Factors Contributing to Preferential Motor Reinnervation in the Primate Peripheral Nervous System

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Received May 3, 1999; revised Sept. 20, 1999; accepted Sept. 30, 1999.

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The major determinant of functional recovery after nerve lesions in the peripheral nervous system is the accurate regeneration of axons to their original target end organs. The definitive measure of such accuracy is physiological proof of functional reinnervation of the distal end organ target. Regenerating axons exhibit a strong preference to grow along Schwann cell basal lamina tubes in the distal nerve stump, i.e., “bands of Büngner” (Büngner, 1891; Cajal, 1928; Holmes, Young, 1942; Ide, 1983; Scherer and Easter, 1984). Myelinating Schwann cells in the normal nerve are associated with just a single axon and form continuous, unbranched basal lamina tubes all the way from a nerve transaction site to the distal end organ target. As long as these basal lamina tubes remain intact they will forcibly direct regenerating axons into the previous terminal nerve branches and will thus determine the ultimate destination of the regenerating axons (Guttmann and Sanders, 1943; Brown and Hopkins, 1981; Brown and Hardman, 1987; Lee and Färel, 1988). The generation of several collateral branches from lesioned axons is one mechanism by which regeneration accuracy is increased. Increasing the number of collaterals per axon at the nerve transaction site increases the chance that at least one collateral will enter a band of Büngner, which will guide it back to its original innervation target.

Preferential motor reinnervation (PMR) refers to the proven ability of regenerating motor axons in rat femoral nerve to preferentially, albeit incompletely, reinnervate muscle versus cutaneous distal nerve branches. Previous work with the rat femoral nerve has revealed that given equal access, regenerating motor axons preferentially reinnervate the terminal muscle branch, i.e., PMR (Brushart, 1988; Madison et al., 1996). Regenerating motor axons initially grow into both nerve branches but over time are pruned from the cutaneous branch (Brushart, 1990). This suggests that immediate mechanical guidance is unlikely to be crucial, and that over time the muscle branch becomes a preferred environment for motor axons because of influences from both the pathway itself as well as end organ reinnervation (Brushart et al., 1998).

Former studies describing PMR have been limited to quantifying regeneration accuracy at the terminal nerve branch level in rodents by using retrograde tracing methods. Correct choices at the nerve branch level are necessary for functional regeneration but may not be sufficient. Just because an axon has chosen the correct nerve branch does not mean that it will be able to reinnervate the correct distal receptor. The current study addressed the following questions: (1) can the functional reinnervation of target muscle, as judged physiologically, be used as an index of PMR; (2) is PMR limited to lower vertebrates, or can it be demonstrated in nonhuman primates; and (3) does the type of nerve repair procedure (e.g., autograft vs nerve guide tube) affect PMR?

As a model system, we used the median nerve of nonhuman primates, which at the wrist level divides into distinct muscle and cutaneous branches. Motor axons innervate the thenar muscles,
whereas sensory cutaneous axons form the digital nerves of digits I-III and the radial half of digit IV. After median nerve transection and repair, we performed “motor unit counting” to determine the likelihood that regenerating motor neurons were reinnervating distal muscle at levels greater than that expected by random regeneration. These studies address the general question of motor neuron regeneration accuracy at the terminal nerve branch level (i.e., muscle vs cutaneous) and their eventual functional connection to muscle, rather than the possibility of specific motor neurons reinnervating specific muscles.

The results of these experiments are the first to show that PMR takes place in the nonhuman primate and expand our understanding of PMR by quantifying the degree to which regenerating motor axons functionally reinnervate their correct distal end organ, i.e., muscle. The extent of PMR was dependent on the type of nerve repair, and although pruning of inappropriate motor axon collaterals from sensory branches can contribute to eventual regeneration specificity, aberrant collaterals can also remain in sensory nerve for extended periods. Understanding the limits of PMR in nonhuman primates will be helpful in designing the most effective nerve repair procedures for humans.

