Original article
https://doi.org/10.18019/1028-4427-2024-30-3-417-426

Evaluation of long-term results of single intraoperative electrical neurostimulation after autologous plastic surgery of a resection defect of the tibial portion of the sciatic nerve in adult rats

N.A. Shchudlo, T.N. Varsegova✉, T.A. Stupina

Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

Corresponding author: Tatyana N. Varsegova, varstn@mail.ru

Abstract

Introduction World literature data indicate the effectiveness of single intraoperative electrical stimulation (IES) of the proximal segment of the damaged nerve to stimulate its regeneration, but there is no data on its effect on the long-term results of autoplasty of resection defects.

The purpose of the work was to evaluate the long-term results of a single IES after autologous plastic surgery of the tibial portion of the sciatic nerve in rats.

Materials and methods Thirty rats after autologous repair of the resection defect of the tibial portion of the sciatic nerve were divided into series 1 (unstimulated control, $n = 16$) and series 2 (single IES for 40 minutes, $n = 14$). At 4 and 6 months after surgery, the static sciatic functional index (SFI) and morphometry of epoxy transverse semithin sections of the tibial nerve at the level of the middle third of the leg were assessed. For comparison with the normal values, the corresponding data from 7 intact rats were used.

Results The number of animals with excellent results of SFI restoration was 12.5 % in series 1 and 50 % in series 2 ($p = 0.05$). The numerical density of regenerated myelinated fibers (MF) exceeded the norm: in series 1 — by 63 % ($p < 0.01$) and 34 % ($p < 0.01$), in series 2 — by 58 % ($p < 0.01$) and 47 % ($p < 0.01$), respectively. In series 2, there were greater values in comparison with series 1: the median diameters of MFs were by 11.7 % and 15.7 %, the median diameters of their axons were by 5.4 % and 11.9 %, the median thickness of the myelin sheath was by 17.0 % and 24.1 %, respectively ($p < 0.05$ 4 months and $p < 0.01$ 6 months after surgery). Four months after surgery in series 1 and 2, the numerical densities of endoneurial vessels exceeded the intact control by 134 % ($p < 0.05$) and 156 % (0.05), their average diameters by 18 % and 16 % ($p < 0.01$) respectively, and lumen diameters increased only in series 2 by 8 % ($p = 0.07$). After 6 months of the experiment in series 1 and 2, the numerical densities of microvessels decreased, but significantly exceeded the control by 66 % ($p < 0.05$) and 83 % ($p < 0.05$), the average diameters — by 14 % and 36 % ($p < 0.05$), lumen diameters — by 26 % ($p < 0.05$) and 50 % ($p < 0.01$), respectively.

Discussion The difference between stimulated and unstimulated animals in all MF size parameters 6 months after surgery was greater than after 4 months, indicating a persistent neuroregenerative effect.

Conclusions A significant increase in the diameters of regenerating nerve fibers in the tibial nerve, as well as the diameters of their axons and the thickness of the myelinated sheaths 4 and 6 months after autoplasty of the tibial portion of the sciatic nerve in the group of animals with a single 40-minute IES of the proximal portion of the sciatic nerve indicates the promoting effect of the applied additive effect on regenerative axono- and myelinogenesis. Increase in the lumens and improvement of blood flow of the endoneurial vessels of the tibial nerve in the series with IES ensured the stability of the neuroregenerative effect. The functional significance of the effects of a single IES is confirmed by a significantly higher percentage of animals with excellent results in restoring the static functional index.

Keywords: rats, sciatic nerve, autoplastic, intraoperative electrical neurostimulation, sciatic functional index, hystomorphometry

For citation: Shchudlo N.A. Varsegova T.N. Stupina T.A. Evaluation of long-term results of single intraoperative electrical neurostimulation after autologous plastic surgery of a resection defect of the tibial portion of the sciatic nerve in adult rats. *Genij Ortopedii*. 2024;30(3):417-426. doi: 10.18019/1028-4427-2024-30-3-417-426

INTRODUCTION

Peripheral nerve injuries occur in 3.3 % of upper extremity [1] and in 1.8 % of lower extremity trauma [2]. They vary significantly in mechanisms of injury, severity of injury, and treatment outcomes. During military conflicts, the incidence of damage to the nerves of the extremities increases significantly; they frequently lead to disability with severe social and personal consequences for the injured [3].

Despite the potential of restoring the functions of damaged peripheral nerves in mammals and humans [4], their regeneration after complete anatomical cutting requiring surgical intervention occurs slowly and usually incompletely: no more than half of the patients achieve good or excellent results in restoring sensitivity and movements [5]. Unsatisfactory results of treatment of nerve injuries in the clinic setting have a fundamental biological basis. These include post-traumatic apoptosis of sensory neurons [6], the latent period of regeneration during which the axons of the proximal segment of the nerve do not grow into the damaged area [7], low rate of regenerative growth, especially in adults [8], scar formation [9, 10] , destruction of denervated target organs [11], including their capillaries [12].

A variety of techniques for electrical stimulation of peripheral nerves may accelerate and improve the recovery of motor and sensory functions [13, 14, 15]. Since a great number of patients do not have the opportunity to receive a course of rehabilitation treatment, the interests of many researchers in recent years have focused on studying the effects of a single intraoperative electrical stimulation (IES).

In a randomized study of 36 patients who underwent reconstructive surgery on the digital nerves, it was found that one hour-long session of low-frequency IES (20 Hz, 1 hour) improved all types of sensitivity by 5–6 months after surgery compared with the unstimulated group, but the difference was not statistically confirmed [16].

