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Neuroprotective Effect of Allogeneic Biomaterial on Rat Neocortex After Its Intramuscular Injection

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Abstract

Background: Forced physical activity causes a violation of all organs and systems interactions. Allogeneic biomaterial has been used for many years for regeneration, but remote exposure hasn't been studied.

Objectives: The aim of this research is the morphological study of experimental animals' precentral gyrus neocortex under conditions of background forced physical activity and after intramuscular injection of allogeneic biomaterial.

Methods: Male Wistar rats were used for the experiment. The Porsolt test, or despair test, was used for 30 days. In the main group (n=10), after all 30 swimming sessions, allogeneic biomaterial (BMA) was injected intramuscularly. In the control group (n=10), the animals were injected with saline in the same volume. 5 and 21 days after the injections, a tolerance load test was performed, after which the animals were taken out of the experiment by insufflation of a lethal dose of chloroform vapors, the brain was removed, and morphological studies were performed.

Result: In the experimental group 5 days after the BMA injection, the median level of the multiplicity of tolerant load was significantly higher than in the control group, respectively, and remained so in the long term. The neocortex of the control group animals was characterized by

the development of pathomorphological changes. After 21 days, these signs persisted. In the early period after the BMA injection, no signs of nervous tissue edema were detected. A clear architectonics of the neocortex neurocytes layers was preserved.

Conclusions: Allogeneic biomaterial has a positive systemic effect on the organism. Also, neuroprotective and immunomodulating effects have been recorded.

Keywords: allogeneic biomaterial, cerebral cortex, forced swimming, neuroprotection, rats.

Introduction

The sport of the highest achievements, as a rule, is associated with extreme physical and emotional stress, which imposes increased demands on health. In most cases, overwork and overtraining are superimposed on each other, giving a symptom complex of an organism's function disturbances, including the central nervous system (CNS), which can be considered a neurosis (Alekhin et al., 2023; Kholodny *et al.,* 2021; Zehtabvar *et al.*, 2023; Meftani *et al.,* 2023). Overfatigue disrupts the coherence of interactions between the cerebral cortex, underlying parts of the nervous system, and internal organs (Ilyin and Alvani, 2016; Trouski and Parham, 2021; Jaber Al-Mamoori *et al.,* 2022). Allogeneic biomaterial is known as a regeneration

stimulator when applied locally, including skeletal muscle tissue (Lebedeva *et al.* 2019 a). However, studies of systemic influence, remote action, and feedback have not previously been conducted. To identify the pharmacological effects of the allogeneic biomaterial on the central nervous system, the behavioral despair test or Porsolt forced swimming test (forced swimming to complete fatigue with a load that reflects the state of depression of the animals) was performed. This test is a complex, severe stress that combines physical and emotional components (Khabibullin *et al.* 2019). The purpose of the research was to study the experimental animals' cerebral cortex structure after forced physical activity and allogeneic biomaterial use.

Materials and methods

In the experiment, mature male Wistar rats weighing 200–240 g (N=20). The rats were kept in plastic cages at a temperature of 22-24ºC, fed with pelleted mixed fodder. Water was supplied ad libitum (to the will). Before the start of the "forced swimming" test, all experimental rats were taught to swim for 3 days without a load for 10 minutes once a day.

The model of anaerobic physical activity was chosen as the method of forced swimming of rats until complete fatigue with a load, the Porsolt test, or the despair test (Zhou *et al.* 2021) in modification (patent for invention No. 261706 dated April 21, 2017). The swimming test was

conducted daily for 30 consecutive days, from 9:00 a.m. to 11:00 a.m. The weight of the animals was measured daily. The weight of the load was selected in accordance with the weighing results and was equal to 10% of the body weight.

In the main group (n=10), after all 30 training sessions, a dispersed allogeneic biomaterial (BMA) suspension was injected intramuscularly. For this, 1 vial (10 mg) was diluted in 5 ml of saline to achieve a 0.2% concentration. A total of 8 intramuscular single injections were made into the muscles of the thoracic limbs (the shoulder biceps (*m. bicepsbrachii*), the superficial muscles of the forearm flexors - the ulnar and radial flexors of the wrist (*m. flexorcarpiulnaris*, *m. flexorcarpiradialis)),* and the pelvic limbs (gastrocnemius (*m. tricepssurae*), quadriceps femoris (*m. quadriceps femoris*)). 0.5 ml of BMA suspension was injected (4 ml in total). The dose of BMA was chosen arbitrarily. As BMA dispersed form the "Alloplant" biomaterial, with a particle size of 50–80 μm, was used. The "Alloplant" biomaterial was developed at the All-Russian Center for Eye and Plastic Surgery, Ministry of Health of the Russian Federation, Ufa. The biomaterial is manufactured in accordance with the technical specifications 42-2-537-87, certified and approved for use in clinical practice by order of the Ministry of Health of the USSR No. 87 901-87 of July 22, 1987. For the present study, BMA was made from the extracellular

matrix of rat tendons. In the control group (n=10), the animals were injected with physiological saline in similar zones and at the same volume.

After 5 and 21 days after the injections, a tolerance load test was performed, and swimming time (minutes) was recorded. After that, the animals were taken out of the experiment by insufflation of a chloroform vapor lethal dose (695 mg/kg). (Recommendation of the EEC No. 33; Material Safety Data Sheet, Chloroform MSDS) Decapitation and extraction of the brain were carried out. For histological examination, tissue pieces of the precentral gyrus were fixed in a 10% neutral formalin solution, dehydrated in a series of alcohols of increasing concentration, and embedded in paraffin according to the generally accepted method. Histological sections were prepared on a LEICA RM 2145 microtome (Germany) and stained with hematoxylin and eosin.

