GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Effect of Transplantation of Embryonic Nervous Tissue on Reorganization of Interneuronal Relationships after Mechanical Damage to Sensorimotor Cortex

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The effect of implanted embryonic nervous tissue on restoration of axonal connections in the cerebral cortex after mechanical injury was studied on albino rats using fluorescent lipophilic probe DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate) and confocal laser scanning microscope. Implantation of embryonic tissue to damaged area promotes the growth of axons through the transplant to adjacent tissue. The damaged area is impenetrable for axons growing without implantation.

Key Words: *transplant; lipophilic probe DiI; neocortex; confocal microscopy*

Local damage to the brain of any etiology (ischemic and hemorrhagic stroke, craniocerebral injury, neurosurgical intervention) activates a complex of patho- and sanogenetical mechanisms in the brain, which lead to replacement of damaged region by gliomesenchymal elements and reorganization of the relationships between survived neurons in various cerebral subdivisions. This reorganization proceeds via activation of growth mechanisms in dendrites, dendritic spines, and axons and the formation of synapses. However, it is unclear how the connections between neighboring cortical regions of the brain separated by focal damage are restored during the posttraumatic period, and whether this process can be directly stimulated. We studied recovery of axonal connections between modules of mechanically damaged sensorimotor cortex under the effect of transplanted embryonic nervous tissue (TENT). The fluorescent lipophilic label DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate) used in our experiments possesses a unique property: it diffuses ortho- and antidromically in plasma membranes of fixed brain tissue without crossing synapses. The ortodromic diffusion of DiI stains the axons up to their terminals, while antidromic transport stains somas of neurons and their dendrites with spines [3,4]. Small DiI crystals were placed at one side and close to the local damage in the cerebral cortex. This allowed to trace stained horizontal connections between cortical segments to the symmetrical subdivisions on the other side of the damage and vice versa.

MATERIALS AND METHODS

Experiments were performed on 15 albino male rats (190-210 g). The rats were anesthetized with ether. Surgery was performed at room temperature without specific therapy. In some rats (n=10), a glass transplantation needle (external and internal diameters 0.7

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and 0.5 mm, respectively, and bevel 45°) was introduced via a trephine opening into the sensorimotor cortex and embryonic neocortex (from 15-17-day embryos) was implanted into the perforated area. The transplantation needle contained 2-3 pieces of embryonic cerebral tissue (2-3 mm^3) in 0.05 ml cold physiological saline. The transplants were transferred into specific cortical area (AP -4.3; L 4.0; H 2.75 mm) using a Gyartasi/sz stereotaxic apparatus. In control rats (n=5) the same procedure was perfomed without TENT. Three weeks after cortical puncture, the rats were sacrificed under ether narcosis and perfused with 4% paraformaldehyde in phosphate buffer (pH 7.4) for 15-20 min. Then a small DiI crystal (0.3-0.5 mm) was introduced into strictly defined area of the sensorimotor cortex at a distance of 3 mm from the left side of the implant or puncture at the depth of external Baillarger strip using a transplantation needle and a mandrin manipulated with stereotaxic apparatus. After a 1-7-day exposure at room temperature, 150-200-μ frontal slices of the sensorimotor cortex were prepared, transferred to slides, and examined under a Bio-Rad MRC 600 confocal scanning laser microscope equipped with an argon laser (514 nm, 25 mW power) and a 550-nm filter. This microscope made it possible to clearly visualize the orthodromically labeled axons and differentiate them from the dendrites of antidromically labeled neurons. The images of serial confocal slices (0.5-2.0-μ intervals) were analyzed by superposition.