Animal experiments have demonstrated the positive effects of a single IES in various models of nerve injury in the femur and tibia. In focal demyelination of the tibial nerve of rats, IES accelerated the clearance of demyelination products and subsequent remyelination [17]. By modeling neuroma in continuum of the sciatic nerve of rats, a single IES of the proximal section of the damaged nerve improved the recovery of limb function after 4 to 8 weeks, but after 3 months differences with the unstimulated group were leveled out [18]. In experiments on transection and suture of the tibial nerve of rats, it was found that even a 10-minute IES accelerates the growth of the suture zone with regenerating axons [19]. Similar results were obtained by other authors when transecting and suturing the sciatic nerve of mice [20], who also proved an increase in the number of neurons entering regeneration in series with electrical stimulation. After transecting and microsurgically suturing the femoral nerve of 10-week-old rats, one-hour IES caused a more rapid recovery of functional parameters in comparison with sham-stimulated animals, reaching the preoperative level at 5 months after injury, which, according to the authors, is associated with an increase in the number of motor neurons that correctly reinnervated target organs [21].

Following autoplasty of the common peroneal nerve in young rats, a single IES of the proximal segment of the nerve provided an increase in the number of sensory and motor neurons entering regeneration, as well as an increase in the number of myelinated nerve fibers regenerating into the distal segment of the damaged nerve 6 weeks after surgery [22].

Following autoplasty of the sciatic nerve, IES improved the motor function of the limb of rats in the period from 2 to 12 weeks compared with unstimulated animals, however, sensory testing and histomorphometry of the distal segment of the nerve and gastrocnemius muscles did not reveal any merits in the series with IES [23].

In the available literature, we did not find data on the effect of single intraoperative electrical stimulation on the long-term results of autoplasty of mixed nerves. This fact determined the purpose of our study.

The purpose of the work was to evaluate the long-term results of a single intraoperative electrical neurostimulation after autologous plastic surgery of a resection defect of the tibial portion of the sciatic nerve.

MATERIALS AND METHODS

The experiment was performed on 30 male laboratory Wistar rats (age 8–15 months, weight 360–460 g). The animals were kept in controlled hygienic conditions and had access to water and food. The experiment was carried out in accordance with the European Convention for the Protection of Vertebrate Animals, Directive 2010/63/EU of the European Parliament and the Council of the European Union for 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 institutional ethics committee (protocol No. 2 (57) dated May 17, 2018). For anesthesia and pain relief, the animals were injected intramuscularly with 0.8 mg of xylazine hydrochloride and 0.4 mg of tiletamine/zolazepam per 100 g of body weight, and the hair on the right thigh and lower leg was cut.

In the operating room, after treating the skin with iodine-alcohol tincture and performing a non-projection skin incision, the access was made to the right sciatic nerve at the level of the middle third of the thigh using a sharp-blunt method through the biceps muscle. Under an 8x magnification of an operating room microscope (OPMI-6, Germany), epifascicular longitudinal epineurotomy incisions were made with a sharp vascular microprobe and iridectomy scissors to isolate the tibial portion of the sciatic nerve. After resection of its 6 mm section, interfascicular autologous repair of the resulting defect was performed using microsurgical suture 9–0/10–0 caliber material. In series 1 of non-stimulated control animals (n = 16), at the end of autoneuroplasty, the wound was sutured layer-by-layer with 3-0 caliber absorbable suture material. In series 2 of the study group (n = 14), immediately after autoneuroplasty, electrodes were installed on the proximal portion of the nerve. Using the system of electrical stimulation of peripheral nerves ElSI.08.ice (registration certificate No. RZN 2017/5382; LLC High Medical Technologies), for 40 minutes. intraoperative stimulation of the proximal segment of the nerve was performed with monopolar electrical pulses of a rectangular shape with an amplitude of 0.25 mA, a frequency of 20 Hz and a duration of 100 μs. The wound was sutured at the end of the electrical stimulation session.

To ensure comparability of the experimental groups by age, a pair of rats underwent surgery on each operating day: one without stimulation, the other with stimulation; one pair of eight-month-old rats were both without stimulation.

In the postoperative period, studies of the static sciatic functional index (SFI) were carried out according to the modified method [24], considering the semiotics of denervation-reinnervation syndrome (Table 1). To do this, each rat was placed in a box made of transparent plastic with holes for air access. The box was fixed on a tripod and placed above the table on which the mirror was placed. Using a fluorescent lamp, the box was illuminated and the reflection of the rat's feet in the mirror was photographed, from 3 to 5 shots from each rat were taken. Digital photos were saved in the computer memory, and then the spread of their toes was measured in the Photofiltre program (Fig. 1): total (between the first and fifth toes) and intermediate (between the second and fourth toes). The ratio of the spread of the toes of the involved foot to the corresponding spread of the contralateral foot in fractions of a unit was used as the SFI. Measurements of the toe spread in animals were not carried out if the foot of the involved limb had no support on the pads of the paws, but on the dorsal and/or lateral surface of the foot if contractures and deformities developed. The SFI rating scale is also presented in Table 1.

Table 1

Semiotics of denervation-reinnervation syndrome and assessment of the static sciatic functional index in the long-term period after autoplasty of the tibial portion of the sciatic nerve

Symptoms	Evaluation
Support on the dorsal and/or lateral surface of the foot in developed contractures and deformities of the metatarsus and toes. Support on the pads of the paws without the speading toes or with partial restoration of the spread and hyperextension in the metatarsophalangeal joints and flexion in the interphalangeal joints	poor
Correct foot position with the presence of intermediary spread and adduction of either the first or fifth toe	fair
The toes are separated symmetrically. Intermediary or total span of 0.8 or more, or in some shots both spans of 0.8 or more	good
Indices of intermediary and total spread 0.8 and more	excellent

