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Морфофункциональная оценка мышц голени и стопы после аутонейропластики резекционного дефекта большеберцовой порции седалищного нерва взрослых крыс и однократной интраоперационной электронейростимуляции

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АННОТАЦИЯ

Введение. В литературе отсутствуют данные о влиянии однократной интраоперационной электростимуляции (ИЭС) на состояние мышц голени и стопы в отдалённые сроки после аутопластики седалищного нерва у взрослых крыс.

Цель. Изучить морфофункциональные характеристики мышц голени и стопы после аутонейропластики резекционного дефекта большеберцовой порции седалищного нерва взрослых крыс и однократной ИЭС.

Материалы и методы. Эксперимент выполнен на 30 крысах линии Wistar, которым после резекции участка большеберцовой порции седалищного нерва была выполнена аутонейропластика (АН). 14 крысам провели 40-минутный сеанс ИЭС (серия АН + ИЭС). 16 крысам ИЭС не проводили (серия АН). Через 4 и 6 месяцев после операции методом анализа следов-отпечатков лап крыс на пешеходной дорожке рассчитали индекс функции большеберцового нерва (англ.: *tibial nerve function index*, TFI). В эти же сроки провели световую микроскопию и гистоморфометрию парафиновых и эпоксидных срезов икроножных и подошвенных межкостных мышц. Условный контроль — мышцы интактных конечностей.

Результаты. В икроножной мышце серии АН + ИЭС по сравнению с серией АН менее выражена атрофия мышечных волокон и фиброз эндомизия, эффект опосредован усилением васкуляризации. В подошвенных межкостных мышцах через 4 месяца после операции объёмная плотность кровеносных сосудов в серии АН + ИЭС составила 7,35 (5,49; 8,69), что больше, чем в серии АН — 3,43 (2,02; 5,59), p = 0,0196. Диаметры мышечных волокон и объёмная плотность эндомизия были сопоставимы. Через 6 месяцев после операции в обеих сериях прогрессировал фиброз эндомизия, однако в серии с АН + ИЭС миопатически измененные мышечные волокна встречались реже. Через 6 месяцев наблюдения в серии АН + ИЭС TFI повысился (-47,95) и стал больше (p = 0,0339), чем в серии АН, в которой TFI стал еще более низким (-93,64), чем был через 4 месяца (-81,95) опыта.

Заключение. Однократная ИЭС позволяет уменьшить связанные с повреждением нерва и взрослением денервационные изменения икроножных и межкостных подошвенных мышц, а также улучшить большеберцовый функциональный индекс в отдалённые сроки после аутонейропластики.

Ключевые слова: *крысы; нерв; аутонейропластика; интраоперационная электростимуляция; икроножные, подошвенные межкостные мышцы; гистоморфометрия*

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Morphofunctional Assessment of Muscles of the Lower Leg and Foot after Autoneuroplasty of Resection Defect of Tibial Portion of the Sciatic Nerve and Single Intraoperative Electrical Neurostimulation in Adult Rats

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ABSTRACT

INTRODUCTION: There are no data in the literature on the effect of a single intraoperative electrical stimulation (IES) on the condition of the muscles of the lower leg and foot in the long-term period after autoplasty of the sciatic nerve in adult rats.

AIM: To study morphofunctional characteristics of muscles of the lower leg and foot after autoneuroplasty of a resection defect of the tibial portion of the sciatic nerve and a single IES in adult rats.

MATERIALS AND METHODS: The experiment was performed on 30 Wistar rats who underwent autoneuroplasty (AN) after resection of the tibial portion of the sciatic nerve. Fourteen rats underwent a 40-munite IES session (AN + IES series). In 16 rats IES was not conducted (AN series). At 4 and 6 months after the operation, the tibial nerve function index (TFI) was calculated by analyzing rats' paw traces on a walking track. At the same time, light microscopy and histomorphometry of paraffin and epoxy sections of gastrocnemius and plantar interosseous muscles were performed. A conventional control was muscles of intact limbs.

RESULTS: Atrophy and endomysial fibrosis were less expressed in the gastrocnemius of the AN + IES series in comparison with AN series, the effect was mediated by enhancement of vascularization. In plantar interosseous muscles at 4 months after the operation, the volume density of blood vessels in the AN + IES series was 7.35 (5.49; 8.69), which was greater than in the AN series — 3.43 (2.02; 5.59), p = 0.0196. Diameters of muscle fibers and volume density of endomysium were comparable. At 6 months after the operation, endomysial fibrosis progressed in both series, but in the AN + IES series, myopathically altered muscle fibers were less common. After 6-month observation, TFI increased (-47.95) in the AN + IES series and became higher (p = 0.0339) than in the AN series, in which TFI became even lower (-93.64) than it was after 4 months (-81.95) of the experiment.

CONCLUSION: A single IES permits to reduce the denervation alterations in the gastrocnemius and plantar interosseous muscles conditioned by damage to the nerve and maturation, and also to improve the tibial nerve function index in the long term after autoneuroplasty.

Keywords: *rats; nerve; autoneuroplasty; intraoperative electrical stimulation; gastrocnemius, plantar interosseous muscles; histomorphometry*

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ECO•VECTOR

LIST OF ABBREVIATIONS

AN — autoneuroplasty
IES — intraoperative electrical stimulation
TFI — tibial nerve function index

INTRODUCTION

Despite the regenerative capacity of the peripheral nervous system fibers and the phenomenon of neural plasticity, injuries to peripheral nerves significantly impair the quality of life of patients causing long-term disorders of sensitivity and movement. Anatomic transections of nerves require surgical interventions, and if the direct suturing of the proximal and distal ends of the transected nerve is unfeasible, the gold standard of the restorative surgery is considered to be the autoneuroplasty [1].

After the primary nerve suture, full functional recovery is achieved in only 10% of cases [2], and after the autoneuroplasty good results are possible in patients under 25 years old [3]. The results of the nerve autoneuroplasty on the lower limb are worse than on the upper limb [4].

Along with the improvement of reconstructive nerve surgery methods, numerous molecular and bioengineering strategies of improving neuroregeneration including methods of electrical stimulation, are being developed in experiments on animals [5]. Of particular interest for clinical practice is a single intraoperative electrical stimulation (IES). Its positive effect on the neuroregeneration and functional recovery has been proven in different nerve damages in a pelvic limb of rats [6].