MATERIALS AND METHODS

Surgery. Forty-one median nerve transections and repairs were performed in Macaca fascicularis adult male monkeys. The use of nonhuman primates for these studies was justified by the need to relate the time course and extent of physiological recovery to human nerve repair procedures. One of the most common sites of peripheral nerve injury in humans involves lesions to the median nerve immediately above the wrist. In addition, some of the materials used in these studies are being evaluated for eventual human use; thus it was necessary to use an animal model that closely simulates the human clinical situation.

All surgical, electrophysiological, and terminal procedures were performed under deep general anesthesia with ketamine (12.0 mg/kg) and acepromazine maleate (1.0 mg/kg). Median nerve transection at the wrist results in sensory loss of the palmar surface and finger tips of digits I, II, and III and the radial aspect of the palmar surface of digit IV. However, radial and ulnar innervation provide protective sensation to the dorsal surface of the hand and digits. Despite these sensory and related motor deficits the animals could easily grasp the cage bars, and normal behaviors such as feeding and grooming were not significantly affected. Surgical procedures, electrophysiological assessments, animal housing, and enrichment programs were approved by the Duke University Animal Care and Use Committee. Animals were monitored by the veterinary staff on a daily basis, and no animal displayed distress or any secondary complications resulting from the median nerve lesions such as self-mutilation, pressure ulcers, sensory neglect, or infection.

For the direct suture group (n = 4 nerves) and 5 mm gap groups (n = 5 nerves each autograft or collagen nerve guide) the median nerve was transected 2 cm above the wrist, and a 5 mm section of nerve was removed. The nerve was then mobilized within the muscle bed for a transverse distance of 10–20 mm from the wrist stimulating electrode, and the stimulus current was increased above that which elicited a maximal CMAP. During the three regeneration periods the wound is markedly elevated, and at this stage a maximal stimulus of 60 mA and a duration of 1 msec was used to ascertain whether the muscle had become reinnervated. After maturation of the regenerated fibers, standard stimulus intensities (e.g., ≤3–8 mA) were adequate to evoke a maximal CMAP response.

Sensory conduction studies. Sensory nerve fibers in digit II were stimulated with needle electrodes at the base and tip of the digit, or a subcutaneous needle electrode was placed at the midlevel of the proximal phalanx (see Fig. 4.4 for electrode placements). The evoked compound sensory potentials (CSAPs) were recorded from the needle electrode that had been optimally situated close to the median nerve at the wrist to evoke the CMAPs (see above). In selected sessions, this recording electrode was then moved to ascertain whether potentials are still evoked at that location. Evoked sensory potentials (CSAPs) were recorded from the needle electrode that had been optimally situated close to the median nerve at the wrist to evoke the CMAPs (see above). In selected sessions, this recording electrode was then moved to ascertain whether potentials are still evoked at that location. 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Motor unit number estimation. Motor unit number estimation (MUNE) was used for the serial counting of the number of motor units in the median nerve innervated hand muscles (Brown, 1972; Sica et al., 1974; Fu and Gordon, 1995a,b). This nondestructive physiological technique detects distinct incremental increases in the amplitude of the evoked CMAP following the gradual incremental increase in the threshold level. Each distinct increase in the CMAP represents the recruitment of a single additional motor unit (see Fig. 1). Stimuli were first delivered at or slightly above the threshold needed to evoke the smallest CMAP. Stimulus intensity was then carefully increased to evoke discrete incre-
ments in the CMAP. These “jumps” in the CMAP were followed on a storage oscilloscope and reflect the combined contributions of successively recruited individual motor units. The total number of motor units was then estimated by dividing the amplitude of the maximally evoked CMAP by the average amplitude of the first (5–15) discrete motor unit potentials. In this manner, the number of evoked motor units from median nerve reinnervated muscles was followed serially from 400–1100 d postoperatively. Systematic MUNE was not initiated until after the killing of the 5 mm group, and the limited data regarding the number of motor units for this group were not included in the analysis.

A well known limitation of the technique of MUNE is that the total number of motor units must be estimated from just the first few discrete increments of the CMAP. It is thus reassuring that the baseline estimates obtained using this technique in a serial nondestructive manner agreed with an estimate obtained after extensive dissection of the hand and stimulation of individual nerve branches under visual guidance (see below). In addition, in most of the recording sessions for reinnervated muscle virtually all of the motor units could be discerned as distinct jumps in the evoked CMAPs, and extrapolation using just the first few distinct CMAPs was not necessary.

