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Motor nerve transplantation

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The motor nerve transplantation (MNT) technique is used to transfer an intact nerve into a denervated muscle by harvesting a neurovascular pedicle of muscle containing motor endplates from the motor endplate zone of a donor muscle and implanting it into a denervated muscle. Thirty-six adult New Zealand White rabbits underwent reinnervation of the left long peroneal (LP) muscle (fast twitch) with a motor nerve graft from the soleus muscle (slow twitch). The right LP muscle served as a control. Reinnervation was assessed using microstimulatory single-fiber electromyography (SFEMG), alterations in muscle fiber typing and grouping, and isometric response curves. Neurofilament antibody was used for axon staining. The neurofilament studies provided direct evidence of nerve growth from the motor nerve graft into the adjacent denervated muscle. Median motor endplate jitter was 13 μ sec preoperatively, and 26 μ sec at 2 months, 29.5 μ sec at 4 months, and 14 μ sec at 6 months postoperatively ($p < 0.001$). Isometric tetanic tension studies showed a progressive functional recovery in the reinnervated muscle over 6 months. There was no histological evidence of aberrant reinnervation from any source outside the nerve pedicle. Isometric twitch responses and adenosine triphosphatase studies confirmed the conversion of the reinnervated LP muscle to a slow-type muscle. Acetylcholinesterase studies confirmed the presence of functioning motor endplates beneath the insertion of the motor nerve graft. It is concluded that the MNT technique achieves motor reinnervation by growth of new nerve fibers across the pedicle graft into the recipient muscle.

Key Words * peripheral nerve * denervation * motor neuron * transplantation * muscle

Conventional surgical techniques for the treatment of peripheral nerve injury involve either direct reapproximation of damaged nerve ends or interpositional nerve grafting. These techniques rely on injured nerve cells to survive, regenerate across the zone of injury, and grow distally in a directed manner to reinnervate the appropriate target organ.[21] The poor results using these techniques are largely due to neuronal death and misdirected growth of regenerating axons at the suture site(s).[7,12,47] To circumvent these problems, the ideal surgical technique for reinnervation is to use intact undamaged nerve fibers and deliver them directly to their target organs. In most human voluntary muscles, the majority of motor endplates are localized to one or more discrete areas close to the point of entry of the

motor nerve.[2,9,10] Because of the topographical location of these motor endplates, intact motor nerves can be harvested from normal muscle by excising the area of muscle containing motor endplates with its motor nerve en bloc. This preparation of largely intact motor nerves can then be implanted into a denervated muscle to restore motor innervation. The concept of motor nerve transplantation (MNT) was first reported in 1970 by Tucker, et al.,[41] who coined the term "nerve muscle pedicle reinnervation" for this technique. Despite success using this technique for reinnervating the human larynx,[41] experimental studies of laryngeal reinnervation have yielded conflicting results: some showing indirect evidence of reinnervation[1,4,8,19,43] and others showing none.[11,17,22,24] None of these studies has definitively demonstrated reinnervation. All previous experimental studies have attempted to reinnervate small intrinsic muscles of the larynx or face. If the technique of MNT is to be considered as an alternative or a complement to existing techniques of peripheral nerve repair, reinnervation must be conclusively demonstrated. To find application in peripheral nerve surgery, its efficacy in innervating peripheral limb musculature must be assessed.

The aims of this study were twofold: 1) to validate the concept of MNT by examining evidence of motor reinnervation produced by this technique; and 2) to attempt to quantify the rate and extent of such reinnervation.

MATERIALS AND METHODS

Animal Model

An animal model that uses the soleus and long peroneal (LP) muscles in adult male New Zealand White rabbits was developed for this study under license from the Irish Department of Health (Licence No. B100/2286). Our technique of reinnervation differs from that of Tucker, et al.,[41] in that we used a vascularized motor nerve graft and attempted reinnervation in relatively large peripheral muscles.