RESULTS

Ortho- and antidromic staining of neuronal network around damaged area in rats subjected to TENT markedly differed from that in the control. After complete engraftement, orhtodromically stained individual transversal axons were clearly seen in the transplant and at both sides of it against the background of unstained neuropil as early as days 1-2 after application of DiI (Fig. 1). At the same time, the antidromically stained neurons were revealed only near the DiI crystal. Increasing the DiI exposure to 5-7 days showed that the fluorescent label crossed the transplant and stained somas and dendrites of the neurons in the adjacent tissue (Fig. 2). The antidromic diffusion of the fluorescent label along the plasmalemma revealed even very small dendritic arbors and multiple spines of the pyramidal neurons. Staining of the neurons located close to the transplant, *i.e.* in the area with disturbed the connections between cortical modules, attests to restoration of these connections. It should be noted that the used fluorescent probe can not be transported to the examined area via other polysynaptic projections because of peculiar localization of DiI (lower third of cortical layer III, *i.e.* lateral Baillarge strip) and pecu-

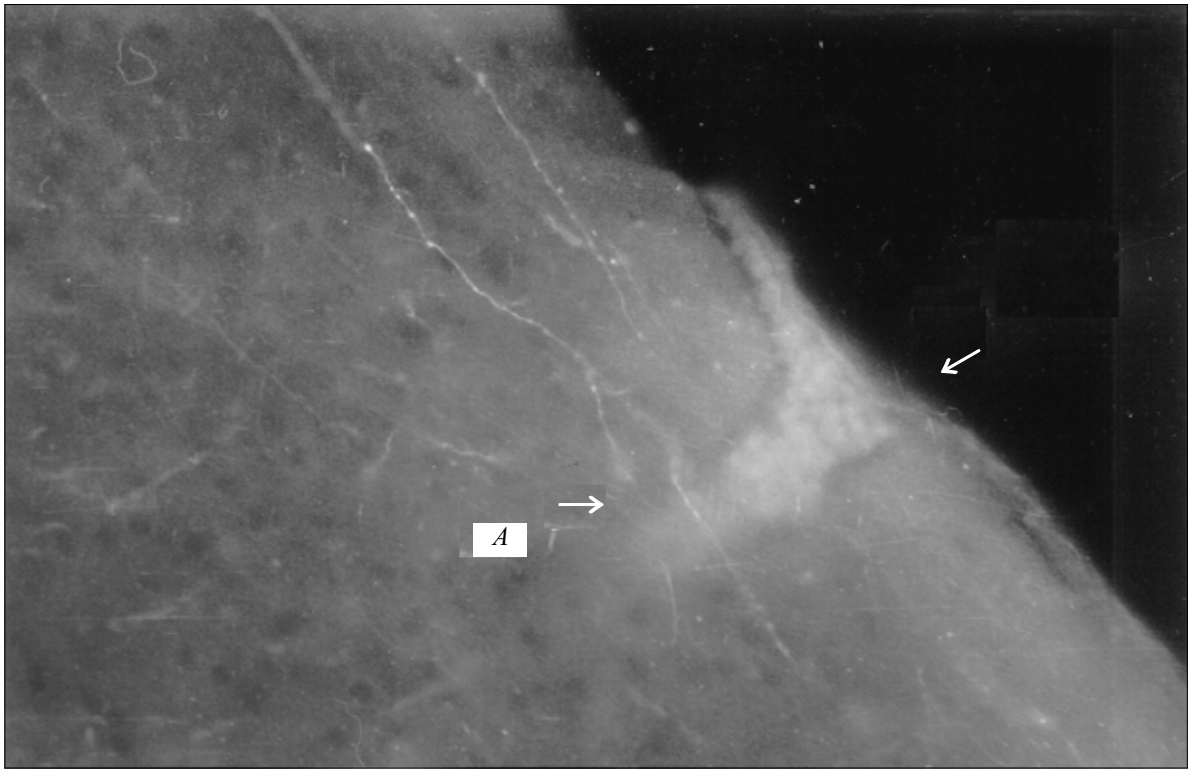

Fig. 1. Orthodromically labeled axons (A) in the transplant (arrows). Fluorescent DiI staining, ×50.