For immunohistochemical studies, paraffin sections 4 μm thick were stained using a LeicaMicrosystemsBond™ immunohistotainer (Germany). The primary antibodies used were CD 68 at a dilution of 1:300 (ED1 clone), Gfap at a dilution of 1:300, and bcl-2 (SantaCruzBiotechnology, USA). The indirect streptavidin-biotin detection system LeicaBOND (Novocastra™, Germany) was used for staining. (Ozawa, 2019; Magaki *et al*., 2019; Sukswai and Khoury, 2019; Moreno *et al*., 2022; Prichard, 2014).

For electron microscopic examination, tissue pieces were fixed in 2.5% glutaraldehyde solution prepared in cacodylate buffer (pH 7.2–7.4) with additional fixation in 1% OsO4 solution in the same buffer. The material was dehydrated in alcohols of increasing concentration and poured into Epon-812 according to the generally accepted method. Semithin sections 1 μm thick were preliminarily prepared and stained with toluidine blue in a 2.5% anhydrous soda solution. On these sections, areas for electron microscopic examination were selected. Semithin and ultrathin sections were prepared on an EM UC 7 ultratome (Leica, Germany). Ultrathin sections were counterstained with a 2% aqueous solution of uranyl acetate and lead citrate, according to Reynolds, and studied under a JEM-1011 transmission microscope (Jeol, Japan) at an accelerating voltage of 80 kV. (Condello *et al.,* 2013; Kim *et al.*, 2021; Klang *et al.,* 2013).

Cells were counted in 20 fields of view for each sample. The examination and visualization of samples were carried out using a Leica DMD 108 microscope (Germany) with specialized software for controlling settings and image capture. Kruskal-Wallace rank analysis of variance was used: median (Me) and quartiles (Q1 - 25%; Q3 - 75%), and the Mann-Whitney test to compare the results of individual observation periods within one series of experiments or between them (Schlattmann *et al.* 2019). Differences were considered statistically significant at $P<0.05$. The statistical software package Statistica 10.0 was used.

The experiment was approved by the ethical committees of Bashkir State Medical University and Saint Petersburg State University of Veterinary Medicine.

Results

In the experimental group after the administration of BMA, after 5 days, the median level of multiplicity was significantly ($Z=2.17$, $P<0.04$) higher than in the control group: Me=1.70 (Q1=1.46, Q3=2.01) and Me=1.00 (Q1=0.62, Q3=1.14) respectively. After 21 days in the experimental and control groups, the level of multiplicity increased insignificantly (Me=1.88; Q1=1.88, Q3=2.02) and Me=1.47; Q1=1.37, Q3=1.56), respectively, but not significant ($Z=0.96$, $P>0.37$ and $Z=1.85$, $P>0.07$). At the same time, in the experimental group, the excess of the multiplicity level over the control group remained and remained statistically significant ($Z=2.13$, $P<0.04$). (Fig.1)

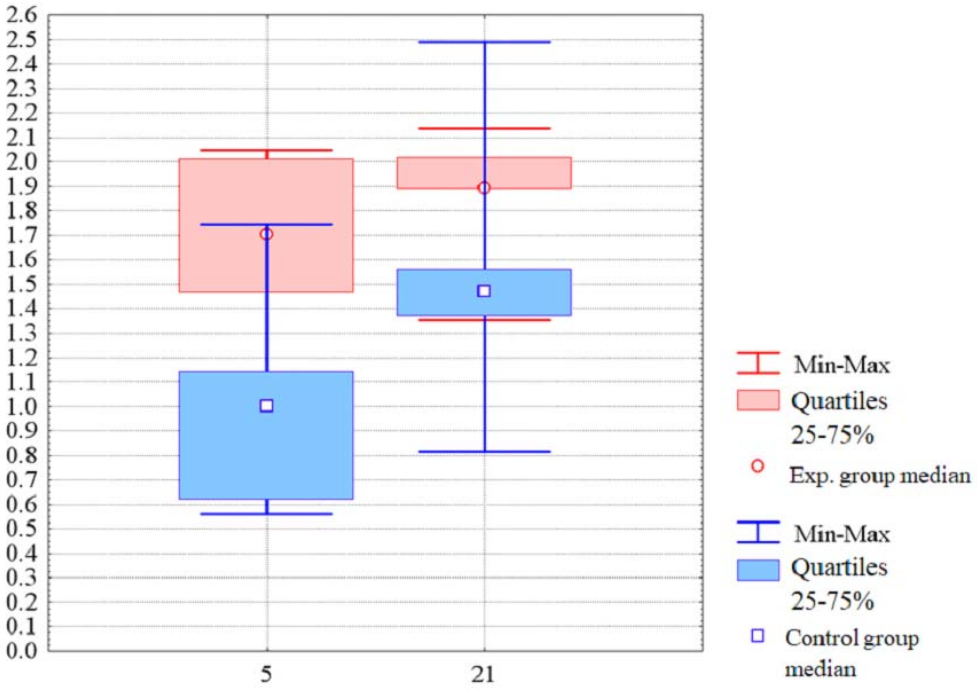


Figure 1. Multiplicity of swimming duration in rats after exhausting physical activity under conditions of intramuscular injection of BMA (experimental group) and saline (control group). The x-axis shows the number of days after BMA administration. On the y-axis, the multiplicity in fractions of a unit.