The effect of IES on the results of nerve defect repair showed ambiguous results. With nerve regeneration via a collagen gel conduit, nerve repair and functional restoration of the limb were improved in the 10- and 60-minute IES series compared to the non-stimulated control over 12 weeks of the experiment [7]. In similar experiments with autoplasty, 10-minute IES revealed a reliable difference in the thickness of regenerated nerve fibers at 6 weeks after surgery, but IES did not influence the severity of gastrocnemius muscle atrophy [8].

In the available literature, we did not find data on the effect of a single IES on the condition of the muscles of the lower leg and foot in long-term periods after autoplasty of the sciatic nerve in adult rats.

The **aim** of this study to morphofunctional characteristics of the muscles of the lower leg and foot after the autoneuroplasty of a resection defect of the tibial portion of the sciatic nerve and a single intraoperative electrical neurostimulation in adult rats.

MATERIALS AND METHODS

In the operating room, 30 Wistar laboratory rats (male gender, 8–15 months old, weighing 360 g–460 g), underwent an extra-projection incision of skin at the level of the middle third of thigh to provide access to the right sciatic nerve through the biceps muscle using a sharp-blunt method. Under 8-fold magnification of the operating microscope (OPMI-6, Germany), using a sharp vascular microprobe and iridectomy scissors, epifascicular longitudinal epineurotonic incisions were performed to isolate the tibial portion of the sciatic nerve. After resection of a 6 mm long portion, interfascicuar autologous plasty of the resulting defect was performed using microsurgical suturing material of 9–0 and 10–0 caliber.

Sixteen rats constituted a non-stimulated control group: after completion of the autoneuroplasty (AN), the wound was sutured layer-by-layer with 3-0 caliber resorbable suture material (***AN series***). In 14 rats, immediately after AN, electrodes were installed on the proximal part of the nerve. Using NeySi-3M (registraition certificate No. FSL 2011/10004), the proximal part of the nerve was electrically stimulated with monopolar square-wave electrical pulses of 0.25 mA amplitude, 20 Hz frequency and 100 μm duration for 40 minutes, after which the wound was sutured — ***AN + IES series***.

The experiment was carried out in accordance with the European Convention for the Protection of Vertebrate Animals, 2010/63/EU Directive of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes and SP 2.2.1.3218-14; GOST 33217-2014; GOST 33215-2014. The study design was approved by the Ethics Committee of the institution (Protocol No. 2 (57) of May 17, 2018). The animals were kept in controlled hygienic conditions, had access to water and food. For anesthesia and pain relief, the animals were administered intramuscularly 0.8 mg of xylazine hydrochloride and 0.4 mg of tiletamine/zolazepam per 100 g of body weight; the hair was cut on the right thigh and the lower leg.

To ensure comparability of the experimental groups by age, on each surgical day a pair of rats was operated on: one rat without stimulation, the other one with stimulation; in one pair of eight-month-old rats, both were without stimulation.

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The animals were withdrawn from the experiment at 4 and 6 months after the operation. Samples of gastrocnemius muscles and foot fragments were fixed in 10% neutral formalin solution with decalcification of feet in a mixture of hydrochloric and formic acids, and were embedded in paraffin according to a standard method. Transverse paraffin sections (5 μm–7 μm) of gastrocnemius muscles and total sections of foot fragments preserving topography and histoarchitecture of the muscles, were prepared on HM 450 microtome (Thermo Scientific, USA), stained with Masson three-color method, with hematoxylin and eosin. After aldehyde-osmium fixation, a part of samples was embedded in araldite; using diamond knives, semi-thin sections (1 μm) were prepared on a Nova LKB ultramicrotome (Sweden) and stained with methylene blue, azure II and basic fuchsine.

Sections were microscopied in an AxioScope.A1 microscope with obtaining full-color digital images using an AxioCam digital camera (Carl Zeiss MicroImaging GmbH, Germany). The average diameters of muscle fibers (200–400 fibers in each animal) were measured in the Video-Test Master Morphology 4.0 software, at 400× magnification. The quantitative ratio (%) of muscle fibers, endomysium, and blood vessels was determined in the PhotoFiltre 7 program at 400× magnification using a test grid of equally spaced points with transparent centers using the point-counting volumetry method.

The ***control group*** included 9 adult intact rats close in age to the operated rats at the time of euthanasia — 16–18 months (in this group there were no statistically significant differences between the studied quantitative parameters).

The functional recovery of the limb was assessed at 4 and 6 months after the operation using walking track analysis (Figure 1) and calculation of tibial nerve function index (TFI). TFI = 0 — normal function, TFI = -100 — complete loss of function. The formula modified by J. R. Bain, et al. was used [9]:

$$TFI = -37.2\,(EPL - NPL)/KPL + 104.4\,(ETS - NTS)/NTS + 45.6\,(EIT - NIT)/NIT - 8.8,$$

where PL is the length of the footprint from the heel to the third toe, TS is the distance between the first and fifth toes, IT is the distance between the second and fourth toes, NPL, NTS and NIT are the data of the non-operated (contralateral) limb, EPL, ETS and EIT are the data of the experimental (operated) limb (Figure 1). For the calculation, manual measurements of the parameters in mm were used.

Statistical data processing was performed using the Attestat computer program, version 9.3.1 (developer I. P. Gaidyshev, Rospatent registration certificate No. 2002611109). Samples were tested for normal distribution of values using Kolmogorov test. Pairwise comparison of animal groups was performed using Mann–Whitney and χ^2 tests. Parameter values were presented as medians and quartiles Me (Q_1; Q_3). Differences were considered significant at $p \le 0.05$.

