NADPH-Positive Neurons in Heterotopic Transplants of Embryonic CNS

E. S. Petrova and V. A. Otellin

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Neurons of rat neocortex and spinal cord express NADPH-diaphorase after heterotopic allotransplantation into the sciatic nerve. These peculiarity of diaphorase expression in transplanted cells in comparison with brain cells developing *in situ* suggest an important role of afferent and efferent relationships in the development of NO mechanisms in neurons.

Key Words: *nitric oxide; neurotransplantation; neocortex; spinal cord; nerve*

Nitric oxide (NO), a regulator of brain activity, is now often referred to new transmitters. In the brain NO is formed from arginine in the reaction catalyzed by NO-synthase (NOS) possessing diaphorase activity. NOS carries electrons from NADPH to a one-electron acceptor [3]. NADPH-diaphorase (NADPH-d) indicates the presence of NO in cells [3]. In the CNS NO is present in vascular endotheliocytes, microglia, astrocytes, and neurons of various brain structures [5-7, 9,10]. The number of NO-positive neurons increases after brain injuries, in hypoxia and ischemia [6,12]. NO is involved in the regulation of cerebral bloodflow [7]. Therefore, it was of interest to study NO-positive cells in the nervous system under conditions of neurotransplantation, which allowed us to trace the histogenetic processes associated with injuries to donor and recipient tissues, changed microenvironment, and formation of atypical afferent and efferent innervation. We studied the development of NADPH-positive neurons of the rat embryonic neocortex and spinal cord in heterotopic transplants.

METHODS

Twenty male and 5 female Wistar rats (200 g) were used in the study. Embryonic neocortex (n=12) and spinal cord (n=6) from a 15-day-old rat were transplanted under ether narcosis as described previously [1,4]. Neocortical neurotransplants were studied 7, 11, 15, and 30-60 days after transplantation, spinal cord transplants after 30-60 days. The respective brain compartments from newborn and 2-month-old rats served as the control. The animals were sacrificed by ether narcosis, the material was fixed in 4% paraformaldehyde (Fluka) in phosphate buffer (pH 7.2) for 2.5 h and washed with 15% sucrose for 24 h. NOS was detected by histochemical test for NADPH-d. Histological sections (30-50 μ) were made on a freezing microtome and stained for 30 min at 37°C in a solution containing 1 mg/ml β-NADPH (Sigma), 0.3 mg/ml NBT (Sigma), and 0.3% Triton X-100 (Serva) (pH 7.2), dehydrated in ascending alcohols, and embedded in Canadian balm. Staining solution without β-NADPH was used as the control.

RESULTS

Neurotransplants of different size were seen in 70-80% longitudinal sections of sciatic nerves of recipient rats at all terms (Fig. 1, *a, b*). Our previous experiments showed that transplanted neuroepithelial cells of embryonic neocortex and spinal cord survived and differentiated into neuroblasts, young and mature neurons within 1-2 months after transplantation into damaged sciatic nerve [1,4]. Uni- and bipolar neurofibroblasts with round light nuclei and 1-2 nucleoli appeared in transplants on day 7 postoperation and then merged into clusters [4]; all these

Department of Morphology, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg. ***Address for correspondence:*** iem@iem.spb.ru. Petrova E. S.