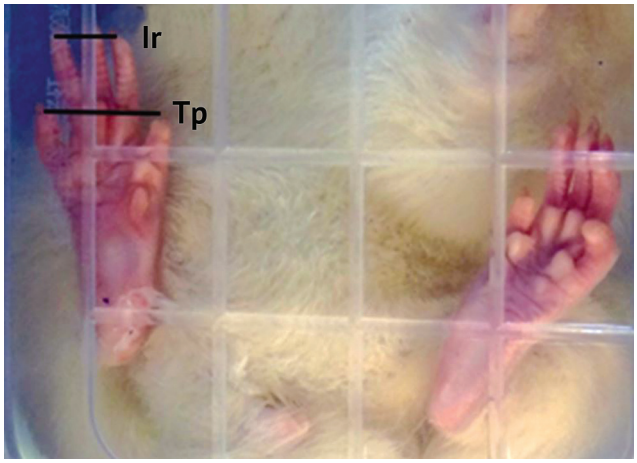

Fig. 1 Photo of the reflection of a rat's feet when it is placed in a plastic box. Normal position of the foot of the contralateral limb with a pronounced Ir — intermediary toe spread and Tp — total toe spread; lack of finger spreading in flexion of the interphalangeal joints of the foot of the involved limb and denervation atrophy of small muscles of the foot

Animals were removed from the experiment. Dissected samples of sciatic and tibial nerves were subjected to aldehyde-osmium fixation and embedded in Araldite to obtain semi-thin sections. Sections were cut with diamond knives on a Nova LKB ultramicrotome (Sweden), stained with toluidine blue and the polychrome method — methylene blue, azure II and basic fuchsin. Microscopy of sections and acquisition of digital images was carried out using an AxioScope. A1 microscope and an AxioCam digital camera (Carl Zeiss MicroImaging GmbH, Germany). 15–30 endoneurial microvessels and 400–500 myelinated nerve fibers were histomorphometrically examined in each animal at 1000× magnification. Their numerical densities in 1 mm^2 of the bundle area were determined, the diameters of the fibers, their axons and the thickness of the myelinated sheaths were measured, and histograms of the distribution of fibers by diameter were constructed with a step of 1 μm. The proportion (%) of destructively altered nerve conductors was calculated. The diameters of microvessels and their lumens were measured, and the modified Kernogan index was determined as the ratio of the lumen diameter to the vessel diameter. For comparison with the norm, we used histomorphometric data from 7 adult intact rats, close in age to the experimental rats at the time of euthanasia, age 16–19 months (in this group there are no interindividual statistically significant differences in the studied quantitative indicators).

Statistical data processing was performed in the Attestat software, version 9.3.1 (developed by I.P. Gaidyshev, certificate of registration with Rospatent No. 2002611109). Samples were checked for normal distribution of values using the Kolmogorov and Shapiro – Wilk tests; pairwise comparison of experimental series with each other and with intact controls was carried out using the Mann – Whitney, Chi-square, and Fisher's exact tests. The values of histomorphometric parameters were presented as medians and quartiles — Me [Q1; Q3]. The significance level of differences is 0.05.

RESULTS

A significant difference was obtained between series 1 and 2 in the rate of excellent results when SFI was assessed in the long term after surgery (Table 2).

Table 2

Distribution of static sciatic functional index scores in the long-term period after autoplasty of the tibial portion of the sciatic nerve

Result	Series 1 — autoplasty (n = 16)		Series 2 — autoplasty + IES (n = 14)		P^{1-2}
	number	%	number	%	
Poor	9	56.25	4	28.57	0.16
Fair	1	6.25	0	0	0.55
Good	4	25.00	3	21.43	1.00
Excellent	2	12.50	7	50.00	0.05

P^{1-2} — Levels of significance of differences by comparing groups of unstimulated and stimulated animals using Fisher's exact test; * — differences significant at $P \leqslant 0.05$

It is important to note that the age of the 7 rats that achieved excellent results in the series 2 varied from 8 to 13 months at the time of the operation.

Microscopic examination of the distal zone of the sutures showed that in series 1 and 2 after 4 and 6 months post- surgery, the endoneurium and epineurium of the graft were abundantly neuroticized by regenerating myelinated and non-myelinated fibers. Some of them were located as part of minifascicles or regeneration clusters (Fig. 2).

Transverse semi-thin sections of the tibial nerve at the level of the middle third of the tibia (Fig. 3) also revealed a large population of regenerated myelinated and unmyelinated fibers and a few regenerative clusters. In the endoneurium of regenerating nerves, fibroblasts, macrophages, perineural and mast cells, as well as nucleated profiles of myelinated and unmyelinated fibers, were more common than in intact nerves (Fig. 3).

At 6 months after surgery, compared with the previous period, the number of large myelinated fibers increased both at the level of the graft and in the tibial nerves at the level of the middle third (Fig. 2, c, d; Fig. 3, c, d), which was visually more noticeable in stimulated animals.

