Comparison of the fastest regenerating motor and sensory myelinated axons in the same peripheral nerve

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Functional outcome after peripheral nerve regeneration is often poor, particularly involving nerve injuries far from their targets. Comparison of sensory and motor axon regeneration before target reinnervation is not possible in the clinical setting, and previous experimental studies addressing the question of differences in growth rates of different nerve fibre populations led to conflicting results. We developed an animal model to compare growth and maturation of the fastest growing sensory and motor fibres within the same mixed nerve after Wallerian degeneration. Regeneration of cat tibial nerve after crush (n = 13) and section (n = 7) was monitored for up to 140 days, using implanted cuff electrodes placed around the sciatic and tibial nerves and wire electrodes at plantar muscles. To distinguish between sensory and motor fibres, recordings were carried out from L6–S2 spinal roots using cuff electrodes. The timing of laminectomy was based on the presence of regenerating fibres along the nerve within the tibial cuff. Stimulation of unlesioned tibial nerves (n = 6) evoked the largest motor response in S1 ventral root and the largest sensory response in L7 dorsal root. Growth rates were compared by mapping the regenerating nerve fibres within the tibial nerve cuff to all ventral or dorsal roots and, regardless of the lesion type, the fastest growth was similar in sensory and motor fibres. Maturation was assessed as recovery of the maximum motor and sensory conduction velocities (CVs) within the tibial nerve cuff. Throughout the observation period the CV was ~14% faster in regenerated sensory fibres than in motor fibres in accordance with the difference observed in control nerves. Recovery of amplitude was only partial after section, whereas the root distribution pattern was restored. Our data suggest that the fastest growth and maturation rates that can be achieved during regeneration are similar for motor and sensory myelinated fibres.

Keywords: nerve; regeneration; laminectomy; cuff electrodes; cat

Abbreviations: CMAP = compound muscle action potential; CNAP = compound nerve action potentials; CRP = compound root action potential; CV = conduction velocity


Introduction

After a nerve injury, both motor and sensory axons have the ability to regenerate and, given a proper pathway, reconnect with targets. Despite this capacity, the functional outcome of peripheral nerve regeneration is often poor, particularly after nerve injuries that sever peripheral nerves far from their targets (Lundborg, 2003). In a long-term study of nerve regeneration in non-human primates the lesion and repair type, through the time to muscle reinnervation, influenced recovery assessed by physiological methods (Krarup et al., 2002). Thus, both the distance and rate of regeneration probably play important roles in determining the outcome of nerve regeneration.

Axonal transport responsible for nerve regeneration is slow (Wujek and Lasek, 1983) for both motor and sensory axons (Braendgaard and Sidenius, 1986). The fastest growth rates approach 4 mm/day depending on the regeneration environment (Fugleholm et al., 1994, 1998, 2000; Sorensen et al., 2001). In the mouse distal stump, Schwann cells previously associated with motor axons retain specialized features (Martini et al., 1992), and this was suggested to contribute to preferential reinnervation of motor pathways by motor axons (Brushart, 1988), which may improve functional outcome of motor regeneration after section (Brushart, 1993). It is unknown whether such environmental specializations
accelerate motor axon growth rates compared with sensory axon growth rates.

Comparison of sensory and motor axon regeneration before target reinnervation is not possible in the clinical setting, and previous experimental studies addressing the question of differences in growth rates of different nerve fibre populations led to conflicting results. In some studies, axons in muscle or mixed nerves were found to regenerate faster than fibres in pure sensory nerves (Jenq and Coggeshall, 1985; Chen and Bisby, 1993). A better motor than sensory regeneration was also suggested in the same mixed nerve (da Silva et al., 1985; Madison et al., 1988). Other studies, however, indicated that sensory fibres dominate in the early stages of regeneration (Sanger et al., 1991; Madorsky et al., 1998; Suzuki et al., 1998; Kawasaki et al., 2000). Nevertheless, the fastest growing motor fibres were found to regenerate as fast as the sensory fibres (Forman and Berenberg, 1978; Forman et al., 1979). The reason for these controversies may be attributed to the technical difficulties of identifying the fastest growing fibres at the front of regeneration.