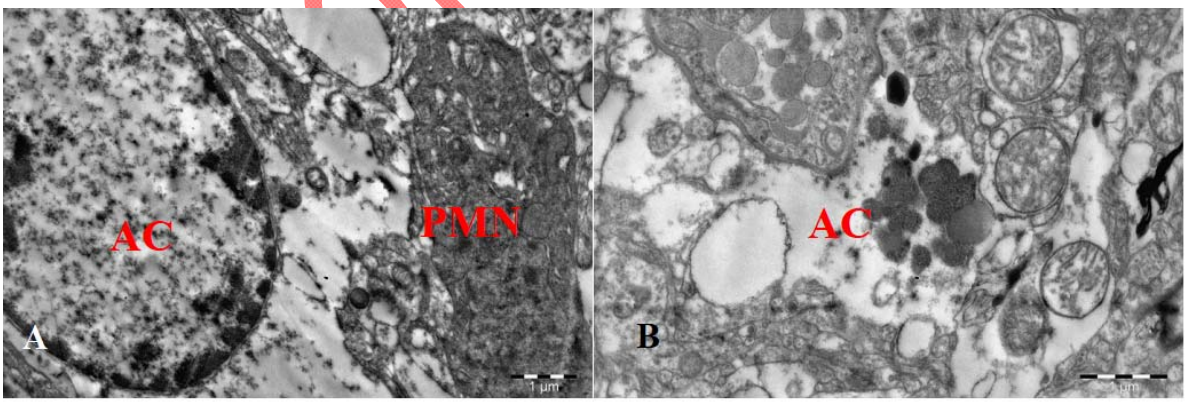
Morphological analysis revealed that in the control group, after 5 days, in the cerebral cortex of the precentral gyrus, all the cell layers characteristic of the cerebral cortex were traced.

However, there were many hyperchromic pycnomorphic nerve cells of medium and small sizes with a dense elongated nucleus and jagged edges, containing a dense osmiophilic nucleoplasm, in which signs of chromatin disorganization and condensation were revealed. The optically dark cytoplasm contained hypertrophied Golgi complexes with dilated canals, signs of secretion stagnation in them, and condensation of the granular cytoplasmic reticulum. These cells were often detected in direct contact with macroglial cells - astrocytes. The perinuclear cytoplasm in this cells was optically clear due to the accumulation of vacuoles and total destruction, followed by the reduction of cytoplasmic organelles. In astrocytes, both the bodies themselves and the terminal processes surrounding the capillaries were edematous and showed signs of organelle destruction. In them, lipofuscin inclusions (aging or "wear-and-tear" pigment) were observed. Also, there was a thickening of certain sections of the basement membrane, a large number of pinocytic vesicles in the cytoplasm of endothelial cells and pericytes, microvesicular bodies,

which is not typical for the norm. The capillary lumen was not free - it was filled with cell fragments and various inclusions (Fig. 2). Consequently, signs of pericellular and perivascular edema were observed, which is evidence of a breach of the blood-brain barrier.

Figure 2. Edema of astrocytes in the cerebral cortex of the precentral gyrus of a rat after forced swimming and intramuscular injection of saline after 5 days. A – Contact of an astrocyte (AC) and pycnomorphic neuron (PMN) B – Swelling of the terminal pedicles of astrocytes (AC). Electronogram.

The neurocyte profiles were sharply expanded, the organelles were vacuolized, the mitochondria were enlarged, rounded with partial fragmentation of the cristae. The synaptic apparatus was poorly developed (Fig. 3).



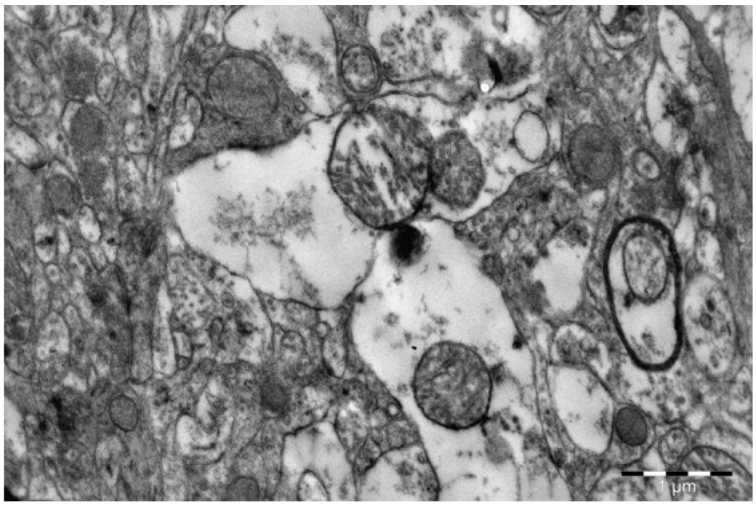


Figure 3. Vacuolization of the neuropile in the precentral gyrus' cerebral cortex after forced swimming and intramuscular injection of saline after 5 days. Electronogram.

In the inner pyramidal layer, along with a small number of hyperchromic neurocytes, light cells were observed - shadows ("melting neurons"), which arose as a result of their chromatolysis (irreversible necrobiotic changes). Microglial cells were often detected in close contact with necrobiotically altered neurocytes - Betz cells.