To verify the origin of the muscles giving rise to the recorded evoked CMAP, a terminal recording session was performed on one unoperated hand. Each muscle fiber group was serially identified by the median nerve innervated thenar and lumbrical muscles and their respective terminal nerve branches. Under visual guidance, a recording electrode was serially inserted into each of the identified muscles, and the median nerve was stimulated at the wrist and then at the terminal nerve branch for that particular muscle. After recording of the number of evoked motor units at each muscle, the terminal nerve branch to that individual muscle was cut. The complete dropout of the CMAP after stimulation at the wrist or terminal nerve verified that the previous potentials were arising exclusively from that particular muscle. The very close agreement between estimates of the number of motor units during the one terminal recording session and the more extensively applied nondestructive closed hand procedures (147 and 155; see Table 1), shows that the area of the recording electrodes used during the nondestructive sessions recorded motor units from all of the median nerve innervated hand muscles in these very small hands. There was thus no detectable bias in terms of the placement of the recording electrode and the source epicenter, as can be demonstrated when recording from much larger extremity muscles.

Statistical estimation of the “expected” number of regenerated motor units and defining the null hypothesis of random regeneration. We estimated the number of myelinated axons at the wrist level that eventually project to the median nerve innervated thenar and lumbrical muscles and their respective terminal nerve branches. Although we considered directly counting the number of myelinated axons in the terminal muscle branches, it has previously been shown that these axons display extensive branching before the discrete anatomical formation of the terminal branches themselves (Wray, 1969; R. D. Madison, S. J. Archibald, and C. Krarup, unpublished observations). Thus it is impossible to obtain reliable unbranched axon estimates at this level by morphometrics alone.

By contrast, the number of α-motor neurons can be estimated by MUNE and in the unlesioned median nerve was found to be 147 ± 14 (this paper; see below). Based on axon diameter and conduction velocity measurements, previous studies have estimated γ motor neurons to make up between 19 and 45% of a total motor neuron pool (McLeod and Wray, 1967; Henneman, 1974; Swett et al., 1986). To be conservative, if one assumes the 45% level, it would add 120 γ motor neurons to the 147 α-motor neurons to bring the total motor neuron pool to these muscles to 267.

Previous denervation studies in the primate have estimated that the number of myelinated sensory fibers to the intrinsic hand muscles is approximately equal to or less than the number of myelinated motor fibers (McLeod and Wray, 1967; Wray, 1969). Thus doubling the number of myelinated axons from motor axons would bring the total number of myelinated axons in the terminal muscle branches to 534. By dividing the number of myelinated axons at the cut nerve face (8449) by the number of myelinated axons eventually projecting to the terminal muscle branches (534), one can estimate the proportion of continuous Schwann cell tubes at the cut face that project directly to terminal muscles (0.06). This resulting fraction was used to multiply the normal number of α-motor units (0.06 × 147) to estimate the average number of α-motor units expected to return to these muscles after random axon regeneration: 8.82. Using binomial statistics, an SD can be placed around this average, 8.82 ± 2.9 (see above; Zar, 1984).

Physiological characteristics of motor units at >900 d after repair and comparison with the expected number of motor units

In an attempt to compare stabilized motor units, we analyzed the number of motor units in the direct CMAP and the recCMAP at time periods of >900 d (i.e., ~2.5 years after surgery). We calculated 147 ± 14 (mean ± SEM) motor units in the normal median nerve innervated hand muscles (n = 16 nerves, 64 independent recording sessions). A single terminal recording session after extensive dissection of the hand revealed 155 distinct motor
Anatomical basis for the recCMAP

When 2% lidocaine was injected into the base of digit II, both the CSAP and the recCMAP evoked by stimulation at the digit tip was abolished, whereas the CMAP evoked by stimulation at the wrist was not affected, thereby ruling out stimulus spread as a basis for the recCMAP (data not shown). To exclude the possibility that the recCMAP might be attributable to reflex activity, the median nerve was blocked in the forearm or upper arm by local injection of lidocaine. A hollow needle with a 3 mm bared tip was placed close to the median nerve (as indicated by a low CMAP threshold when used for stimulation) and then used to inject 2% lidocaine. The local anesthesia caused block of conduction along the nerve in the forearm but had no effect on the recCMAP elicited by digit II stimulation (Fig. 4B), thus excluding the possibility that the recCMAP represented a CNS reflex.