The aim of this study was to compare, in a large animal model, the fastest rates of regeneration that are achieved by sensory and motor fibres within the same mixed nerve both after crush and section. We previously developed an electrophysiological method in cat to monitor growth of axons in the tibial nerve through a series of electrodes implanted around the nerves in a silicone cuff and confirmed by histological studies (Fugleholm et al., 1994, 1998; Sorensen et al., 2001). In this study, to distinguish between motor and sensory fibres, we recorded evoked action potentials from all the ventral and dorsal roots containing fibres projecting to the tibial nerve. To compare rates of growth, we mapped the distances reached within the tibial nerve cuff by fibres belonging to different spinal roots. Furthermore, to compare maturation, recovery of motor and sensory conduction velocities (CVs) within the tibial nerve cuff was investigated for up to 5 months of regeneration.

Material and methods

Animals, experimental design and anaesthesia

Electrodes were implanted in both legs in the plantar muscles and around the sciatic and tibial nerves of 15 adult female cats (2.5–3.2 kg, Ifa-Credo, France). After implantation, all animals regained normal movements and gait and stayed healthy during the experiment. Data from four legs were excluded from the analysis because the cat destroyed the cabling connecting the electrodes.

The study was performed in five consecutive steps: (i) surgery for electrode implantation and nerve lesions; (ii) electrophysiological validation of the nerve lesion; (iii) serial recordings to identify the outgrowth of the tibial nerve fibres; (iv) terminal laminectomies to compare growth of sensory and motor fibres in the tibial cuff after crush \((n = 5)\) and section \((n = 4)\); (v) terminal laminectomies to compare maturation of sensory and motor axons after target reinnervation after crush \((n = 8)\) and section \((n = 3)\). Recordings from roots were performed in six unlesioned tibial nerves for control 1 week after the electrode implantation.

Electrode implantations and nerve lesions

Procedures of nerve lesions and electrode implantation (Krarup and Loeb, 1988) were described in detail previously (Fugleholm et al., 1994, 1998; Sorensen et al., 2001). Briefly, after exposure of the tibial nerves over ~70 mm, crush lesions were carried out by clamping a silicone coated forceps for 2 min, and complete transection lesions were immediately co-adapted using microsurgical techniques by four epineural sutures (10-0 Taper Point, S&T Neuhausen, Switzerland). Eight-lead semicircular cuff electrodes (Larsen et al., 1998) with leads spaced 7.5 mm apart were implanted around the tibial nerves (Fig. 1A) and positioned so that the first electrode (T1) was 10 mm distal to the lesion site (marked with a 6-0 suture). Six-lead cuff electrodes were implanted around the sciatic nerves (two pairs of three leads, with 18 mm between the centre recording leads). The electrode placed around the tibial nerve had an internal diameter of 3 mm and the cuff around the sciatic nerve had an internal diameter of 4 mm, which was at least 30% larger than the diameter of the nerves to avoid compression. Wire electrodes for recording of plantar muscle activity were sutured to the fascia of the flexor digitorum brevis muscle and subcutaneously on the dorsum of the paw. A ground wire electrode was implanted subcutaneously. The connecting teflon-coated stainless steel wires (AS 631, Cooner WIRE Co., Chatsworth, CA, USA) connecting the electrodes were passed subcutaneously to the back, resurfaced through a skin excision and mounted on an integrated circuit board protected by an aluminium shield.

The integrity of the implanted electrodes and electrical connections was tested in situ by measuring the impedance between the leads and ground. Complete axonal loss after the nerve lesion was ascertained electrophysiologically 1 week after the lesion.

