Changes in Crossed Spinal Reflexes After Peripheral Nerve Injury and Repair

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Valero-Cabrè, Antoni and Xavier Navarro. Changes in crossed spinal reflexes after peripheral nerve injury and repair. J Neurophysiol 87: 1763–1771, 2002; 10.1152/jn.00305.2001. We investigated the changes induced in crossed extensor reflex responses after peripheral nerve injury and repair in the rat. Adults rats were submitted to non repaired sciatic nerve crush (CRH, n = 9), section repaired by either aligned epineurial suture (CS, n = 11) or silicone tube (SIL4, n = 13), and 8 mm resection repaired by tubulization (SIL8, n = 12). To assess reinnervation, the sciatic nerve was stimulated proximal to the injury site, and the evoked compound muscle action potential (M and H waves) from tibialis anterior and plantar muscles and nerve action potential (CNAP) from the tibial nerve and the 4th digital nerve were recorded at monthly intervals for 3 mo postoperation. Nociceptive reinnervation to the hindpaw was also assessed by plantar algesimetry. Crossed extensor reflexes were evoked by stimulation of the tibial nerve at the ankle and recorded from the contralateral tibialis anterior muscle. Reinnervation of the hindpaw increased progressively with time during the 3 mo after lesion. The degree of muscle and sensory target reinnervation was dependent on the severity of the injury and the nerve gap created. The crossed extensor reflex consisted of three bursts of activity (C1, C2, and C3) of gradually longer latency, lower amplitude, and higher threshold in control rats. During follow-up after sciatic nerve injury, all animals in the operated groups showed recovery of components C1 and C2 and of the reflex H wave, whereas component C3 was detected in a significantly lower proportion of animals in groups with tube repair. The maximal amplitude of components C1 and C2 recovered to values higher than preoperative values, reaching final levels between 150 and 245% at the end of the follow-up in groups CRH, CS, and SIL4. When reflex amplitude was normalized by the CNAP amplitude of the regenerated tibial nerve, components C1 (300–400%) and C2 (150–350%) showed highly increased responses, while C3 was similar to baseline levels. In conclusion, reflexes mediated by myelinated sensory afferents showed, after nerve injuries, a higher degree of facilitation than those mediated by unmyelinated fibers. These changes tended to decline toward baseline values with progressive reinnervation but still remained significant 3 mo after injury.

INTRODUCTION

Axotomy of peripheral nerves severs sense organs and muscles from their innervating sensory and motor nerve fibers, respectively. Similarly, the spinal cord reflexes evoked from intact sensory afferents may be abolished. Spinal cord synapses are detached (Brännström et al. 1992a) by the action of glial cells reacting to the injury (Aldskogius and Kozlova 1998) so that spinal cord reflexes are completely abolished thereafter (Mendell 1984). When adequate repair procedures are applied, axons regrow distally reestablishing functional connections with peripheral organs. In the spinal cord, motoneuron dendritic arborization is reextended, synaptic button stripping is reverted and sensory-motor synapses become functional again (Brännström et al. 1992b; Mendell 1984; Valero and Navarro 2001). However, distal organ reinnervation by regenerating axons does not guarantee the restitution of normal spinal cord reflex function. Motor and sensory neurons show lower dopaminergic thresholds after injury and regeneration evoking excitatory postsynaptic potentials of higher amplitude than controls after afferent stimulation (Eccles et al. 1958; Gustafsson 1979; Horch and Linsay 1981). Moreover, central reorganization induced by nerve lesion changes the laminar projections of afferent fibers (Shortland and Fitzgerald 1994; Woolf et al. 1992) and alters the somatotopy of the body representation at the spinal cord level (Fitzgerald 1985; Koerber et al. 1994). In adult animals, all these changes persist for a long time after reinnervation and may impair motor unit recruitment and control of movement (Cope and Clark 1993; Milner-Brown et al. 1974) and account in part for the poor clinical outcome achieved after severe nerve injuries (Cope and Clark 1993; De Medinaceli 1988; Gramsbergen et al. 2000; Wasserschaff 1990).