To test whether the recCMAP represented dispersed compound motor activity evoked by stimulation of the digital nerve or whether a single fiber or a few fibers carried the afferent activity, the effect of double stimulation was evaluated. The recCMAP shown in Figure 4B consisted of three different spikes (Pot 1–3), which were evoked at twice the stimulus intensity that elicited a maximal CSAP (Fig. 4C). To record the responses (R1 and R2) to the two stimuli (S1 and S2), the responses R1 + R2 were recorded digitally, and the response R1 to S1 alone was then electronically subtracted. The three components of the recCMAP had different absolute refractory periods of 1.3, 2.0, and 1.45 msec, respectively (Fig. 4D). The full amplitudes of the individual spikes were reached at the absolute refractory period, and at longer time intervals there was no further change in amplitude (Fig. 4F). In contrast, the CMAP evoked by stimulation of the median nerve at the wrist had an absolute refractory period of 0.9 msec, which was ∼30% shorter than the shortest refractory period for the recCMAP (Fig. 4E). Moreover, the amplitude of the CMAP gradually increased (Fig. 4F) during the relative refractory period at longer S1–S2 intervals, as expected from a response that was the sum of several motor units. The abrupt rise of the recCMAP to its maximal amplitude at the absolute refractory period suggests that only a few digital nerve fibers with all-or-nothing behavior contributed to the afferent pathway along which the activity giving rise to the recCMAP was propagated. When recorded at threshold the recCMAP in some instances also suggested an all-or-nothing behavior. The latencies of both the recCMAP and the CMAP gradually decreased during the relative refractory period (data not shown).

Stimulation of mechanoreceptors using a pulsatile tactile stimulator applied to the distal pad or proximal phalanx of digit II resulted in a sensory potential that could be recorded at the wrist; however, even after averaging up to 1000 traces no activity was detected in muscle (data not shown).

The effect of cooling and local intravenous lidocaine on the recCMAP was examined to explore the possibility of ephaptic transmission (data not shown). Ephaptic transmission usually occurs from unmyelinated fibers requiring higher stimulus intensities to larger myelinated fibers with lower stimulus thresholds. Ephaptic responses are considered to have a lower safety margin of transmission compared with direct electrical conduction, and thus their occurrence can be increased by prolonging the amount of time that action potentials traverse a repair site (Rasminsky, 1980). When the skin temperature of the hand and forearm was reduced from 35 to 20ºC by packing the arm in ice, the latency of the CMAP increased by 40%, and the amplitude decreased by 25%. The latency of the recCMAP increased by 65–70%, whereas the amplitude did not change systematically. No further recCMAP components were recruited at low temperature.

Lidocaine (10 ml of 0.2%) was injected intravenously distal to a blood pressure cuff. Within 10 min, the CMAP and recCMAP were each reduced by ∼20%. The cuff was released, and the recCMAP followed until it returned to baseline. Ischemia was again induced, and 10 ml of 0.2% lidocaine was injected. Within 10 min, the CMAP and recCMAP were each reduced by ∼50%.
Table 1. Characteristics of motor units in the direct (CMAP) and the recurrent motor response (recCMAP) in regenerated median nerves at times >900 d after repair