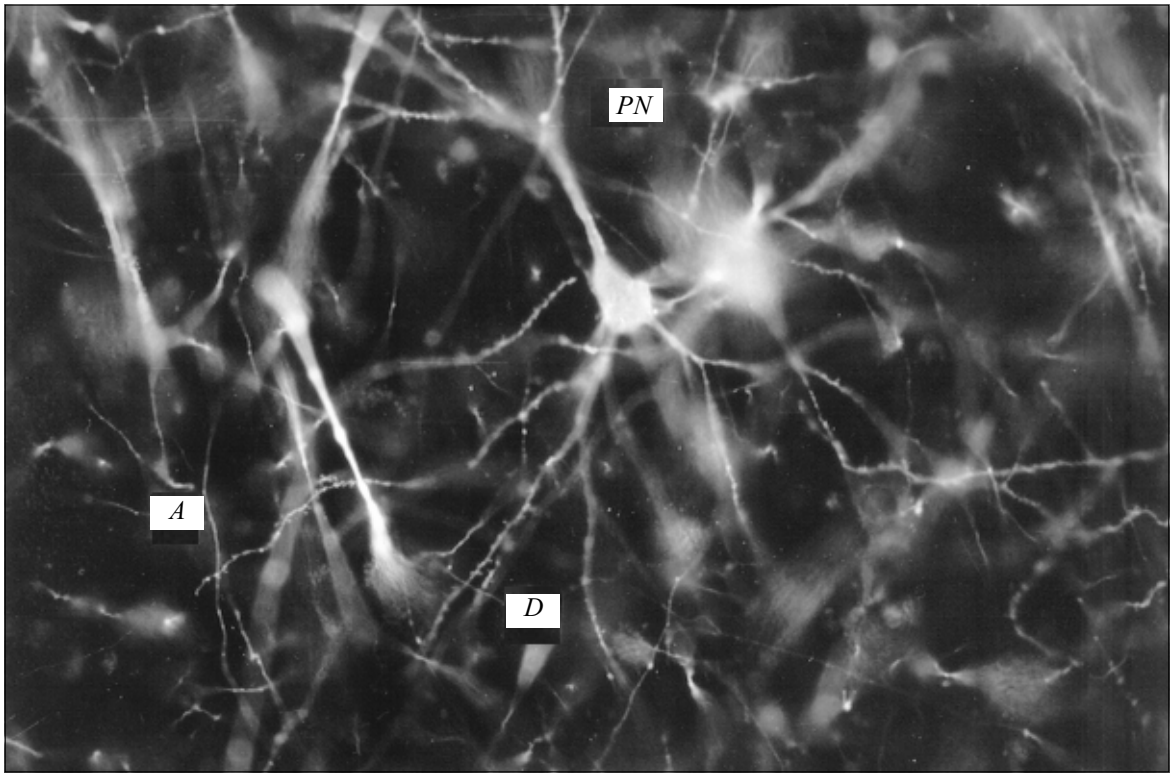

Fig. 2. Antidromically labeled pyramidal neurons (PN) in layer III of rat sensorimotor cortex. Fluorescent DiI staining, ×600. D: dendrites, A: axon.