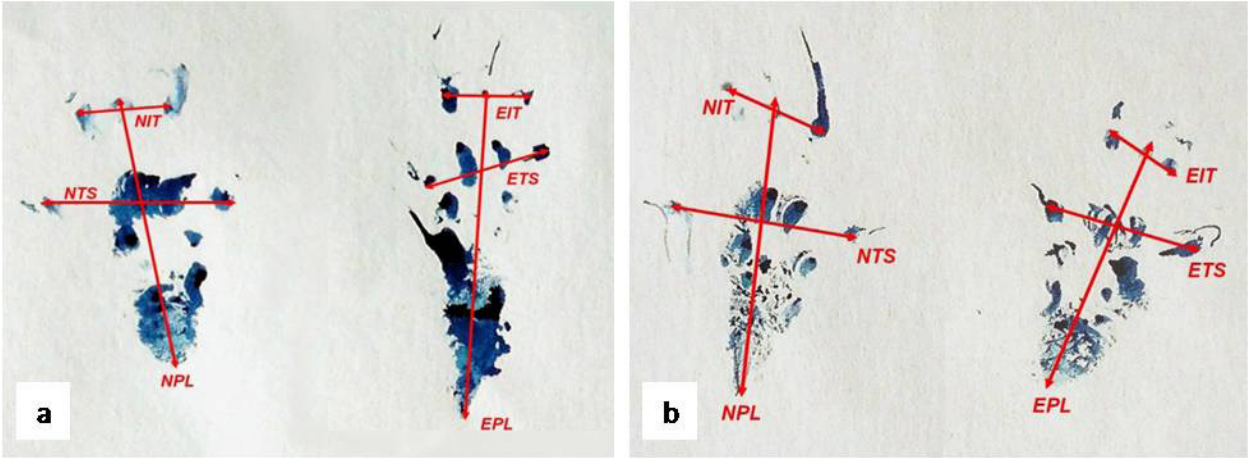

Fig. 1. Footprints of rats on a walking track: (a) AN series, (b) AN + IES series.
Notes: PL is the distance from the heel to the third toe, TS is the distance between the first and fifth toes, IT is the distance between the second and fourth toes; EPL, ETS and EIT are the measurements on the experimental limb, NPL, NTS and NIT are the measurements on the non-operated limb; AN is autoneuroplasty, IES is intraoperative electrical stimulation.

RESULTS

In the gastrocnemius and plantar interosseous muscles of the operated rats, similar alterations of muscle fibers and connective tissue sheaths were identified associated with a long denervation-reinnervation process. In the gastrocnemius muscle, perimysium and endomysium layers were thickened in comparison with the intact gastrocnemius muscle of rat (Figures 2, 3). In a part of nerve trunks, epi- and endoneural edemas were present and also nerve fibers with reactive-destructive alterations (Figure 2c).

Muscle fibers of gastrocnemius muscles and plantar interosseous muscles were variable in size and shape of cross-sectional profiles: atrophied and hypertrophied fibers were encountered, a part of them lost polygonality and acquired rounded shape, some fibers acquired angular shape (Figures 2–5), alterations in shape were more often recorded in the plantar interosseous muscles. Reactive-destructive alterations in some muscle fibers included the appearance of small vacuoles, irregular contours, disappearance of striated pattern, non-uniform staining (Figures 2c, 2f), displacement of nuclei toward the center (Figures 2b, 2e, 4b, 4c, 5b).

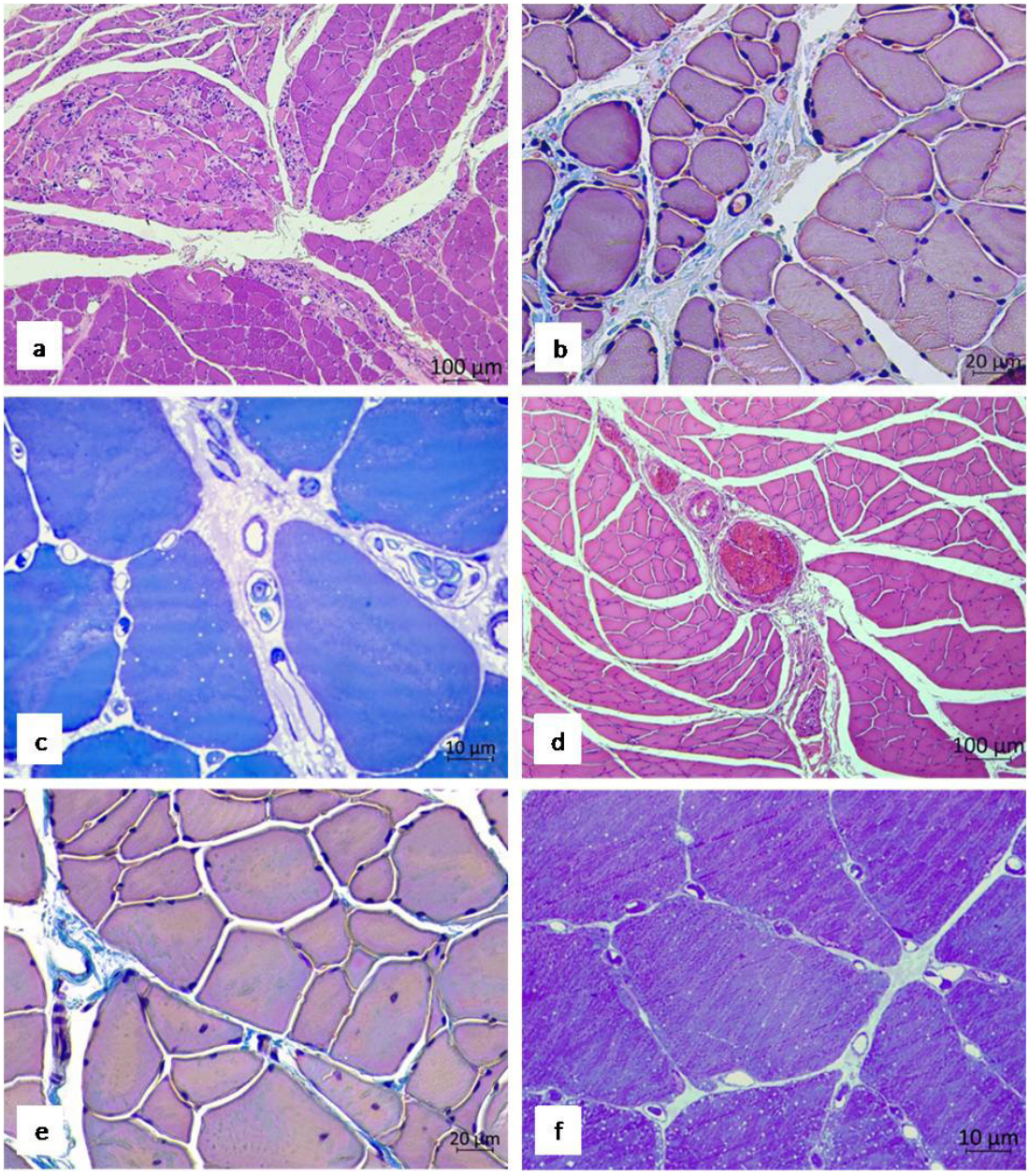

Fig. 2. Gastrocnemius muscle of rats at 4 months of the experiment: (a–c) AN series; (d–f) AN + IES series.
Notes: transverse paraffin sections, stained with hematoxylin and eosin, 100× magnification (a, d), stained with Masson three-color method, 400× magnification (b, e) transverse semi-thin sections, stained with methylene blue, azure II and basic fuchsine, 1000× magnification (c, f).

