A comparative morphological study of direct nerve implantation and neuromuscular pedicle methods in cross reinnervation of the rat skeletal muscle

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Abstract

Purpose: The study was undertaken to evaluate the recovery of rat skeletal muscles reinnervated by crossed direct nerve implantation and crossed neuromuscular pedicle graft.

Material and methods: The animals (157) were divided into 3 groups. In the first group – direct nerve implantation – (DNI) 52 animals, the peroneal nerve was transected and implanted into the gastrocnemius muscle of the hind limb of the rats. In the second group (53 animals) – the neuromuscular pedicle (NMP) of the peroneal nerve was elevated and transferred to the gastrocnemius muscle. The third group consisted of 52 healthy animals. Muscle function was examined by electrophysiological methods (evoked electromyography and muscle isometric tetanic tension) and by morphological methods (reinnervated muscle weight, microscopic examinations, morphometric and histochemical examinations).

Results: The weight of reinnervated muscles in the first 3 weeks decreased. The lowest values of muscle weights were noted at 4 weeks. At the end of the experiment muscle weight in the first group was 64.3% of the control group and in the second group 65.2%. Morphometric, histological and histochemical analysis were performed after 12 and 36 weeks. At 12 weeks of the experiment the diameter of reinnervated muscle fibers in the second group was statistically higher than in the first group. At that time the process of reinnervation was more advanced than in the first group. At 36 weeks there were no statistical differences between the two groups. An increase in the number of muscle fibers was noted as the processes of reinnervations progressed.

At the site of nerve (or pedicled nerve-muscle) implantation new motor end plates were formed.

Conclusions: We consider that reinnervation of the experimentally paralyzed muscle is possible after crossed DNI and after NMP neurotization. The reinnervation with the NMP technique is quicker than with the DNI. Based on the morphological examinations – both methods guarantee only a partial recovery of the function of the paralyzed muscle.

Key words: denervation, direct neurotization, neuromuscular pedicle, muscle reinnervation, motor end plates.

Introduction

When the nerve is injured near its entrance to the muscle belly we cannot perform conventional methods of nerve repair (nerve suture, or nerve grafting) because we do not have the distal stump of the injured nerve. One useful method in such a situation is direct implantation of nerve fibers into the paralyzed muscle (direct nerve implantation method – DNI) [1-15]. We call such procedures direct neurotization of the muscle. The first experimental investigations of direct nerve implantations into paralyzed muscles were performed by Heinecke and Erlacher [16,17]. Steindler, Aitkin, Hoffman and Miller also contributed to investigating the processes of nerve regenerations. All these investigations showed that an injured proximal nerve stump can grow into the paralyzed muscle and establish functional connections (motor end plates). Direct nerve neurotization such as the nerve grafting procedures are inevitably connected with processes of retrograde Wallerian degeneration of the injured nerve [18,19]. Such problems can be avoided when we transpose intact undamaged nerve fibers

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to target organ [20,21]. In most human muscles the end plates are localized at places of nerve entrances into the muscle belly. This enables excision of the area of muscle containing the motor end plates with its motor nerve and transferring such a „nerve muscle pedicle" onto another paralyzed muscle. The concept of motor nerve transposition (MNT) was first reported in 1970 by Tucker et al. [1,22-29]. These authors called such a procedure "neuromuscular pedicle reinnervation" (NMP). This technique was successfully used for reinnervation of the human larynx [25-27]. Experimental studies of muscle reinnervation with motor nerve transplantation technique showed conflicting results. Some authors wrote about evidenced signs of motor reinnervation [1,24,30] other authors [31,32] doubted whether this method can really give successful reinnervation of paralyzed muscles. Most of the experiments made previously were performed on small paralyzed muscles of the larynx or face [33-35]. Only demonstration of satisfactory paralyzed extremity muscle reinnervation can confirm the technique of motor nerve transplantation to be another reliable technique of muscle reinnervation.

We performed our study to answer the following questions:

1. Is it possible to reinnervate a paralyzed hind limb rat muscles with the method of „motor nerve transplantation"?
2. What is the efficacy of reinnervation of a paralyzed muscle with the technique of neuromuscular pedicle (NMP) as compared with the method of direct nerve implantation (DNI)?
3. What is the extent of reinnervation of paralyzed muscles as evaluated by morphological methods?

Materials and methods

157 Wistar rats with an average weight of 250 gm were used. Anesthesia was induced with an intra-peritoneal injection of Thiopental at the dosage of 40 mg/kg of body weight. A curved incision was made on the posterior surface of the right thigh, a posteriori-based skin flap raised and biceps femoris muscle was pushed aside to expose the popliteal fossa. The tibial, common peroneal and sural nerves were identified. A 1.5 cm segment of tibial nerve was resected where it entered the gastrocnemius muscle and the proximal stump of the tibial nerve was implanted into the underlying adductor muscle. The whole sural nerve was also resected and the nerve implanted into the adductor muscle. The animals were operated on using microinstruments (Aesculap) and an operating microscope OPM-1.