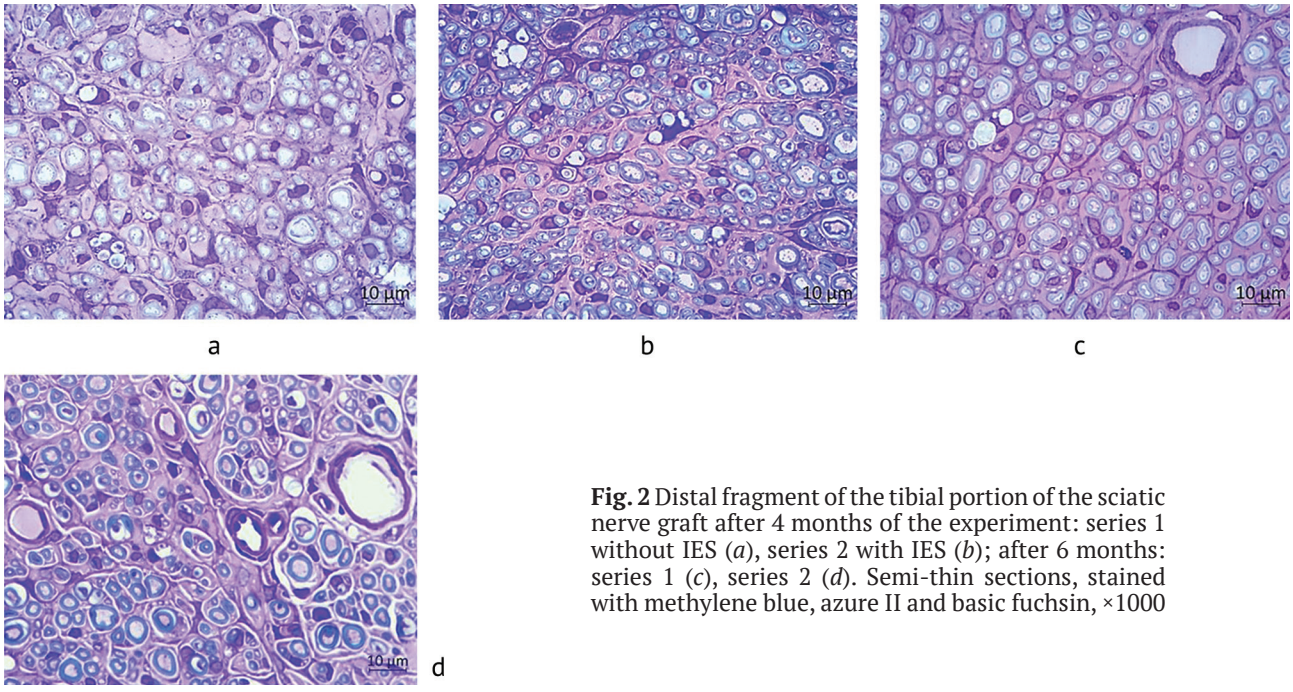

Fig. 2 Distal fragment of the tibial portion of the sciatic nerve graft after 4 months of the experiment: series 1 without IES (*a*), series 2 with IES (*b*); after 6 months: series 1 (*c*), series 2 (*d*). Semi-thin sections, stained with methylene blue, azure II and basic fuchsin, ×1000

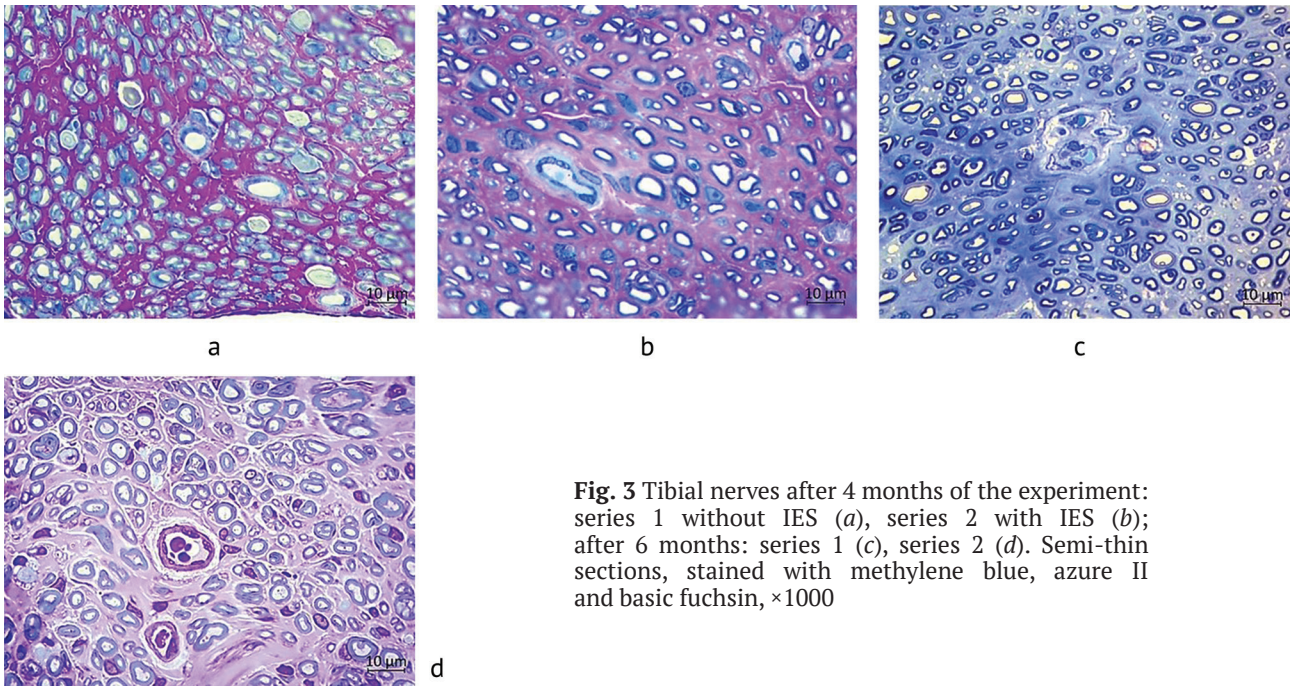

Fig. 3 Tibial nerves after 4 months of the experiment: series 1 without IES (*a*), series 2 with IES (*b*); after 6 months: series 1 (*c*), series 2 (*d*). Semi-thin sections, stained with methylene blue, azure II and basic fuchsin, ×1000

A histomorphometric study of the tibial nerve showed that the numerical density of regenerated fibers after 4 and 6 months post-surgery significantly exceeded the norm: in series 1 by 63 % and 34 %, in series 2 by 58 % and 47 %, respectively (Table 3). Proportion of destructively changed conductors after 4 and 6 months post-surgery in experimental animals was significantly lower than the values of intact nerves (Table 3).

