Experimental Validation of Direct Long Electrostimulation after Neurotransplantation of the Peripheral Nerves

R. P. Gorshkov, V. G. Ninel', D. K. Dzhumagishiyev, and G. A. Korshunova

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Effects of direct long-term electric stimulation on the growth of the sciatic nerve regenerating axons through autoneurotransplants were studied in rabbits and an optimal electrostimulation method was developed.

Key Words: *sciatic nerve; electrostimulation; Wallerian degeneration; regeneration*

Plastic repair of the nerves with a free graft is a well known method, investigated not once; however, the problem is still far to be solved. The longer is the fragment of the transplanted nerve, the worse are conditions for the transplant take, mainly because of its cicatricial regeneration [1]. Electrostimulation (ES) is a method stimulating the development of blood vessels in the neurotransplant [2].

We studied the effects of pulsed electric current on the growth of regenerating axons through the autotransplant and developed a method for its ES.

MATERIALS AND METHODS

Experiment was carried out on 75 normal adult Russian Chinchilla rabbits. Fragments (2-3 cm) of the sciatic nerve were resected and then grafted back. All animals were divided into 2 groups. Control rabbits (n=30) received no pulsed ES of the transplant after neurotransplantation. Experimental animals (n=45) received 30-min sessions of direct ES of the neurotransplant after autoneuroplasty 3 times daily through implanted electrodes (rectangular pulses 0.1-0.2 msec long, 50-60 Hz frequency, and 12-25 mA amplitude). Three series of experiments (15 rabbits per series) were carried out in order to specify the location of electrodes in this group. In series 1, one electrode was placed on the proximal end of the nerve and the other on the transplant. In series 2, the electrodes were implanted so that their active parts were in contact with the transplant and the proximal and distal portions of the nerve. In series 3, one electrode was placed on the transplant and the other on the distal end of the nerve.

Denervation and reinnervation processes in the zone of the sciatic nerve innervation in rabbits were evaluated by the data of stimulation and needle EMG. The amplitude of evoked muscle response (M-response) and rate of pulse propagation (VPP) in the motor fibers of the sciatic nerve were registered. The mean values of M-response amplitude and VPP were determined in a preliminary study in 20 normal rabbits of the same strain. Electroneuromyography (ENMG) was carried out 1, 3, and 6 months after surgery.

Macro- and microstructural changes in the autotransplants and the number of axons regenerating through the proximal and distal portions of neurorrhaphy were evaluated in morphological studies. Transverse sections were made at a distance of 1 cm from the site of the autotransplant neurorrhaphy with the proximal fragments of the sciatic nerves. The number of axons grown into the autotransplant was determined by examining the transverse sections of autotransplants at the site of their suturing to the distal portions of the sciatic nerves. The

Saratov Institute of Traumatology and Orthopedics, Russian Ministry of Health. ***Address for correspondence:*** v.ninel@mail.ru. V. G. Ninel

animals of both groups were sacrificed 7, 30, 90, and 180 after surgery. Nerve fragments resected in these animals were fixed in 10% neutral formalin, delipidated, dehydrated in ascending alcohols, and embedded in paraffin. Micropreparations were stained with hematoxylin, eosin, and picrofuchsin after Van-Gieson for studies of the nerve trunk connective tissue structures. Morphohistological changes in the nerve tissue were studied in frozen sections impregnated with silver by the method of Bilshovskii—Gross. Histological studies were caried out using morphometrical methods, including count of degenerative nerve fibers and regenerating axons. G. G. Avtandilov's ocular measuring gridnet was used for evaluating the volume occupied by Wallerian degeneration products and newly formed nerve fibers.

The data were statistically processed with estimation of the means and evaluation of the significance of differences between the means using Student's t test.