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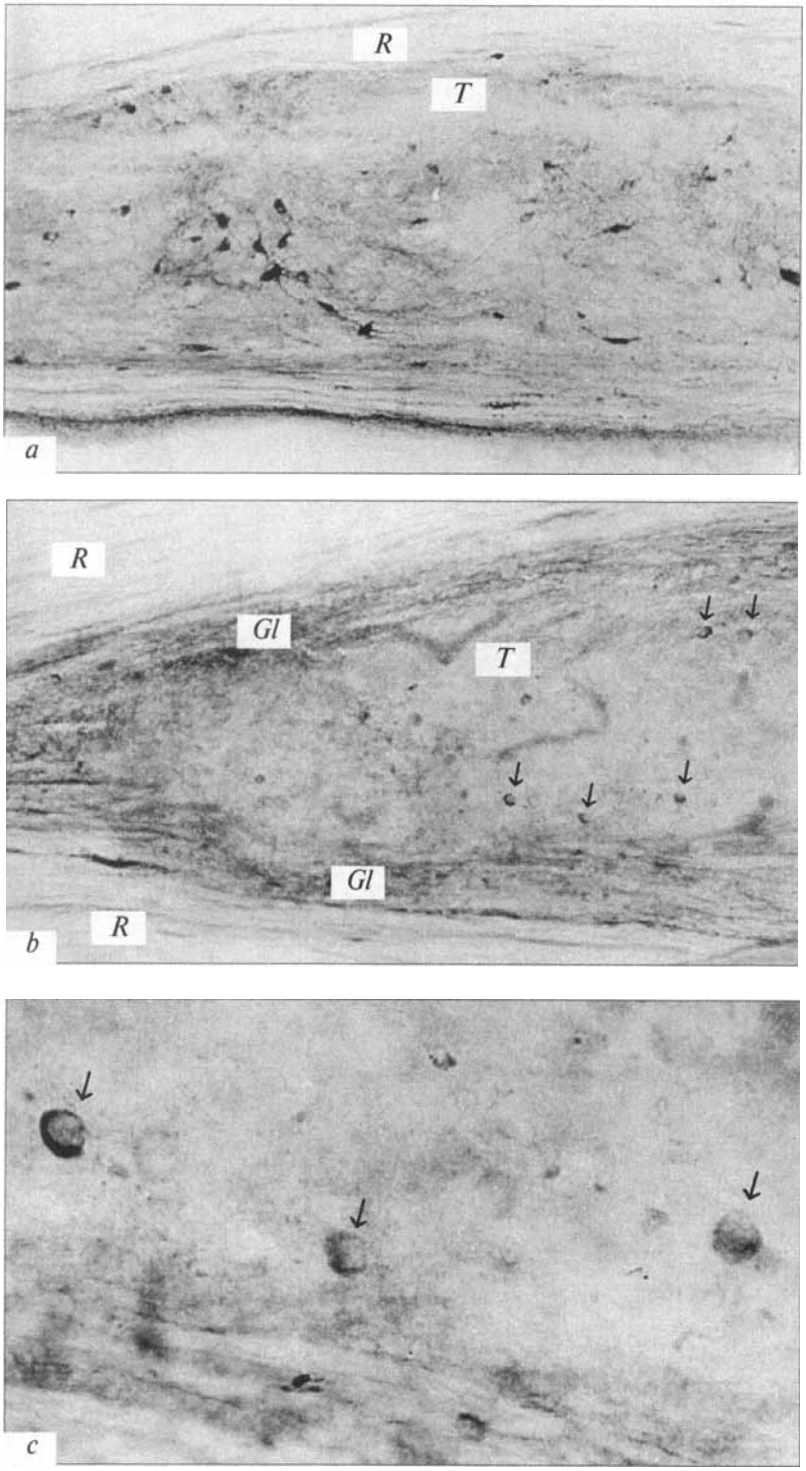

Fig. 1. Fragments of embryonic spinal cord (E15; *a*) and neocortex (*b*, *c*) 2 months after heterotopic transplantation into sciatic nerve of adult rats. Reaction to NADPH-diaphorase, ×100 (*a*, *b*), ×400 (*c*). Arrows show NADPH-positive nerve cells in the neocortex transplant. *R*: recipient nerve fibers; *Gl*: glial lining; *T*: transplant.

neuroblasts were NADPH-d-negative. At this term, only capillary endotheliocytes in the transplants and endotheliocytes and vascular smooth-muscle cells in the recipient nerve were NADPH-positive. In vascular smooth-muscle cells, NOS is regulated by cytokins, interleukin-1, and some other substances [11]. The appearance of NADP-positive smooth-muscle cells in blood vessels of the nerve is apparently due to the presence of interleukin-1 synthesized in nerve trunk after injury [13].

It was demonstrated that neurogenesis in embryonic transplants is synchronous with *in situ* development and differentiation [2,8]. Morphological analysis showed that differentiation of some neural elements (NE) in transplants 7 days after transplantation was similar to cell differentiation in the corresponding