Table 3

Numerical densities and proportions (%) of destructively altered myelinated nerve fibers of the tibial nerve in 1 mm^2 of section area after 4 and 6 months post-surgery, Me [Q1; Q3]

Series/experiment term	Numerical density of myelinated fibers		Proportion of destructively altered myelinated fibers	
	4 months	6 months	4 months	6 months
Series 1 — autoplasty (n = 16)	24444 [19182; 28280] P^{1-2} = 0.319465 P^{1-K} = 0.0000002*	20207 [18140; 22618] P^{1-2} = 0.187249 P^{1-K} = 0.000573*	4,46 % [2,79; 5.73] P^{1-2} = 0.072117 P^{1-K} = 0.001244*	4,05 % [2,38; 5.06] P^{1-2} = 0.99999 P^{1-K} = 0.00046*
Series 2 — autoplasty + IES (n = 14)	23786 [22142; 24882] P^{2-K} = 0.0000001*	22142 [20498; 23411] P^{2-K} = 0.000289*	5,58 % [3,85; 6.05] P^{2-k} = 0.030766*	4,05 % [2,38; 5.06] P^{2-K} = 0.00014*
Intract controls (n = 7)	15040 (12859; 15499)		6,75 % (5,70; 8,13)	

P^{1-2} — significance levels of differences in compared groups of unstimulated and stimulated animals using the Mann – Whitney test: P^{1-K}, P^{2-K} — significance levels of differences between each group of operated animals and intact controls according to the Mann – Whitney test; * — differences are significant at $p < 0.05$

The dimensional characteristics of myelinated regenerating fibers were significantly greater in series 2 compared to series 1 (Table 4) after 4 and 6 months post-surgery: median diameters of fibers by 11.7 % and 15.7 %, median diameters of their axons by 5.4 % and 11.9 %, median thickness of the myelin sheath by 17.0 % and 24.1 %, respectively. However, even at the end of the experiment, all dimensional characteristics of the fibers in both groups of experimental animals were significantly smaller than the intact nerve.

The distribution of myelinated fibers in regard to the diameter of series 1 and series 2 was significantly different from the distribution in the intact nerve (Fig. 4). Even after 6 months post-surgery it remained unimodal, the number of histogram classes was reduced. However, at both 4 and 6 months after surgery, in series 2, the main peak of the histogram was shifted to the right compared to series 1, and the number of histogram classes was one class more; and this difference between the series after 6 months was confirmed statistically (Fig. 4).

Table 4

Dimensional parameters of myelinated fibers of the tibial nerve at 4 and 6 months post-surgery, Me [Q1; Q3]

Parameter	Series 1 — autoplasty (n = 16)		Series 2 — autoplasty+IES (n = 14)		Intact controls (n = 7)
	4 months (n = 7)	6 months (n = 7)	4 months (n = 8)	6 months (n = 8)	
Fiber diameter (μm)	3.24 [2.52; 4.03] P^{1-K} = 0.00119* P^{1-2} = 0.00267*	3.50 [2.79; 4.04] P^{1-K}= 0.00119* P^{1-2} = 0.00982*	3.62 [2.91; 4.37] P^{2-K} = 0.00014*	4.05 [3.14; 5.12] P^{2-K} = 0.00268*	6.73 [5.50; 8.75]
Axon diameter (μm)	2.23 [1.65; 2.87] P^{1-K} = 0,.00119* P^{1-2} = 0.02914*	2,42 [1,84; 2,84] P^{1-K} = 0,00119* P^{1-2} = 0,00450*	2,35 [1,82; 2,94] P^{2-K} = 0,00174*	2,71 [2,02; 3,53] P^{2-K} = 0,0027*	4.34 [3.54; 5.18]
Thickness of myelinated membranes (μm)	0.53 [0.40; 0.63] P^{1-K} = 0.00119* P^{1-2} = 0.02831*	0.54 [0.44; 0.62] P^{1-K} = 0.00119* P^{1-2} = 0.0027*	0.62 [0.50; 0.75] P^{2-K} = 0.00038*	0.67 [0.53; 0.83] P^{2-K} = 0.00255*	1.02 [0.72; 1.30]

P^{1-2} — significance levels of differences in compared groups of unstimulated and stimulated animals using the Mann – Whitney test: P^{1-K}, P^{2-K} — significance levels of differences between each group of operated animals and intact controls according to the Mann – Whitney test; * — differences are significant at $p < 0.05$