The rats were divided into the three groups for the subsequent experimental procedures.

Group I (DNI) (52 animals). The common peroneal nerve was transected at the place where it traversed the fibular head. The proximal stump was transposed into the proximal third of the simultaneously denervated gastrocnemius muscle (cross neurotization). The epimysium of gastrocnemius muscle was incised and the peroneal nerve stump was implanted into the bluntly created muscular pocket (*Fig. 1*, *2*). If there was bleeding when creating a pocket another muscular pocket was prepared. A single 10/0 nylon suture, transfixing the epimysium and epineurium of the peroneal nerve stump was used to stabilize the nerve. The biceps femoris was replaced and the wound closed.

Group II (NMP) (53 animals). The peroneal nerve of the right leg was exposed around the fibular head and further into the muscle belly. A small piece of tibial anterior muscle (0.2/0.2 cm) – containing intact motor endplates with the entering peroneal nerve fibers was sharply excised (nerve muscle pedicle) and transposed to the proximal third of the simultaneously denervated gastrocnemius muscle (*Fig. 3*, *4*). At that site, where the small block of pedicled muscle was approximated – the epimysium from the gastrocnemius was removed. Two stay nylon sutures 8/0 between the peroneal nerve muscle island and epimysium of the denervated gastrocnemius muscle were used to stabilized the muscle. The wound was closed in layers.

Group III (52 animals). The group of healthy non-operated rats served as the control.

Morphological, histological and enzyme histochemical assessment

After electrophysiological and functional assessment (36) the GAST-PL-SOL muscle group was excised en bloc, the macroscopic appearance of neurotized muscles was evaluated and, the wet weight of the muscles estimated. With the use of a computer program "Imager-512" morphometric measurements (a mean muscle fiber count per 1 mm^2 area and size of muscle fibers) of reinnervated muscles was performed.

Small muscle blocks from the reinnervated gastrocnemius with the pedicle were fixed in buffered 10% formalin solution and than embedded in parafin blocks. Microscopic examinations of cross-sections of the reinnervated muscles stained by the hematoxylin-eosin method were performed.

Muscle blocks collected with a pedicle (common peroneal muscle or pedicled muscle island) were immersed in liquid nitrogen, cut in a cryostat into pieces of 10 μm thickness and stained for demonstration of acetylcholinesterase activity by the method of Tsuji [37]. By this method it was possible to demonstrate motor end plates beneath the nerve pedicle graft.

The results obtained were statistically assessed. Differences between mean values were analyzed using Student's t-test (data of normal distribution) or the Mann-Whitney U test (data without normal distribution). The differences were recognized as statistically significant when $p<0.05$. Statistical analysis was performed with a computer using Statistica program.

Results

During the experiment 157 rats were operated on with the two methods mentioned above. Five rats died in the perioperative period (probably because of overdosing of the anesthetic drug), 2 rats died during the later periods of the experiment. 150 animals were examined and assessed.

In the first weeks of the experimental study, the weights of the reinnervated muscles in the DNI and NMP groups decreased (*Tab. 1*). In the second week, the muscle weight in the DNI group was 1490 (±65) mg, and in the second group 1798 (±75) mg – 75.7% and 91.3% of the controls respectively.

Figure 1. The proximal stump of the common peroneal nerve was transposed and implanted into the simultaneously denervated gastrocnemius muscle

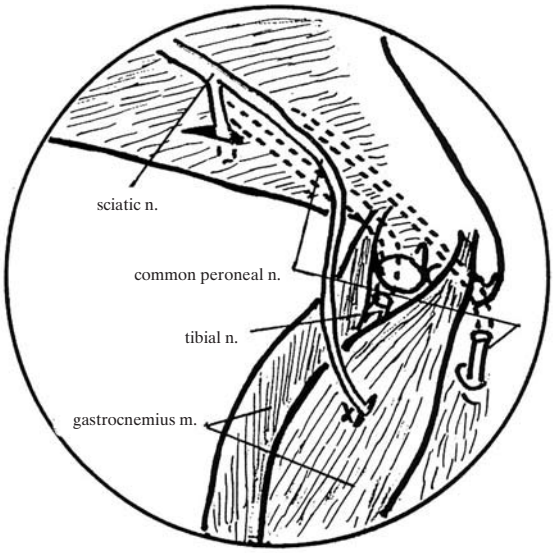


Figure 2. Intraoperative view – the common peroneal nerve is inserted into the pocket of the gastrocnemius muscle

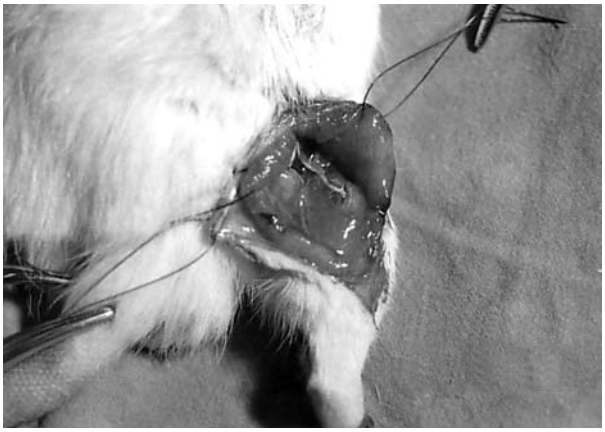


Figure 3. The neuromuscular pedicle was excised, transposed and implanted into the denervated gastrocnemius muscle