A detailed description of the animal model will be given in a separate publication; however, a summary is provided here. After general anesthesia is induced in the animal with 1% halothane in an N₂O and O₂ vehicle, a linear incision is made below the knee over the peroneal compartment of the leg and is deepened to expose the common peroneal nerve (CPN) and the LP and soleus muscles. Microstimulatory single-fiber electromyography (SFEMG) recordings (see **Electrophysiological Assessment of Motor Reinnervation**) are then obtained from the intact LP muscle. The CPN is excised, denervating all muscles of the peroneal and extensor compartments, including the LP, in the following manner. The nerve is divided proximally high in the thigh and is excised distally, sectioning the motor branches flush with their muscles and the sensory branches flush with the skin. A small block of muscle containing intact motor endplates and motor neurons is then harvested from the soleus muscle in continuity with its pedicle of motor nerve and blood vessels. The presence of functioning motor endplates in the harvested muscle block and their continuity with the motor nerve is confirmed by observing twitch contractions of the muscle pedicle on bipolar stimulation of the soleus nerve. After removing the epimysium over the surface of the denervated LP muscle, the motor nerve preparation is sutured to the denuded surface of the muscle using No. 10-0 Ethilon. This whole preparation is then isolated from the surrounding muscles using 1-mm-thick sheets of presterilized Silastic. A technique of differential muscle closure is used to isolate the preparation further from aberrant reinnervation by the CPN stump at its origin at the sciatic nerve. A schematic representation of the surgical procedure is shown in Fig. 1.

Fig. 1. Drawings showing normal muscle and muscle with grafted motor nerve. Left: Normal muscle with its motor endplate zone highlighted. Right: Motor nerve graft, consisting of the motor nerve and motor endplates with some attached muscle, has been harvested from the motor endplate zone of the donor muscle. The motor nerve graft has been transposed and is inserted into the recipient denervated muscle. The recipient muscle has been denervated by excising its original motor nerve down to the motor point (hatched line).

Electrophysiological Assessment of Motor Reinnervation

Single-fiber electromyography parameters were recorded in the LP muscle on microstimulation in an anesthetized model by means of the technique described by Stålberg and Trontelj and their colleagues.[33,36,39] An electromyographic system, modified for SFEMG recording, was used for this study. Automatic data logging and analysis were performed with a 286 personal computer using commercially available software. The intramuscular terminal branches of motor nerves received bipolar stimulation an optically isolated stimulator. A digital programmer was used to trigger the SFEMG recording system 2 msec before stimulation so that the time interval between the stimulus potential and the resulting single-fiber potential could be accurately recorded. The interpotential interval (IPI) was

measured from the stimulus artifact to the selected point on the single-fiber potential. Using suprathreshold stimuli, as recommended by Stålberg, Trontelj, and colleagues[37,40] to avoid liminal stimulation, 200 consecutive stimuli were given, and the mean consecutive difference (MCD) between IPIs was calculated by the computer. This MCD or "neuromuscular jitter" is a measure of the stability of the motor endplate complex and is increased in reinnervation as the growth of unmyelinated nerve twigs and the formation of new immature motor endplates occur. The animal and the operative field were kept at a constant temperature by means of a radiant heat source on the operating surface situated close to the animal because cooling artificially increases neuromuscular jitter.[29] The stimulation frequency was kept constant at 10 Hz for all recorded single-fiber potentials. An average of 10 neuromuscular junctions were sampled in each muscle.

Functional Assessment of Motor Reinnervation

Bipolar stimulation was accomplished by inserting the cathode into the pedicle and the anode into the subcutaneous tissues 1 to 2 cm laterally. The animal was grounded by means of a subcutaneous electrode distant from the stimulating site. The leg was fixed in place with two tibial screws and the detached tendon of LP muscle was connected using a No. 2-0 silk tie to a 100-G isometric transducer; the muscle was restored to its resting length prior to stimulation. Isometric responses to single and tetanic 80-V supramaximal stimuli were recorded using an oscillograph. The tetanic stimulus was applied to all muscles at a frequency of 10 Hz.