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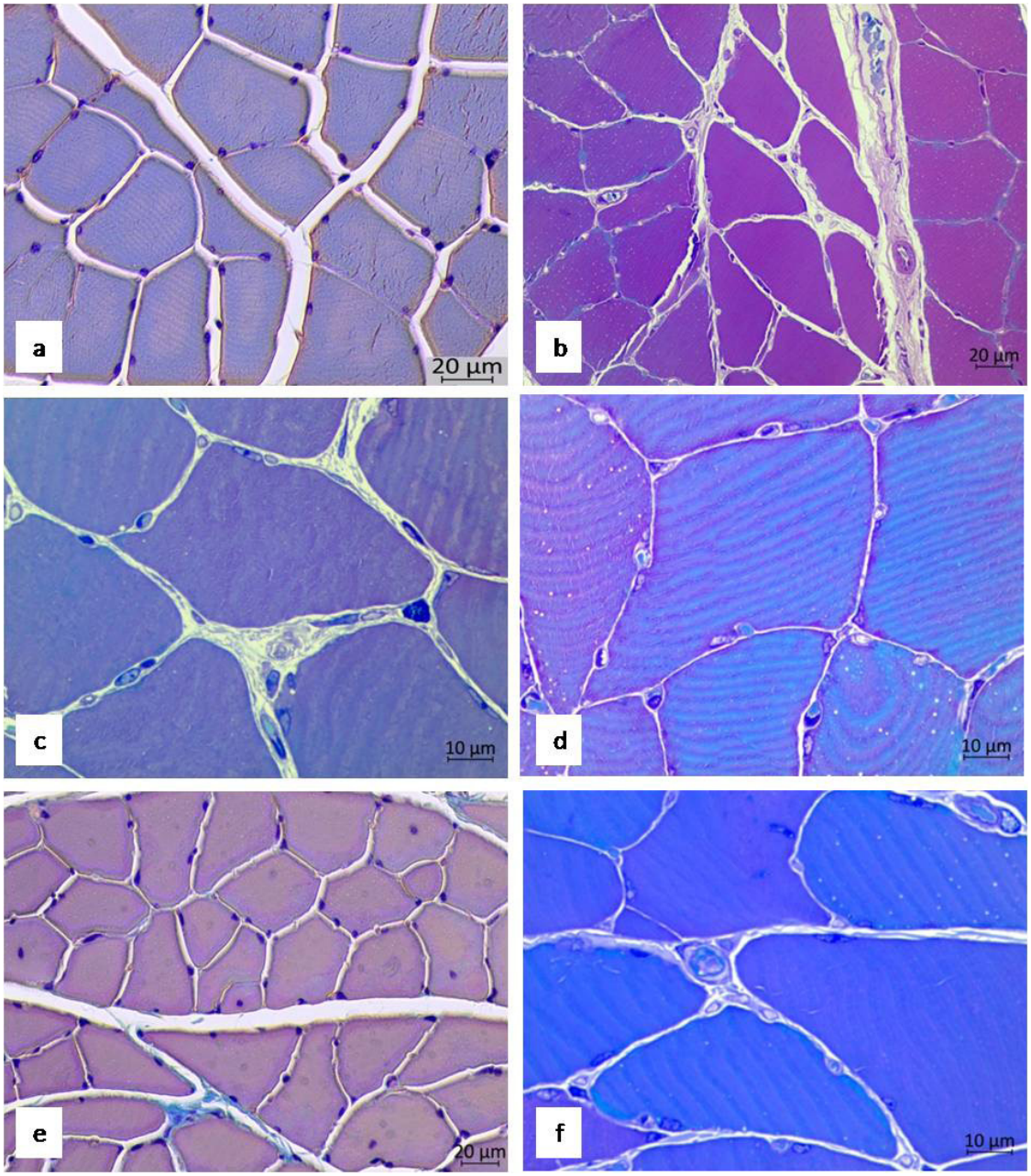

Fig. 3. Gastrocnemius muscle of rats at 6 months of the experiment: (a, d) control; (b, c) AN series; (e, f) AN + IES series.
Notes: transverse paraffin sections, stained with Masson's three-color method, 400× magnification (a, e); transverse semi-thin sections, stained with methylene blue, azure II and basic fuchsine, 400× (b), 1000× magnification (c, d, f).

At 4 months, individual muscle areas with pronounced atrophy and necrobiotic degradation of a number of muscle fibers, edema of the sheaths, an increased number of microvessels, individual hemorrhagic foci, accumulation of fibroblastic cells and lymphocytes were noted. Such areas (Figures 2a, 4b) were more typical of the AN series and were found much less frequently in the muscles of rats of the AN + IES series.

At 6 months of the experiment, signs of fibrous replacement of muscle fiber bundles progressed in the plantar interosseous muscles in both series (Figures 5a, 5c). Histomorphometric study of the gastrocnemius muscles (Table 1) showed that at 4 months after the autoneuroplasty of the tibial portion of the sciatic nerve, the decrease in the average diameter of muscle fibers compared to the control was more pronounced in the

