Neuroprotective Effect of Mesenchymal and Neural Stem and Progenitor Cells on Sensorimotor Recovery after Brain Injury

R. A. Poltavtseva[1], D. N. Silachev[2], S. V. Pavlovich[1], M. I. Kesova[1], K. N. Yarygin[3], A. Yu. Lupatov[3], L. V. Van'ko[1], M. P. Shuvalova[1], and G. T. Sukhikh[1]

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We studied the effect of systemic administration of multipotent stem cells on impaired neurological status in rats with brain injury. It was found that transplantation of multipotent mesenchymal stromal cells of the bone marrow or human neural stem and progenitor cells to rats with local brain injury promoted recovery of the brain control over locomotor function and proprioceptive sensitivity of forelegs. The dynamics of neurological recovery was similar after transplantation of fetal neural stem and progenitor cells and multipotent mesenchymal stromal cells. Transplantation of cell cultures improved survival of experimental animals. It should be noted that administration of neural stem and progenitor cells prevented animal death not only in the acute traumatic period, but also in delayed periods.

Key Words: *local brain injury; neurological status disturbances; rat survival; intravenous transplantation of fetal multipotent mesenchymal stromal cells and human neural stem and progenitor cells*

Traumatic brain injury is one of the major causes of death and disability among working population in many countries in the world. In view of low efficiency of modern neuroprotective drugs, the search for new methods for the therapy of brain injuries is a pressing problem. Numerous studies showed that SC transplantation ensures long-lasting clinical effect during treatment of various neurodegenerative pathologies and ischemic and traumatic injuries of the spinal cord and brain [7]. Evaluation of the possibility of using cell technologies, in particular, transplantation of various cell cultures for posttraumatic brain tissue recovery, is a promising trend. Pathophysiological mechanisms of traumatic brain damage are related to primary disturbances (physical and biomechanical effects of the trauma) and secondary neurodegenerative changes progressing after trauma. Modulation of secondary death of brain cells after trauma and posttraumatic recovery of the nervous tissue seems to be most promising aspects for application of cell technologies.

Here we studied the effect of transplantation of fetal multipotent mesenchymal stromal cells (MMSC) and neural stem and progenitor cells (NSC) on correction of neurological dysfunctions (dysregulation of motor and proprioceptive functions of forelegs and pathological changes in the brain of rats with experimental local traumatic brain injury (TBI).

[1]V. I. Kulakov Research Center of Obstetrics, Gynecology, and Perinatology, Ministry of Health and Social Development of the Russian Federation; [2]A. N. Belozerskii Institute of Physicochemical Biology, M. V. Lomonosov Moscow Medical University; [3]V. N. Orekhovich Research Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** rimpol@mail.ru. P. A. Poltavsteva

MATERIALS AND METHODS

Experiments were carried out on outbred albino male rats weighing 300-350 g. The study was performed with strict adherence to EU Directive 86/609/EEC on Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The animals were divided into 3 groups. Group 1 comprised rats with TBI (control; n=11); groups 2 and 3 (n=11 each) included rats with TBI receiving transplantation of MMSC and NSC, respectively, on the next day after TBI. The rats were daily weighed for monitoring of their physiological state.

Before TBI modeling, the animals were narcotized with chloral hydrate (300 mg/kg, intravenously). TBI was modeled by the method of graded and reproducible focal cortical contusion [4]. To this end, the animal was placed into stereotaxis, the skin on the head was shaved and cut along the medial longitudinal line. The skull above the sensorimotor cortex (2.5 laterally and 1.5 mm caudally from the bregma) was drilled with a cutter (diameter 5 mm). The trauma infliction setup was positioned above the dura matter so that the striker was located 3 mm below it. A cylindrical striker was placed into the drilled hole above the dura matter. A load (50 g) was dropped from a height of 10 cm to the striker for TBI modeling. During these manipulations, the body temperature was maintained at 37±0.5°C.