Histological Assessment of Motor Reinnervation

The entire LP muscle with its motor nerve graft was excised en bloc, sectioned transversely proximal and distal to the attached nerve pedicle, and immediately immersed in super-cooled isopentane in a liquid nitrogen bath. These blocks were sectioned transversely, and 5- μ sections were incubated with myosin adenosine triphosphatase (ATPase) at 4.2, 4.6, and 9.4 pH to differentiate muscle fiber types. The block of muscle with the nerve pedicle attached was sectioned longitudinally through the pedicle interface and immunostained using the avidin-biotin complex (ABC) technique and neurofilament antibody (70- and 200-kD polypeptides of neurofilament) to demonstrate nerves crossing the interface between the motor nerve pedicle and the target muscle. Two of these blocks taken from muscles 6 months after MNT were studied by using enzyme histochemical analysis for acetylcholinesterase to demonstrate motor endplates beneath the nerve pedicle grafts.

A limited morphometric analysis was performed on a whole-muscle cross section of an MNT-reinnervated LP muscle, stained with ATPase preincubated at a pH of 4.6 obtained in each operative group (see Study Design) at 2, 4, and 6 months. Measurements were made using an image analysis system running appropriate software, and the minimum fiber diameter was recorded to minimize errors due to any tangential cuts through muscle fibers.

Study Design

The histochemical and SFEMG indices of the normal LP muscle were obtained in 58 of these muscles after the animals had been anesthetized.

The study population consisted of 36 adult male New Zealand White rabbits. In all animals the left LP muscle was denervated and immediately underwent MNT using a graft from the nearby soleus muscle. The study population was divided into three groups according to the control operation performed on the right LP muscle. Twelve animals underwent a sham operation on the right LP muscle (sham controls), 12

underwent sectioning of the right CPN (denervation controls), and 12 were subjected to a standard crush injury to the right CPN (reinnervation controls). All animals had SFEMG readings taken from each LP muscle (right and left) before operative interference with its nerve supply.

Four animals were killed from each group at 2, 4, and 6 months after the initial operation. Before this occurred, SFEMG readings were again taken from the LP muscle distal to the site of the motor nerve graft (on the left side). The tendon of LP was then severed and attached to a 100-G isometric transducer for isometric stimulation studies. An identical set of procedures was performed on the right-sided control muscle. The entire LP muscle with its motor nerve graft was then excised en bloc and prepared for histochemical studies.

Sources of Supplies and Equipment

The Neuromatic 2000 electromyographic system, which we modified for use as an SFEMG system in this study, was obtained from Dantec Electronics Ltd., Bristol, England. We used computer software from SES (Uppsala, Sweden) to log and analyze data. Digitimer (Hertfordshire, England) manufactured the DS2 optically isolated stimulator and the D100 programmer used to trigger the SFEMG recording system. Harvard Apparatus (Kent, England) provided the isometric transducer and the Universal Oscillograph used in functional assessment of motor reinnervation. The source for the 70- and 200-kD peptides of neurofilament was Monosan, Uden, The Netherlands. The Kontron image analysis system and the Video Plan version 3.0 software used in the morphometric analysis were obtained from Kontron Elektronik, GmbH, Eching, Germany.

RESULTS

Macroscopic Appearances

At the time of reexploration, no signs of infection or inflammatory reaction were noted in the legs of any of the animals. The Silastic sheeting was successful in isolating the operative preparation from the surrounding tissues. There was no macroscopic evidence of aberrant reinnervation of the LP muscle or the motor nerve graft. Dissection of the resutured femoral biceps from the lateral head of the gastrocnemius muscle showed no evidence of any regenerating CPN reaching the peroneal compartment. Monopolar stimulation of the track previously occupied by the CPN revealed no visible or electrical activity in the LP.