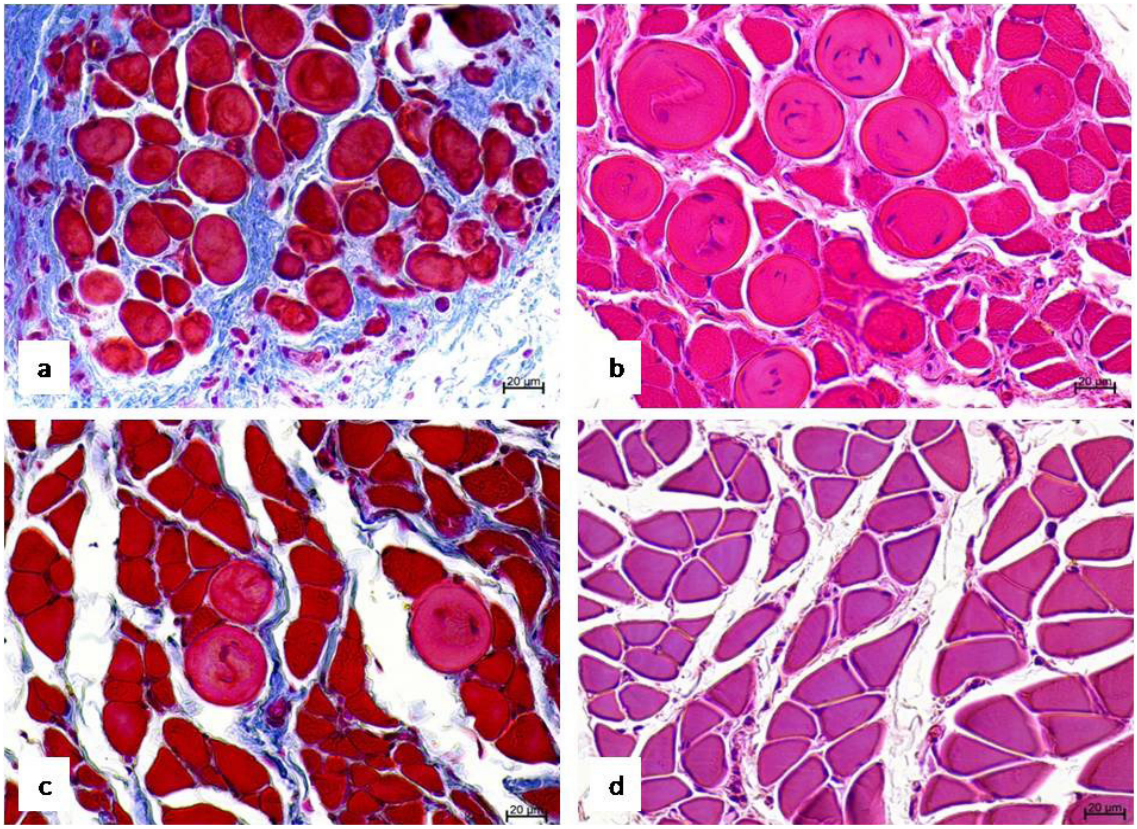

Fig. 4. Plantar interosseous muscle of rats at 4 months of the experiment: (a, b) AN series; (c, d) AN + IES series.
Notes: transverse paraffin sections, stained with Masson's three-color method (a, c), hematoxylin and eosin (b, d); 400× magnification.

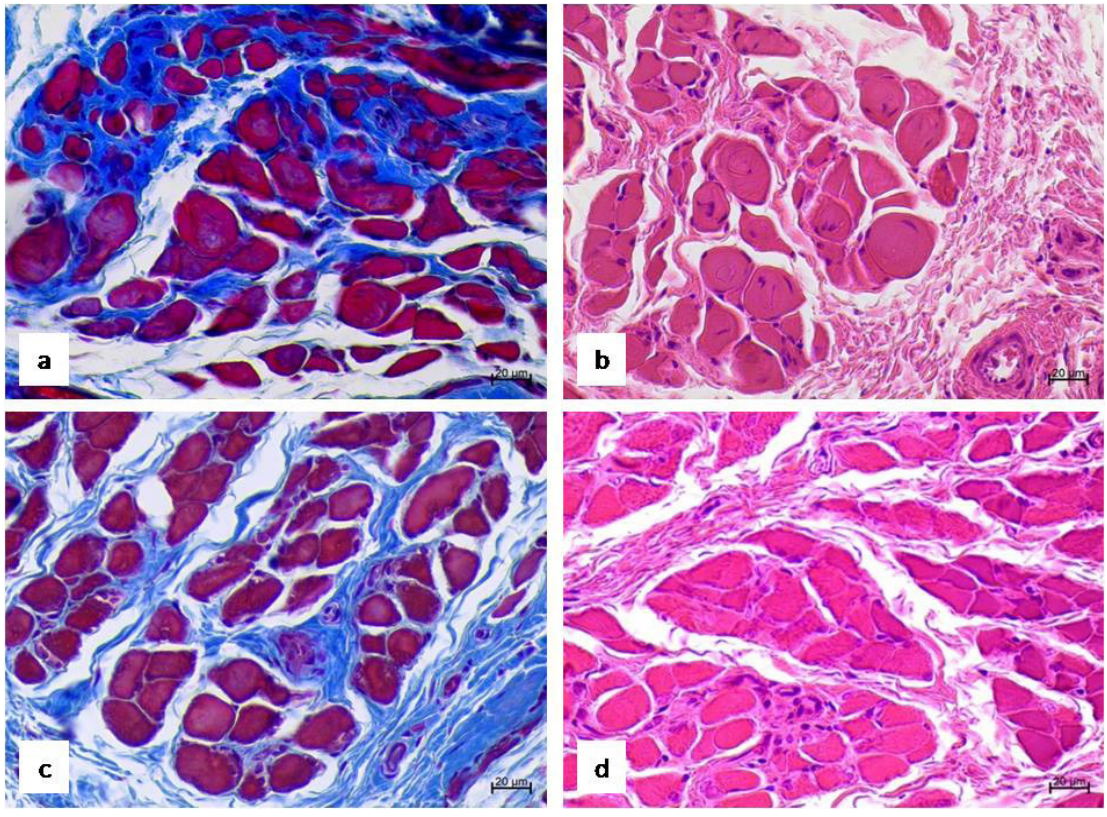

Fig. 5. Plantar interosseous muscle of rats at 6 months of the experiment: (a, b) AN series; (c, d) AN + IES series.
Notes: transverse paraffin sections, stained with Masson's three-color method (a, c), with hematoxylin and eosin (b, d); 400× magnification.

AN series by 43% ($p < 0.05$), and by 24% in the AN + IES series ($p < 0.05$). The volume density of capillaries in the series with stimulation twice exceeded the control and the AN series (Table 1), indicating better blood supply to the muscles. The volume density of endomysium compared to the control was increased in both series ($p < 0.05$), 2.2 times in AN series and only 1.4 times in the AN + IES series.

At 6 months after the operation, the mean diameters of muscle fibers remained reduced relative to the control (Table 1). The volume density of capillaries in stimulated animals, as at 4 months of the experiment, twice exceeded the values in control and non-stimulated animals. The volume density of endomysium in the gastrocnemius muscle exceeded the control only in the AN series, and in stimulated animals the parameter did not differ from the control (Table 1).

