Astrogliogenesis in Heterotopic Allotransplants of Rat Embryonic Neocortex

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We studied gliogenesis in transplants of rat embryonic neocortex (E14-15) in 3, 7, 15, and 30 days and 12-13 months after transplantation into the sciatic nerve of adult animals. Immunogistochemical reactions to intermediate filament proteins nestin, vimentin, glial fibrillary acidic protein were used. In transplants, vimentin- and nestin-positive precursor cells differentiate into astrocytes earlier that in the developing rat neocortex (*in situ*). One year after transplantation, some astrocytes start to express nestin and vimentin, which attests to the development of reactive gliosis in the transplants.

Key Words: *nestin; vimentin; glial fibrillary acidic protein; astrocytes; neurotransplantation*

In 1980-90s, transplantation of embryonic CNS anlages into the brain and spinal cord was widely used for the development of approaches to the treatment of neurodegenerative diseases. In modern studies in this filed, neural stem/progenitor cells [1,9] and multipotent stromal cells of the bone marrow (BM) [3,11], rather then fragments of embryonic anlages are used. This is also true for neurotransplantation into the peripheral nerve. This is an urgent problem in view of growing interest in transplantation of embryonic brain anlages [12] and various SC [14] for stimulation of regeneration of nerve trunks. In our previous experiments on transplantation of embryonic brain into the peripheral nerve of adult animals we primarily studied the development of neuronal cells. For instance, we observed degenerative changes in some nerve cells and their death that in delayed terms after transplantation [5,8]. Expression of glial fibrillary acidic protein (GFAP) in cells of neurotransplants was detected [7].

Here we used immunohistochemical methods for studying of astrogliogenesis in allotransplants of rat embryonic neocortical anlages transplanted into the sciatic nerve of adult animals.

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MATERIALS AND METHODS

Experiments were carried out on 30 Wistar rats weighing 200-250 g. The dorsolateral wall of the prosencephalon from Wistar rat embryos (perinatal days 14-15) was transplanted into the sciatic nerve of adult animals as described previously [10]. The material was fixed in Bouin fixative and zinc-formalin-ethanol mixture in 3, 7, 15, and 30 days and 12-13 months after surgery, dehydrated, embedded in paraffin, and 5-μ sections were prepared. For detection of nestin, the protein typical of neural stem/progenitor cells, monoclonal murine antibodies (clone Rat-401, BD Pharmingen, 1:400); astrocyte marker GFAP was detected using polyclonal rabbit antibodies (Dako, RTU); vimentin, the protein typical of rat radial gliocytes and ependymocytes [4], was visualized using monoclonal murine antibodies (clone V-9), Dako, RTU). The preparations were washed with phosphate buffer (pH 7.4) and incubated with biotinylated second antibodies and then with streptavidin conjugated with horseradish peroxidase (LSAB2 kit). Peroxidase label was visualized using DAB$^+$ chromogen (Dako). Some sections were poststained with toluidine blue.

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RESULTS

Dorsolateral wall of rat prosencephalon on perinatal days 14-15 contains neocortex anlage and consists of precursors of neural and glial cells and radial glia. The results of immunohistochemical assay of the original material with antibodies against nestin showed that most cells of the anlage were nestin-positive, which agreed with published data [2]. Nestin was present in both radial glia cells and neuronal and glial precursor cells. Evaluation of the dynamics of nestin expression by neurotransplant cells showed that the number of nestin-positive precursor cells gradually decreased over the first 7 days after transplantation. In 3 days, nestin-positive cells were similar to cells of the original material: 7-8 μ in diameter with small nuclei and rather short processes (Fig. 1, *a*). Some cells of the transplants at this term were immunonegative and most likely represent differentiated neuroblasts. In 15 and 30 days after transplantation, no nestin-positive precursor cells were detected. Only endothelial cells of forming blood vessels demonstrated nestin-positive staining (Fig. 1, *b*), which was typical of developing brain [2]. We previously reported that the transplants underwent degeneration 12-13 months after surgery: some neurons died, cells of some vascular walls and numerous neurons underwent degenerative changes, amyloid deposition were detected in the transplants [5,8]. In our study, changes in the expression of cytoskeletal intermediate filament proteins were detected at these terms: nestin-positive cells again appeared in the transplants (Fig. 2, *c*). These hypertrophied cells with processes can be classified by their morphological characteristics as activated astrocytes. Similar cells were described in rat brain during some pathologies [15].