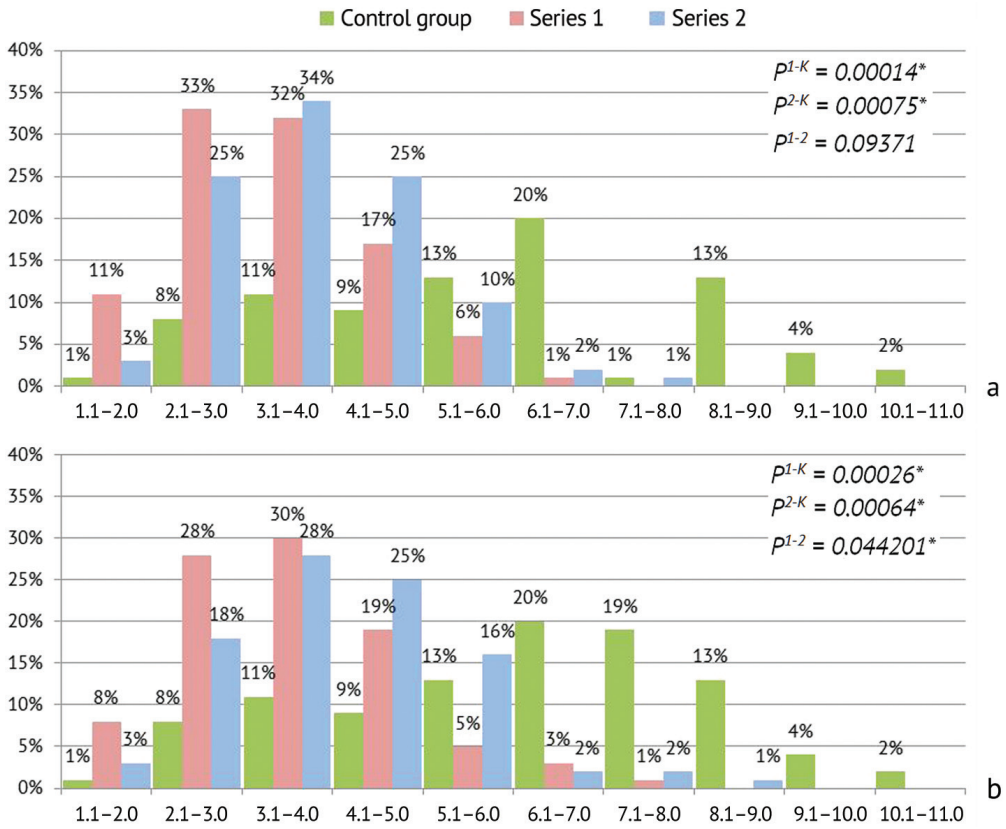

Fig. 4 Histograms of the distribution of myelinated fibers by diameter: *a* — 4 months after operation; *b* — 6 months after operation. The abscissa axis is the diameters of the fibers in microns, the ordinate axis is their percentage in the sample; P^{1-2} — significance levels of differences between unstimulated and stimulated animals of series 1 and 2; P^{1-K}, P^{2-K} — significance levels of differences in each series with intact control using the Chi-square test

A study of endoneural vascularization showed that at 4 months after surgery in series 1 and 2, the numerical densities of endoneurial vessels significantly exceeded the intact control by 134 % and 156 %, their average diameters by 18 % and 16 %, respectively, and the lumen diameter increased only in series 2 by 8 % (Table 5) . A decrease in the Kernogan index parameter in series 1 at this time relative to the control indicates a deterioration in the throughput of blood vessels. After 6 months in series 1 and 2, the numerical densities of microvessels decreased, but significantly exceeded the control by 66 % and 83 %, the average diameters — by 14 % and 36 %, the diameters of the lumens — by 26 % and 50 %, respectively (Table 5). At the same time, there were no significant differences in the Kernogan index parameter between the series at this period of the experiment (Table 5).

Table 5

Dimensional parameters of the endoneurial vessels of the tibial nerve after 4 and 6 months of the experiment, Me [Q1; Q3]

Parameter	Series 1 — autoplasty (n = 16)		Series 2 — autoplasty + IES (n = 14)		Intact controls (n = 7)
	4 мес. (n = 7)	6 мес. (n = 7)	4 мес. (n = 8)	6 мес. (n = 8)	
Numerical density of vessels	200 [109; 219] P^{1-K} = 0.01935* P^{1-2} = 0.25751	142.13 [130.69; 168.27] P^{1-K} = 0.00618* P^{1-2} = 0.715001	219 [109; 274] P^{2-K} = 0.00695*	156.31 [132;183] P^{2-K} = 0.03213*	85.64 [82.21; 127.88]
Vessel diameter (μm)	14.49 [12.56; 16.88] P^{1-K} = 0.00206* P^{1-2} = 0.854221	14.08 [11.91; 17.82] P^{1-K} = 0.00138* P^{1-2} = 0.01454*	14,29 [11.86; 17.53] P^{2-K} = 0.00743*	16,76 [13.45; 19.14] P^{2-K} = 0.00007*	12,32 [9.31; 15.30]
Lumen diameter (μm)	4.81 [1.75; 6.72] P^{1-K} = 0.355172 P^{1-2} = 0.126962	5.97 [3.64; 7.77] P^{1-K} = 0.06917 P^{1-2} = 0.00692*	5,10 [3.62; 7.29] P^{2-K} = 0.49948	7.09 [5.30; 10.01] P^{2-K} = 0.00001*	4,72 [3.28; 5.92]
Kernogan index	0.30 [0.15; 0.43] P^{1-K} = 0.00075* P^{1-2} = 0.002664*	0,42 [0.30; 0.44] P^{1-K} = 0.14051 P^{1-2} = 0.05255*	0,39 [0.28; 0.46] P^{2-K} = 0.29573	0.42 [0.39; 0.52] P^{2-K} = 0.31603	0,41 [0.32; 0.48]

P^{1-2} — significance levels of differences in compared groups of unstimulated and stimulated animals using the Mann – Whitney test: P^{1-K}, P^{2-K} — significance levels of differences between each group of operated animals and intact controls according to the Mann – Whitney test; * — differences are significant at $p < 0.05$

DISCUSSION

Compensation for functional deficits caused by nerve damage occurs through three mechanisms: reinnervation of denervated target organs through regeneration of damaged axons, reinnervation through collateral sprouting of undamaged axons, and remodeling of the central nervous system circuits related to lost functions [25].

To assess the severity of the regenerative component of morphofunctional recovery of nerve damage in animal models, the most informative method is histomorphometry of transverse semi-thin sections distal to the damage zone, which has found wide use in preclinical studies of neuroregeneration.

Thus, Oliveira et al noted correlation between the sciatic functional index and the numerical density of regenerating fibers in sciatic nerve damage of rats in the early stages of regeneration (up to 2 months after surgery), which the authors assessed as an adequate tool for assessing functional deficits [26]. According to Martins et al, of the 17 histomorphometric and electrophysiological parameters studied, only the average diameter of myelinated fibers proximal and distal to the injury zone correlated with the sciatic functional index at six months after surgery [27].

The noted pattern is apparently not accidental. Radial growth of axons, increasing the diameter of regenerated fibers, initiates only after contact of growth cones with target organs [28]. However, restoration of the normal size of regenerating fibers does not occur until 6 months after injury, even in the rat nerve compression model, when axonal regeneration occurs inside endoneural tubes that have preserved their integrity [29]. According to Ikeda et al, 7 months after transection and suturing of the sciatic nerve of rats, the average diameter of the regenerated nerve fibers did not exceed 50 % of the value of the intact nerve [30].