Table 1. Quantitative Characteristics of Gastrocnemius Muscles

4 months of experiment		6 months of experiment		Control, n = 9
Series 1 (AN), n = 8	**Series 2 (AN and IES), n = 7**	**Series 1 (AN), n = 8**	**Series 2 (AN and IES), n = 7**	
Muscle fiber diameter, Me (Q_1; Q_3), μm				
28.68 (19.91; 37.68) $p^{1-к} = 0.00052^*$ $p^{1-2} = 0.00195^*$	39.92 (33.24; 46.90) $p^{2-к} = 0.00146^*$	37.22 (29.27; 41.91) $p^{1-к} = 0.00053^*$ $p^{1-2} = 0.04914^*$	41.10 (34.33; 47.15) $p^{2-к} = 0.00085^*$	52.80 (44.20; 60.93)
Volume density of blood vessels, Me (Q_1; Q_3), %				
2 (2; 3) $p^{1-к} = 0.023845$ $p^{1-2} = 0.004078$	4 (3; 5) $p^{2-к} = 3.27 \times 10^{-7}$	2 (1; 3) $p^{1-к} = 0.09758$ $p^{1-2} = 0.004026$	4 (3; 5) $p^{2-к} = 0.000017$	2 (1; 3)
Volume density of endomysium, Me (Q_1; Q_3), %				
11 (8; 14) $p^{1-к} = 7 \times 10^{-9}$ $p^{1-2} = 2 \times 10^{-9}$	7 (5; 8) $p^{2-к} = 0.015757$	6 (5; 11) $p^{1-к} = 0.001678$ $p^{1-2} = 0.00193$	5 (4; 7) $p^{2-к} = 0.76819$	5 (3; 6)

Notes: Mann–Whitney test was used; $p^{к-1}$ — comparison of control and series 1 (AN); $p^{к-2}$ — comparison of control and series 2 (AN and IES); p^{1-2} — comparison of series 1 and series 2; AN — autoneuroplasty; IES — intraoperative electrical stimulation

Table 2. Quantitative Characteristics of Plantar Interosseous Muscles

4 months of experiment		6 months of experiment		Control, n = 9
Series 1 (AN), n = 8	**Series 2 (AN and IES), n = 7**	**Series 1 (AN), n = 8**	**Series 2 (AN and IES), n = 7**	
Muscle fiber diameter, Me (Q_1; Q_3), μm				
17.07 (13.54; 22.87) $p^{к-1} = 2 \times 10^{-10}$ $p^{1-2} = 0.4532$	19.01 (13.81; 24.39) $p^{к-2} = 3 \times 10^{-9}$	17.01 (11.43; 20.52) $p^{к-1} = 0.0001$ $p^{1-2} = 7 \times 10^{-5}$	14.1 (9.41; 16.79) $p^{к-2} = 0.0001$	21.52 (17.13; 25.31)
Volume density of blood vessels, Me (Q_1; Q_3), %				
3.43 (2.02; 5.59) $p^{к-1} = 0.0007$ $p^{1-2} = 0.0196$	7.35 (5.49; 8.69) $p^{к-2} = 0.3088$	6.66 (5.01; 7.51) $p^{к-1} = 0.0266$ $p^{1-2} = 0.2234$	5.72 (4.06; 6.56) $p^{к-2} = 0.0006$	8.18 (6.51; 11.45)
Volume density of endomysium, Me (Q_1; Q_3), %				
28.51 (19.32; 41.58) $p^{к-1} = 0.0009$ $p^{1-2} = 0.0121$	20.51 (17.99; 29.29) $p^{к-2} = 0.0708$	40.68 (30.66; 56.68) $p^{к-1} = 2.01 \times 10^{-5}$	46.33 (44.25; 54.33) $p^{к-2} = 1.98 \times 10^{-6}$ $p^{1-2} = 0.3544$	18.15 (17.01; 19.79)

Notes: Mann–Whitney test was used; $p^{к-1}$ — comparison of control and series 1 (AN); $p^{к-2}$ — comparison of control and series 2 (AN and IES); p^{1-2} — comparison of series 1 and series 2; AN — autoneuroplasty; IES — intraoperative electrical stimulation

Histomorphometric analysis of the plantar interosseous muscles showed that at 4 months after the operation, the diameter of muscle fibers in the AN series was 20.6% reduced relative to the control, and 11.6% reduced in the AN + IES series, with no statistical differences between the series (Table 2). At 6 months after the operation, this parameter remained below the control in both series, in non-stimulated animals, the diameter of muscle fibers was greater ($p < 0.05$) than in stimulated rats (Table 2), which is associated with hypertrophy of most part of fibers (Figures 5a, 5b). The volume density of microvessels in the AN + IES series was higher than in the AN series only at 4 months after the operation, at 6 months the differences in this parameter were statistically insignificant (Table 2). The volume density of endomysium at 4 months after the operation was lower in stimulated animals compared with stimulated ones, and at 6 months, the parameter significantly increased in both groups, more than twice exceeding the control (Table 2).

Analysis of rat footprints on a walking track at 4 months after the autoneuroplasty of the tibial portion of the sciatic nerve, showed a significant disturbance in the function of the operated limbs of both series, more pronounced ($p = 0.1649$) in non-stimulated animals: in the AN series, the functional index was -81.95 ± 15.85, and in the AN + IES series it was -69.19 ± 8.57. At 6-months follow-up, the functional index in the AN + IES series increased to -47.95±20.76, which is regarded as positive dynamics, and became reliably higher ($p = 0.0339$) than in the AN series, where the functional index appeared to be even lower than at 4 months, and was -93.64 ± 12.51, which evidences preservation of regress of the motor function in non-stimulated animals.

DISCUSSION

In most experimental developments of novel nerve reconstruction methods, either young [10], or very old rats are used [11]. According to the epidemiological analysis of nerve injuries in the clinic, the mean age of victims exceeds 40 years with two peaks at the age of 24 and 56 years [12]. The periodization of the age of rats relative to the human age has not been studied in detail, however, it is known that 12-month-old Wistar rats compared to 8-month-old rats, have reduced antioxidant blood activity [13] and pronounced metabolic syndrome [14]. To improve the translation of the results of experimental studies into the clinical practice, we tried to take these data into account in determining the age composition of the experimental groups.

The clinical relevance of investigating the regeneration of the sciatic nerve in the experimental animals is conditioned by its frequent injury in combat situations [15] and in hip arthroplasty surgeries [16, 17].