Previous studies have investigated spinal cord reflexes after peripheral nerve injuries in newborn and postnatal animals. Despite the substantial rearrangement of innervation after neonatal injuries, withdrawal reflexes easily recovered and attained a near to normal spatial organization (Holmberg and Schouenborg 1996a). Spinal reflexes appeared to be facilitated, yielding higher amplitude and longer lasting bursts than controls after neonatal nerve crush (Navarrete et al. 1990; Vejsada et al. 1991, 1999). However, findings of facilitation of spinal reflexes reported after neonatal nerve crush do not necessarily infer that spinal reflexes are facilitated after injury to the adult nerve in light of evidence of more extensive neuronal death in newborn than in adult animals (Romanes 1946; Vejsada et al. 1999), more accurate distal reinnervation in postnatal animals (Aldskogius and Molander 1990), and the higher capability of postnatal animals to reorganize central projections of misdirected collaterals, suggesting that functional recovery and spinal reorganization after nerve injury are age dependent (Holm-
berg and Schouenborg 1996a,b). The aims of this work were to study the restitution of crossed extensor reflex responses conveyed by different types of sensory fibers after peripheral nerve injuries in adult animals and to compare the effects of nerve injuries of varying severity: nerve compression, section, and resection, that result in different levels of peripheral reinnervation.

METHODS

Sixty-four adult female Sprague-Dawley rats [260 ± 4 (SD) g] were used in the study. All rats were kept on standard laboratory food and tap water ad libitum with a light-dark cycle of 12 h. The experimental procedures were approved by the ethical committee of our institution and were conducted in conformity with the Guiding Principles for Research Involving Animals and Human Beings, taking adequate measures to minimize pain and animal discomfort during surgery and testing procedures.

Surgical procedures

Animals were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg); the right sciatic nerve was then exposed at the midhigh and injured at a constant point, 90 mm from the tip of the third toe, well proximal to the sciatic trifurcation. Rats were divided into five groups depending on the lesion made to the sciatic nerve. In one group the right sciatic nerve was crushed during 30 s in three different orientations by means of a fine forceps (CRH, n = 9). In two other groups, the sciatic nerve was sharply transected and repaired either by epineurial 10-0 sutures (CS, n = 11) or by tubulization with a silicone guide (6 mm long, 2 mm ID, 0.5-mm wall thickness) sutured between both nerve ends leaving a 4 mm gap (SIL4, n = 13). In another group an 8-mm segment of the sciatic nerve was resected, and the gap was bridged with a silicone tube (10 mm long; SIL8, n = 12). The original alignment of the tibial and peroneal fascicles of the sciatic nerve trunk was preserved. Immediately after injury, the distal sciatic nerve was mechanically stimulated by gently pinching to guarantee complete lesion, as the normal reflex response consisting in contraction of muscles of the trunk was completely absent. To avoid collateral reinnervation of the denervated sciatic territory by other nerves supplying neighboring areas, the caudal cutaneous branch of the saphenous nerve was sectioned at the midhigh and a long distal segment resected to prevent regeneration. A control group of intact rats (CNT, n = 9) was submitted to sham injury of the sciatic nerve plus resection of saphenous and caudal cutaneous nerves and evaluated in parallel. All the animals were kept during 90 days postoperation (dpo) to allow for nerve regeneration and target reinnervation.