<table>
<thead>
<tr>
<th>Procedure</th>
<th>CMAP: no. of motor units (mean ± SD)</th>
<th>No. of motor units in CMAP vs expected</th>
<th>recCMAP: no. of motor units (mean ± SEM)</th>
<th>Motor unit amplitude (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, closed hand (n = 64; 16)</td>
<td>147 ± 14</td>
<td></td>
<td>6</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Normal, open surgery (n = 1)</td>
<td>155</td>
<td></td>
<td></td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td>Direct suture (n = 19; 4)</td>
<td>18.4 ± 3.2</td>
<td>p &lt; 0.001</td>
<td>2.7 ± 0.5</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Graft, 20 mm (n = 29; 8)</td>
<td>14.9 ± 1.9</td>
<td>p &lt; 0.02</td>
<td>3.1 ± 0.3</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Graft, 50 mm (n = 25; 6)</td>
<td>16.3 ± 2.9</td>
<td>p &lt; 0.01</td>
<td>3.5 ± 0.5</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Tube, 20 mm (n = 38; 8)</td>
<td>12.5 ± 1.9</td>
<td>NS</td>
<td>2.3 ± 0.4</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Tube, 50 mm (n = 15; 3)</td>
<td>9.7 ± 2.2</td>
<td>NS</td>
<td>1.7 ± 0.5</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Expected</td>
<td>8.8 ± 2.9</td>
<td></td>
<td></td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

The number of independent physiological assessments as well as the number of nerves sampled for this period (>900 d) is shown for each repair group (e.g., for the normal baseline measurements 64 independent assessments were made from 16 different nerves). The expected number of regenerated motor units was derived from calculations using binomial statistics to model “random” motor axon regeneration (see Results and Discussion for details).

*Compared with the amplitude of motor units in normal muscle (0.2 ± 0.1 mV), the amplitude of the direct CMAP motor units was significantly greater for all repair groups (ANOVA, p < 0.05).

**Within each of the repair groups, the motor unit amplitude of recCMAPs was significantly smaller than that of the direct CMAP (p < 0.001, paired t tests).

The cuff was released, and the response was allowed to recover. Ischemia was again induced, and 7.5 ml of 0.5% lidocaine was injected. The recCMAP and CMAP were both extinguished within 3 min.

The response of the recCMAP to refracted doses of lidocaine injected intravenously suggested that it was as stable as the CMAP, rather than demonstrating a lower safety margin of transmission. The results of cooling and lidocaine injections strongly suggest the recCMAP arises from an aberrant axon collateral of an α-motor neuron, which also sends an axon to muscle.

Appearance and maturation of the recCMAP

In the 5 mm repair group, the recCMAP could first be recorded 25 d (range, 0–42 d) after a CSAP was first present (~100 d), and it was associated with a visible contraction of the muscle. The stimulation threshold of the recCMAP was initially much higher than that of the CSAP but gradually decreased during maturation; however, even at 350–500 d after repair, the current necessary to evoke a maximal recCMAP remained on average 4.1 ± 0.3 (mean ± SEM) times that needed to evoke a maximal CSAP (p < 0.001; data not shown). Similar results were found in all repair groups, although as expected, there was a correlated increase in the amount of time postoperatively until responses could be first detected depending on the initial nerve gap distance.

Throughout the observation period the latency of the recCMAP decreased exponentially but remained significantly greater than that of the CMAP or CSAP (p < 0.001; data not shown). The conduction velocity of fibers along the same path in the digit that respectively give rise to the CSAP or the recCMAP was calculated by stimulating the digital nerve at the tip or the base of digit II (Fig. 54; see Materials and Methods) and dividing this measured distance by the differences in the recorded latencies. Both conduction velocities along digit II increased gradually along S-shaped curves with similar slopes; however, the CSAP reached a higher level than the recCMAP. At 341–679 d after repair the CSAP was 34 ± 2 m/sec, whereas the conduction...
velocity of the recCMAP was only 13 ± 1 m/sec (Fig. 5A; p < 0.001, paired t test).

The effect of ischemia was also consistent with the aberrant collaterals having a small diameter (Parry and Brown, 1982). Ischemia was induced for periods of 15–20 min by inflating a blood pressure cuff placed around the forearm above the systolic blood pressure, and the effect on the CMAP and recCMAP was continuously monitored. The recCMAP was stable for 10 min and then declined rapidly and was no longer detectable by 15 min. After 17 min of ischemia the amplitude of the CMAP had decreased by 10%, and the latency had increased by 5%. Within 2 min after deflating the cuff, the recCMAP had fully recovered. The total conduction time in milliseconds for the recCMAP from the digit to the muscle was directly determined by its latency.