Serial investigations to identify the growth front of the tibial nerve fibres

During the first month after the lesion, serial electrophysiological studies were carried out every 4–6 days. An illustration of the recording set-up is presented in Fig. 1A. Supramaximal stimulation (10 mA at 0.5–3 Hz) was delivered consecutively to the T1–T7 leads in the tibial cuff with the anode placed one lead distal to the cathode. Biphasic rectangular negative-positive pulses (duration = 0.1 ms) were generated from a photo-isolated battery-powered, constant current stimulator. The ascending evoked compound nerve action potentials (CNAPs) were recorded (10C02 Dantec, 0.2–6 KHz) in two tripolar electrode configurations (R1 and R2) in the sciatic cuff. The stimulus was first applied to T1, and if a clearly
identifiable CNAP (at least twice the background noise after averaging of at least 200 repetitions on a Nicolet 20 Pro Digital Oscilloscope) could be recorded at R1 and R2 the stimulus site was stepwise moved distally. The front of growth was identified at the most distal cathode where the CNAP had a longer latency than the CNAP elicited at the next cathode in the proximal direction (Fig. 1B and C). Our electrophysiological set-up allowed identification of the growth front with single-fibre resolution (Fig. 2).

**Root recordings during terminal laminectomies**

Before the terminal laminectomy, the peroneal nerves were cut bilaterally through a small skin incision below the knee to exclude...
erroneous conduction to the roots. The cat was placed in a stereotaxic frame and the vertebrae were exposed by stepwise removal of fascia, muscles and ligaments adjacent to os sacrum and the vertebrae. The L6–S2 (Highland et al., 1990; Bailey et al., 1992) ventral and dorsal roots were separated (polished forceps, type FRS-15 & RM-8; S&T Neuhausen, Switzerland) under optic magnification and cut as close to the spinal cord as possible (∼3 cm free length). In order to ensure fixed geometry of the recording electrodes, cuffs rather than hooks were used. Each ventral or dorsal root was pulled gently through the tubular cuff (3 circular leads 7.5 mm apart) by a thin thread (5–0) attached to its free tip. The internal cuff diameter was 1.0 mm (around S1 and S2 roots) and 2.0 mm (around the L7 and L6 roots), owing to the differences in root thickness. The contact between the root and the leads in the cuff was ensured by

Fig. 2 The potential evoked at the growth front in Fig. 1 had triphasic morphology and amplitude <0.5 μV, suggesting that stimulation at T5 evoked a response from a single fibre. (A) During double stimulation (s1 and s2) the unconditioned response r1 and the test response r2 had similar shape and amplitude with occlusion at the absolute refractory period. (B) The difference between the interresponse (ΔR) and the interstimulus (ΔS) interval (ΔR − ΔS) increased at smaller ΔS as a result of relative refractory period. Stippled line indicates the absolute refractory period. (C) Amplitude of r2 relative to r1 (100%) as a function of ΔS. The sudden complete loss of the single-fibre response for ΔS < 0.3 ms may be noted.
physiological saline solution maintained by the capillarity of the cuff. In order to prevent tissue dry-out the remaining exposed spinal cord was covered with gauze soaked in saline.

The ascending ventral and dorsal compound root action potentials (CRPs) were recorded (10C02 Dantec, 0.2–6 kHz) in tripolar electrode configurations identical to the R1 and R2 sciatic nerve recording sites. The integrity of the root recordings was ascertained by stimulation in the sciatic cuff at R2 and R1 positions. Sciatic nerve stimuli were first applied at T1–T2 (cathode proximally) and were then stepwise moved distally until no clearly identifiable CRPs—at least twice the background noise after averaging 500 repetitions—could be recorded at each of the investigated roots. The motor growth front was identified at T4 by S1V recording. The sensory growth front was identified at T5 by L6D recording. It may be noted that on the basis of the refractory period measurements in Fig. 2 sensory growth front at T5 was represented by a single fibre that could be identified by CRP recordings.