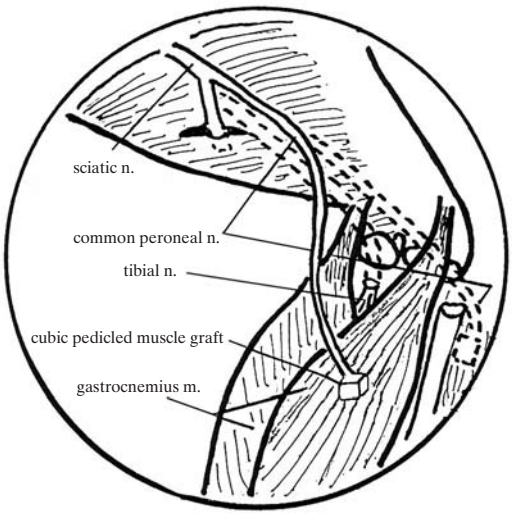


Figure 4. Intraoperative view – the prepared pedicled muscle pad is inserted into the gastrocnemius muscle

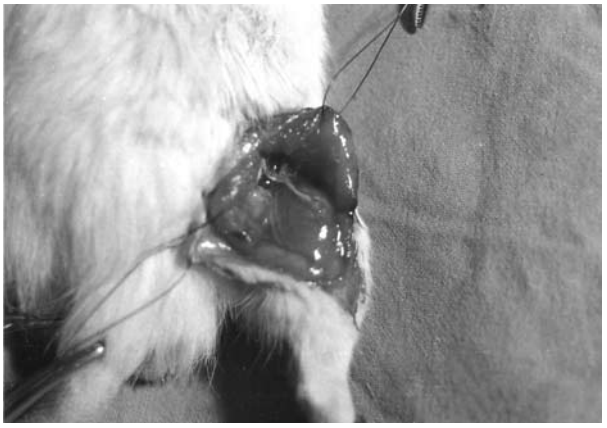


Table 1. Mean data of reinnervated GASTR-PL-SOL muscle weights. In the lower right-handcorner the number of rats from which the mean value was calculated is shown. # Data estimated before experimental study. * Statistically significant difference in comparison with the NMP and controls ($p<0.01$). **Statistically significant difference in comparison with the controls ($p<0.05$). *** Statistically significant difference in comparison with the DNI and controls ($p<0.01$)

Time after surgery (weeks)	Group I Direct nerve implamation	Group II Neuromuscular pedicle	Control group
	Muscle weight (mg)	Muscle weight (mg)	Muscle weight (mg)
0#	1954±77 N=20	1950±62 N=20	1951±42 N=10
2	1490±65 * N=8	1798±75 *** N=8	1969±118 N=8
4	1014±118 * N=8	1531±76 *** N=7	2217±164 N=8
12	1245±117 * N=7	1634±118 *** N=8	2674±213 N=8
16	1332±69 * N=8	1674±162 *** N=8	2819±190 N=8
24	1752±178 ** N=8	1825±249 ** N=8	2993±190 N=8
36	2135±195 ** N=8	2165±123 ** N=8	3321±374 N=7

Figure 5. **Appearance of muscle after 36 weeks of experimental study**

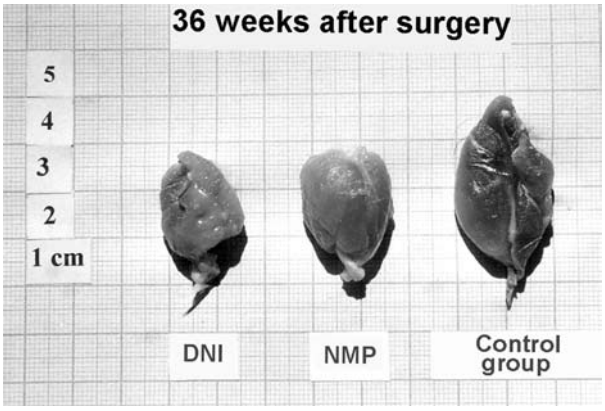


Figure 6. **Computer muscle weight curves of experimental groups**

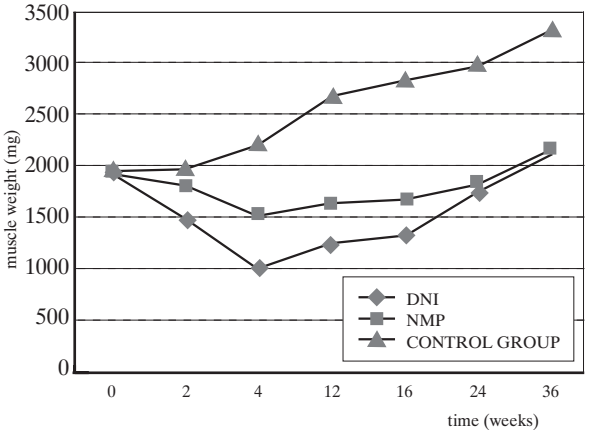


Table 2. **Muscle fiber counts and diameter in the experimental groups. In the lower right-hand corner the number of animals from which the mean value was estimated is shown. * Significant differences in comparison with the DNI and control group (p<0.01). **Significant differences in comparison with the NMP and the control group (p<0.01)**