The conduction time was significantly longer than that for the latency of the CSAP (also expressed in milliseconds) obtained by stimulating at the same site in the digit and recording directly at the same site in the wrist (Fig. 5B). The recCMAP conduction time from the digit to the wrist was then subtracted from the total conduction time from digit to muscle (APB latency) to give the “residual” conduction time along the efferent pathway from the repair site in the wrist to the muscle. This residual conduction time of the recCMAP (in milliseconds) was not significantly different from that for the latency (in milliseconds) of the direct CMAP response evoked by stimulation at the same site in the wrist and recorded at the muscle (Fig. 5B). All of the above conduction velocity measurements were taken at 300 d after repair in the 5 mm lesion groups, a time when the latencies had stabilized.

Change in the number of motor units contributing to the CMAP and recCMAP from 400 to 1100 d postoperatively (Figs. 6, 7) In the direct suture group the correlation between time and the number of motor units contributing to the CMAP was nonsignificant, indicating that the number of motor units was not significantly changing over this period. Over the same period, however,
the number of motor units contributing to the recCMAP was significantly decreasing ($p < 0.02$; Fig. 6). A similar pattern was seen in the 20 mm sural cable graft group, with no significant change in the number of motor units for the CMAP but a significant reduction in the recCMAP ($p < 0.01$; Fig. 7A). Conversely, in the 50 mm nerve graft group the number of motor units for the direct CMAP significantly decreased, and the number for the recCMAP significantly increased over time (Fig. 7B).

All of the nerves in the 20 mm nerve guide group displayed successful regeneration; however two of the nerves in the 50 mm nerve guide group failed to show any signs of regeneration. Thus the data for the number of motor units in the 50 mm nerve guide group are derived from the three animals that showed regeneration. For both nerve guide groups there were no significant changes in the number of motor units for either the direct or recCMAP (Fig. 7C, D).

**DISCUSSION**

These results suggest several major points concerning preferential motor reinnervation after primate median nerve lesions: (1) under certain circumstances regenerating motor neurons reinnervate muscle at levels that are significantly greater than expected from random regeneration; (2) there is a significant negative correlation between the initial length of time of muscle denervation and the much later final motor unit amplitudes; (3) aberrant motor axon collaterals enter cutaneous pathways and remain for extended periods, although they remain small and immature; and (4) pruning of the aberrant collateral from the cutaneous pathway over time is seen under certain conditions.

**Number of motor units reinnervating muscle (CMAP)**

MUNE revealed significantly more $\alpha$-motor units for the direct suture and the sural cable graft groups than expected if regeneration were random, but not in the nerve guide repair groups. These findings suggest that PMR, which has previously been documented in rodents, also occurs in primates after certain lesions. The severity of the nerve lesion, including distance and the presence of initial bridging material, may determine whether PMR takes place. In the direct suture and the graft groups, regenerating axons encounter nerve environment immediately after crossing the suture lines. In the nerve guide repair groups, axons must regenerate across an initially empty space, resulting in fewer regenerating motor units eventually contacting muscle.

The statistical analysis to determine the expected number of regenerated motor units after random regeneration assumes that all $\alpha$-motor neurons regenerate and send an axon into the distal stump. It should be stressed that if fewer motor neurons regenerated, the statistically expected number of regenerated motor
units would decrease proportionately. Thus our criteria for whether PMR has occurred is conservative.

The average number of motor units in the CMAP in each repair group did not significantly change over the observation period, except for a significant decrease in the 50 mm graft group. This decline is difficult to fully explain; however, the following possibilities must be considered. We have previously found that a nerve graft in the primate median nerve stimulates significant axonal branching as measured in the distal nerve stump (Archibald et al., 1995). Motor unit dropout could be attributable to increased metabolic stress on the motor neurons as a result of maintaining numerous long axon collaterals within the 50 mm nerve graft and distal nerve stump as well as innervating muscle. Excessive metabolic stress has been suggested as a reason for the dropout of motor units observed in patients with post-polio syndrome (Dalakas, 1995). The rationale for this happening in the 50 but not the 20 mm graft groups would be that the greater graft length represents a more severe lesion. Support for this is given by the significantly longer muscle denervation time in the 50 compared with the 20 mm graft group, as well as a smaller final motor unit size. Alternatively, both the decline in the number of motor units over time and the smaller motor unit size may represent an inability of the long-term denervated muscle and distal nerve sheaths to support the regenerated axons, as has recently been reported after denervation periods of ≥6 months in rodents (Fu and Gordon, 1995b). There would also be a greater time delay before regenerating axons in the 50 mm graft group begin to grow within basal lamina tubes of the distal nerve, and over time there is a fragmentation of basal lamina tubes in denervated nerve that adversely affects regeneration (Giannini and Dyck, 1990).