The culture of fetal stem and progenitor cells was isolated from human fetuses (abortion autopsy material) obtained from institutions licensed by Ministry of Health and Social Development of the Russian Federation acting under the laws of the Russian Federation.

Fetal MMSC cells were washed out from long bones with DMEM (PanEko) containing 2 mM EDTA as the anticoagulant. The suspension was fractionated by centrifugation (30 min at 200g; Jouan) in Ficoll-urografin density gradient (1.077 g/ml; PanEko). Mononuclear cell fraction at phase interface was collected, resuspended in the medium, and re-centrifuged at 150g for 5 min. The pellet was resuspended again in culture medium containing DMEM/F-12 (1:1, Gibco), 10% ECS (PanEko), 2 mM L-glutamine (PanEko), and 20 μg/ml gentamicin (PanEko), transferred to flasks (25 cm^2, Corning), and cultured in an incubator at 37°C and 5% CO_2 for 9 days.

Isolation and culturing of NSC were performed as described previously [1]. NSC were isolated from fetal brain autopsy material (9-10 week gestation). The cells (2×10^6/ml) were cultured for 3 weeks in flasks with DMEM/F-12 (1:1), N2 additive (1:100, Gibco), 20 ng/ml bFGF-2 (Sigma), 20 ng/ml EGF (Sigma), 8 μg/ml heparin (PanEko), 2 mM L-glutamine, and 20 μg/ml gentamicin.

Before transplantation, the cells were dissociated with 0.25% trypsin (PanEko) and centrifuged in DMEM (5 min at 200g) after trypsin inactivation. The final concentration of viable cells before transplantation was 10^6/ml in 0.9% NaCl. Cell viability was evaluated by trypan blue or propidium iodide staining. Cells demonstrating 95% survival after dissociation were used for transplantation. The cell culture was transplanted to rats into the right jugular vein [8] under isofluorane narcosis 1 day after TBI modeling. Immunosuppression was not used.

Neurological status was evaluated before TBI modeling and on days 3, 5, 7, 14, and 21 in limb stimulation test and on day 21 in the cylinder test.

Asymmetry of forelimb using was evaluated in the cylinder test during spontaneous exploration of the cylinder walls [9].

Sensorimotor recovery of the limbs was evaluated using a modified version of limb stimulation test [5] by the response of the forelimb and hind limbs to tactile, proprioceptive, and visual stimulation. The test consisted of 5 trials for the left and right limbs. Disorders in limb functioning were scored using a 3-point scale: complete performance (2), delayed (by more than 2 sec) and/or incomplete performance (1), and no response to stimulation (0).

The animals were sacrificed with high chloral hydrate doses. Brain tissue samples were fixed in 10% neutral formalin, embedded in paraffin, and the sections were stained with hematoxylin and eosin.

The data are presented as arithmetic mean±standard error of the mean. The normality of sign distribution in the sample was evaluated using Shapiro–Wilk test. For evaluation of statistical significance of differences in behavioral tests, 95% confidence interval (CI) for relative incidences, Mann–Whitney U test for independent variables, and Wilcoxon test for dependent variables were used. The data were significant at 0.05.

RESULTS

Local brain trauma not only destroys the sensorimotor area of the cortex responsible for motor activity of the forelimbs, but also produces a pronounced traumatizing effect on the whole organism [2,4,13,10]. In our experiments, most cells died on day 3 after trauma. In group 1, 8 rats died on day 13 (95% CI 0.060-0.610; Fig. 1). Systemic administration of NSC in group 3 completely prevented animal death (95% CI 0.715-1.00; Fig. 1). The effect of MMSC on animal survival was also positive, but less pronounced. In group 2, 6 rats died on day 21 (95% CI 0.234-0.883; Fig. 1), the rest died but later than in group 1. This attests to a positive effect of SC transplantation.