21 days after the intramuscular injection of physiological saline, signs of trophic disorders, the blood-brain barrier persisted. Destruction of lamellar structures and accumulation of aging pigment were determined in neurons. Apoptotic-altered cells and glial proliferation were noted. All layers of neurons in the cerebral cortex were viewed. The outer granular layer

was thinned and sparse. Zones of local accumulation of glial cells - glial scars - formed in the surrounding brain tissue. Neurocytes were detected in a state of programmed cell death - apoptosis. The nuclei of these cells were hyperchromic with condensed clumps, and euchromatin was absent. The cytoplasm was marked by an increased electron density due to ribosomal dispersion. The cisternae of the Golgi complex are vacuolated, sharply dilated, and elongated. Their hypertrophy was observed. Mitochondria are dark, destroyed, cristae were not determined. The cytoplasm contained numerous phagosomes and residual bodies (Fig. 4).

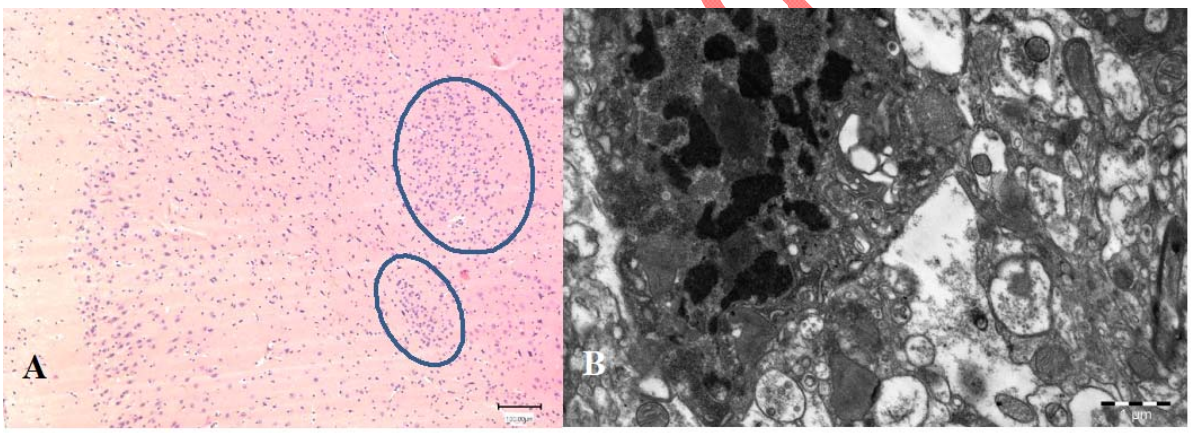


Figure 4. Morphological changes in the nervous tissue in the precentral gyrus after forced swimming and intramuscular injection of saline after 21 days. A- Focal proliferation of glial

cells. Hematoxylin and eosin staining. B-Apoptosis of the hyperchromic neurocyte. Electronogram.

After 5 days, no pathomorphological signs in the form of cell edema and inflammatory cell infiltration were detected in the autopsy material of the cerebral cortex of rats in the experimental group. On histological samples it was possible to identify all the layers characteristic of the cerebral cortex. Pyramidal normochromic neurocytes in this period showed signs of mosaic structural and functional activity. On the one hand, the nucleus was large, outlined by a clear double karyolemma, in which nuclear pores were revealed. Euchromatin uniformly filled the entire karyoplasm. In the cytoplasm, mitochondria of various sizes were found, from rounded to elongated. The lamellar cristae were clearly parallel. On the other hand, they were swollen and destroyed in places. The mitochondrial matrix is light, moderately edematous. Signs of hyperplasia were determined in the lamellar Golgi complex. Also, both ribosomal rosettes and free ribosomes were observed in the cytosol. The morphological characteristics of the nuclear elements and cytoplasm of these cells linked to their enhanced functional activity. These neurons formed contacts with each other (Fig. 5).

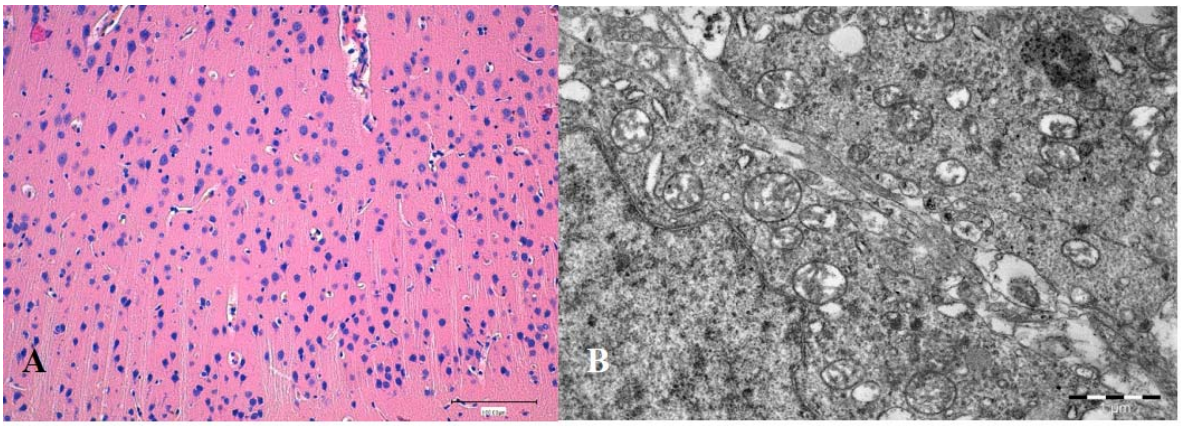


Figure 5. Structure of the rat precentral gyrus 5 days after forced swimming and BMA injection. A - Cerebral cortex. Hematoxylin and eosin staining. B - Interneuronal contacts. Electronogram