The muscle in the motor nerve graft was atrophied in all animals irrespective of the time interval since initial operation. The sham-operated muscles looked normal and were not atrophied. The MNT muscles and those that sustained a crush injury to the CPN showed some evidence of atrophy. Muscles that had been denervated were very atrophic, especially those at 4 and 6 months postdenervation. Spontaneous fasciculations could be seen in the surrounding denervated musculature.

Single-Fiber Electromyographic Studies

Thirty-six adult New Zealand White rabbits were examined by using 215 recordings made from 58 LP muscles. The mean jitter (MCD) was 13.6 μ sec with a gaussian distribution and a standard deviation (SD) of 4.4 μ sec (Fig. 2). Low jitter values due to direct muscle fiber stimulation were ignored. There was no significant difference between the left (13.6 ± 4.7 μ sec) and right (13.7 ± 4.01 μ sec) muscles (two-sample t-test, $t = -0.16$, $p = 0.87$). Preoperative MCD recordings in all 36 animals (72 LP muscles) undergoing MNT showed a mean MCD of 13.5 ± 4.04 μ sec.

Fig. 2. Frequency histogram showing MCD values from microstimulatory SFEMG studies of the normal LP muscle.

The overall median MCDs and interquartile ranges (IQRs) from all reinnervated LP muscles at 2, 4, and 6 months were compared with their preoperative MCDs and the MCDs of the normal population MCD (Fig. 3). Because the postoperative distribution of MCDs was asymmetrical and nonnormal, the use of medians was more appropriate than means and the Mann-Whitney procedure was used for two sample comparisons pre- and postoperation. The Wilcoxon test was not used because the motor endplates sampled preoperatively were different from the motor endplates sampled postoperatively in the same muscle and, therefore, the samples could not be considered as truly paired. Compared with its preoperative value, the median MCD for an LP muscle fiber was significantly increased at 2 and 4 months with values of 26 μ sec (IQR 27 μ sec; Mann-Whitney test, $W = 2566$, $p < 0.00005$) and 29.5 μ sec (IQR 37 μ sec; $W = 6345$, $p < 0.00005$), respectively. A return to a more "normal" value was observed by 6 months with a median MCD of 14 μ sec (IQR 13 μ sec; $W = 3255$, $p = 0.478$).

Fig. 3. Graph displaying median MCD values with interquartile ranges before and after MNT. PREOP = data from the entire preoperative population; PRE2M, PRE4M, and PRE6M = preoperative data from the 2-, 4-, and 6-month groups, respectively; 2MTHS, 4MTHS, and 6MTHS = postoperative data from the 2-, 4-, and 6-month groups, respectively, after MNT.

The median MCD recorded from the right LP muscle 2 months after crush denervation was 31 µsec at 2 months, falling to 18 µsec at 4 months, and 20.5 µsec at 6 months. Compared with their preoperative values, the MCDs were significantly increased at 2 months ($W = 437$, $p < 0.00005$) but less so at 4 months ($W = 413$, $p = 0.027$) and 6 months ($W = 267$, $p = 0.01$). There was no significant difference between the preoperative MCDs recorded in the sham-operated LP muscles and those recorded at 2, 4, and 6 months ($p > 0.05$). The median MCDs recorded in denervated LP muscles at 2 and 4 months were 3 and 2 µsec, respectively, which is typical of direct muscle fiber stimulation showing no motor endplate activity. By 6 months postdenervation, no single-fiber action potentials were recordable and the "muscle" was macroscopically very atrophied.

Isometric Studies

The isometric tensions generated by LP muscles from a 10-Hz tetanic 80-V stimulus, are shown in Fig. 4. There is a progressive rise in isometric tension generated by the MNT muscles during a 2- to 6-month period (analysis of variance [ANOVA], $p < 0.0005$, pooled SD 11.72 G). The tension generated by the sham-control LP muscles remained unchanged and that of the denervated LP muscles remained low. At 10-Hz tetanic stimulation, the reinnervation control LP muscles (nerve crush injury group) generated a low isometric tension at 2 months, which was similar to the denervated control muscles. By 4 months

this tension had not changed appreciably, but by 6 months the tension had increased sharply (ANOVA, $F = 19.48$, $p = 0.001$, pooled SD 6 G).