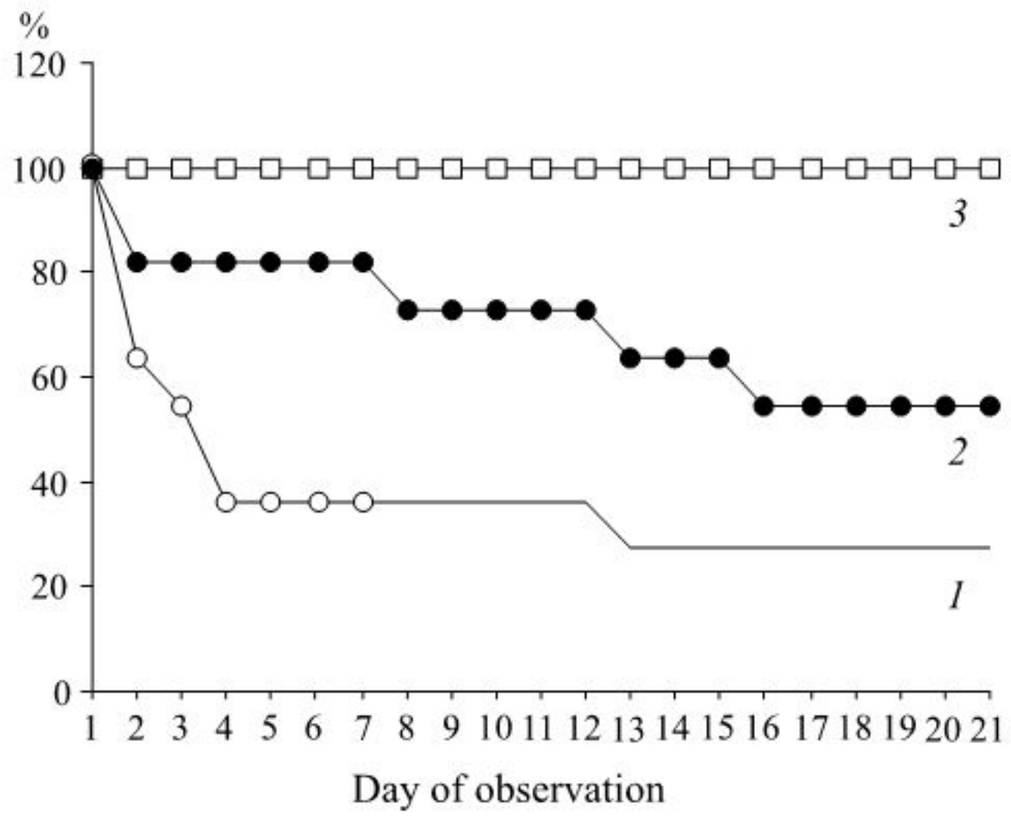

Fig. 1. Dynamics of rat survival after local brain trauma. Here and in Fig. 2: *1*) trauma; *2*) trauma+MMSC; *3*) trauma+NSC.

In the cylinder test (Fig. 2, *b*) before ischemia induction, independent use of the contralateral and ipsilateral forelimbs constituted 20% of all touches to the cylinder wall. Analysis of forelimb-use asymmetry on day 21 after surgery showed that control rats 2-fold less used forelimbs ($p<0.05$; to 8.6% in comparison with the corresponding value before surgery). The use of contralateral forelimb compensatory increased to 45% (in comparison with the value before surgery). Transplantation of SC and progenitor cells promoted recovery of disturbed sensorimotor functions. In groups with MMSC and NSC transplantation, the compensatory use of ipsilateral forelimb decreased ($p<0.05$) and the use of contralateral forelimb returned to the baseline value (Fig. 2, *b*). These findings attest to appreciable recovery of impaired functions starting from day 3 after TBI modeling.