In our study, we obtained the first evidence of the effect of a single intraoperative electrical stimulation on the morphofunctional characteristics of the lower leg and foot muscles in the long term (4 and 6 months) after autoplasty of the resection defect of the tibial portion of the sciatic nerve. It was found that in the gastrocnemius muscle of stimulated animals, compared to non-stimulated ones, muscle fiber atrophy and endomysial fibrosis were less pronounced, the effect being mediated by increased vascularization. The most clinically significant result obtained in this study is that in stimulated animals, the sciatic functional index was improved in comparison with non-stimulated animals during up to 6-month period after surgery, despite the fact that at this time many animals either approach the age of aging-related sarcopenia or even exceed it. It is known that in Wistar rats, a decrease in the mass of the calf muscles, dysfunction of neuromuscular junctions, gait disturbances and changes in the numerical and dimensional composition of muscle fibers are detected already at 18 months of age [18], and similar changes are detected in the calf muscles in humans [19].

The sciatic functional index depends on the condition of not only of the lower leg muscle, but also of muscles of foot. At 4 months after the operation, the volume density of blood vessels in the plantar interosseous muscles, as in the gastrocnemius muscle, was reliably higher in the stimulated animals than in non-stimulated ones, but the diameters of muscle fibers and the volume density of endomysium were comparable. At 6 months after the operation, endomysial fibrosis progressed in both series. However, in the series with electrical stimulation, muscle fibers with myopathic alterations were less common.

Progression of the endomysial fibrosis of the plantar interosseous muscles at 6 months was probably associated with the development of age-related sarcopenia. According to other authors, in adult rats, the expression of neural cell adhesion molecule NCAM1 is reduced, and the level of Gadd45α protein regulating biogenesis of mitochondria and differentiation of preadipocytes, is increased compared to young rats, which documents maturation-related denervation of plantar muscles [20], however, it was proven that high-intensity exercises prevent age-related denervation atrophy.

CONCLUSION

A single intraoperative electrical stimulation permits to reduce denervation changes in the gastrocnemius muscles and plantar interosseous muscles associated with nerve injury and maturation, and also to improve

the tibial function index in the long-term period after autoneuroplasty.

The authors consider further searches for new methods of increasing the effectiveness of the results of autoneuroplasty of peripheral nerve defects to be relevant. Further developments of a complex of rehabilitation measures aimed at improving the morphofunctional parameters of the foot muscles and the results of autoneuroplasty are promising.

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СПИСОК ИСТОЧНИКОВ

1. Kornfeld T., Vogt P.M., Radtke C. Nerve grafting for peripheral nerve injuries with extended defect sizes // Wien. Med. Wochenschr. 2019. Vol. 169, No. 9–10. P. 240–251. doi: 10.1007/s10354-018-0675-6
2. Scholz T., Krichevsky A., Sumarto A., et al. Peripheral nerve injuries: an international survey of current treatments and future perspectives // J. Reconstr. Microsurg. 2009. Vol. 25, No. 6. P. 339–344. doi: 10.1055/s-0029-1215529
3. Kuffler D.P., Foy C. Restoration of Neurological Function Following Peripheral Nerve Trauma // Int. J. Mol. Sci. 2020. Vol. 21, No. 5. P. 1808. doi: 10.3390/ijms21051808
4. Matejcik V., Steno J., Benetin J., et al. Results of peripheral nerve reconstruction by autograft // Bratisl. Lek. Listy. 2001. Vol. 102, No. 2. P. 92–98.
5. Grinsell D., Keating C.P. Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies // Biomed Res. Int. 2014. Vol. 2014. P. 698256. doi: 10.1155/2014/698256
6. Roh J., Schellhardt L., Keane G.C., et al. Short-Duration, Pulsatile, Electrical Stimulation Therapy Accelerates Axon Regeneration and Recovery following Tibial Nerve Injury and Repair in Rats // Plast. Reconstr. Surg. 2022. Vol. 149, No. 4. P. 681e–690e. doi: 10.1097/prs.0000000000008924
7. Calvey C., Zhou W., Stakleff K.S., et al. Short-term electrical stimulation to promote nerve repair and functional recovery in a rat model // J. Hand Surg. Am. 2015. Vol. 40, No. 2. P. 314–322. doi: 10.1016/j.jhsa.2014.10.002
8. Koh G.P., Fouad C., Lanzinger W., et al. Effect of Intraoperative Electrical Stimulation on Recovery after Rat Sciatic Nerve Isograft Repair // Neurotrauma Rep. 2020. Vol. 1, No. 1. P. 181–191. doi: 10.1089/neur.2020.0049
9. Bain J.R., Mackinnon S.E., Hunter D.A. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat // Plast. Reconstr. Surg. 1989. Vol. 83, No. 1. P. 129–138. doi: 10.1097/00006534-198901000-00024
10. Contreras E., Bolívar S., Nieto–Nicolau N., et al. A novel decellularized nerve graft for repairing peripheral nerve long gap injury in the rat // Cell Tissue Res. 2022. Vol. 390, No. 3. P. 355–366. doi: 10.1007/s00441-022-03682-1
11. Kaneko A., Naito K., Nakamura S., et al. Influence of aging on the peripheral nerve repair process using an artificial nerve conduit // Exp. Ther. Med. 2021. Vol. 21, No. 2. P. 168. doi: 10.3892/etm.2020.9599
12. Aman M., Zimmermann K.S., Thielen M., et al. An Epidemiological and Etiological Analysis of 5026 Peripheral Nerve Lesions from a European Level I Trauma Center // J. Pers. Med. 2022. Vol. 12, No. 10. P. 1673. doi: 10.3390/jpm12101673
13. Kumar D., Rizvi S.I. Age-dependent paraoxonase 1 (PON1) activity and LDL oxidation in Wistar rats during their entire lifespan // ScientificWorldJournal. 2014. Vol. 2014. P. 538049. doi: 10.1155/2014/538049
14. Ghezzi A.C., Cambri L.T., Botezelli J.D., et al. Metabolic syndrome markers in Wistar rats of different ages // Diabetol. Metab. Syndr. 2012. Vol. 4, No. 1. P. 16. doi: 10.1186/1758-5996-4-16
15. Jones P.E., Meyer R.M., Faillace W.J., et al. Combat Injury of the Sciatic Nerve — An Institutional Experience // Mil. Med. 2018. Vol. 183, No. 9–10. P. e434–e441. doi: 10.1093/milmed/usy030