Time after surgery (weeks)	Group I Direct nerve implantation DNI		Group II Neuromuscular pedicle NMP		Control group	
	Muscle fiber counts (mm^2)	Muscle fiber diamater (μm)	Muscle fiber counts (mm^2)	Muscle fiber diamater (μm)	Muscle fiber counts (mm^2)	Muscle fiber diamater (μm)
12	605±65.2* N=6	12.1±4.11* N=6	721±78.1** N=6	18.5±4.85 N=6	998±78.3	24.6±1.8
36	882±67.5 N=7	22.8±4.55 N=7	901±69.4 N=7	25.9±7.21 N=7	N=8	N=8

At that time the mean muscle weight of the DNI group was significantly lower than in the NMP (p< 0.01) and control group (p< 0.05). There were also significant differences between the NMP and control group (p<0.05). After 4 weeks, the muscle weight in both groups reached its lowest value (DNI group 45.7% of the normal group, and NMP group 69.1% of the normal group). There were statistical differences between the DNI and NMP groups at that time (p<0.01).

From the 4th week onwards the muscle weight in two groups successfully increased (*Fig. 6*). Between the 12th week and the end of the experimental study the increase in muscle weight was less dynamic. In the 16th week the muscle weight in the DNI group was 1332 (±69) mg and in the NMP group 1674 (±162) mg. The differences between the DNI and NMP groups (p<0.01), DNI and control (p<0.01), and NMP and control (p<0.01) were found to be significant. At the end of the experimental study (36 weeks) the muscle weight of the DNI group was 64.3% and NMP group 65.2% of the control rats (*Fig. 5*, *6*). No statistical differences between DNI and NMP groups were noted, although the muscle mass from the DNI group was significantly lower than in the control (p<0.01), and similarly the NMP muscle weight was significantly lower than in the control group (p<0.01).

Morphometric analysis was performed after 3 and 6 months (*Tab. 2*). The number of muscle fibers present in a cross-section of 1 mm^2 reinnervated muscle taken from the site of nerve (island) implantation and the diameter of the reinnervated muscle fibers were estimated. After 12 weeks the mean fiber count on the cross-sections in the DNI group was 605/mm^2, and in the NMP group 721/mm^2. At that time the number of muscle fibers in the control group was 998/mm^2 (*Fig. 7*). There were significant differences between the DNI and NMP groups (p<0.01), DNI and control rats (p<0.01) and NMP and controls (p<0.01). After 36 weeks the number of muscle fibers was greater in the NMP than in the DNI group, although these values did not change significantly. The number of muscle fibers in the control group was greater than in the DNI and NMP groups, no significant differences between the controls and DNI or control and NMP groups were noted.

After 12 weeks the muscle fiber diameter in the DNI group was 12.1 μm and in the NMP group 18.5 μm. The mean fiber diameter in the control rats was 24.6 μm. There were significant differences between the DNI and NMP groups (p< 0.01), and the DNI and control groups (p< 0.01). After 36 weeks the mean fiber diameter in the DNI was smaller than in the NMP group (*Fig. 8*). These values did not change significantly (p< 0.01). No

Figure 7. **Fiber counts of reinnervated muscle in the groups examinated. * Significant differences in comparison with the NMP and control group (p<0.01). Significant differences in comparison with the DNI and controls (p<0.01)**

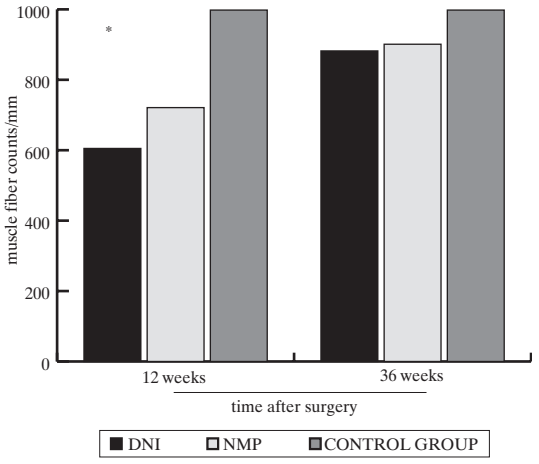


Figure 8. **Computer muscle fiber diameter of experimental groups. * Significant differences in comparison with the NMP and control group (p<0.01)**