Electrophysiological evaluation

Before injury, 1 wk after operation, and thereafter on a monthly basis, motor and nerve reinnervation and reflex recovery were assessed by means of nerve conduction tests. During the test, the rat body temperature was kept constant between 34 and 36°C by means of a thermostated flat coil, and anesthesia was maintained steady after the initial induction (40 mg/kg ip) by injection of additional pentobarbital bolus (10 mg/kg ip) every 60 min. The right sciatic nerve was stimulated with single electrical pulses (100-μs duration and supramaximal intensity) delivered by monopolar needles percutaneously placed proximal to the injury site, the cathode at the sciatic notch and the anode one centimeter proximally to prevent regeneration. The compound muscle action potentials (CMAPs) of the tibialis anterior and plantar muscles were recorded during 20 ms by means of needle electrodes (27G), the active electrode inserted on the muscle belly and the reference at the fourth toe, and displayed in an oscilloscope (Sapphyre 4 M, Vickers). Likewise, the compound nerve action potential (CNAP) was recorded by needle electrodes inserted near the tibial nerve at the ankle and the 4th digital nerve. To ensure reproducibility, the recording needles were placed at the microscopic magnification to secure the same placement on all animals guided by anatomical landmarks. The active recording electrode was placed subcutaneously at the first third of the distance between knee and ankle on the belly of the tibialis anterior muscle and at the third metatarsal space for plantar muscle recording, and the maximal amplitude CMAP elicited was chosen for each animal at each test day. The latency from the stimulus to the onset of the first negative deflection and the maximal amplitude from baseline to the negative peak of the evoked action potentials were measured (Navarro et al. 1994). The stretch reflex, elicited by electrical stimulation of large myelinated afferent fibers, was evaluated from the late H wave recorded in the motor nerve conduction tests described in the preceding text. The latency from stimulus to the negative peak and the amplitude from the onset to the peak of the H wave were measured (see Navarro et al. 1996, 1999; Valero-Cabrè and Navarro 2001 for detailed traces of M and H waves).

The crossed extensor reflex was elicited by stimulating the right tibial nerve with single electrical pulses (500-μs duration and supramaximal intensity for each component) with wide interstimuli intervals (30 s or longer) through needles inserted the cathode at the ankle under the Achilles tendon (distal to the injury) and the anode 1 cm distally on the medial side of the paw. Crossed reflex responses were recorded from the left tibialis anterior muscle by means of monopolar needle electrodes. This muscle cooperates in the fixation of the contralateral ankle joint when ipsilateral flexor reflex is elicited. The reflex response was recorded up to 350 ms after stimulation, rectified, and displayed in the oscilloscope at a scale between 20 and 500 μV per division. The latency from the stimulus to the onset of each burst of activity, the maximal amplitude from baseline to peak, and the number of peaks were measured (Navarro et al. 1996, 1999). To reduce variability, reflexes were evoked eight times (with varying intervals of at least 30 s in between), and the highest amplitude recorded for each reflex component was measured.

At the time points during follow-up, the amplitude of each response in all rats of each group, either with or without recorded responses, was averaged to reflect the degree of recovery. To study reflex facilitation, the amplitude of each reflex component was normalized by the degree of peripheral regeneration. For the stretch reflex, the H/M amplitude ratio was calculated for each muscle tested, whereas for crossed extensor reflexes, the maximal amplitude of each component was divided by the amplitude of the CNAP of the tibial nerve at the ankle. As an estimate of the central latency of the reflex responses, we subtracted the latency of the peripheral afferent (from ipsilateral ankle to sciatic notch) and efferent pathways (from contralateral sciatic notch to muscle) to the total latency from stimulus to response. Thus the calculated central latency is an estimate of the impulse conduction from ipsilateral sciatic notch to contralateral sciatic notch, i.e., including a segment of peripheral conduction at the dorsal ipsilateral and ventral contralateral lumbar roots.

Assessment of nociception recovery

Reinnervation of the plantar skin by C nociceptive fibers was evaluated every month during 90 dpo by a heat-radiation method using a modified plantar algesimeter (Hargreaves et al. 1988). Rats were placed into a metacrylate box with an elevated glass floor. The surface of the glass was kept at 30 ± 1°C before starting each test. From the bottom of the box, the light of a projection lamp was focused directly onto the plantar surface of a hindfoot. The time spent until raising the heated hindpaw was measured through a time meter coupled with infrared (IR) detectors directed to the plantar surface. The value for a test was the mean of three trials.
RESULTS

Functional reinnervation

The results of electrophysiological tests for control rats (group CNT) did not show significant changes over the 3 mo follow-up after sham injury (Fig. 1, Table 1). There were evidences of a slight and progressive increase in the amplitude of CMAPs and CNAPs, attributed to an increase of muscle size and nerve fiber size with age, and of a slight decrease in latencies (indicating an increase in nerve conduction velocity), which is attributable to mild increase in myelin thickness and axonal caliber during adult age (see Verdu et al. 2000 for a review). These findings indicate that adult rats 10–12 wk old have not reached yet a steady state of development with regard to peripheral nerve function.