21 days after the intramuscular injection of BMA, the layers of neurocytes of the cerebral cortex were clearly visible. There were no signs of edematous phenomena in the perivascular and pericellular spaces. The neuropil is dense, not sparse. The hemocapillaries had a dense, well-defined basement membrane that formed splits. Pericytes were located in these duplications. The perivascular space was free from pathological changes and inclusions. The end legs of astrocytes adjoined to the basement membrane, tightly covering the hemocapillary and forming a sleeve. Numerous axon-dendritic and axon-axon synapses were noted in the perivascular field (Fig. 6).

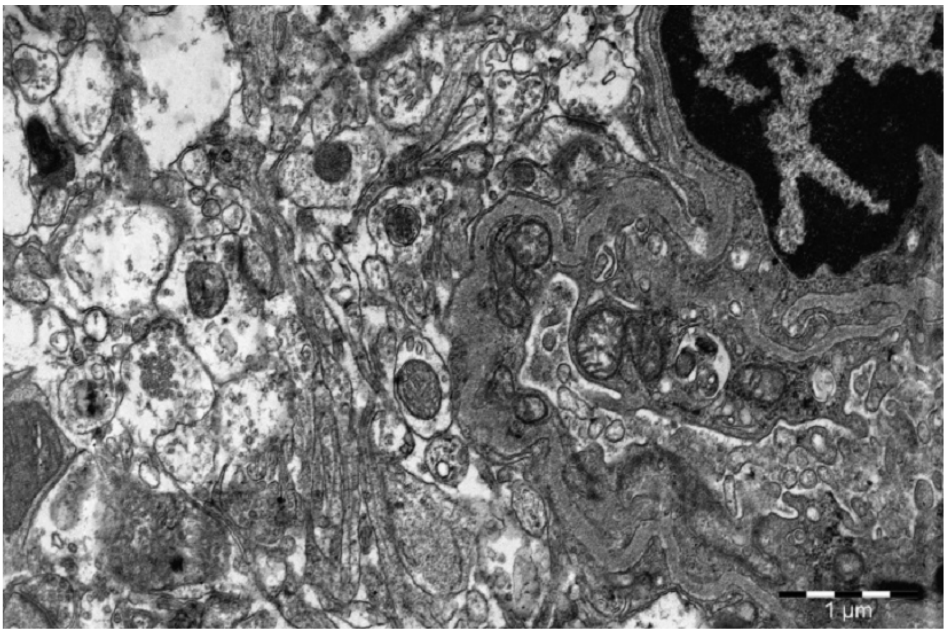


Figure 6. Neuropile and hemocapillary 21 days after forced swimming and intramuscular injection of BMA. Electronogram.

Immunohistochemical determination of some functionally significant cells revealed that on the fifth day of observation, the number of GFAP+ cells in the main group was significantly lower than in the control groups ($Z=3.7$ ($P<0.0001$)). On the 21st day, in the experimental and control groups, there was a significant, sometimes double, ($Z=2.1 \div Z=4.92$, $P<0.04 \div P<0.0001$) decrease in the number of GFAP-cells. However, when using BMA, the level of GFAP cells was

remarkably lower than in the control group (Z=3.0 (P<0.002)). GFAP is manifested in the cytoplasm of astrocytes, which perform a trophic, supporting role, form the glial membrane and the blood-brain barrier, isolate the receptive surfaces of neurons from extraneous afferent influences, but don't conduct nerve impulses (Flanagan *et al.* 2017; Aziz Anah and Aziz Anah, 2023). Reparative function consists in proliferation and replacement of dead nerve cells. Therefore, it is a marker of gliosis in the cerebral cortex.

After 5 days, the levels of Bcl-2+ cells in the main group were significantly (Z=3.5 ÷ Z=4.25, P<0.0005 ÷ P<<0.0001) higher than in the control groups. On the 21st day, the number of Bcl-2+ cells in the main group increased (Z=4.0 (P<<0.0001)). In the control group, the number of Bcl-2+ cells, on the contrary, remarkably decreased (Z=2.18 (P<0.03). Bcl-2 - suppresses apoptosis in many cellular systems, including lymphohematopoietic and neuronal cells. These cells regulate cell death by controlling mitochondrial membrane permeability (Elgendy *et al.* 2022).

On the fifth day, the number of CD-68+ cells in the main group was significantly (P<0.004) higher than in the control group. On the 21st day in the main group, the number of CD-68+ cells increased remarkably (Z=5, P<<0.0001). In the control group, on the contrary, there were no significant changes (Z=1.1 ÷ Z=0.1, P>0.24 ÷ 0.93) and, as a result, remarkable

excess (Z=5.49 ÷ Z= 5.83, P<0.0001) of the main group data over the control group became even more contrasting. CD-68 is a phagocytic macrophage marker. They are microsialin, an enzyme of secondary lysosomes. CD-68 in the cerebral cortex was found in microglial cells (Stankov *et al.* 2015).