Fig. 3 Comparison of distances attained by motor and sensory fibres for the tibial nerve in Fig. 1 was carried out during a terminal laminectomy (A). Tripolar recordings were carried out from cuffs placed around ventral (B) and dorsal (C) roots L6–S2. The integrity of root recordings was ascertained by stimulating in the sciatic cuff at R2 and R1 positions. Tibial nerve stimuli were first applied at T1–T2 (cathode proximally) and were then stepwise moved distally until no clearly identifiable CRPs—at least twice the background noise after averaging 500 repetitions—could be recorded at each of the investigated roots. The motor growth front was identified at T4 by S1V recording. The sensory growth front was identified at T5 by L6D recording. It may be noted that on the basis of the refractory period measurements in Fig. 2 sensory growth front at T5 was represented by a single fibre that could be identified by CRP recordings.
recorded from S2 dorsal root at tibial stimulation in any of the investigated nerves. The largest CRP amplitudes were at least as large as the corresponding R1 and R2 recordings and allowed identification at the root level of the single fibre(s) at the front of regeneration (Fig. 3).

**Motor and sensory growth fronts**

Laminectomies were carried so that the growth front approached but did not exceed the distal end of the tibial cuff. Stimuli were first applied at T1–T2 (cathode proximally) and then stepwise moved distally until no clearly identifiable CRP (at least twice the background noise after averaging 500 repetitions) could be recorded at each of the investigated roots (Fig. 3). The motor growth front was identified at the most distal cathode position where stimulation evoked ventral root CRPs (Fig. 3B). Similarly, the sensory growth front was identified at the most distal cathode position where stimulation evoked dorsal root CRPs (Fig. 3C). The stability of the growth front was ascertained after the 6–10 h of recordings during laminectomy. The actual growth distance, and subsequently the growth rates, could only be determined at the completion of the study when the nerves were exposed and T1 position in respect to the lesion mark was reassessed to allow corrections due to the sliding of the cuff. At the completion of the experiment, the distances varied from 8 to 14 mm, and the measured distance was used to calculate growth rates. The slight displacement probably occurred during the first week of the experiment when collagen formed around the electrode (Fugleholm et al., 1994).

**Motor and sensory CVs**

Maturation of the fastest axons was compared by investigating recovery in motor and sensory CVs after target reinnervation. Laminectomies were performed between 52 and 139 days after the lesion, when compound muscle action potentials (CMAPs) could be recorded (10C02 Dantec, 0.01–10 kHz) after tibial stimulation. The latencies of the CNAPs and CRPs were measured to the first positive peak and latencies of the CMAPs were measured to the take-off of the negative phase to calculate the CVs of the fastest fibres. Because of known distances between electrodes relative to T1, the CV along the nerve segment in the tibial cuff could be estimated from the slope of the latency versus distance linear fit without measuring the distances to the recording sites. The motor CV was estimated as the fastest CV among all ventral roots. Similarly, the sensory CV was estimated as the fastest CV to all dorsal roots. This method of CV calculation was validated by comparing the motor CV with the plantar CV in the same nerve both in regenerated (Fig. 4) and control nerves (data not shown).

**Motor and sensory amplitudes**

The amplitudes of CRPs and CNAPs were measured peak to peak. Amplitude measurements depend on the number, distribution and temporal dispersion of the different single-fibre potentials contributing to the compound response. Since we could not compare the ‘control’ and ‘regenerated’ amplitudes in the same nerve, we limited our investigations to the recovery and distribution of the maximum sensory and motor amplitude that could be attained (among all stimulation sites and among all roots).

**Statistics**

Results are given as mean ± standard error of measurement (SEM). Owing to the small sample size, comparisons were performed using non-parametric statistics (SPSS Inc. SPSS for Windows, release 13, 2005, Chicago). Individual tests are mentioned where used.