16. Пронских А.А., Харитонов К.Н., Корыткин А.А., и др. Тотальное эндопротезирование у пациентов с последствиями переломов вертлужной впадины // Гений ортопедии. 2021. Т. 27, № 5. С. 620–627. doi: 10.18019/1028-4427-2021-27-5-620-627
17. Шнайдер Л.С., Голенков О.И., Тургунов Э.У., и др. Укорачивающая подвертельная остеотомия бедренной кости при эндопротезировании тазобедренного сустава у пациентов с врожденным вывихом бедра // Гений ортопедии. 2020. Т. 26, № 3. С. 340–346. doi: 10.18019/1028-4427-2020-26-3-340-346
18. Pannérec A., Springer M., Migliavacca E., et al. A robust neuromuscular system protects rat and human skeletal muscle from sarcopenia // Aging (Albany NY). 2016. Vol. 8, No. 4. P. 712–729. doi: 10.18632/aging.100926
19. Janssen I., Heymsfield S.B., Wang Z.M., et al. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr // J. Appl. Physiol. (1985). 2000. Vol. 89, No. 1. P. 81–88. doi: 10.1152/jappl.2000.89.1.81
20. Tayebi S.M., Siahkouhian M., Keshavarz M., et al. The Effects of High-Intensity Interval Training on Skeletal Muscle Morphological Changes and Denervation Gene Expression of Aged Rats // Monten. J. Sports Sci. Med. 2019. Vol. 8, No. 2. P. 39–45. doi: 10.26773/mjssm.190906

REFERENCES

1. Kornfeld T, Vogt PM, Radtke C. Nerve grafting for peripheral nerve injuries with extended defect sizes. *Wien Med Wochenschr*. 2019;169(9–10):240–51. doi: 10.1007/s10354-018-0675-6
2. Scholz T, Krichevsky A, Sumarto A, et al. Peripheral nerve injuries: an international survey of current treatments and future perspectives. *J Reconstr Microsurg.* 2009;25(6):339–44. doi: 10.1055/s-0029-1215529
3. Kuffler DP, Foy C. Restoration of Neurological Function Following Peripheral Nerve Trauma. *Int J Mol Sci.* 2020;21(5):1808. doi: 10.3390/ijms21051808
4. Matejcik V, Steno J, Benetin J, et al. Results of peripheral nerve reconstruction by autograft. *Bratisl Lek Listy.* 2001;102(2):92–8. (In Engl., Slovak.).
5. Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int.* 2014;2014:698256. doi: 10.1155/2014/698256
6. Roh J, Schellhardt L, Keane GC, et al. Short-Duration, Pulsatile, Electrical Stimulation Therapy Accelerates Axon Regeneration and Recovery following Tibial Nerve Injury and Repair in Rats. *Plast Reconstr Surg.* 2022;149(4):681e–90e. doi: 10.1097/prs.0000000000008924
7. Calvey C, Zhou W, Stakleff KS, et al. Short-term electrical stimulation to promote nerve repair and functional recovery in a rat model. *J Hand Surg Am.* 2015;40(2):314–22. doi: 10.1016/j.jhsa.2014.10.002
8. Koh GP, Fouad C, Lanzinger W, et al. Effect of Intraoperative Electrical Stimulation on Recovery after Rat Sciatic Nerve Isograft Repair. *Neurotrauma Rep.* 2020;1(1):181–91. doi: 10.1089/neur.2020.0049
9. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. *Plast Reconstr Surg.* 1989;83(1):129–38. doi: 10.1097/00006534-198901000-00024
10. Contreras E, Bolívar S, Nieto–Nicolau N, et al. A novel decellularized nerve graft for repairing peripheral nerve long gap injury in the rat. *Cell Tissue Res.* 2022;390(3):355–66. doi: 10.1007/s00441-022-03682-1
11. Kaneko A, Naito K, Nakamura S, et al. Influence of aging on the peripheral nerve repair process using an artificial nerve conduit. *Exp Ther Med.* 2021;21(2):168. doi: 10.3892/etm.2020.9599
12. Aman M, Zimmermann KS, Thielen M, et al. An Epidemiological and Etiological Analysis of 5026 Peripheral Nerve Lesions from a European Level I Trauma Center. *J Pers Med.* 2022;12(10):1673. doi: 10.3390/jpm12101673
13. Kumar D, Rizvi SI. Age-dependent paraoxonase 1 (PON1) activity and LDL oxidation in Wistar rats during their entire lifespan. *Scientific World Journal.* 2014;2014:538049. doi: 10.1155/2014/538049
14. Ghezzi AC, Cambri LT, Botezelli JD, et al. Metabolic syndrome markers in Wistar rats of different ages. *Diabetol Metab Syndr.* 2012;4(1):16. doi: 10.1186/1758-5996-4-16
15. Jones PE, Meyer RM, Faillace WJ, et al. Combat Injury of the Sciatic Nerve — An Institutional Experience. *Mil Med.* 2018;183(9–10):e434–41. doi: 10.1093/milmed/usy030
16. Pronskikh AA, Kharitonov KN, Korytkin AA, et al. Total hip arthroplasty in patients with acetabular fractures. *Genij Ortopedii.* 2021;27(5):620–7. (In Russ). doi: 10.18019/1028-4427-2021-27-5-620-627
17. Shnaider LS, Golenkov OI, Turgunov EU, et al. Shortening subtrochanteric osteotomy of the femur in total hip arthroplasty in patients with congenital hip dislocation. *Genij Ortopedii.* 2020;26(3):340–6. (In Russ). doi: 10.18019/1028-4427-2020-26-3-340-346
18. Pannérec A, Springer M, Migliavacca E, et al. A robust neuromuscular system protects rat and human skeletal muscle from sarcopenia. *Aging (Albany NY).* 2016;8(4):712–9. doi: 10.18632/aging.100926
19. Janssen I, Heymsfield SB, Wang ZM, et al. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol (1985).* 2000;89(1):81–8. doi: 10.1152/jappl.2000.89.1.81
20. Tayebi SM, Siahkouhian M, Keshavarz M, et al. The Effects of High-Intensity Interval Training on Skeletal Muscle Morphological Changes and Denervation Gene Expression of Aged Rats. *Monten J Sports Sci Med.* 2019;8(2):39–45. doi: 10.26773/mjssm.190906

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