Vimentin in the original material was synthesized by a considerable number of rat embryonic neocortical anlage cells at the specified term of perinatal ontogeny. Similarly to nestin, vimentin was present in the radial glia and in other precursors. Three days after transplantation, vimentin-positive cells were detected in depth of the transplants and along the periphery at the boundary with recipient tissues (Fig. 3, *a*). The number of vimentin-positive precursors in the central zone of the transplant decreased during the first week after surgery. At the periphery of the transplants, vimentin-positive cells with long thin processes and with signs of radial gliocytes were seen over 15 days. The fate of vimentin-positive precursors in the central and peripheral zones of the transplant was different. The former gradually differentiated into GFAP-positive astrocytes, the latter after 30 days formed a multilayer glial lining consisting of vimentin-positive cells at the boundary with tissues of the recipient nerve. The formation of this lining in neurotransplants of the neocortex transplanted into the peripheral nerve has been previously reported by us [6,7,10]. A special study comparing the development of transplants from rat embryonic neocortex in the brain, anterior chamber of the eye, and peripheral nerve showed that this lining consisting of ependyma-like cells is typical only of transplants developing in the nerve [7]. Here we showed immunohistochemically that most cells of the glial lining are presented by ependymocytes: it is known that the ependyma of cerebral ventricles in intact rats contain vimentin [4]. After 12-13 months, some astrocytes in the central zones of the transplants again expressed vimentin (Fig. 2, *b*). These astrocytes usually concentrated in sites of vascular cell degeneration and near amyloid-like depositions. Similar vimentin-positive astrocytes

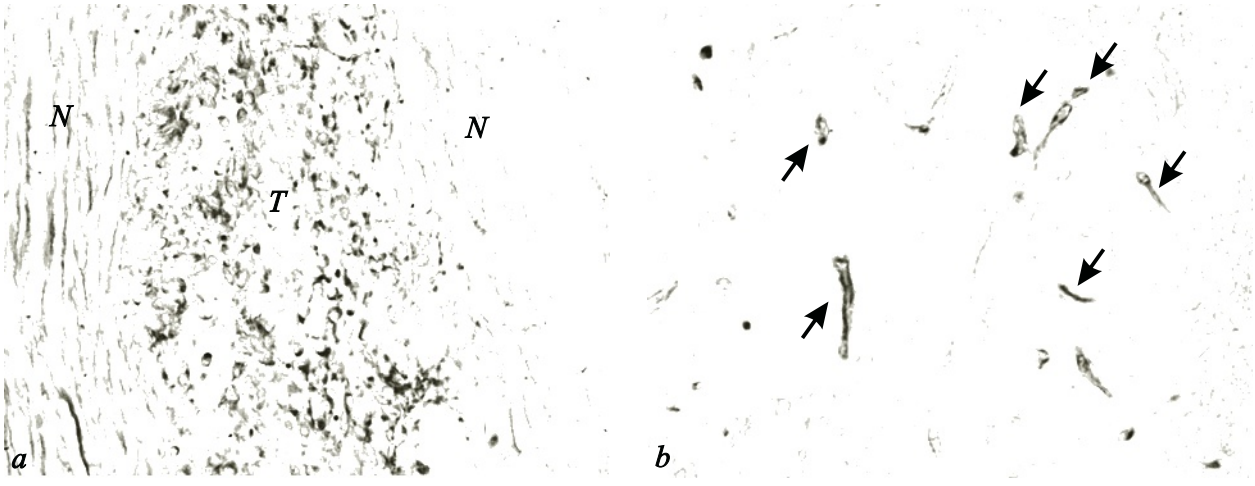

Fig. 1. Nestin-immunopositive cells in rat embryonic neocortex fragments transplanted into the nerve of adult animals 3 (*a*) and 30 days (*b*) after surgery. Immunohistochemical reaction for nestin. Arrows show blood vessels in the transplant central zone. Magnification: *a*) ×200, *b*) ×400. Here and in Fig. 2: *T*: transplant; *N*: recipient nerve.