In our study, the median diameter of regenerated myelinated fibers at the level of the middle third of the leg was 52 % of the value of the intact group in unstimulated rats and exceeded 60 % in stimulated rats. This gives reason to believe that reinnervation of target organs in our experiments continued actively in both stimulated and unstimulated animals, but by 6 months after autoneuroplasty was not completed. The most significant result of our study is a significant difference in all dimensional parameters of regenerating nerve fibers in the groups of unstimulated and stimulated animals, proving the effectiveness of a single 40-minute session of IES of the proximal nerve segment. It should also be noted that the difference between stimulated and unstimulated animals in all dimentional parameters of myelinated fibers 6 months after surgery was greater than after 4 months, and indicates a persistent neuroregenerative effect.

The assessment of endoneural vascularization of the tibial nerve performed in our study at 4 and 6 months after autoneuroplasty of the tibial portion of the sciatic nerve also indicates the revascularizing IES effect.

Studies on vascularization of regenerating nerves are few, but they show an increase in the size of intraneural vessels under the influence of neuropeptides, as well as vasodilation and neoangiogenesis associated with increased secretion of vasogenic factors by resident and recruited macrophages [31]. In our study, the numerical density of endoneurial vessels and their diameters significantly exceeded the corresponding parameters of intact nerves in both groups of experimental animals. However, four months after surgery, the lumen diameter increased only in the stimulated rats by 8 %, which, along with changes in the Kernogan index, indicates a better flow rate in endoneurial vessels in this series compared to unstimulated controls. Six months after surgery, hypervascularization of the endoneurium persisted in both series, but in the stimulated rats, the diameters of endoneurial vessels and their lumens significantly exceeded the values of unstimulated rats by 19 %.

The obtained histomorphometric data are consistent with the results of the functional assessment.

According to clinical studies, good nerve function recovery after autoneuroplasty may only be obtained in patients under 25 years of age [32]. Our experiments used rats of age groups that, as reported, are characterized by a decrease in the level of antioxidants and the development of metabolic syndrome [33]. Thus, the results of our study may be translated into clinical practice for not only young but also middle-aged patients.

CONCLUSION

A significant increase in the diameters of regenerating nerve fibers in the tibial nerve, as well as the diameters of their axons and the thickness of the myelinated sheaths 4 and 6 months after autoplasty of the tibial portion of the sciatic nerve in the group of animals with a single 40 minute IES of the proximal portion of the sciatic nerve indicates the promoting effect of the applied additive effect on regenerative axono- and myelinogenesis.

Increase in the lumens and improvement in the blood flow rate of the endoneurial vessels of the tibial nerve in the series with IES ensured the stability of the neuroregenerative effect.

The functional significance of the effects of a single IES is confirmed by a significantly higher percentage of animals with excellent results in restoring the static functional index (50 % vs 12.15 % in unstimulated controls).

Funding *This scientific study was financed in the framework of state assignment.*

REFERENCES

1. Huckhagel T, Nüchtern J, Regelsberger J, et al. Nerve injury in severe trauma with upper extremity involvement: evaluation of 49,382 patients from the TraumaRegister DGU® between 2002 and 2015. *Scand J Trauma Resusc Emerg Med*. 2018;26(1):76. doi: 10.1186/s13049-018-0546-6
2. Huckhagel T, Nüchtern J, Regelsberger J, et al. Nerve trauma of the lower extremity: evaluation of 60,422 leg injured patients from the TraumaRegister DGU® between 2002 and 2015. *Scand J Trauma Resusc Emerg Med*. 2018;26(1):40. doi: 10.1186/s13049-018-0502-5
3. Litvinenko IV, Odinak MM, Zhivolupov SA, et al. Clinical and instrumental characteristics of traumatic lesions of peripheral nerves of limbs. *Bulletin of the Russian Military Medical Academy*. 2018;20(3):50-56. (In Russ.) doi: 10.17816/brmma12231
4. Ferguson TA, Son YJ. Extrinsic and intrinsic determinants of nerve regeneration. *J Tissue Eng*. 2011;2(1):2041731411418392. doi: 10.1177/2041731411418392
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. Raginov IS, Chelyshev YA. Post-traumatic survival of sensory neurons of different subpopulations. *Neurosci Behav Physiol*. 2005;35(1):17-20. doi: 10.1023/b:neab.0000049647.19397.30
7. Gordon T. Peripheral Nerve Regeneration and Muscle Reinnervation. *Int J Mol Sci*. 2020;21(22):8652. doi: 10.3390/ijms21228652
8. Lamoureux PL, O'Toole MR, Heidemann SR, Miller KE. Slowing of axonal regeneration is correlated with increased axonal viscosity during aging. *BMC Neurosci*. 2010;11:140. doi: 10.1186/1471-2202-11-140
9. Lemke A, Penzenstadler C, Ferguson J, et al. A novel experimental rat model of peripheral nerve scarring that reliably mimics post-surgical complications and recurring adhesions. *Dis Model Mech*. 2017;10(8):1015-1025. doi: 10.1242/dmm.028852