The number of shadow cells in the main group decreased significantly with time (Z=3.1 (P<0.002)). And it turned out to be a multiple and significantly lower than in the control group at all times of observation (Z=5.56 ÷ Z=5.68, P<0.0001) (Table 1).

Table 1. The number of GFAP+, Bcl-2+, CD-68+ cells and shadow cells on the 5th and 21st days of observation.

Group / cell type	**GFAP$^+$**	**Bcl-2$^+$**	**CD-68$^+$**	**shadow cells**
Experimental group (5th day)	Me=15 (13, 18)	Me=22,5 (20, 25)	Me=1 (0 , 2)	Me=14 (12, 18)
Experimental group (21st day)	Me=9 (7, 11)	Me=31 (27, 34)	Me=3 (2 , 3)	Me=3 (3, 5)
Control group (5th day)	Me=26 (21, 28)	Me=8,5 (6, 12)	Me=0,5 (0 , 1)	Me=21 (10, 36)

Control group (21st day)	Me=13 (11, 15)	Me=6 (5, 7)	Me=1 (0 , 2)	Me=18 (9, 23)

Disscusion

It is known that BMA is a cell-free extracellular matrix of allogeneic origin and contains collagen, glycosaminoglycans, and proteoglycans (Lebedeva *et al.* 2019). Its pharmacological action is to stimulate tissue regeneration when applied locally, which activates angiogenesis, causes chemoattraction of mesenchymal stem cells and stimulation of their differentiation, followed by induction into tissue-specific tissues. Also, it is an inhibitor of fibrosis during the healing of various tissues (Lebedeva *et al.* 2021, Lebedeva *et al.* 2018). BMA exerts its range of influences through the system of mononuclear phagocytes – M1 macrophages, which migrate in response to biomaterial implantation and phagocytize it, acquiring a special phenotype. That is, BMA is an immunostimulator of the cellular link of immunity (Lebedeva *et al.* 2019 b). The remote effect of BMA biodegradation products has not been studied to date. It can be assumed that in response to the introduction of BMA into the muscle tissue of the upper and lower extremities, which were subjected to intense physical activity, they showed an actoprotective effect, which is confirmed by an increase in the level of the tolerant load multiplicity compared

to the control group (Baryshev *et al.* 2022, Kuznetsov *et al.* 2022, Ponamarev *et al.* 2022). Normoxic muscle tissues can also contribute to the immune-adaptive defense of various tissues, including nervous. CD-68 macrophages in nervous tissue have a similar effect by suppressing tissue gliosis. (Badi *et al.,* 2022; Chukwu *et al.,* 2023; Prusakova *et al.*, 2023a; Prusakova *et al.*, 2023b) Recovery, stabilization or stimulation of angiogenesis takes place, which is observed in the main group. Due to this, neurohumoral homeostasis is maintained, it has a neuroprotective effect and leads to a decrease in chromatolysis in neurocytes, which is confirmed by an increase in the number of Bcl-2+ cells in the main group, and, consequently, a decrease in the number of GFAP+ and shadow cells.

Conclusion

Forced anaerobic physical activity contributed to the development of pathomorphological changes in the nervous tissue of the cerebral cortex in the precentral gyrus. In the control group, signs of cell edema, neuropil, perivascular pool, and synaptic apparatus inactivation were determined. Pycnosis and chromatolysis of neurocytes ended with focal gliosis. Moreover, these signs persisted for 21 days. The number of GFAP+ cells and shadow cells increased. And the number of Bcl-2+ cells was reduced compared to the main group.

Five days after the injection of BMA under conditions of prolonged forced swimming with a load, no signs of edema of the nervous tissue were detected. The layers of neurocytes of the cerebral cortex retained a distinct architectonics. Synaptic activity was noted. Structural features of neurocytes also indicated their functional viability: restoration of energy balance, activation of biosynthetic processes and secretory activity, as well as their cooperation. The content of CD68+ macrophages was increased.

Therefore, on the basis of the results obtained, it can be concluded that the allogeneic biomaterial has a systemic effect on the animal organism. When locally injected into muscles, it has actoprotective, neuroprotective and immunomodulatory effects.

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Conflict of interest. The authors declare no explicit or potential conflicts of interest related to the publication of this article.

References

Alekhin, Yu.N., Popova, O.S., Ponamarev, V.S., Parshin, P.A. (2023). Effect of a Farnesoid X-receptor Agonist on Postprandial Lipemia in Rats Fed a Supraphysiological Fat Dozes. *Drug development & registration,* 12(2), 174-184. https://doi.org/10.33380/2305-2066-2023-12-2-174-184

Aziz Anah, S., Aziz Anah, S. (2023). New Recording of Toxoplasma gondii in Wild Tortoise Testudo graeca Using Nested PCR Method. *Archives of Razi Institute*, 78(3): 1029-1034. doi: 10.22092/ari.2022.360398.2579

Badi, B., Fazelipour, F., Naji, T., Babaei B, Kalantari Hessari, A. (2022). Histomorphometric and Biochemical Study of Liver and Thyroid Hormones Following Administration of MoO3 Nanoparticles in Female Rats. *Iranian Journal of Veterinary Medicine*, 16(2), 188-201. http://dx.doi.org/10.22059/IJVM.2021.330872.1005196

Baryshev, VA., Popova, OS., Ponamarev, VS. (2022). 'New methods for detoxification of heavy metals and mycotoxins in dairy cows'. *Online Journal of Animal and Feed Research*, 12(2), 81-88. https://dx.doi.org/10.51227/ojafr.2022.11