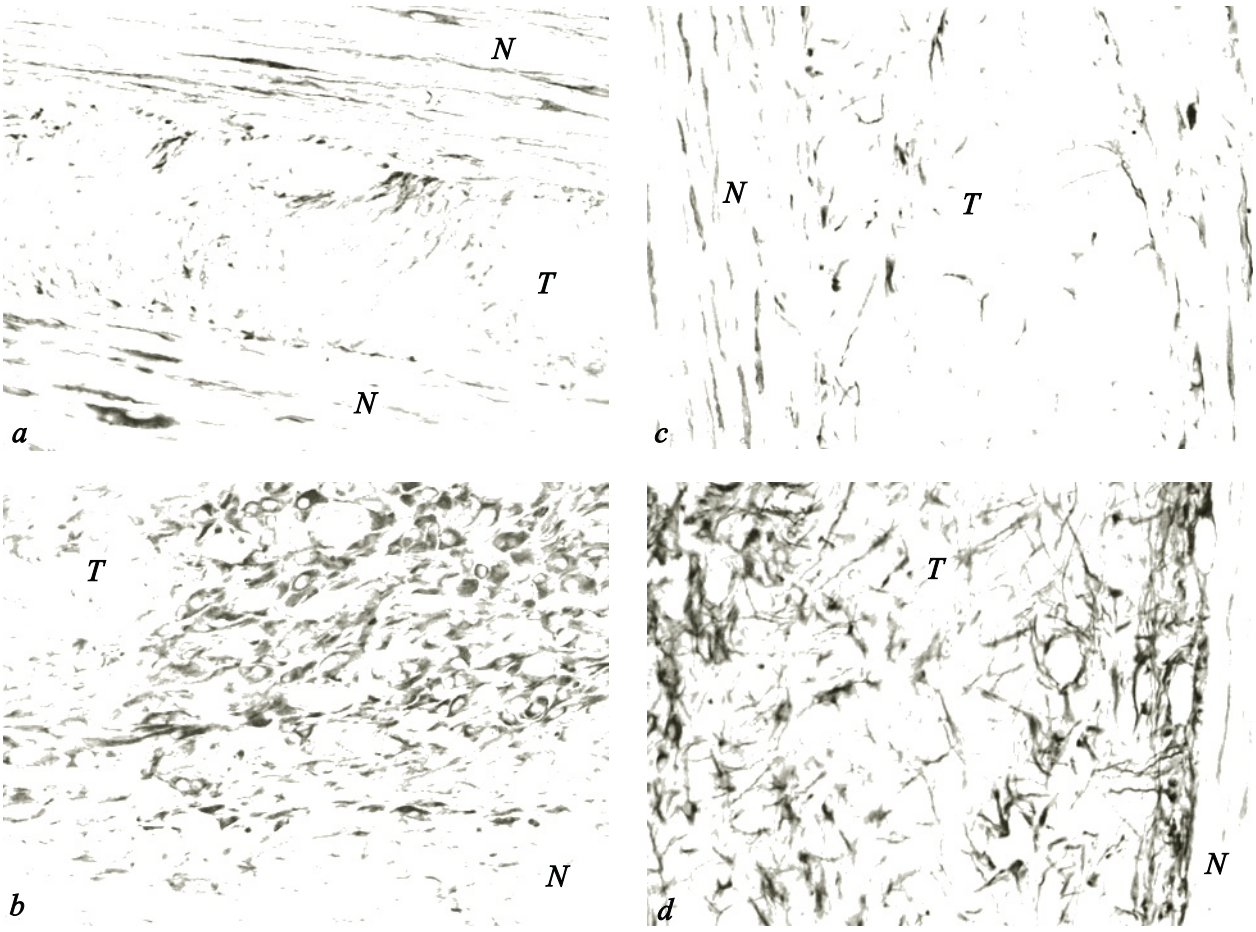

Fig. 2. Reactive astrocytes in transplants+ of rat embryonic neocortex 12-13 months after transplantation into the sciatic nerve of adult animals, ×400. Immunohistochemical reaction to GFAP (*a*), vimentin (*b*), and nestin (*c*).

were described in experimental brain ischemia [4].

Immunohistochemical assay of GFAP expression showed that this protein was not expressed in cell of the original material used for transplantation. In rodents, expression of GFAP normally appears during the postnatal ontogeny. In neurotransplants, GFAP-positive first appeared on day 7 after transplantation (Fig. 3, *c*). These were small cells of different shape with thin and often long processes. In 15 days, many GFAP-positive cells with processes formed a dense net throughout the transplant thickness (Fig. 3, *d*). The intensity of their staining was higher than in the intact neocortex of adult rats. In 30 days, the transplants also contained many activated astrocytes. One year later, intensively stained GFAP-immunopositive cells were seen both in the central zone of the transplant between the neurons (Fig. 2, *a*) and at the boundary with the recipient nerve tissues.