Theoretically, two pathways of regeneration of damaged brain tissues are possible. Replacement therapy implies that intravenously transplanted SC are incorporated into various structures of the damaged brain, differentiate into specialized neural cells, and integrate into the microenvironment with the expression the corresponding neurotransmitters and receptors and formation of axonal outgrowths [8]. According to the paracrine stimulation model, transplanted SC stimulate remaining neurons or induce regeneration of damaged neurons via production and release of neurotransmitters and neurotrophic factors or induction of endogenous neurotrophic factors [12]. MMSC and NSC differ by differentiation directions and the repertoire of produced growth factors, cytokines, and other bioactive substances [6,11,13].

Our findings suggest that MMSC primarily affect regeneration of vessels and produce some factors exhibiting anti-inflammatory and cytoprotective effects [11]. NSC have primarily neuroprotective effects. Paracrine secretion of these factors by the transplants stimulates growth factor production in brain cells. Thus, experimental trauma was accompanied by enhanced production of neurotrophin 3 by nerve cells, while accumulation of VEGF was observed in cortical neurons and vascular plexuses [2].

It is now accepted by the majority of researchers that neuroprotective effects of donor SC and progenitor cells are determined by stimulation of regeneration of own cells, rather than replacement of lost cells [3,7].

We should not overestimate the role of local destruction of this or that brain area in the development of traumatic syndrome. In our experiments we dealt with both local brain trauma and systemic reaction of the organism.