All of these unfavorable factors would apply to the nerve guide groups; thus one might expect those groups to have also displayed a reduction in the number of motor units in the CMAP over time. A possible explanation for not detecting such a decline is that the lesions may be so severe that only a few motor neurons display robust enough regeneration to cross the initially empty nerve guide tube in the first place, but once they have innervated muscle they remain relatively stable. Two of five animals in the 50 mm tube group failed to show any signs of regeneration, highlighting the difficulty of regeneration in the nerve guide groups.

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Figure 7. Number of motor units contributing to the CMAP (open circles, left y-axis) and the recCMAP (filled circles, right y-axis) with time after nerve repair by sural cable graft (A, B) or collagen nerve guide tube (C, D). Each data point represents the mean ± SEM of the measurements for that time point. Linear curves were calculated from the least squares method. n, Number of time points used to calculate the curve; r, correlation between time and number of motor units; p, statistical significance of the correlation (two-tailed test); ns, nonsignificant.
Aberrant motor axon collaterals in cutaneous nerve

In all of the regenerated nerves, stimulation of digit II resulted in an aberrant recCMAP. Studies were performed to rule out the possibility of stimulus spread, CNS reflex, or ephaptic transmission. All of the evidence suggests that the most parsimonious explanation for the aberrant recCMAP is that it represents a collateral of an α-motor axon, which also innervates muscle; afferent conduction from the digit would occur along the aberrant axon collateral, and after reaching a branching point localized at or distal to the repair site at the wrist, the electrical activity would be conducted efferently along the main axon that innervates muscle.

The conduction velocity along the aberrant motor collateral in the sensory territory initially increased at the same rate as conduction in the sensory fibers giving rise to the CSAP but reached a final level of ~10–15 m/sec, whereas the CSAP increased to 30–40 m/sec. These conduction velocities are within the range of myelinated fibers and indicate that both aberrant motor axon collaterals and sensory fibers matured to the point of becoming myelinated. However, the relative conduction velocities also suggest that the aberrant motor axon collaterals only reached a caliber approximately one-third of that of the regenerated sensory fibers in the same digital nerve [(e.g., 3–4 vs 8–9 µm, assuming a conversion factor of 4–5 m·sec⁻¹·µm⁻¹ (Buchthal et al., 1984)]. Thus the digital nerve was capable of supporting large, well myelinated fibers, but the aberrant motor axon collaterals remained quite small.

The absolute refractory period of fibers giving rise to the recCMAP remained 1.5–2.5 times longer than that of the direct motor response, also suggesting that the aberrant fibers had small diameter. The long refractory period could not be used to distinguish whether the diameters of the fibers in both the afferent and the efferent branches differed in size, because the measurement included the entire conduction path. The respective sizes of the axons giving rise to the recCMAP within the aberrant afferent portion (digit to wrist) and the normal efferent motor portion of the pathway (i.e., wrist to muscle) were estimated by comparing the conduction time along both of these pathways. In contrast to the stunted development of the aberrant motor axon collateral within the sensory digital nerve, the conduction velocity of its sibling projecting to muscle was similar to that of the most developed motor axons (i.e., fastest conducting). These refractory period and conduction time results suggest that collaterals from the same motor axon mature to different levels depending on whether they project appropriately to muscle or aberrantly to sensory nerve and suggest greater maturational support when projecting to muscle.