RESULTS

Control animals refused from food and were inert. They spared the injured limb (did not stand on it). By the beginning of week 2 postoperation, all rabbits developed trophic disorders (edema, crusts and ulcers on the skin) in the distal part of the operated on limb. Then trophic disorders augmented, the hair disappeared, the ulcers grew in size and pus discharge appeared on their surfaces; all animals died after 5-6 weeks.

Study by ENMG 30 days after surgery showed muscle response in only 2 of 8 control rabbits. The amplitude of M-response was far below the normal (0.06±0.1 mV), VPP did not exceed 25 m/sec. These data indicated recovery of conduction in just solitary nerve axons. Repeated ENMG could not be carried out because of animal death.

In experimental animals, characteristic external changes similar to those in controls were seen on operated limb on day 1 and later. By the beginning of month 3 the ulcers healed, the first signs of motor function recovery (small muscle jerks, trembling, and small movements of the paws) were noted, and pain sensitivity appeared. After 6 months the animals stood on the operated limbs, but atrophy of the hips and shins was still seen.

The appearance of low-amplitude polyphase potentials of motor units in the muscles indicated the beginning reinnervation of muscle fibers by regenerating axons. After 3 months, the amplitude of M-response increased 6.1 times, of VPP less significantly (Table 1). The dynamics of ENMG indicated the progress of regeneration, remyelinization, and reinnervation of axons. Wallerian degeneration was more active in experimental animals, which was seen from greater number of degenerating fibers (86.27±1.45%; $p<0.01$) and greater volume of their degradation products (48.34±2.56%; $p<0.01$). More mature (in comparison with preparations from the controls) connective tissue structure was formed in the zone of the autotransplant connection to the proximal and distal ends of the sciatic nerve in preparations from experimental animals. This structure was presented by mature granulation tissue forming bundles of numerous collagen fibers, the majority of them being orderly located, oriented parallel to the nerve trunk and constituting an integral structure with the connective tissue membranes of the transplant. No foci of hemorrhages or destruction, characteristic of controls, were seen. The number of inflammatory cells, located mainly perivascularly, was significantly lower (10.21±0.13%; $p<0.01$). Signs of irritation and destruction (irregular diameter, varicosities, partial degradation) of the ends of crossed axons were detected in 25% preparations in the proximal and distal portions of the nerve trunk. Foci of nerve fiber degeneration with partial degradation and replacement by fibrous connective tissue were detected in the central zone of the autotransplant along with nerve fibers with intact macrostructure. Comparative analysis of specimens from the two groups showed 3.58 times less number of signs of Walle-

TABLE 1. Dynamics of ENMG Values in Rabbits before and after Operation ($M±m$)

Parameter	Normal value	Month postoperation			
		1		3	6
		control	experiment	experiment	experiment
M-response amplitude, mV	12.4±0.4	0.06±0.1	0.8±0.02	4.9±1.3	5.0±0.04
VPP in sciatic nerve motor fibers, m/sec	44.7±1.4	25.0	45.0±3.7	52.0±2.5	54.0±2.3

rian degeneration of nerve fibers in experimental animals (15.28±1.05%; $p<0.001$).

After 1 month the number of nerve fibers grown into the autotransplant was negligible in controls (19.5±0.48%) and reached 58.83±0.64% in experimental animals ($p<0.001$). After 6 months the number of grown axons in experimental group was 55.66±1.29%. By this period the regenerating axons were united into fine bundles, separated from each other by potent layers of fibrous connective tissue, similar by its structure to the connective tissue membranes of the nerve trunk. Analysis of morphological preparations from experimental animals, which received sessions of direct ES of the autoneurotransplant, showed that location of the electrodes in the immediate vicinity of the zone of the transplant connection to the sciatic nerve ends was the optimal. This mode allowed simultaneous electric pulse exposure of the autotransplant and the proximal and distal portions of the nerve.

Hence, direct long ES of autotransplants stimulated the quantitative and qualitative growth of regenerating axons through the autotransplant. The optimal length of the autotransplant for effective neuroplasty in animals should be no longer than 3 cm.

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