Fig. 4. Graph showing isometric tensions generated by the MNT group and control groups at 2, 4, and 6 months after the initial operation. Each group is represented by the mean, with bars showing the 95% confidence interval based on the pooled SD.

Figure 5 shows a superimposition of typical isometric twitch response curves from a normal LP muscle, a reinnervation control LP muscle at 6 months, and an LP muscle 6 months after soleus MNT. This clearly demonstrates that the MNT LP muscle has acquired the characteristics of a slow twitch muscle.

Fig. 5. Tracings showing superimposition of twitch responses from a normal LP muscle (A), a reinnervation control muscle at 6 months (B), and an LP muscle 6 months after MNT (C). Note the progressive change from a fast to a slow twitch muscle.

Figure 6 shows the mean contraction times (time from onset of twitch response to peak tension) of the reinnervation controls, the sham controls, and the MNT muscles at 2, 4, and 6 months. The MNT LP muscles had significantly longer contraction times (ANOVA, $F = 8.11$, $p < 0.0005$, pooled SD 27.9 msec) consistent with a muscle consisting of predominantly slow twitch fibers.

Fig. 6. Graph showing mean contraction times (time from onset of contraction to achievement of maximum tension) for the MNT group, the reinnervation control group, and the sham-control group at 2, 4, and 6 months after the initial operation. Each group is represented by the mean, with bars showing the 95% confidence interval based on the pooled SD.

Histochemical Studies

ATPase Studies. The normal LP muscle is a fast twitch muscle composed of predominantly type 2 fibers with a random mosaic of slow twitch type 1 fibers (Fig. 7).

Fig. 7. Photomicrograph of a cross section of normal LP muscle, stained with ATPase after preincubation at pH 4.6, showing a predominance of type 2 fibers (light staining) with a random mosaic of type 1 fibers (dark staining). Original magnification X 40.

In the MNT muscles there was a progressive increase in the number of type 1 fibers from Month 2 to 6 with a prominent fiber type grouping consistent with reinnervation by a slow twitch motor nerve supply (Fig. 8 upper). In the reinnervation controls there was also an increase in the type 1 fiber population (Fig. 8 lower), but to a much lesser extent than the MNT muscles.

Fig. 8. Photomicrographs. Upper: Cross section of an LP muscle 6 months after MNT. Note the almost complete reversal of fiber type to type 1 (dark staining). Lower: Reinnervation control LP muscle at 6 months. There is evidence of reinnervation with fiber type grouping (especially of lightly stained type 2 fibers); however, the predominant fiber type remains type 2 in contrast to the MNT muscle in the upper panel. ATPase staining after preincubation at pH 4.6, original magnification X 50.

Prominent grouping of type 1 and type 2 fibers was evident, which is consistent with reinnervation. These findings were quantified by a morphometric analysis of fiber type and size in randomly selected whole-muscle transverse sections, one taken from each group at 2, 4, and 6 months. The proportion of

type 1 fibers rose progressively in the MNT group from 10% in a normal LP muscle to 17% at 2 months, 49% at 4 months, and 73% at 6 months (chi-squared = 2972, $p < 0.00005$) (Fig. 9). The impression of a greater proportion of type 1 fibers in MNT muscles compared with reinnervation controls was quantified in a single animal by measuring the fiber type proportions in a cross section of the left LP muscle 6 months post-MNT and in the right LP muscle 6 months after the crush reinnervation injury. This showed 36% type 1 fibers in the reinnervated muscle compared with 74% in the MNT muscle (chi-squared = 848, $p < 0.00005$). All denervation control muscles showed progressive atrophy with no evidence of reinnervation even at 6 months.