Chukwu, O. O., Emelike, C. U., Konyefom, N. G., Ibekailo, S. N., Ekakitie, O. O., Ghasi, S., Iyare, E. E. (2023). Histological Studies of the Heart and Biochemical Changes Due to the Perinatal Consumption of Hibiscus sabdariffa (Flavonoid-rich Extract) to Feed-restricted Rats on

Offspring. *Iranian Journal of Veterinary Medicine*, 17(1), 37-46. http://dx.doi.org/10.32598/ijvm.17.1.1005272

Condello, M., Caraglia, M., Castellano, M., Arancia, G., Meschini, S. (2013). Structural and functional alterations of cellular components as revealed by electron microscopy. *Microscopy research and technique*, 76(10): 1057–1069. https://doi.org/10.1002/jemt.22266

Elgendy, W., Swelem, R., Aboudiba, N., Elwafa, RA. (2022). Role of MicroRNA-326 and its Target Genes Bcl-xL and Bak as Potential Markers in Platelet Storage Lesion in Blood Banks. *Indian Journal of Hematology and Blood Transfusion,* 38(4), 731-738. https://doi.org/10.1007/s12288-022-01542-0

Fahimi Trouski, F ., Parham, A. (2021). Exosomes Derived From Mesenchymal Stem Cells in the Treatment of Animal Tendon Injuries: A Review on Their Isolation and Application. *Iranian Journal of Veterinary Medicine*, 15(3), 259-274. http://dx.doi.org/10.22059/IJVM.2021.322050.1005168

Flanagan, EP., Hinson, SR., Lennon, VA., Fang, B., Aksamit, AJ., Morris, PP., Basal, E., Honorat, JA., Alfugham, NB., Linnoila, JJ., Weinshenker, BG., Pittock, SJ., McKeon, A. (2017). 'Glial fibrillary acidic protein immunoglobulin G as a biomarker of autoimmune astrocytopathy: analysis of 102 patients', *Ann Neurol*, 81, 298–309. https://doi.org/10.1002/ana.24881

Ilyin, VN., Alvani, AR. (2016). 'Prevalence and formation of chronic fatigue in qualified athletes', *Pedagogics, psychology, medical-biological problems of physical training and sports*, 3, 11-17.

Jaber Al-Mamoori, A., Abdulameer Almustafa, H. M. S., & Alsaffar, Y. (2022). Overexpressed Toll-Like Receptor 2 and the Influence on the Severity of Acute Ischemic Stroke. *Archives of Razi Institute*, 77(6), 2379–2384. https://doi.org/10.22092/ARI.2022.358615.2265

Khabibullin, R., Khabibullin, I., Yagafarov, R., Bakirova, A., Fazlaev, R., Karimov, F., Mussina, L., Ismagilova, E., Fazlaeva, S., Tuktarov, V. (2019). The influence of dietary supplements on the adaptive processes in animals after physical stress. *Bulgarian Journal of Agricultural Science*, 25 (Suppl. 2), 105–118

Kholodny, YuI., Malakhov, DG., Orlov, VA., Kartashov, SI., Aleksandrov, YuI., Kovalchuk, MV. (2021). Study of neurocognitive processes in the paradigm of information concealment. *Experimental psychology*, 14(3), 17-39. https://doi.org/10.17759/exppsy.2021140302

Kim, H., Murata, M. M., Chang, H., Lee, S. H., Kim, J., Lee, J.H., Rho, W.Y., Jun, B.H. (2021). Optical and Electron Microscopy for Analysis of Nanomaterials. *Advances in*

experimental medicine and biology, 1309: 277–287. https://doi.org/10.1007/978-981-33-6158-4_12

Klang, V., Valenta, C., Matsko, N.B. (2013). Electron microscopy of pharmaceutical systems. *Micron (Oxford, England: 1993)*, 44: 45–74. https://doi.org/10.1016/j.micron.2012.07.008

Kuznetsov, YuE., Lunegov, AM., Ponamarev, VS., Romashova, EB. (2022). 'Correlation relationships between the content of total bile acids and the main biochemical parameters of blood in minks (Mustela vison Schreber, 1777)', *Agricultural biology*, vol. 57, no. 6, pp. 1217-1224. https://doi.org/10.15389/agrobiology.2022.6.1217eng

Lebedeva, AI., Muslimov, SA., Afanasiev, SA., Kondratieva, DS. (2019). 'The role of macrophages in the regeneration of muscle tissue induced by allogeneic biomaterial', *Russian Journal of Immunology*, 13(22), 2, 849-851. https://doi.org/10.31857/S102872210006676-8

Lebedeva, AI., Muslimov, SA., Gareev, EM., Popov, SV., Afanasiev, SA. (2018). 'Stimulation of autologous progenitor and committed cells in ischemic damaged myocardium', *Russian Journal of Cardiology*, 23(11), 123–129. https://doi.org/10.15829/1560-4071-2018-11-123-129

Lebedeva, AI., Muslimov, SA., Gareev, EM., Popov, SV., Afanasiev, SA., Kondratieva, DS. (2021). 'Allogeneic biomaterial as an inducer of regeneration of ischemic myocardium in the experiment', *Pathological Physiology and Experimental Therapy*, 65(1), 60-69. https://doi.org/10.25557/0031-2991.2021.01.60-69

Lebedeva, AI., Muslimov, SA., Vagapova, VSh., Shcherbakov DA. (2019). 'Morphological aspects of regeneration of skeletal muscle tissue induced by allogeneic biomaterial', *Practical Medicine*, 17(1), 98-102.