Here we studied the dynamics of astroglia development from transplanted precursor cells under conditions of ectopic neurotransplantation and detected the peculiarities of the expression of cytoskeletal intermediate filament proteins at delayed terms after transplantation. It was shown that differentiation of transplanted precursor cells into astrocytes occurs during the period from day 7 to day 15 after surgery. During normal ontogeny, GFAP-positive astrocytes from vimentin-positive radial gliocytes in rat neocortex are formed by a few days later than in the analyzed transplants [2,13]. Acceleration of astrocyte differentiation in neurotransplants did not depend on the site of transplantation. The same regularity was observed in homotypic transplants of rat embryonic neocortex [13]. The presence of vimentin-positive cells in mature transplants can be explained as follows: either some transplanted radial gliocytes did not differentiate into astrocytes or differentiated astrocytes started to express vimentin [13]. Our experiments proved the latter. Studying the dynamics of the expression of intermediate filament proteins in cells of the transplants over a long time we found that the number of vimentin-positive cells in the transplants decreased at early terms, but at later terms resumption of vimentin expression was observed in some cells. Vimentin was expressed by reactive astrocytes, which was seen from their morphological characteristics. It should be noted that the method of

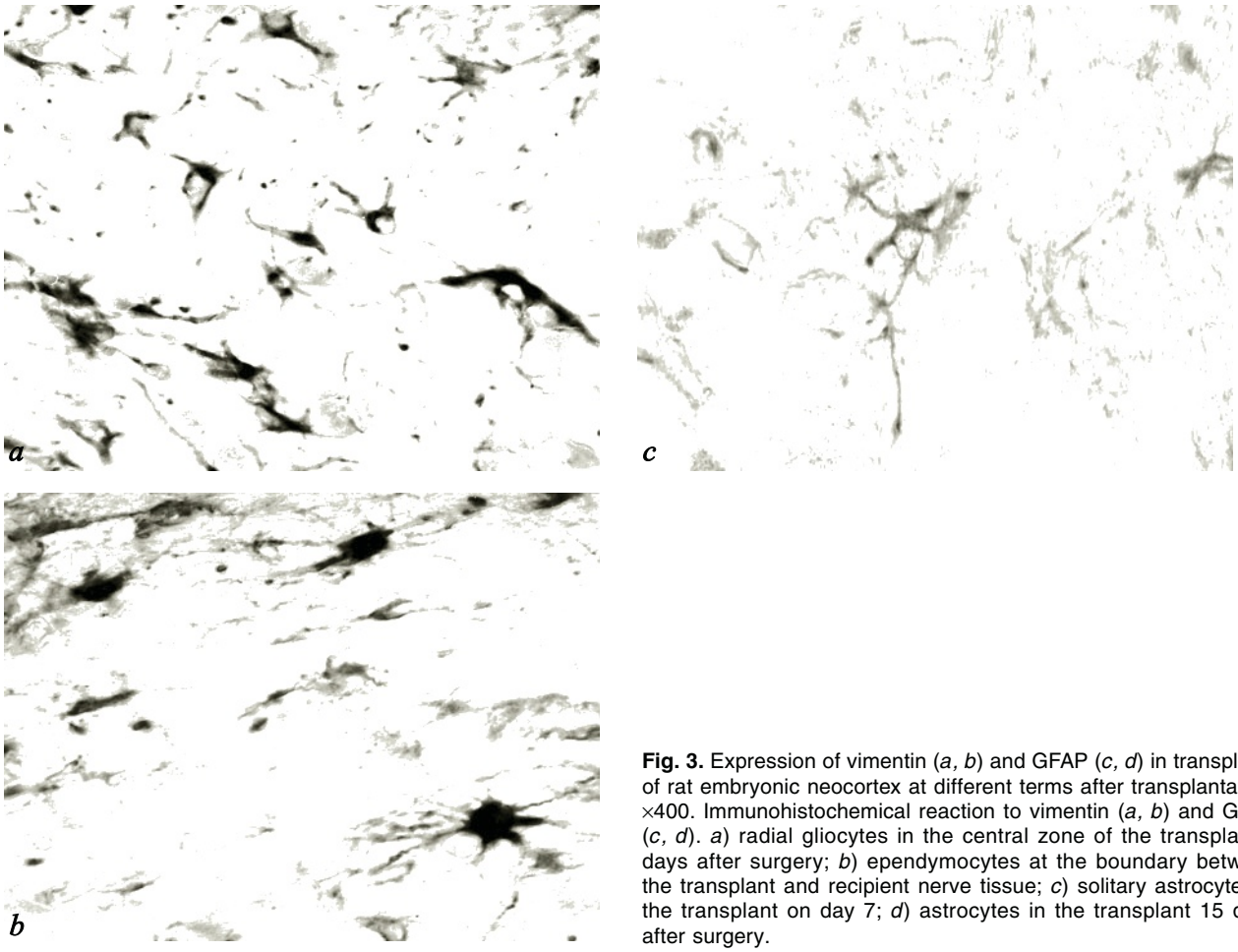

Fig. 3. Expression of vimentin (*a, b*) and GFAP (*c, d*) in transplants of rat embryonic neocortex at different terms after transplantation, ×400. Immunohistochemical reaction to vimentin (*a, b*) and GFAP (*c, d*). *a*) radial gliocytes in the central zone of the transplant 3 days after surgery; *b*) ependymocytes at the boundary between the transplant and recipient nerve tissue; *c*) solitary astrocytes in the transplant on day 7; *d*) astrocytes in the transplant 15 days after surgery.

transplantation of rat embryonic neocortex into the nerve provides the possibility of studying one more process: differentiation of vimentin-positive ependymocytes at the boundary of the transplant with recipient tissues. Under conditions of ectopic neurotransplantation, some of them can express GFAP [7]. A peculiarity of long-living transplants is the formation of a large number of hypertrophied GFAP-positive astrocytes and vimentin- and nestin-containing astrocytic cells. The presence of these astrocytes in the brain after neurotrauma and in neurodegenerative pathologies attests to the development of reactive gliosis [15].