Fig. 9. Bar graph depicting fiber type counts from cross sections of a normal control LP muscle and sections taken from muscles after MNT at 2, 4, and 6 months. Note that only a sample of 1000 fibers was measured in the normal LP muscle. Complete cross sections were measured at 2, 4, and 6 months to eliminate any sampling error.

Acetylcholinesterase Studies. Serial sectioning and acetylcholinesterase enzyme histochemical analysis of the normal soleus muscle confirmed the highest concentration of motor endplates where the motor nerve entered the muscle. In the MNT group, examination of the LP muscle beneath the motor nerve graft at 4 months showed strong localized acetylcholinesterase activity that was consistent with functioning motor endplates.

Neurofilament Studies. All eight LP muscles sectioned through the motor nerve graft-LP muscle

interface showed axons traversing the scar tissue and coursing out into the muscle beneath the motor nerve graft (Fig. 10).

Fig. 10. Photomicrographs of sections passing through nerve graft (pedicle [PED])-LP muscle interface stained with antibody to neurofilament. Left: Note that the muscle fibers in the PED are sectioned transversely, whereas those (MF) in the LP muscle are sectioned longitudinally. Reaction product-staining axons (arrows) can be seen crossing the scar tissue. Note the No. 10-0 prolene suture (S) confirming the location of the section. Right: Note again the cross sectioning through the muscle fibers of the PED and the longitudinal sectioning of the muscle fibers of the LP muscle (musculus peroneus longus [PL]). Reaction product can be seen staining multiple axons (arrows) running from the PED into the LP muscle.

DISCUSSION

Normal LP and Soleus Muscles

Characterization of the LP muscle as a fast twitch muscle was supported by the fiber type distribution on ATPase staining (type 2/type 1 ratio of 9.6:1) and a mean contraction time of 85 μ sec ($\pm$ 13 μ sec). The soleus is well documented as a slow twitch muscle,[27, 44] and this was also confirmed on ATPase staining. Microstimulatory SFEMG studies demonstrated a stable population of motor endplates in the normal LP muscle that were similar to normal MCD values obtained from striated fast muscles in humans.[31] Acetylcholinesterase histochemical analysis of the normal soleus muscle showed acetylcholine activity at motor endplates in the area harvested as a motor nerve graft. The observation of contractions in the muscle fibers of the motor nerve graft on stimulation of the motor nerve confirmed the presence of motor endplates that had not been denervated by harvesting of the motor nerve graft.

Reinnervation by MNT

In the early stages of denervation, the time lapse between normal transmission across the neuromuscular junction and total block is very short (days). When the muscle is denervated and wallerian degeneration has started, extrajunctional receptors develop outside the original endplate area, the muscle fibers' sensitivity to acetylcholine increases, and muscle fiber membrane parameters change.[34] Many fibers show spontaneous activity and are easily stimulated. However, with supraliminal stimulation, the jitter of these stimulated denervated muscle fibers is small (2-5 μ sec),[38] and this small jitter is used to distinguish them from neuromuscular jitter. By 2 months the original motor nerve would have disappeared. Regenerating terminal nerve fibers are unmyelinated and small in diameter. These nerve fibers transmit electrical impulses poorly and are blocked easily, especially with continuous activity, and the motor endplates formed by these nerves are also immature.[32] Increased neuromuscular jitter recorded after 2 months is therefore attributable to immature nerve twigs and motor endplates and is consistent with early reinnervation.[40] Studies of reinnervation in free muscle grafts[18] have confirmed the first functional contacts at 1 month, with highly increased jitter during the first 3 months, which returns to normal by 6 months. The results in this study are similar, with increased jitter at 2 and 4 months after MNT grafting. The jitter rarely becomes completely normalized in the later stages of reinnervation,[30] which was confirmed in this study by the fact that the median jitter (MCD) recorded at 6 months had returned to near normal, although the range was more variable. This return toward normal jitter is attributed to myelination of the terminal nerve twigs and maturation of the new motor endplates.