Lebedeva, AI., Muslimov, SA., Vagapova, VSh., Shcherbakov, DA. (2019). Morphological aspects of regeneration of skeletal muscle tissue induced by allogeneic biomaterial. *Practical Medicine,* 17(1), 98-102. https://doi.org/10.32000/2072-1757-2019-1-98-102

Magaki, S., Hojat, S. A., Wei, B., So, A., Yong, W. H. (2019). An Introduction to the Performance of Immunohistochemistry. *Methods in molecular biology (Clifton, N.J.)*, *1897*: 289–298. https://doi.org/10.1007/978-1-4939-8935-5_25

Material Safety Data Sheet Chloroform MSDS. ScienceLab.com Chemicals and Laboratory Equipment.

https://www.unil.ch/cig/files/live/sites/cig/files/FAQ/Safety/PDF/MSDS/Chloroform_msds.pdf (access date: 06.01.2024)

Meftahi, G. H., Hatef, B., Pirzad Jahromi, G. (2023). Creatine Activity as a Neuromodulator in the Central Nervous System. *Archives of Razi Institute*; 78(4): 1169-1175. https://doi.org/10.32592/ARI.2023.78.4.1169

Moreno, V., Smith, E. A., Piña-Oviedo, S. (2022). Fluorescent Immunohistochemistry. *Methods in molecular biology (Clifton, N.J.)*, 2422: 131–146. https://doi.org/10.1007/978-1-0716-1948-3_9

Ozawa, H. (2019) [Principles and basics of immunohistochemistry]. *Nihon Yakurigaku Zasshi*,154(4):156-164. https://doi.org/10.1254/fpj.154.156. PMID: 31597893.

Ponamarev, V., Yashin, A., Prusakov, A., Popova, O. (2022). 'Influence of Modern Probiotics on Morphological Indicators of Pigs' Blood in Toxic Dyspepsia', *Agriculture Digitalization and Organic Production: Proceedings of the Second International Conference*, St. Petersburg, Springer, 133-142. https://doi.org/10.1007/978-981-19-7780-0_12

Prichard J.W. (2014). Overview of automated immunohistochemistry. *Archives of pathology & laboratory medicine*, 138(12): 1578–1582. https://doi.org/10.5858/arpa.2014-0083-RA

Prusakova, A. V., Zelenevskiy, N. V., Prusakov, A. V., Yashin, A. V., Ponamarev, V. S. (2023). Ultrastructural organization of liver hepatocytes of the Anglo-Nubian goat. *Veterinarski Glasnik*, 77(2), 176-187. https://dx.doi.org/10.2298/VETGL230615007P

Prusakova, A., Zelenevskiy, N., Prusakov, A., Yashin, A., Ponamarev, V. (2023). Organization of histo-hematic barriers of the liver in Anglo-Nubian goat. *Online J. Anim. Feed Res.*, 13(4), 242-245. https://dx.doi.org/10.51227/ojafr.2023.36

Recommendation of the EEC dated November 14, 2023 No. 33 "On the Guidelines for working with laboratory (experimental) animals when conducting preclinical (non-clinical) studies." Customs documents. (n.d.). Alta-Soft. https://www.alta.ru/tamdoc/23rk0033/?ysclid=lr25q6aq8d307332511 (access date: 06.01.2024)

Schlattmann, P., Scherag, A., Rauch, G., Mansmann, U. (2019).The role of biostatistics in institutional review boards. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz*. 62(6), 751-757. https://doi.org/10.1007/s00103-019-02951-9

Stankov, A., Belakaposka-Srpanova, V., Bitoljanu, N., Cakar, L., Cakar, Z., Rosoklija, G. (2015). 'Visualization of Microglia with the use of Immunohistochemical Double Staining Method for CD-68 and Iba-1 of Cerebral Tissue Samples in Cases of Brain Contusions', *Pril*

(Makedon Akad Nauk Umet Odd Med Nauki), 36(2), https://doi.org/141-145. 10.1515/prilozi-2015-0062

Sukswai, N., Khoury, J. D. (2019). Immunohistochemistry Innovations for Diagnosis and Tissue-Based Biomarker Detection. *Current hematologic malignancy reports*, 14(5): 368–375. https://doi.org/10.1007/s11899-019-00533-9

Zehtabvar, O., Vajhi, A. R., Rostami, A., Vosoogh Afkhsmi, A. R., Davudypoor, S., Gholikhani, M., Modarres, S. H. (2023). Morphometric and Normal Two-dimensional Computerized Tomography Anatomic Study of the Vertebral Column of the European Pond Turtle (Emys Orbicularis). *Iranian Journal of Veterinary Medicine*, 17(1), 53-64. http://dx.doi.org/10.32598/ijvm.17.1.1005235

Zhou, Y., Yan, M., Pan, R., Wang, Z., Tao, X., Li, Ch., Xia, T., Liu, X., Chang, Q. (2021). Radix Polygalae extract exerts antidepressant effects in behavioral despair mice and chronic restraint stress-induced rats probably by promoting autophagy and inhibiting neuroinflammation. *Journal of Ethnopharmacology*. 265, 113317. https://doi.org/10.1016/j.jep.2020.113317