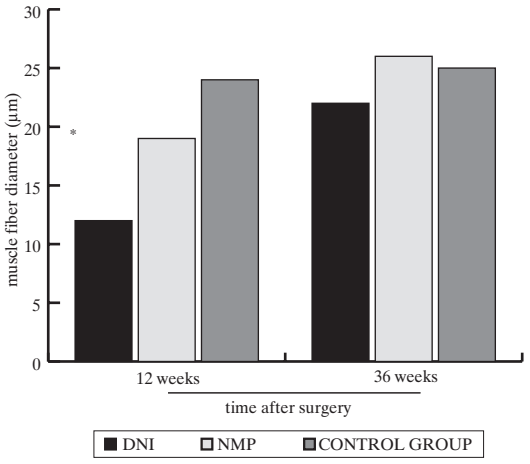


Figure 9. **Histological pattern in the DNI group (most muscle fibers are placed in regular fascicules, the diameter of the fibers are similar, Transvers sections of fibers appear to be oval and polygonal, site of nerve implantation, DNI method 36 weeks after surgery, H+E stain, x200)**

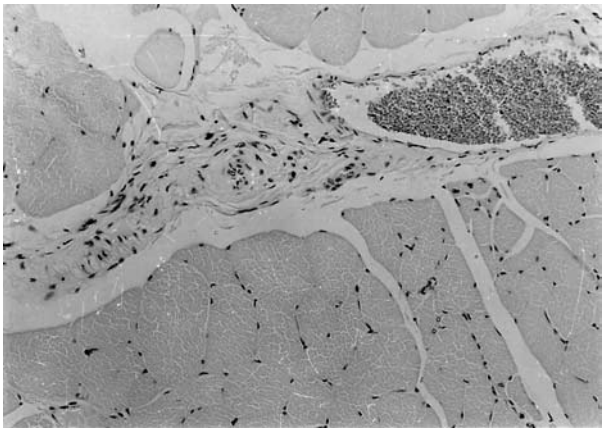
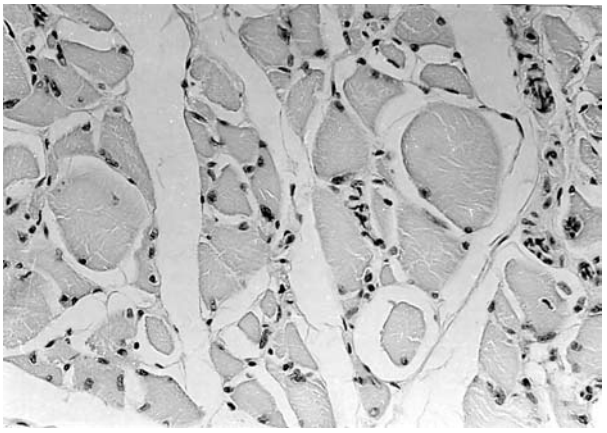


Figure 10. **Histological pattern in the NMP group (most fibers are large, hypertrophied, another fibers are polygonal atrophied, site of nerve-muscle pedicle aproximation, NMP method, 36 weeks after surgery, H+E stain, x200)**



significant differences were found between the DNI and control groups, and the NMP and control groups at that time.

Histological studies

In the 4th week muscle fibers of different shape and diameter were seen in the DNI group. Some of them were rounded, others polygonal shaped. Small branches of nerves were evident on the cross-sections of the reinnervated muscle. In the later periods of reinnervation (12th week) most of the round or polygonal-shaped muscle fibers with similar diameters gathered in regular fascicules (*Fig. 9*).

In the NMP group, in the late periods of reinnervation, fascicules of various shapes were seen (*Fig. 10*). Some fibers were large, oval shaped, while others were polygonal with signs of degeneration.

Histochemical examination

In NMP group after 4 weeks, motor end plates were demonstrated at the place of muscle island approximation. No acetylcholinesterase staining was seen in the DNI group at that time. After 6 weeks motor end plates of various shapes, arranged in irregular lines, sometimes incompletely stained, were observed in both groups. At the end of the experimental study, the completely-stained clusters of motor end plates were more rounded in the DNI as well as in the NMP group (*Fig. 11*, *12*).

Figure 11. **Motor end plates evident in the DNI group (irregular and linear forms of well stained motor end plates, 36 weeks, AChE method by Tsuji, x400)**