The MCD recordings obtained in sham controls at 2, 4, and 6 months were unchanged from their preoperative values, confirming that the operative dissection and the Silastic sheeting had no effect on the motor nerve supply to the LP muscle. Muscle fibers belonging to one motor unit have similar structural and functional properties[6,16] because the contractile and metabolic characteristics of mammalian muscles are determined by the motor nerve.[45] Alterations in the motor nerve supply lead to a predictable rearrangement in the enzyme[5,14,23] and histochemical patterns[15,20,26] of the affected muscle. Thus, reinnervating a fast twitch muscle with a slow nerve causes the reinnervated muscle fiber to change its histochemical type from a fast fiber to a slow one. This process of fiber type conversion is not immediate but occurs with some delay after establishment of functional neuromuscular connections by regenerating axons.[46] Reorganization of the normal motor unit architecture is also reflected in the occurrence of local groups of histochemically similar fibers in reinnervated muscle, a phenomenon known as "fiber-type grouping." It is presumed that the clustering of muscle fibers innervated by one motor axon is due mainly to intramuscular sprouting of regenerating motor axons taking over adjacent denervated muscle fibers that previously belonged to different motor units. In this study a fast twitch muscle was reinnervated by a slow twitch nerve so that reinnervation could be inferred from an alteration in fiber typing from type 2 to type 1 in the reinnervated muscle. This phenomenon was strongly evident in the MNT reinnervated LP muscle, in which the fiber typing ratio was almost reversed at 6 months to being a predominantly slow twitch muscle. This is supported by the whole-muscle isometric twitch response curves, which showed conversion to a slow twitch response with a prolonged contraction time. Reinnervation is also supported by the progressive increase in isometric tetanic tension generated by the LP muscle compared with the absence of such an increase in the denervated group. It is important to realize that the muscle was stimulated directly in this study and, therefore, the resultant force was generated by both reinnervated and denervated muscle fibers. The relative contribution from innervated and denervated fiber will change over time as denervated fibers atrophy, generating less tension, and reinnervated fibers mature, generating more tension.

Thus far we have amassed evidence that the LP muscle exhibited progressive reinnervation over the

6-month study period. The next issue to address must be whether this reinnervation came from the transplanted motor nerve, an aberrant nerve supply, or both. The most direct evidence comes from the neurofilament studies. The antibody stains the 70- and 200-kD polypeptides of neurofilament. Neurofilament is a nervous tissue-specific cytoskeletal protein that is manufactured in the nerve cell body and transported down the axon by slow axonal transport. Neurofilament is necessary for the cytoskeletal stabilization of axons,[28] specifically for increasing their cross-sectional diameter. The synthesis of neurofilament in surviving nerve cell bodies decreases after axotomy and returns to normal late in the regenerative process.[3] The presence of strong neurofilament staining, reaching its maximum in the motor nerve graft and streaming out across the interface into the LP, is strongly supportive of reinnervation from the motor nerve graft. Neurofilament staining from the original nerve supply would have disappeared 21 days after axotomy and would have displayed a granular staining pattern.[35] Indirect evidence comes from the finding of motor endplates confined to the area beneath the motor nerve graft in the LP muscle at 4 months. The muscle fiber type distribution of LP muscles that underwent self-reinnervation after a crush injury to the CPN was very different from that of the MNT LP muscles, again implying that reinnervation by regrowth of the CPN was unlikely. Finally the Silastic isolation of the LP muscle appeared effective since the denervation control muscles showed no evidence of reinnervation even at 6 months.

The differences between the behavior of the MNT and reinnervation control LP muscles are interesting and deserve further comment. The reinnervation control LP muscles become self-reinnervated over time by regrowth of their original nerves because the connective tissue framework of the motor nerves is not completely disrupted by a crush injury. The different fiber type distribution of the nerve crush injury LP muscles is consistent with reinnervation by a greater proportion of fast twitch nerves than that seen in the MNT muscles. However, the self-reinnervation was not entirely complete because the type 1 fiber proportion was increased. This may be due to misdirected growth by other nerves in the CPN or to a greater ability of slow twitch motor nerves to achieve successful reinnervation.[13] It seems that reinnervation is completed earlier in the nerve crush injury control muscles because the MCD values have begun to normalize at 4 months with a median jitter of 18 μ sec, and this stability is maintained at 6 months.