Pruning of aberrant motor collaterals from cutaneous nerve

Numerous axon collaterals are formed during regeneration (Bray and Aguayo, 1974; Toft et al., 1988). The traditional view has been that once a target (e.g., muscle), is innervated only a single collateral remains (Aitken et al., 1947; Mackinnon et al., 1991; Vrbova et al., 1995; Fu and Gordon, 1997). In our experiments, we would only be able to detect an aberrant motor collateral in the sensory nerve after it acquired functional properties allowing conduction of action potentials and after reinnervation of muscle by its correctly growing sibling axon. A significant decline over time was seen for the number of recCMAPs in the direct suture and 20 mm graft groups, whereas the number of motor units contributing to the CMAP remained constant. These results suggest a preferential maintenance of the axon collateral that had contacted muscle and a pruning of the sibling collateral in the inappropriate sensory nerve. In all repair groups the motor unit size of the CMAP was significantly greater than the size of the recCMAPs. The consistently smaller size of the recCMAP would suggest that axons with sibling collaterals in the sensory nerve were at a disadvantage in terms of competing during reinnervation of muscle fibers (Colman et al., 1997), even though their conduction properties from wrist to muscle were similar to directly projecting motor axons.

Selective pruning has been suggested to be the anatomical mechanism by which PMR occurs in the rodent femoral nerve (Brushart, 1990), and the present data suggest that a similar mechanism exists in the primate. Interestingly, previous work in the rodent has shown that for pruning to take place the motor neuron must have axon collaterals in both the appropriate muscle branch as well as the inappropriate cutaneous branch. The number of rodent motor neurons that projected exclusively to the inappropriate cutaneous branch did not significantly change over time. This suggests that the active process of pruning requires the ability of the neuron and/or axon to be able to distinguish between the two distinct environments. This process of favoring one axon collateral over all others has been termed “sibling bias,” and it has been suggested that it represents the ability of the neuron to compare two different environments in terms of the level of trophic support (Smalheiser and Crain, 1984; Crutcher and Saffran, 1990). There was no evidence of pruning in the 50 mm graft group or either of the nerve guide repair groups. In these more severe nerve lesions pruning of aberrant collaterals may not occur or may be markedly delayed. One explanation for the lack of pruning is that there was not enough of a difference between the two distal nerve or target environments to allow sibling bias to result in detectable pruning.

In the 50 mm graft group there was actually an increase in the number of recCMAP motor units. Both the lack of pruning in this group and the increase in the number of recCMAP motor units may be related. In this group there was a significant postoperative time delay to the first detectable CMAP. This delay suggests that the regenerating axons had a markedly slow advancement; thus aberrant collaterals may have continued to grow into the sensory nerve territory over the period examined. The same rationale would be expected to apply to the nerve guide repair groups, which also had long muscle denervation times, except that these lesions may be so severe that regeneration is significantly blunted overall; thus the number of regenerating motor neurons simply remains quite low.

It is important to stress that because our experimental protocol monitored responses from reinnervated muscle, pruning of aberrant collaterals could occur as well, or even predominantly, during early reinnervation without being detected. Furthermore, a reduction in the number of motor unit potentials in the aberrant motor response may be a conservative estimate of the pruning process, because several collaterals may have been formed, and dropout of an α-motor unit potential would require that all conducting collaterals were pruned. Partial pruning would not be discerned as long as even a single collateral could conduct an action potential.

In summary, our results suggest that more motor neurons than expected by random axon regeneration reinnervate muscle after median nerve lesions repaired by direct suture or by nerve grafts of up to 50 mm. In the less severe lesions (i.e., direct suture and 20 mm graft), pruning of aberrant motor collaterals from sensory
nerve may be one factor contributing to reinnervation specificity. In all of the repair groups, although aberrant motor axon collaterals remained in sensory nerve territory for long periods, they remained relatively immature, and there was no evidence of innervation of low-threshold mecanoreceptors.

There have been findings of aberrant sensory–motor connections in severe long-term lesions of human nerve (Krarup et al., 1990; Montserrat and Benito, 1990). However, the frequency of such aberrant responses in less severe lesions, their development and changes over time, and the underlying anatomical basis for the response have been unclear. The results of the current experiments suggest that such aberrant connections are quite common. A better understanding of the mechanisms that lead to the pruning of such aberrant collaterals would aid therapeutic intervention strategies designed to increase regeneration specificity and ultimate functional recovery.

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