**Results**

**Fastest rate of growth in the tibial nerve**

By serial observations during the first month after the lesion, the progression of the growth front without distinction between sensory and motor fibres could be followed along the tibial nerve (Fig. 1). At the completion of the experiments, the growth rate in the tibial cuff was calculated from the slope of the linear fit of the growth distance versus time after lesion plot (Fig. 5). The crushed fibres had a growth rate through the cuff of ~4 mm/day (Fig. 5A) and the sectioned fibres slightly lower at 3.7 mm/day (Fig. 5B). The intercept was ~8 days both after crush and section, indicating a similar delay between lesion and sprouting (Fig. 5A and B). These values were in agreement with previous findings on the same model (Krarup et al., 1989; Fugleholm et al., 1994).

**Motor and sensory growth fronts**

To compare the distances reached by sensory and motor fibres within the tibial cuff a laminectomy was performed between 18 and 21 days after the lesion (Fig. 3). The distances reached by the motor and sensory fibres at the growth front were similar both after crush (Fig. 5C) and section (Fig. 5D). The differences between motor and sensory growth fronts never exceeded one stimulation level (7.5 mm).

At the termination of the experiment after measurement of the tibial cuff placement in respect to the lesion mark, differences between the motor and sensory fibre growth were compared with the variability of the overall fastest growth rate in the tibial cuff (Fig. 5A and B). After crush, the difference between the motor and sensory growth fronts remained within the 95% confidence limits (±7.3 mm, alpha = 0.05) of the growth front estimated from serial observations. After section, the variability was greater but still no significant differences between motor and sensory fibres could be detected.

**Parent fibre CV**

Parent fibre CV of the axons stimulated in the tibial cuff could be measured between the R1 and R2 sciatic recording sites (Fig. 1E). Since only one recording site was feasible at the root cuff, it was not possible to distinguish the parent fibre CV of sensory and motor fibres in our study. In controls, sciatic nerve CV was 85 ± 1 m/s. During early outgrowth (Fig. 1E) and during post-reinnervation maturation (data not shown), parent fibre CVs at the sciatic nerve were in the normal range. Nevertheless, when single fibres were activated at the tip of the regeneration front, their parent fibre CVs were lower than the parent velocities of fibres activated at more proximal stimulation sites with lower growth rates (Figs 1E and 2). These observations indicated that it was not the fastest fibres (parent fibre CV) that constituted the growth front.
Recovery of CV along the tibial nerves

In control nerves, average motor CV (77 ± 6 m/s; n = 6) was lower than the sensory CV (95 ± 6 m/s, P < 0.05, Wilcoxon). During outgrowth through the tibial cuff the motor and sensory CVs along the regenerating nerve segments were <3 m/s both after crush and section (Fig. 1D). At this early time point, latency changes were uneven as different axons with different CVs reached different cathodes (Fig. 1A) and no consistent differences were detected between the sensory and motor mean conduction velocities estimated from the linear slopes. After 7 weeks of regeneration, the latency–distance relationships in the tibial cuff were linear both after crush and section, and CVs could be accurately estimated (Fig. 4). The recovery of CV from pooled observations collected at different laminectomies could best be described by a logarithmic function (Fig. 6A) both for motor CV ($R^2 = 0.94$) and sensory CV ($R^2 = 0.98$). The slope seemed steeper for sensory (42 m/s/day) than for motor (35 m/s/day), indicating that the absolute difference between the motor and the sensory CVs increased with time.

Recovery of CV along the tibial nerves

In control nerves, average motor CV ($77 \pm 3$ m/s; n = 6) was lower than the sensory CV ($95 \pm 4$ m/s, $P < 0.05$, Wilcoxon). During outgrowth through the tibial cuff the motor and sensory CVs along the regenerating nerve segments were <3 m/s both after crush and section (Fig. 1D). At this early time point, latency changes were uneven as different axons with different CVs reached different cathodes (Fig. 1A) and no consistent differences were detected between the sensory and motor mean conduction velocities estimated from the linear slopes. After 7 weeks of regeneration, the latency–distance relationships in the tibial cuff were linear both after crush and section, and CVs could be accurately estimated (Fig. 4). The recovery of CV from pooled observations collected at different laminectomies could best be described by a logarithmic function (Fig. 6A) both for motor CV ($R^2 = 0.94$) and sensory CV ($R^2 = 0.98$). The slope seemed steeper for sensory (42 m/s/day) than for motor (35 m/s/day), indicating that the absolute difference between the motor and the sensory CVs increased with time.
For example, at 52 days after crush, motor CV was 13 m/s and sensory CV was 15 m/s, whereas at 139 days after crush motor CV was 49 m/s and sensory CV was 56 m/s.