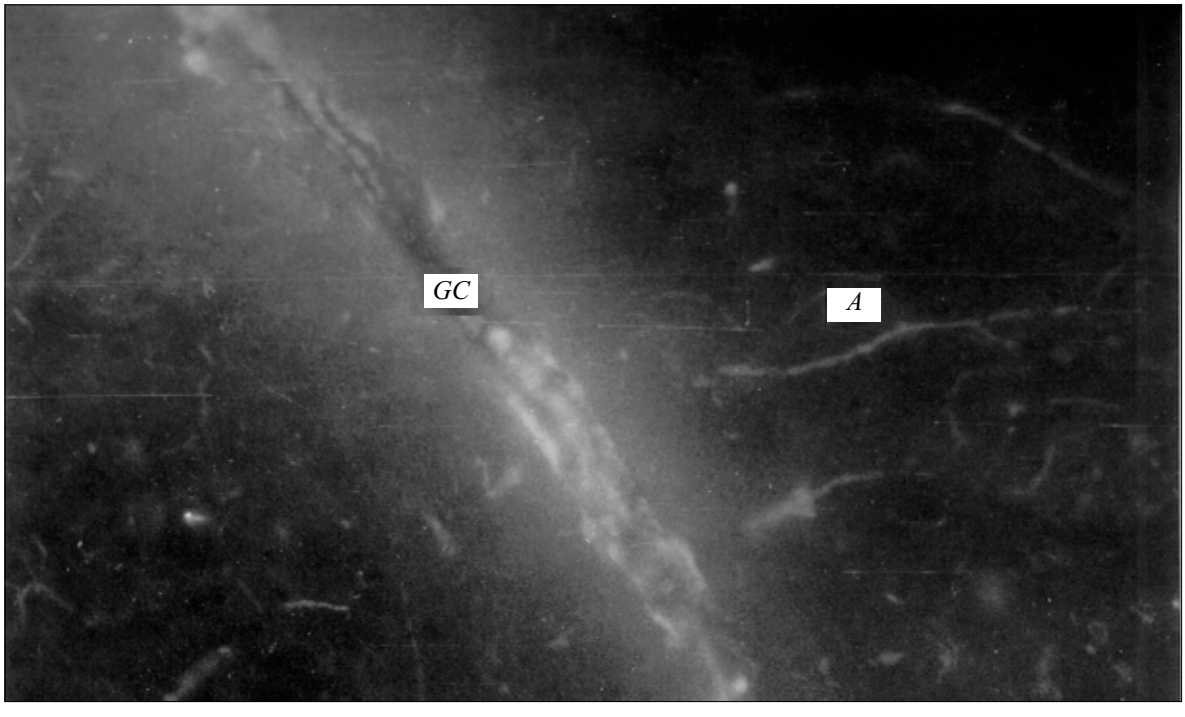

Fig. 3. Orthodromically labeled axons (A) outside glial cicatrix (GC) formed after perforation with a transplantation needle. Fluorescent DiI staining, ×50.

liarities of staining (probe diffuses only along the plasma membranes).

The density of antidromically stained neurons in layer III of the sensorimotor cortex depended on their localization. The density of labeled neurons was minimum in the area separated from the label by the transplant and maximum around the DiI crystal (Fig. 3). The density of antidromically stained neurons in front

of the transplant 2-3-fold surpassed that immediately behind it, which attests that only few damaged axons in layer III grow through the transplant and contact with neurons in neighboring modules. The density of antidromically stained neurons decreased even more at a distance from the transplant. It indicates that only axons of the neurons adjacent to the transplant reach the area of DiI localization. We observed no antidromically-stained neurons in the transplant. Probably, even if the transplant axons penetrate into recipient brain, the area of their growth is limited, and they cannot reach the level occupied by DiI crystal.

In cerebral cortex of the control rats or in rats with unsuccessful transplantation, the gliomesenchymal cicatrix formed after puncture was insurmountable for tangentially growing cortical axons. This was confirmed by complete absence of orthodromically labeled axons in the cicatrix area and at the side opposite to location of DiI crystal. Moreover, the density of antidromically labeled axons around the cicatrix was characterized by more pronounced asymmetry compared to rats with TENT. After a 5-7-day exposure, no labeled neurons were detected near the cicatrix at the site opposite to the DiI crystal.

Therefore, our data provide additional arguments in favor of active influence of TENT on post-traumatic reorganization of interneuronal relationships of neighboring structural and functional modules of the cerebral cortex. TENT induces partial restoration of connections between damage-separated cortical regions due to creation of favorable conditions for growth of the axons in the area affected by neurotrophic factors of the transplant. The existence of such influence is experimentally proved and vigorously discussed in literature as indication of efficient plastic capacity of the damaged brain in sexually mature animals [1]. Consequently, cell transplantation is now considered is a new and perspective therapeutic strategy aimed at functional recovery of CNS in humans [5, 7-9]. The possibilities of cellular transplantation and its clinical application are now actively studied [6].

Our findings on the use of embryonic cerebral tissue as a medium for axon growth attest to prospectiveness of the development of communication bridges between intact brain regions.

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