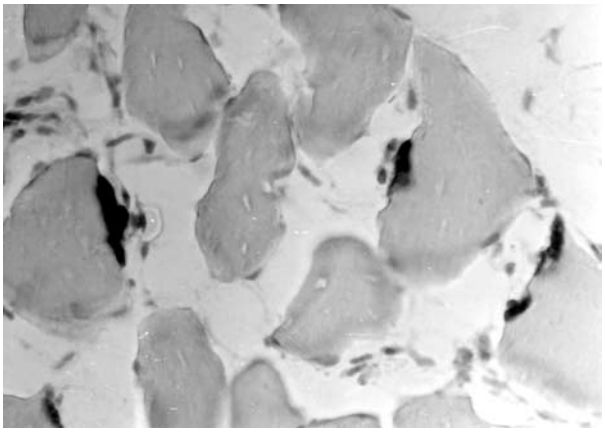
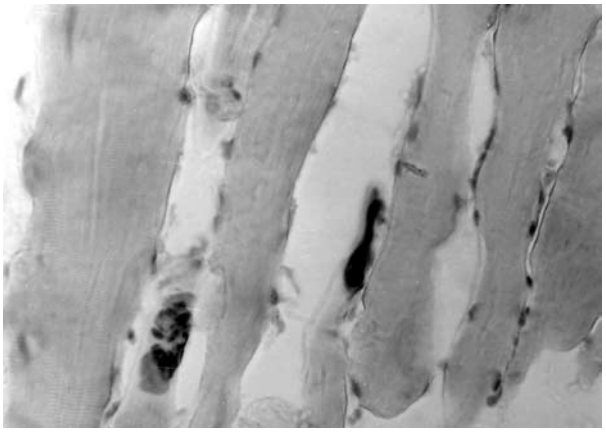


Figure 12. **Motor end plates evident in the NMP group, 36 weeks (oval and linear shaped motor end plates, well stained in histochemical examination, AChE activity – Tsuji method, x400)**



Discussion

The direct nerve implantation method (DNI) was popularized in Europe by Giorgio Brunelli [2-5,38,39]. The author divided the proximal stump of the injured nerves into fascicules to implant them into denervated muscle bellies. The neuromuscular pedicle – NMP technique was introduced by Tucker H in otolaryngological practice at the beginning of 1970 [25-29,37]. Tucker postulated that simple healing of the pedicle muscle into the denervated muscle belly is the primary prerequisite for transferring evoked active potentials from the island to the denervated muscle. What are the differences between these two techniques?

The DNI method is connected with retrograde Wallerian degeneration of transected nerve fibers. During excision of the pedicle muscle graft (NMP technique) there is complete transection injury to the terminal nerve branches at the cut margins of the muscle pad, where axons travelling to more distant muscle fibers are divided. The muscle island contains however a large amount of undivided axons with functional motor plates. The NMP technique enables the transferring of motor fibers with these functional end plates [20]. The retrograde Wallerian degeneration in such nontransected fibers is avoided.

In our experimental study, we neurotized the gastrocnemius muscle with common peroneal nerve (nerve stump or pedicle muscle). That means there was a crossed neurotization of the simultaneously paralyzed muscle. The reinnervation of a muscle with a "foreign" nerves always gave inferior results to reinnervations with native nerves [40]. Reports in the literature vary as regards the period of time from implantation of a "foreign" nerve to the first functional signs of its reinnervation that is from 2 to 12 weeks. The reinnervation was always more effective (in laboratory study and in clinical practice – when the proximal nerve stump was divided into fascicules). Resection of the epineurium surrounding the nerve stump reduces the possibility of connective tissue barrier formation, that can prevent nerve regeneration [41,42]. Brunelli and other authors divide the proximal nerve stump into as many fascicules as possible and implant them into various depths of the muscle belly to increase the counts of reinnervated muscle fibers [5,29,38,43,44].

In our experiment the proximal stump of the common peroneal nerve (CPN) was not divided into fascicles, which resulted in a decrease in the number of reinnervated muscle fibers. On the other hand, the three-dimensional pedicle muscle in the NMP technique contains the transected axons, which are considerably separated from each other. The NMP is similar to the originally described nerve implantation techniques dividing the proximal stump of an injured nerve into fascicules.

The crucial question connected with pedicle muscle technique is its blood supply. Can the muscle and nerve cells survive the transposition? In our experiment the CPN was separated with the alveolar tissue (sleeve), an effort was made to preserve as many small blood vessels in the pedicle as possible. We treated the muscle island as vascularized on a nerve and elevated with the nerve alveolar tissue. We did not determine how many muscle fibers survived such a procedure. In previous studies a special and considerable regenerative potential of autogenous muscle transplants was noted [45-48]. This also applies to the muscle island in the NMP method. A piece of muscle in the NMP method would be a source of growth of new muscle tissue and a considerable regeneration will occur in a muscle graft [45,46,49].

Pedicle muscle fibers can be a source of fibronectin and laminin, which create an excellent medium for the growth of regenerating nerve sprouts [20].

The structural basis for neuromuscular conduction properties are motor end plates. In our experiment, the "fast" nerve (CPN) reinnervated the paralyzed gastrocnemius muscle. The gastrocnemius muscle in contrast to the soleus muscle has no strict asymmetrically located native zone plate [50]. End plates in the gastrocnemius muscles are distributed widely throughout the muscle belly. According to Brooke [51] the gastrocnemius

muscle consists of type B fascicules (there are fewer type A and IIA fascicles than in the soleus muscle). A similar distribution of fascicules is found in the anterior compartment of the hind limb. The transposed neuromuscular pad contained similar fibers to those in the gastrocnemius muscle. We did not perform typical ATP-se staining. No conversion of muscle fascicles was anticipated.