During post-reinnervation maturation we found that the sensory CV was faster at any time point (Fig. 6A). Nevertheless, the relative recovery in conduction was similar in motor and sensory axons. For example, at 52 days conduction recovered ~16% and at 139 days conduction recovered ~60% relative to the respective motor and sensory controls. In fact, in all nerves investigated after target reinnervation we found a high correlation ($R^2 = 0.98$) between motor CVs and the corresponding sensory CVs (Fig. 7A), indicating that sensory CVs were ~14% faster than the corresponding motor CVs. This finding was also consistent in the sectioned...
nerves and was similar to the findings in control nerves (Fig. 7A). A likely explanation for these findings was that at any time point CV recovery was proportional with that of the parent fibre (Fig. 7B). Thus, with respect to the parent fibre, the rate of maturation was similar in motor and sensory sprouts.

**Rate of amplitude recovery**

In control nerves, the largest ventral CRPs were 641 ± 116 μV and the largest dorsal CRPs were 191 ± 30 μV (P < 0.01, Wilcoxon). During the third week of regeneration the CRPs were dispersed and of low amplitude (<5 μV) both after crush and section (Fig. 1A) and no differences could be detected between sensory and motor fibres. The recovery of motor and sensory amplitudes after target reinnervation is presented in Fig. 6B. For example, at 52 days after crush, the highest motor amplitude was 176 μV (27% of control) and the highest sensory amplitude was 108 μV (56% of control).

At 139 days after crush the motor amplitude was 518 μV (80% of control) and the sensory amplitude was 169 μV (88% of control).

The amplitude recovery rate was 3.3 μV/day for motor fibres, higher (Wilcoxon P < 0.05) than the 1.2 μV/day recovery rate of the sensory fibres. Thus, both after crush and section we found that most of the recovery in motor amplitude along the tibial nerve occurred at later stages during maturation (Fig. 6B). While we cannot exclude differences in CRP dispersion and distribution between motor and sensory fibres, it is possible that growth of motor axons was more abundant at later stages during regeneration, as seen in rat (Kawasaki et al., 2000). Our electrophysiological comparison
was, however, aimed at the fastest regenerating motor and sensory axons that were not influenced by such regenerative differences within the motor and sensory fibre populations.

**Recovery of fibre distribution between different roots**

In control nerves, the tibial nerve CVs of fibres projecting to different ventral roots were similar. However, the sensory CVs to the L7 root (95 ± 1 m/s) were significantly (Wilcoxon, \( P < 0.05 \)) faster than sensory CVs to the S1 root (84 ± 5 m/s) (Fig. 8A). Distances from T1 to the root recordings (calculated from the intercept of the latency versus distance linear fit) were ~187 mm to all dorsal roots. It was therefore possible that the different sensory fibre populations (with different CVs) projected to different roots.

In spite of a large variation of absolute amplitudes between the different nerves, the relative amplitude distribution between the different roots in control nerves was, however, consistent: L7 dorsal CRPs contained 61 ± 8% of the total sensory amplitude (\( P < 0.05 \), Wilcoxon), whereas S1 ventral CRPs contained 88 ± 2% of the total motor amplitude (\( P < 0.05 \), Wilcoxon) (Fig. 8B). Thus, myelinated alpha-motor fibres in the tibial nerve originated mostly from S1 root and large sensory fibres mainly from L7 root. This distribution was maintained during outgrowth through the tibial cuff in all investigated nerves, both after crush and section. The motor elongation front contained fibres from S1 in four of five crushed nerves and in two of four sectioned nerves, and the sensory elongation front contained fibres from L7. After target reinnervation (Fig. 8C), the S1 and L7 regained the largest relative amplitude both after crush and after section. Thus, it is likely that for sensory fibres, as for motor fibres, the regeneration pattern for a root was dictated by the number of fibres before the lesion and not by specialized fibre-type projection between roots.