10. Wang ML, Rivlin M, Graham JG, Beredjiklian PK. Peripheral nerve injury, scarring, and recovery. *Connect Tissue Res*. 2019;60(1):3-9. doi: 10.1080/03008207.2018.1489381
11. Mu L, Chen J, Li J, et al. Immunohistochemical Detection of Motor Endplates in the Long-Term Denervated Muscle. *J Reconstr Microsurg*. 2018;34(5):348-358. doi: 10.1055/s-0038-1627463
12. Borisov AB, Huang SK, Carlson BM. Remodeling of the vascular bed and progressive loss of capillaries in denervated skeletal muscle. *Anat Rec*. 2000;258(3):292-304. doi: 10.1002/(SICI)1097-0185(20000301)258:3<292::AID-AR9>3.0.CO;2-N
13. ElAbd R, Alabdulkarim A, AlSabah S, et al. Role of Electrical Stimulation in Peripheral Nerve Regeneration: A Systematic Review. *Plast Reconstr Surg Glob Open*. 2022;10(3):e4115. doi: 10.1097/GOX.0000000000004115
14. Khudiayev AT, Martel II, Samylov VV, et al. Little-invasive techniques of treating peripheral nerve injuries. *Genij Ortopedii*. 2012;(1):85-88. (In Russ.)
15. Bazhanov SP, Shuvalov SD, Bakharev RM, et al. Comparison of short-term surgical outcomes of patients with closed brachial plexus traction injury. *Genij Ortopedii*. 2022;28(5):631-635. doi: 10.18019/1028-4427-2022-28-5-631-635
16. Wong JN, Olson JL, Morhart MJ, Chan KM. Electrical stimulation enhances sensory recovery: a randomized controlled trial. *Ann Neurol*. 2015;77(6):996-1006. doi: 10.1002/ana.24397
17. McLean NA, Verge VM. Dynamic impact of brief electrical nerve stimulation on the neural immune axis-polarization of macrophages toward a pro-repair phenotype in demyelinated peripheral nerve. *Glia*. 2016;64(9):1546-1561. doi: 10.1002/glia.23021
18. Shapira Y, Sammons V, Forden J, et al. Brief Electrical Stimulation Promotes Nerve Regeneration Following Experimental In-Continuity Nerve Injury. *Neurosurgery*. 2019;85(1):156-163. doi: 10.1093/neuros/nyy221
19. 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-690e. doi: 10.1097/PRS.0000000000008924
20. Sayanagi J, Acevedo-Cintrón JA, Pan D, et al. Brief Electrical Stimulation Accelerates Axon Regeneration and Promotes Recovery Following Nerve Transection and Repair in Mice. *J Bone Joint Surg Am*. 2021;103(20):e80. doi: 10.2106/JBJS.20.01965
21. Raslan A, Salem MAM, Al-Hussaini A, et al. Brief Electrical Stimulation Improves Functional Recovery After Femoral But Not After Facial Nerve Injury in Rats. *Anat Rec* (Hoboken). 2019;302(8):1304-1313. doi: 10.1002/ar.24127
22. Zuo KJ, Shafa G, Antonyshyn K, et al. A single session of brief electrical stimulation enhances axon regeneration through nerve autografts. *Exp Neurol*. 2020;323:113074. doi: 10.1016/j.expneurol.2019.113074
23. Koh GP, Fouad C, Lanzinger W, Willits RK. Effect of Intraoperative Electrical Stimulation on Recovery after Rat Sciatic Nerve Isograft Repair. *Neurotrauma Rep*. 2020;1(1):181-191. doi: 10.1089/neur.2020.0049
24. van Neerven SG, Bozkurt A, O'Dey DM, et al. Retrograde tracing and toe spreading after experimental autologous nerve transplantation and crush injury of the sciatic nerve: a descriptive methodological study. *J Brachial Plex Peripher Nerve Inj*. 2012 A;7(1):5. doi: 10.1186/1749-7221-7-5
25. Navarro X, Vivó M, Valero-Cabré A. Neural plasticity after peripheral nerve injury and regeneration. *Prog Neurobiol*. 2007;82(4):163-201. doi: 10.1016/j.pneurobio.2007.06.005
26. Oliveira EF, Mazzer N, Barbieri CH, Selli M. Correlation between functional index and morphometry to evaluate recovery of the rat sciatic nerve following crush injury: experimental study. *J Reconstr Microsurg*. 2001;17(1):69-75. doi: 10.1055/s-2001-12691
27. Martins RS, Siqueira MG, da Silva CF, Plese JP. Correlation between parameters of electrophysiological, histomorphometric and sciatic functional index evaluations after rat sciatic nerve repair. *Arq Neuropsiquiatr*. 2006;64(3B):750-756. doi: 10.1590/s0004-282x2006000500010
28. Regeneration of Neural Tissues. In: Stocum DL. *Regenerative Biology and Medicine* (Second Edition). San Diego: Academic Press Publ.; 2012:67-97.
29. Muratori L, Ronchi G, Raimondo S, et al. Can regenerated nerve fibers return to normal size? A long-term post-traumatic study of the rat median nerve crush injury model. *Microsurgery*. 2012;32(5):383-387. doi: 10.1002/micr.21969
30. Ikeda M, Oka Y. The relationship between nerve conduction velocity and fiber morphology during peripheral nerve regeneration. *Brain Behav*. 2012;2(4):382-390. doi: 10.1002/brb3.61
31. Caillaud M, Richard L, Vallat JM, et al. Peripheral nerve regeneration and intraneural revascularization. *Neural Regen Res*. 2019;14(1):24-33. doi: 10.4103/1673-5374.243699
32. Kuffler DP, Foy C. Restoration of Neurological Function Following Peripheral Nerve Trauma. *Int J Mol Sci*. 2020;21(5):1808. doi: 10.3390/ijms21051808
33. 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

The article was submitted 02.12.2022; approved after reviewing 17.01.2023; accepted for publication 08.04.2024.

Information about the authors:

Natalya A. Shchudlo — Doctor of Medical Sciences, leading researcher, nshchudlo@mail.ru;

Tatyana N. Varsegova — Candidate of Biological Sciences, senior researcher, varstn@mail.ru, https://orcid.org/0000-0001-5430-2045;

Tatyana A. Stupina — Doctor of Biological Sciences, leading researcher, StupinaSTA@mail.ru, https://orcid.org/0000-0003-3434-0372.