CMAPs and CNAPs were completely abolished after nerve injury in all groups. The first evidence of motor reinnervation appeared by 30 dpo in the proximal tibialis anterior muscle and by 60 dpo in the distal plantar muscle with polyphasic CMAPs of longer latency and lower amplitude than those recorded prior to surgery (Fig. 1, A and B). CMAPs were recorded during follow-up in all the rats except in the plantar muscle of 2 of the 12 animals of group SIL8. On the other hand, CNAPs were recorded in the tibial nerve in all the rats from 60 dpo, whereas recovery of digital CNAPs was more compromised (Fig. 1, C and D). We were unable to record CNAPs in the digital nerves in 1 rat of groups CS and SIL4 and in 11 of the 12 of group SIL8.

CMAPs and CNAPs reached significantly higher amplitude values in group CRH than in all other injured groups. The longer the gap between both nerve stumps, the lower amplitude of compound action potentials; CS and SIL4 groups showed significantly higher amplitudes of both CMAPs and CNAPs than group SIL8. Notwithstanding, group CS had only significantly higher values for the tibial CNAP with respect to group SIL4 (Table 1, Fig. 1). The latency of motor and nerve action potentials decreased over follow-up, although in all groups it remained longer than preoperative and CNT values at the end of follow-up. CRH animals showed the best recovery, with significant differences with respect to the other groups especially in the latency of CNAPs (Table 1).

Data analysis

All data are presented as the group means ± SE of percentages with respect to presurgery values of each animal. The results have been compared between groups by nonparametric Kruskall-Wallis test followed by Mann-Whitney U tests. χ² test has been used to compare proportions of animals presenting a given response.

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Percentage recovery with respect to preoperative values of compound muscle action potential (CMAP) amplitude recorded in tibialis anterior (A) and plantar (B) muscles and compound nerve action potential (CNAP) recorded at the tibial nerve (C) and the 4th digital nerve (D) along the 90 days follow-up. Values are the mean for each group at each interval with SE indicated as bars.

**TABLE 1.** Percentages with respect to preoperative values of the amplitude and the latency of CMAPs of tibialis anterior and plantar muscles and CNAPs of tibial and 4th digital nerves at 90 days postoperation

<table>
<thead>
<tr>
<th>Compound Muscle Action Potential (CMAP)</th>
<th>Compound Nerve Action Potential (CNAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis anterior muscle</td>
<td>Plantar muscle</td>
</tr>
<tr>
<td>Percent amplitude</td>
<td></td>
</tr>
<tr>
<td>CNT</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>CRH</td>
<td>97 ± 1*</td>
</tr>
<tr>
<td>CS</td>
<td>56 ± 5*†</td>
</tr>
<tr>
<td>SIL4</td>
<td>51 ± 3*†</td>
</tr>
<tr>
<td>SIL8</td>
<td>35 ± 9*‡†</td>
</tr>
<tr>
<td>Percent latency</td>
<td></td>
</tr>
<tr>
<td>CNT</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>CRH</td>
<td>112 ± 2*</td>
</tr>
<tr>
<td>CS</td>
<td>135 ± 9*</td>
</tr>
<tr>
<td>SIL4</td>
<td>132 ± 6*</td>
</tr>
<tr>
<td>SIL8</td>
<td>149 ± 8*</td>
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</table>

CNT, control; CRH, group with sciatic nerve crushed; CS and SIL4, sciatic nerve sharply transected and repaired by sutures or by tubulization with a silicone guide leaving a 4-mm gap, respectively; SIL8, 8-mm segment of sciatic nerve was resected with its gap bridged by a silicone tube. P < 0.05, * vs. CNT; † vs. CRH; ‡ vs. CS; § vs. SIL4.
Withdrawal responses to plantar heat stimulation reappeared by 60 dpo in all the rats, except in 5 of 12 of group SIL8, which, however, had also a positive response by 90 dpo. Group CRH had the best recovery of pain sensibility, with significantly lower withdrawal time to heat than groups CS and SIL4, which in turn showed shorter withdrawal time than group SIL8 (Fig. 2).

