulates their functional activity even under conditions of atypical afferentation. It can be hypothesized that homeostatic plasticity of neurons that manifests in this case at the postsynaptic level serves as this mechanism [8].

According to morphometric data, the mean area and perimeter of the presynaptic terminals in normal septal brain area were greater than in neurografts. At the same time, in the neurografts, the presynaptic terminals more actively contacted with surrounding astrocyte processes. On average, the length of the perisynaptic glia in the grafts was slightly higher than in the control (Table 1). However, taking into account lesser total perimeter of axonal terminals, the proportion of

TABLE 1. Morphometric Parameters of Synapses in Intraocular Septal Grafts and Septal Region of Rat Brain (*M*±*m*)

Parameters of synaptic endings	Neurografts (*n*=100)	Septal area (control) (*n*=103)
Length of PSD, μ	0.366±0.014	0.347±0.013
Area of presynaptic terminals, μ²	0.520±0.034*	0.650±0.038
Perimeter of presynaptic terminals, μ	2.14±0.09**	2.72±0.10
Length of astrocyte processes in contact with presynaptic terminals, μ	0.96±0.06	0.84±0.06
Coefficient K	0.450±0.025**	0.245±0.026

Note. *n*: number of synapses. *$p \leq 0.01$, **$p \leq 0.001$ in comparison with the control.

contacts with surrounding astrocytic processes is very significant. The coefficient K introduced by us in the grafts was by 1.8 times higher than in the control. This parameter attests to an important role of perisynaptic glia in the organization and maintenance of synaptic contacts with ectopic neuronal targets. Moreover, the perisynaptic processes of astrocytes in intraocular neurografts isolated from the brain, apparently, protect the excitatory synaptic contacts from hyperexcitation. It is known that chronically deafferentiated nervous tissue is characterized by increased seizure readiness [12]. We previously reported a correlation between the decrease in the content of perisynaptic glia and the development of epileptiform activity in intraocular neurografts [3].

Thus, intraocular transplantation of the nervous tissue as the model of denervated brain region demonstrated structural peculiarities of aberrant functional connections formed with inappropriate neuronal targets. Ultrastructural and morphometric analysis of synaptic terminals in the nervous tissue that develops without natural afferents and compensates them by formation of internal interneuronal connections showed that they have lesser area and perimeter of terminals than in normal tissue, but do not differ by the length of PSD, an important parameter of functional strength of the synaptic contact. In addition, important data were obtained on the paramount role of perisynaptic processes of astrocytes in the formation and long-term maintenance of nonspecific synaptic connections under conditions of abnormal afferentation.

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REFERENCES

1. Zhuravleva ZN, Zhuravlev GI, Mugantseva EA. Transsynaptic Coordination of the Formation of Morphofunctional Contacts between the Brain and the Neurotransplant: an Ulrastructural Study. Bull. Exp. Biol. Med. 2015;160(1):91-95.
2. Zhuravleva ZN, Mugantseva EA, Zhuravlev GI. Microscopic Study of Nervous System Plasticity: Interactions of Sympathetic Nerves with Neurons of Intraocular Hippocampal Transplants. Bull. Exp. Biol. Med. 2018;164(5):680-684.
3. Zhuravleva ZN, Khutsyan SS, Zhuravlev GI. Ultrastructure of excitatory synaptic contacts in epileptiform activity focus: experiments on intraocular neurotransplants. Zh. Vyssh. Nervn. Deyat. 2016;66(6):742-750. Russian.
4. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 1999;22(5):208-215.
5. Choo M, Miyazaki T, Yamazaki M, Kawamura M, Nakazawa T, Zhang J, Tanimura A, Uesaka N, Watanabe M, Sakimura K, Kano M. Retrograde BDNF to TrkB signaling promotes synapse elimination in the developing cerebellum. Nat. Commun. 2017;8(1):195. doi: 10.1038/s41467-017-00260-w.
6. Chung WS, Allen NJ, Eroglu C. Astrocytes control synapse formation, function, and elimination. Cold Spring Harb. Perspect. Biol. 2015;7(9):a020370. doi: 10.1101/cshperspect.a020370.
7. Colom LV, Castaneda MT, Reyna T, Hernandez S, Garrido-Sanabria E. Characterization of medial septal glutamatergic neurons and their projection to the hippocampus. Synapse. 2005;58(3):151-164.
8. Fernandes D, Carvalho AL. Mechanisms of homeostatic plasticity in the excitatory synapse. J. Neurochem. 2016;139(6):973-996.
9. Kennedy MB. Signal-processing machines at the postsynaptic density. Science. 2000;290:750-754.
10. Kuśmierczak M, Lajeunesse F, Grand L, Timofeev I. Changes in long-range connectivity and neuronal reorganization in partial cortical deafferentation model of epileptogenesis. Neuroscience. 2015;284:153-164.
11. Magavi SS, Lois C. Transplanted neurons form both normal and ectopic projections in the adult brain. Dev. Neurobiol. 2008;68(14):1527-1537.
12. Nita DA, Cissé Y, Timofeev I, Steriade M. Increased propensity to seizures after chronic cortical deafferentation in vivo. J. Neurophysiol. 2006;95(2):902-913.
13. Uesaka N, Uchigashima M, Mikuni T, Hirai H, Watanabe M, Kano M. Retrograde signaling for climbing fiber synapse elimination. Cerebellum. 2015;14(1):4-7.
14. Verkhratsky A, Nedergaard M, Hertz L. Why are astrocytes important? Neurochem. Res. 2015;40(2):389-401.
15. Zhuravleva ZN, Saifullina VN, Zenchenko CI. Morphometric analysis of the hippocampal neurons in the grafts developing in the anterior eye chamber of young and aged rats. Neural Plasticity. 1997;6(1):49-57.