Because there is no strictly well-located clear native zone plate in the gastrocnemius muscle – an increase in accumulated end plates around the implantation site of a nerve was the sign of reinnervation. At the 4th week after nerve (nerve-muscle) implantation, the motor end plates were seen in cross-sections in both groups. In the DNI group the plates were seen in those animals, in which electrophysiological responses were noted. Their elongated shapes, diffuse granular form little resembled a typically found "en grape" form. That's why we treat those plates as new (ectopic) motor end plates. At that time in the NMP group motor end plates resembling those of the DNI group, were seen, but also "en grape" forms that could be "old" or transposed with the muscle pad functional motor end plate. Without electron microscopy it is not possible to define the actual stem of such neuromuscular junctions. In the late periods of observations (36th week) there was not so great a diversity in the form and size of the motor end plates, the plates of both groups had a rounder disc-like appearance, they were clustered together. It was obvious that in the early stages of reinnervation there were more motor end plates in the NMP operated group. We know that new regenerating motor axons preferably reinnervate original motor end plates, which are located on only 0.1% of the surface of a muscle fiber [52]. On the basis of our study, we have found that regenerating fibers in two of the above – mentioned methods innervated old end plates, in the NMP method of reinnervation consideration must be given to functional motor end plates that were transferred with a pedicle neuromuscular graft.

On the basis of our experimental study we conclude that:

1. Reinnervation is possible by means of two methods of neurolization, DNI and NMP methods.
2. Reinnervation of muscles with the crossed DNI and NMP method only partially restores the morphology of the paralyzed muscles.

Acknowledgment

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References

1. Ancones CK, Patterson HC, Trachy RE, Gordon AM, Cummings ChW. Reinnervation of skeletal muscle with a neuromuscular pedicle. Otolaryngol Head Neck Surgery, 1985; 93(1): 48-57.

2. Brunelli G, Monini L, Brunelli F. La neurotisation musculaire directe. Ann Chir Main, 1990; 4: 290-5.

3. Brunelli G, Monini L, Brunelli F. Problems in nerve lesions surgery. Microsurg, 1985; 6: 187-98.

4. Brunelli G. Bridging nerve defects with combined skeletal muscle and vein conduits. Microsurg, 1993; 14: 247.

5. Brunelli G. Direct neurotization of severely damaged muscles. J Hand Surg, 1982; 7(6): 572-9.

6. May M. Muscle-nerve pedicle larygeal reinnervation. Laryngoscope, 1986; 96(11): 1196-200.

7. Monach J, Drozdowski W, Skowroński J. Elektroneurograficzna i elektromiograficzna ocena reinerwacji mięśni szkieletowych szczura po mikrochirurgicznej implantacji nerwu w brzusiec mięśniowy. Neurol Neurochir Pol, 1994; 28 (1): 13-25.

8. Monach J, Skowroński J, Kuźma K, Tałałaj M, Drozdowski W. Czy miejsce implantacji nerwu w mięsień ma wpływ na skuteczność neurotyzacji nerwowo-mięśniowej. Badania doświadczalne na szczurach. Pol Hand Surg, 1994; 1-2/19: 41-9.

9. Monach J, Skowroński J, Zimnoch L. Neurotyzacja mięśni szkieletowych szczura. II Badania morfologiczne. Pol Hand Surg, 1991; 2/15: 33-43.

10. Monach J. Neurotyzacja mięśni szkieletowych szczura. Badania czynnościowe. Pol Hand Surg, 1991; 2(15): 11-32.

11. Erlacher P. Direct and muscular neurotization of paralyzed muscles. Am J Orthop Surg, 1915; 13: 22-35.

12. Sakellarides HT. Bridging long nerve defects: A review of microscopic nerve grafting standards and practices. Medscape Orthopaedics and Sports Medicine eJournal 2001; 5(3): 1-12.

13. Sato F, Ogura JH. Reconstruction of laryngeal function for recurrent laryngeal nerve paralysis: historical view, advancement of latest investigations and a preliminary experiment. Laryngoskop, 1978; 88: 689-96.

14. Skowroński J. Nerve repair in extensive open injuries. European Instructional Course Lectures. 2001, Vol 5: 78-82.

15. Tucker HM, Harvey J, Ogura JH, Louis St. Vocal cord remobilisation in the canine larynx. Arch Otolaryngol. 1970; 92: 530-3.

16. Erlacher P. Hyperneurotisation, musculare neurotisation. Z Chir, 1914; 15: 625-33.

17. Heinecke H. Einpflanzung des Nerven in den Muskel. Arch Klin Chir, 1913; 105: 517-30.

18. Lundborg G. Nerve injury and repair, Churchill Livingstone, 1988.

19. Broniatowski M, Ilyes LA, Sorensen K, Rosenthal DI, Nose Y, Maniglia AJ. Direct nerve implantation vs. nerve-muscle pedicle: a comparative study of reinnervation in the rabbit. Otolaryngol Head Neck Surg, 1989; 100(2): 126-33.