**Discussion**

In humans the rate of regeneration after peripheral nerve lesions cannot be directly measured. The delay between nerve lesion or repair and functional recovery of target organs, which has been converted to a growth rate of 1–2 mm/day (Seddon et al., 1943; Hodes et al., 1948), consists of the delay before fibre growth, the time used to cross the lesion site, the rate of growth through the distal nerve stump, the reinnervation of target organs and the outcome measure to ascertain reinnervation. The time to reinnervation was the single-most important factor to influence the level of outcome after different types of nerve lesions in monkeys (Krarup et al., 2002), and in humans there is a general agreement on the improved functional recovery when time to reinnervation is short and that surgical treatment when needed should be carried out as quickly as possible after a nerve lesion (Lundborg, 2000). Functional recovery after axonal degeneration is probably dependent on the number, accuracy and growth rate of axons and reinnervation of target organs, and these factors may be linked to the progressive atrophy and loss of Schwann cell tubes in the distal nerve stump (Weinberg and Spencer, 1978) and target organs (Gutmann and Young, 1944). Hence, the rate of growth of

![Fig. 8](image-url)
motor and sensory axons may have direct implications on functional recovery. Since the reinnervation of sensory receptors is considerably more difficult to ascertain than muscle fibre reinnervation in humans and non-human primates owing to the small sensory nerve and receptor responses (Buchthal and Kühl, 1979; Krarup et al., 1990, 2002), direct comparison of motor and sensory regeneration rates is inaccurate. We therefore extended a method to monitor growth of axons during regeneration after Wallerian degeneration to directly compare elongation and maturation of motor and sensory fibres within the same mixed tibial nerve. Both after crush and section we found that similar growth and maturation rates were attained by myelinated motor and sensory fibres.

**Growth of sensory and motor axons**

We could not directly measure the rate of growth of sensory and motor axons in serial studies. We compared instead the distances reached by the motor and sensory fibres at specified times during regeneration in different nerves by stimulating in the tibial cuff and recording from the L6–S2 dorsal and ventral roots. The spatial and temporal resolutions of our method were determined by the length of the tibial cuff, the inter-electrode spacing and the intervals between studies. During serial studies we found that fastest outgrowth started after ~8 days and progressed at a rate of ~4 mm/day, which was largely in agreement with our previous observations (Fugleholm et al., 1994). Under these circumstances, to discriminate a distance difference of one stimulation level (7.5 mm) after 20 days of regeneration, the difference in growth rates should have been >0.5 mm/day (4 versus 3.5 mm/day).

We found that the fastest growth rates achieved by myelinated fibres were similar in motor and sensory fibres populations and fell within the confidence intervals of the mixed nerve growth rates (Fig. 3). This indicated that growth rates of motor and sensory fibres were similar within 0.5 mm/day accuracy. While it was not possible to increase the length of the cuff owing to anatomical constraints, one would expect that an even greater accuracy could have been obtained by reducing the inter-electrode distance. We previously verified by electron microscopy that the 10 mA stimulation current required to excite the immature axons could accurately predict the growth front within 7.5 mm resolution (Fugleholm et al., 1994). By reducing inter-electrode distance, however, stimulus spread could have been a source of error.

**Axons contributing to the growth front**

We stimulated the immature axons at the growth front in the tibial and recorded the evoked responses in cuff electrodes. Our electrophysiological approach was constrained by the methodological limitation that action potentials could be recorded from single myelinated fibres with diameters larger than 5–7 μm (Rindos et al., 1984; Krarup and Loeb, 1988).