Thus, differentiation of nestin- and vimentin-immunopositive precursor cells into astrocytes in transplants of embryonic neurocortex developing in the nerve occurs earlier than in rat neurocortex during normal ontogeny. A peculiarity of long-living transplants is the formation of a large number of hypertrophied GFAP-positive astrocytes and vimentin- and nestin-containing astrocytic cells. We believe that the development of reactive gliosis in neurotransplants engrafted into the nerve and previously reported [5,8] destructive processes typical of long-living ectopic transplants should be taken into account during the development of experimental models using neurotransplantation for stimulation of regeneration of peripheral nerve system organs.

REFERENCES

1. M. A. Aleksandrova, A. V. Revishchin, R. A. Poltavtseva, *et al., Ontogenez*, **34**, No. 3, 167-173 (2003).
2. A. V. Gilyarov, D. E. Korzhevskii, and V. A. Otellin, *Zh. Evoluts. Biokhim. Fiziol.*, **45**, No. 1, 130-137 (2009).
3. A. S. Grigoryan, E. G. Gilerovich, N. N. Pavlichenko, *et al., Klet. Transplantol. Tkan. Inzhener.*, No. 4, 58-67 (2009).
4. D. E. Korzhevskii, M. I. Lentsman, O. V. Kirik, and V. A. Otellin, *Morfologiya*, 132, No. 5, 23-27 (2007).
5. V. A. Otellin and E. S. Petrova, *Morfologiya*, 113, No. 2, 39-44 (1998).
6. E. S. Petrova, *Morfologiya*, **136**, No. 6, 8-19 (2009).
7. E. S. Petrova and V. A. Otellin, *Thitologiya*, **42**, No. 8, 750-757 (2000).
8. E. S. Petrova and V. A. Otellin, *Byull. Eksp. Biol. Med.*, **136**, No. 9, 343-347 (2003).
9. G. T. Sykhikh and V. V. Malaitsev, *Byull. Eksp. Biol. Med.*, **131**, No. 3, 244-255 (2001).
10. E. I. Chumasov and E. S. Petrova, *Byull. Eksp. Biol. Med.*, **108**, No. 8, 198-201 (1990).
11. K. N. Yarygin, I. V. Kholodenko, A. A. Konieva, *et al., Byull.*

Eksp. Biol. Med., **148**, No. 12, 621-627 (2009).
12. A. Tuba, L. Kallai, and M. Kalman, J. *Neural. Transplant Plast.*, **6**, No. 1, 21-29 (1997).
13. G. Xiong, N. Ozaki, and Y. Sugiura, *Arch. Histol.Cytol.*, **72**, No. 2, 127-138 (2009).
14. S. Walsh and R. Midha, *Neurosurg. Focus*., **26**, No. 2, 1-8 (2009).
15. A. Widestand, J. Faijerson, U. Wihelmsson, *et al., Stem Cells*, **10**, No. 1, 2619-2627 (2007).