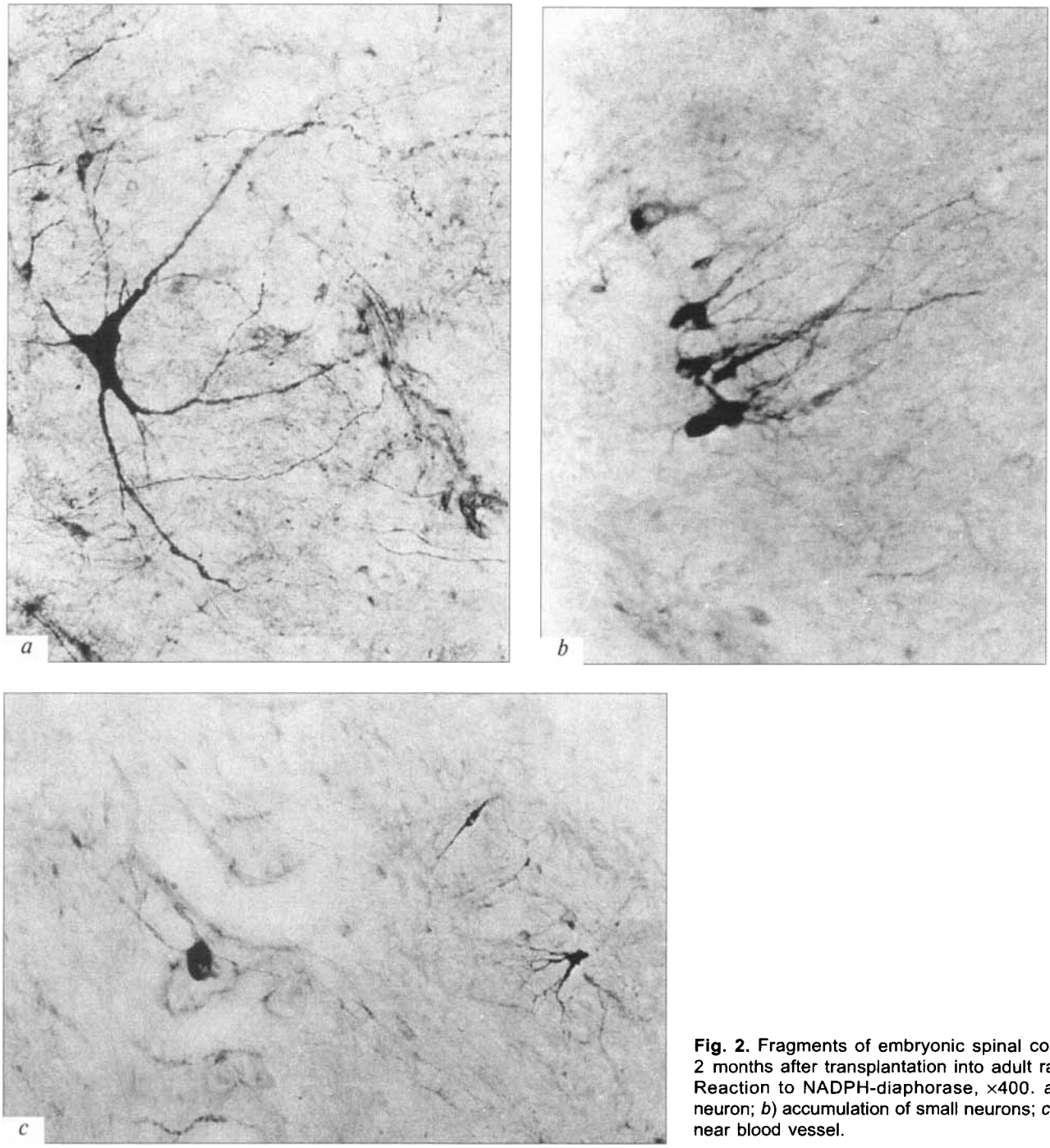

Fig. 2. Fragments of embryonic spinal cord (E15) 2 months after transplantation into adult rat nerve. Reaction to NADPH-diaphorase, ×400. *a*) motoneuron; *b*) accumulation of small neurons; *c*) neuron near blood vessel.

brain structure in newborn rats [2]. In contrast to 7-day transplants, both the neocortex and spinal cord of newborn rats contained NADPH-positive NE. In the neocortex these were some small cells (9×13 μ) with processes. The spinal cord of newborn rats contained much more NADPH-positive NE that the neocortex. There were bi-, multipolar, and triangular NE. Many cells were larger than in the neocortex (13×18 μ), some of them were located near blood vessels. Sometimes thin branching processes of these cells reached the capillary walls, their length of some processes was 70-90 μ.

On days 10-11 after transplantation, solitary NADPH-positive cells (9×9 μ) appeared in the neocortical transplants. These cells were characterized by a thin cytoplasic rim and the absence of processes. The intensity of specific staining in the transplants was much lower than in the neocortex developing *in situ*.

After 15 days the number, shape, and intensity of NADPH-positive staining of NE in the neocortical transplants did not differ from those at the previous term. However, glial elements at the periphery of the neocortical transplants between recipient nerve fibers

showed a weak NADPH-d-specific staining. Our previous studies showed that these cells are morphologically similar to ependymocytes, the majority of them express glial fibrillary acidic protein typical of astrocytes [1]. Positive reaction to NADPH-d in these cells agrees with previous reports on NO synthesis in the ependyma and astrocytes after brain injuries and during tumor growth [10].

After 1-2 months NADPH-positive neurons of the neocortical transplant grew in size in comparison with the previous terms. They differed from NADPH-positive neurons in the corresponding brain structures by size, processes, and intensity of histochemical reaction (Fig. 1, *b, c*); large cells attained 18×13 μ. These cells typically had round shape and carried no processes, while the majority of NADPH-positive neurons in the brain were 18×45 μ, intensely stained, with branching processes up to 100 μ long. Glial elements at the transplant periphery were more intensely stained for NADPH-d than at the previous term.