Thus, our method allowed us to investigate alpha-motor fibres but was limited to only the larger sensory fibres. A functional study in rat showed that responses from nociceptive and sudomotor fibres occur slightly earlier and to a greater extent than recordings of compound muscle and nerve action potentials from large myelinated fibres (Navarro et al., 1994). It is, however, unlikely that small sensory fibres regenerate strikingly faster than large sensory fibres since a recent retrograde tracer study showed that regeneration of unmyelinated sensory fibres and large sensory fibres in rat had similar rates (Lozeron et al., 2004).

However contraintuitive, our study did not support the assumption that the largest fibres have the fastest growth rate. While our method favoured detection of regeneration of the largest fibres with the largest action potential amplitudes, we found evidence that within the respective sensory and motor fibre populations it was not the largest and fastest conducting fibres that grew first (Figs 1E, 2 and 8A), and that motor and sensory fibres made similar contributions to the growth front (Fig. 5). It may therefore be speculated that growth rates were maximized for an ‘optimal’ relationship between radial growth and longitudinal growth, which is similar in sensory and motor axons.

**Maturation of sensory and motor axons**

Maturation (the increase in axonal calibre and myelination) occurs slower (Wujek et al., 1986; Zhu et al., 1997) and independently (Fried et al., 1991; Macias et al., 1998) of growth. In regenerating cat axons (Fugleholm et al., 2000) CV remains proportional with the fibre diameter and we could compare maturation of sensory and motor axons by investigating recovery in CV. After target reinnervation, the change in latency along the tibial cuff was linear and we could calculate the average CV along the tibial nerve as the latency versus distance linear fit and then determine the fastest motor and sensory CV among the different roots. This method of calculation was validated by comparing the motor CV estimated to the roots with the CV to the plantar muscles. Furthermore, this method of calculation was able to detect the slightly faster sensory than motor CV corresponding to the differences in largest diameters of sensory and motor fibres (Hursh, 1939).

We found that at various times during maturation, the sensory CV was slightly faster than the motor CV (Fig. 6A). This did not indicate that sensory maturation occurred at a faster rate, because a similar difference in CV was also seen in control nerves, and the ratio between sensory and motor CV in control nerves was preserved during regeneration. It was more likely that maturation occurred at a rate proportional to the diameter of the parent fibre (Wujek et al., 1986). Furthermore, early studies in rat sensory fibres showed that CV of the sprout was proportional with CV of its parent fibre (Devor and Govrin-Lippmann, 1979). Thus, in respect to the parent fibre diameters, maturation rate was similar between sensory and motor fibres.
Crush versus section

While the theoretical rate-limiting factor of axonal regeneration may be the speed of up to 6.5 mm/day at which slow axonal transport moves cytoskeletal elements involved in the motility of the growth cone and elongation of the axon (Wujek and Lasek, 1983), the linear growth rate is much slower, dependent on the growth cone environment (Fugleholm et al., 1994, 1998, 2000; Sorensen et al., 2001). The fastest rates of regeneration could be attained after crush, where growth cones move along their original Schwann cell tubes. We found that in these ideal conditions the growth rates were similar between motor and sensory fibres. In the clinical situation of a co-adapted nerve section, regenerating fibres are more likely to grow through unfamiliar pathways that may impair the growth rates. In the mouse motor axons preferentially reinnervated motor pathways (Brushart, 1988) after section. It was therefore possible that such environmental specialization may favour the growth of motor fibres. Our data suggests that after section, as after crush, the fastest growth rates could be attained by motor and sensory fibres were similar. Thus, even in the clinically relevant regenerative environment some motor and sensory axons retain similar growth capacity and will simultaneously compete for target selection.

Acknowledgements

The project was supported by Lundbeck Foundation, the Novo Nordisk Foundation, the Danish Medical Research Council, the Ludvig and Sarah Elsass Foundation and the Foundation for Research in Neurology.

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Regeneration of sensory and motor axons