20. Gray WP, Koehane K, Kitwan W. Motor nerve transplantation. J Neurosurg, 1997; 87: 615-24.

21. Lundborg G. Nerve regeneration and repair, a review. Act Orthop Scand, 1987; 58: 145-69.

22. Applebaum EL, Allen GA. Human laryngeal reinnervation: the northwestern experience. The Laryngoscope, 1979; 89: 1984-7.

23. Eisele DW, Hillel D, Trachy RE. Reinnervation of the trapezius muscle. Otolaryngol Head Neck Surg, 1988; 98: 34-44.

24. Ogura J, Harvey JE, Mogi G, Ueda N, Ohyama M, Tucker H. Further experimental observations of transplantation of canine larynx. Laryngoscope, 1970; 1231-43.

25. Tucker HM. Human laryngeal reinnervation. Laryngoscope, 1975; 769-79.

26. Tucker HM. Human laryngeal reinnervation: Long-term experience with the nerve-muscle pedicle technique. Arch Otolaryng 1978; 88: 598-604.

27. Tucker HM. Nerve/muscle pedicle reinnervation of the larynx: avoiding pitfalls and complications. Ann Otol Rhinol Laryngol, 1982; 91: 440-4.

28. Tucker HM. Reinnervation of the unilaterally paralyzed larynx. Ann Otol, 1977; 86: 789-94.

29. Tucker HM. Restoration of function in long-standing facial paralysis. Otolaryngol Clin North Am, 1982; 15: 69.

30. Prewitt MA. Effects of cross innervation on biochemical characteristics of skeletal muscle. Am J Physiol, 1967; 213: 295-300.

31. De Medinaceli. How exact should nerve stump coaptation be? J Hand Surg (Br), 1991; 16: 495-500.

32. Rochel S. Effects of partial denervation. J Neurosci, 1988; 8: 332.

33. Goding GS, Cummings ChW. Extension of neuromuscular pedicles and direct nerve implants in the rabbit. Arch Otolaryngol Head Neck Surg, 1989; 115: 217-23.

34. Hall SJ, Trachy RE, Cummings ChW. Facial muscle reinnervation: a comparison of neuromuscular pedicle with direct nerve implant. Ann Otol Rhinol Laryngol, 1988; 97: 229-33.

35. Johnson J, Tucker H. Selective experimental reinnervation of paralysed facial muscles. Arch Otolaryngol, 1977, 103: 22-5.

36. Bielecki M, Kochanowicz J. Ocena elektrofizjologiczna neurotyzacji nerwowo-mięśniowej i uszypułowanego transferu wyspy mięśniowej w reinerwacji mięśni szkieletowych – badania doświadczalne, Pol Hand Surg, 1998; 23(1): 57-80.

37. Tsuji S. On the chemical basis of thiocholine methods for demonstration of acetylocholinesterase activities. Histochem, 1974; 42: 99-110.

38. Brunelli GA, Brunelli GR. Direct muscle neurotization. Reconstr Microsurg, 1993; 9(2): 81-9.

39. Brunelli GB, Monini L. Direct muscular neurotization. J Hand Surg, 1985; 10A(6): 993.

40. Chiu DTW, Chen L, Spielholtz N, Beasley RW. A comparative electrophysiological study on neurotization in rats. J Hand Surg, 1991; 16B(5): 505-10.

41. Millesi H. Forty-two years of peripheral nerve surgery. Microsurg, 1993; 14: 228-33.

42. Thompson NA. A review of autogenous skeletal muscle graft and their clinical application. Clinic in Plastic Surgery 1974; 1: 349-403.

43. Skowroński J, Bielecki M. Direct neurotization of muscle in irreparable nerve injuries. SIROT, Shanghai-Beijing, China 2000, abstract p. 118.

44. Skowroński J, Latosiewicz R, Monach J. Direct neurotization of the muscle with using free nerve graft. SIROT, Shanghai-Beijing, China 2000, abstract p. 89.

45. Carlson BM, Gutmann E. Development of contractile properties of minced muscle regenerates in the rat. Exp Neurol, 1972; 36: 239-49.

46. Carlson BM. Regeneration of completely excised gastrocnemius muscle in the frog and rat from minced muscle fragments. J Morphol, 125: 447-72.

47. Studitsky AN. Free auto- and homografts of muscle tissue in experiments on animals. Ann NY Acad Sci, 1964; 120: 789.

48. Holloszy JO. Skeletal muscle regeneration and plasticity of grafts. Excer Sport Sci Rev, 1993; 21: 263-95.

49. Carlson BM, Gutmann E. Regeneration in free grafts of normal and denervated muscles in the rat: Morphology and histochemistry. Anat Rec, 1975; 183: 47-53.

50. Payne SH, Brushart TM. Neurotisation of the rat soleus muscle: A quantitative analysis of reinnervation. J Hand Surgery, 1997, 22A(4): 640-3.

51. Brooke MH, Willamson E, Kenneth K, Kaiser K. The behavior of four fiber types in developing and reinnervated muscle. Arch Neurol, 1971; 25: 360-6.

52. Sanes JR, Covault J. Axon guidance during reinnervation of skeletal muscle. Trends Neuro Sci, 1985; 8: 523-8.