The spinal cord transplants contained more NADPH-positive neurons than neocortical transplants at this term (Fig. 1, *a*). They were similar to spinal cord neurons in rats of the corresponding age by staining intensity and shape. The transplants contained large NADPH-positive motoneurons of different shape lying separately and having numerous dendrites (Fig. 2, *a*). Sometimes small NADPH-positive neurons formed clusters similar to developing nuclei of the spinal cord (Fig. 2, *b*). Some neurons lie near blood vessels (Fig. 2, *c*).

The appearance of NADPH-positive neurons in the transplants did not coincide with the formation of blood vessels (vascular network in the transplants formed much earlier, during the first days after transplantation). This does not exclude the involvement of NOS-positive neurons in the regulation of cerebral blood flow [7], but further studies are needed to elucidate the mechanisms of interactions between NADPH-positive neurons and elements of the vascular wall.

Our experiments showed that under conditions of heterotopic transplantation the neurons transplanted into the peripheral nerve synthesize NADPH-d. The spinal cord transplants contain more NADPH-positive neurons than neocortical transplants. The synthesis of NO in transplanted neurons starts later than in neurons developing *in situ*. Published data suggest that NOS-synthesizing cells appear in the CNS during the prenatal ontogeny [5,9]. The role of NO in developing brain is not yet clear. Previous experiments on rat cerebellar cortex showed that NO can be involved in the formation of synaptic contacts in the developing brain [9]. The presence of NOS-positive afferent nerve fibers stimulate migration and differentiation of neurons by promoting the formation of normal cytoarchitectonics. NOS-positive afferent fibers induce NO synthesis in cerebellar neuroblasts [9]. Other authors also demonstrated the important role of NO in the synaptogenesis. C. V. Williams *et al.* [15] showed that the appearance of NOS-positive cells in the tectum coincided with the formation of retinal pathways and that NOS-positive neurons were connected to the retina. Presumably the detected delay in the formation of NADPH-positive neurons in the transplants can be caused by insufficient trophic effect of afferent fibers. In our transplantation model nerve tissue developed in the absence of specific afferent innervation. During transplantation into the nerve the synaptic contacts between the recipient nerve fibers penetrating into transplant and the transplanted neurons are insignificant and their pattern is unknown [14].

Hence, neurons of the neocortex and spinal cord express NADPH-d under conditions of heterotopic transplantation. The differences in diaphorase expression between transplanted and *in situ* developing cells suggest that afferent and efferent connections play an important role in the development of NO mechanisms in nerve cells.

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REFERENCES

1. E. S. Petrova and V. A. Otellin, *Tsitologiya,* **42,** No. 8, 749-756 (2000).
2. L. V. Polezhaev, M. A. Aleksandrova, V. N. Vitvitskii, and L. V. Cherkasova, *Cerebral Tissue Transplantation in Biology and Medicine* [in Russian], Moscow (1993).
3. V. P. Reutov, E. G. Sorokina, V. E. Okhotin, and N. S. Kositsin, *Cyclic Transformations of Nitrous Oxide in Mammals* [in Russian], Moscow (1998).
4. E. I. Chumasov and E. S. Petrova, *Byull. Eksp. Biol. Med.,* **110,** No. 8, 198-201 (1990).
5. D. S. Bredt and S. H. Snyder, *Neuron,* **13,** No. 2, 301-313 (1994).
6. S. Chen and G. Aston-Jones, *Exp. Neurology,* **126,** No. 2, 270-276 (1994).
7. P. Cuevas, F. Carceller, R. Martinez-Murillo, *et al., Neurol. Res.,* **16,** No. 2, 113-115 (1994).
8. G. D. Das and D. N. Ross, *Int. J. Dev. Neurosci.,* **4,** No. 1, 63-79 (1986).
9. C. R. Dermon and A. Stamatakis, *Neuroreport,* **5,** No. 15, 1941-1945 (1994).
10. D. L. Feinstein, E. Galea, J. Germak, *et al., J. Neurochem.,* **62,** No. 2, 811-814 (1994).
11. Y. J. Geng, M. Almqvist, and G. K. Hansson, *Biochim. Biophys. Acta,* **1218,** No. 3, 421-424 (1994).
12. H. Kato, K. Kogure, Y. Liu, *et al., Brain Res.,* **652,** No. 1, 71-75 (1994).
13. D. Lindholm, R. Heuman, M. Meyer, and H. Thoenen, *Nature,* **330,** No. 6077, 658-659 (1987).
14. P. M. Richardson and V. W. K. Issa, *Brain Res.,* **298,** No. 1, 146-148 (1984).
15. C. V. Williams, D. Nordquist, and S. C. McLoon, *J. Neurosci.,* **14,** No. 3, 1746-1755 (1994).