Neurobiology of MNT

The neurobiology of motor nerve graft reinnervation differs from that of reinnervation following direct nerve suture in several important respects. During harvesting of the nerve muscle preparation, there is a complete transection injury to terminal nerve branches at the cut margins of the muscle pad where axons traveling to more distant muscle fibers are divided. However, the nerve muscle preparation also contains a large number of intact axons synapsing on muscle fibers within the donor pedicle (Fig. 11).

Fig. 11. Schematic diagram of the edge of a motor nerve graft showing the different populations of motor nerves it contains. A: This motor nerve is completely intact and suffers a trophic injury due solely to the tenotomy of its muscle fibers. B: The trophic injuries to this nerve are division of a portion of its terminal branches and tenotomy of its muscle fibers. C: This motor nerve has no motor endplates on the pedicle muscle fibers. The heavy dashed line represents the edge of the nerve muscle pedicle with severed axons and muscle

fibers at its margins. Note that vascular supplies are not shown to diminish clutter. MEP = motor endplates.

These muscle fibers have an intact blood supply because the pedicle is vascularized; however, because they are detached from their fixed points of origin and insertion, they are tenotomized and do not exert their normal positive trophic influences on axons. The fact that these axons are intact obviously contrasts with the interrupted axons in the proximal stump of a transected nerve. Also, the transected axons in the cut margins of the pedicle muscle pad differ from the transected axons in a peripheral nerve stump in that the interruption is much more distal, thereby retaining a greater proportion of the original nerve. This is important because one of the major determinants of reinnervation is the ability of the injured nerve to survive and subsequently mount a regenerative response. Another important difference is that the transected axons in the motor nerve graft are considerably separate from each other in three-dimensional space compared with the density of axons in a peripheral nerve. Finally, the well-vascularized motor nerve graft, rich in fibronectin and laminin from the pedicle muscle fibers, provides an excellent medium for the growth of regenerating nerve sprouts. These differences should confer benefits on motor regeneration from a nerve muscle preparation. The muscle fibers of the motor nerve graft provide the regenerating axons with a road map to the recipient muscle. The innervated pedicle muscle fibers, with motor endplates close to the pedicle-recipient muscle interface, may support their attached nerves for a sufficient period to allow them to bypass negative trophic influences and rapidly establish fully functional connections with adjacent intact muscle fibers in the recipient muscle. This "piggy backing" could provide a substantial advantage over peripheral nerve suture. However, in this study, degeneration within the transplanted motor nerve was not examined and this "piggy backing" remains speculative. As with any method of reinnervation the rate of new motor endplate formation will eventually fall as the number of available muscle fibers diminishes, the distance the nerve has to travel before connection increases, the remaining denervated fibers become severely atrophic, and natural barriers such as thick fibrous septa limit nerve growth.[25]

Clinical Applications

Certain criteria need to be fulfilled before the MNT technique can be successfully applied to a clinical situation. 1) The procedure must be anatomically possible. Tension on the sutured pedicle will compromise nutrition and mechanically stress the preparation. 2) The donor nerve should closely match the nerve it is replacing in terms of the proportion of muscle fiber types it induces and in terms of its function. There is little functional benefit in reinnervating a muscle that needs tonic activity (for example, a sphincter) with a phasically active nerve. 3) The motor endplate distributions in the donor and recipient muscles should be known to harvest an optimum pedicle and implant it in an optimum site. 4) The donor muscle must be dispensable because it will be denervated.

CONCLUSIONS

From this study we conclude that motor reinnervation is achieved by the MNT technique. This reinnervation is achieved by growth of new nerve fibers across the pedicle muscle interface and into the recipient muscle.

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