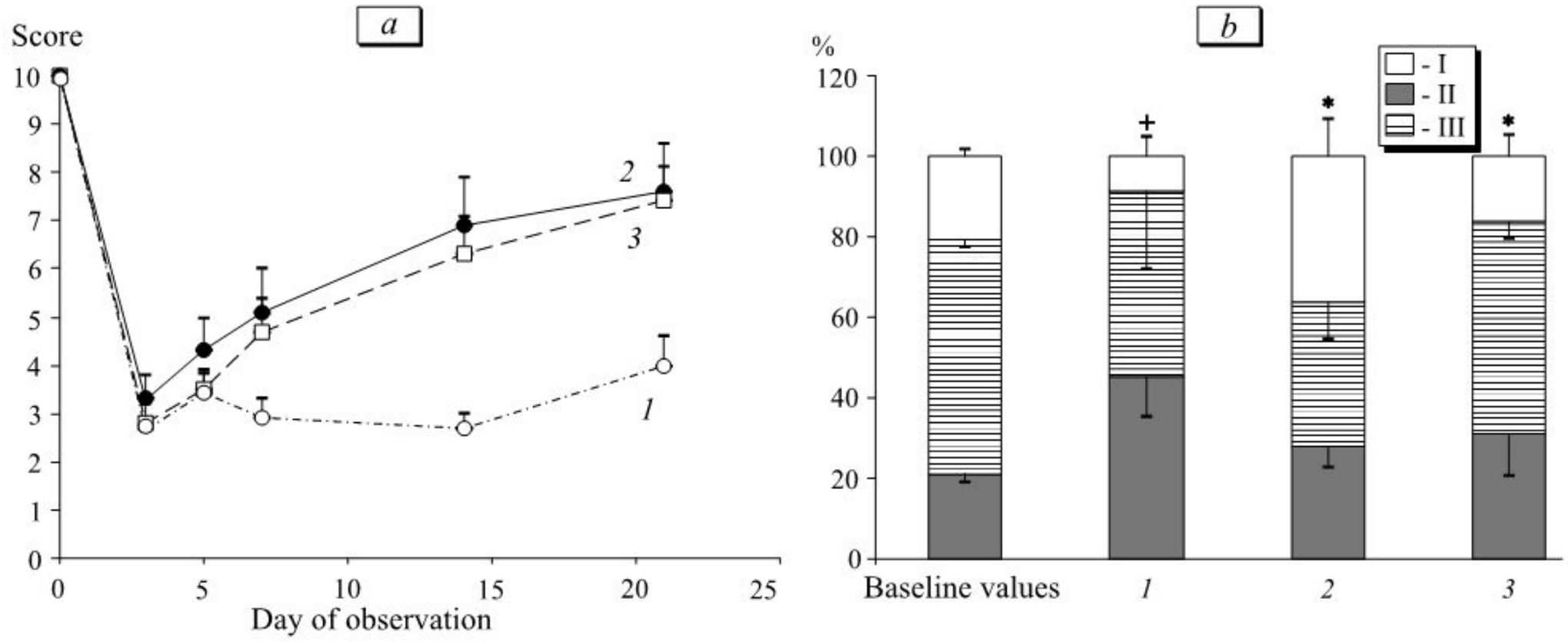

Fig. 2. Evaluation of brain status in limb stimulation (*a*) and cylinder (*b*) tests. I: contralateral limb; II: ipsilateral limb; III: both limbs simultaneously. $p<0.05$ in comparison with: *control group, +baseline values.

The choice of behavioral tests for evaluation of the neurological status of the damaged brain was dictated by predominant influence of the destruction of a certain brain area on the development of traumatic syndrome. The tests were aimed at detection of dysregulation movements and proprioceptive sensitivity. The neurological status gradually decreased on days 1-3; this period coincided with the start of brain tissue regeneration, preceding recovery of neurological status in all three groups of experimental animals (Fig. 2, *a*). The positive effect of SC transplantation was observed starting from day 7. Complete recovery was not attained to the end of the experiment (Fig. 2, *a*). No significant differences in the effect of MMSC and NSC transplantation were found.

Pathomorphological disturbances in the brain tissue after trauma and during therapy were analyzed only within the area of traumatic injury (Fig. 3). In all rats, serious destructive changes of the brain tissue were seen on day 21 of the experiment. They were most pronounced in group 1 (Fig. 3, *a*). Destruction involved all cortical layers and almost reached the corpus callosum. The cell detritus was washed out during preparation processing; fragments of damaged dura mater and pia matter were seen above the formed cavity. The walls of this cavity were coated with a thin layer of vacuolated neuropil. No neuronal processes and neurons were detected at the lesion boundary. Signs of local inflammation were seen (in particular, phagocytes), but considerable hemorrhages were absent.

On day 21 after trauma, destruction of the brain tissue was less pronounced in rats of groups 2 and 3 (Fig. 3, *b, c*); first of all, the formed cavity was less deep. The signs of the inflammatory process were less pronounced. The cavity bottom was filled with loose granulation tissue with numerous thin-walled vessels. A thin layer of vacuolated neuropil with incorporated normally looking neurons and glial cells was found under the granulation layer.

The signs of nerve tissue recovery in the damaged area were most pronounced in group 3 rats: on day 21 the depth of tissue destruction did not exceed 1-3 cortical layers (Fig. 3, *c*). In this group, no signs of inflammation were seen and phagocytic infiltration was absent. The observed differences in morphological picture of brain injury in animals of different

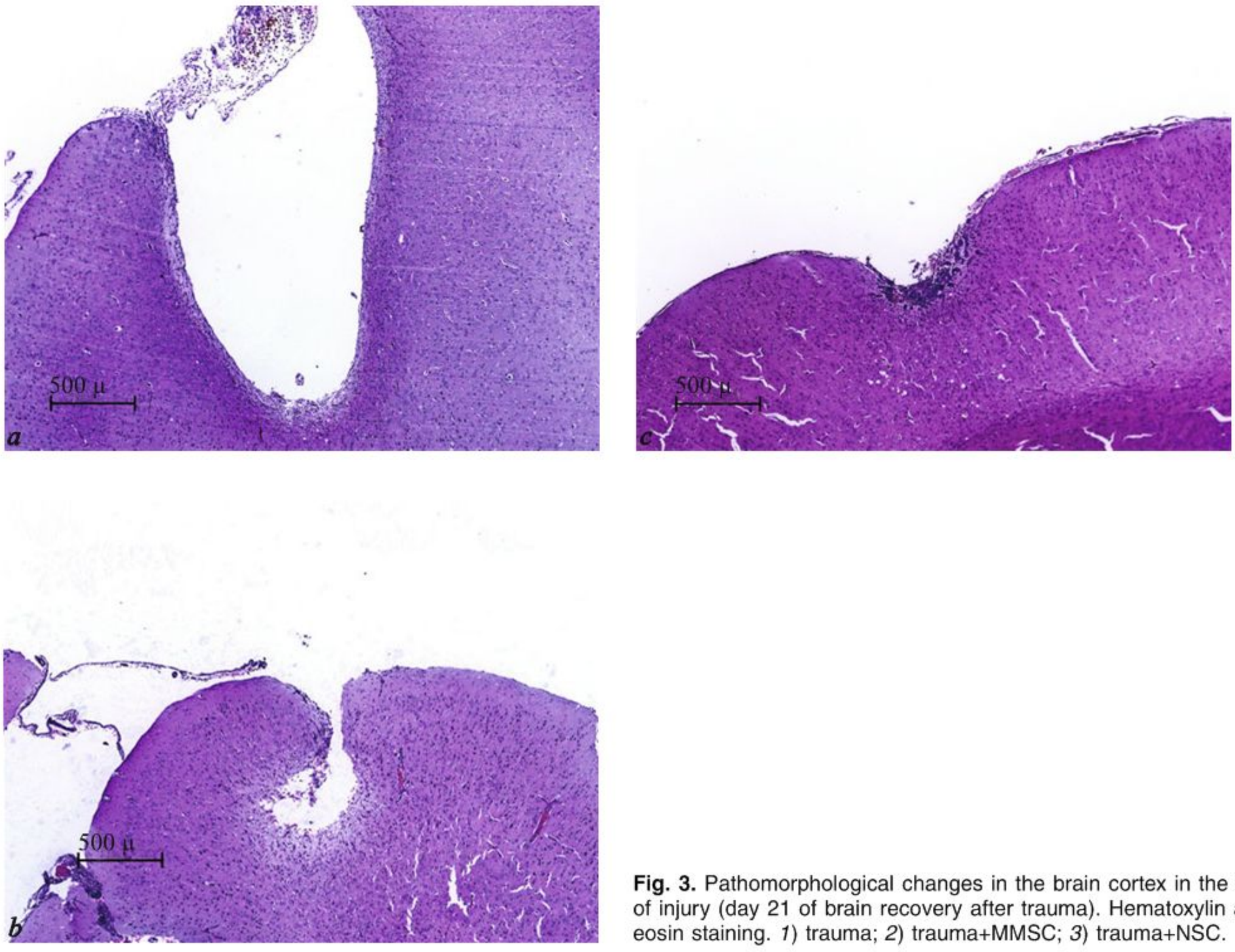

Fig. 3. Pathomorphological changes in the brain cortex in the site of injury (day 21 of brain recovery after trauma). Hematoxylin and eosin staining. *1*) trauma; *2*) trauma+MMSC; *3*) trauma+NSC.

groups can be attributed to the effect of transplanted cells, because the trauma was inflicted similarly in all cases.

We demonstrated that morphological and sensorimotor regeneration of the damaged rat brain is possible after transplantation of both MMSC and NSC. Both transplanted cell types to a greater or lesser extent restored the morphology and function of the damaged brain. Differences in the effects on animal survival can be explained by the mechanisms of neurological sensorimotor deficit restoration. The factors released by MMSC produce primarily anti-inflammatory and neuroprotective effects, but cannot protect from secondary contusion damage causing animal death. In our experiments, NSC produced more pronounced positive effect on animal survival, which can be explained by the effect of neurotrophic factors that protected not only from the primary damage of the brain tissue, but also prevented contusion changes and edema and considerably reduced animal mortality.

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