**Reflex responses**

In a large group of control rats (n = 205), stimulation of the right tibial nerve at the ankle consistently yielded a contralateral crossed reflex response in the left tibialis anterior muscle, recorded as bursts of motor unit action potentials grouped in aeral crossed reflex response in the left tibialis anterior muscle, right tibial nerve at the ankle consistently yielded a contralateral response (2 peaks), and had an average maximal amplitude of about 650 μV. C1 was easily habituated with repeated stimulation. The second component (C2) appeared with longer latency (about 23 ms) and had longer duration (20 ms, 5–7 peaks) but lower amplitude (300 μV). The third component (C3) was detected starting 120–135 ms after trigger, with long duration (170 ms, around 30 peaks) and low amplitude (50–250 μV). The threshold and latencies measurements confirm that each of the three components is likely mediated by peripheral fibers (Aβ, Aδ, and C fibers for C1, C2, and C3, respectively) conveyed by different populations of sensory dorsal root ganglion (DRG) neurons. In comparison, the ipsilateral H wave recorded in the right tibialis anterior muscle showed shorter latency (6 ms) and duration (1 ms) but much higher amplitude (mean 3.3 mV; Fig. 4, Table 2). When thresholds were determined for each spinal reflex at different stimulus duration, the H wave showed the lowest threshold levels. For the crossed extensor reflex, C1 was evoked at slightly lower stimulus intensity than C2, whereas C3 needed about 15 times higher stimulation intensity (Table 2).

The three crossed extensor reflex components showed different patterns of recovery after nerve injury (see Figs. 3 and 4). C1 and C2, as well as the H wave, reappeared in all the rats of the experimental groups during the 90 dpo. However, C3 failed to be evoked in one rat of groups CRH (10%) and CS (10%), in two of SIL4 (15%), and in four of SIL8 (33%). In all groups, the H wave reached values, between 72 and 88%.

![FIG. 2. Recovery of the latency until withdrawal in the operated right hindpaw vs. the intact left paw found in the algesimetry test along the 90 days follow-up. Final values were significantly (P < 0.05) increased in all injured groups vs. controls, in group SIL4 vs. group CRH, and in group SIL8 vs. all the other injured groups. Values are the mean for each group at each interval with SE indicated as bars.](http://jn.physiology.org/)

![FIG. 3. Single sample recordings of crossed extensor reflexes recorded at the contralateral tibialis anterior muscle after stimulation of the right tibial nerve in representative animals at preoperation (A), 30 (B), 60 (C), and 90 days after sciatic nerve crush (D and E). C1 is the 1st component of the reflex response to reappear, followed by C2, and later and in not all cases (see example in D) by C3. Horizontal scale: 25 ms/div; vertical scale: 100 μV/div.](http://jn.physiology.org/)
TABLE 2. Characteristics of the three components (C1–C3) of the crossed extensor reflex

<table>
<thead>
<tr>
<th>Component</th>
<th>Stretch Reflex H</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency, ms</td>
<td>6.2 ± 0.01</td>
<td>12.1 ± 0.1</td>
<td>23.5 ± 0.1</td>
<td>129.4 ± 0.9</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>1.1 ± 0.04</td>
<td>2.8 ± 0.2</td>
<td>19.9 ± 1.1</td>
<td>472.6 ± 48.6</td>
</tr>
<tr>
<td>Peaks, number</td>
<td>1.1 ± 0.05</td>
<td>1.71 ± 0.06</td>
<td>6.4 ± 0.1</td>
<td>28.9 ± 1.3</td>
</tr>
<tr>
<td>Amplitude, μV</td>
<td>3260 ± 90</td>
<td>651 ± 60</td>
<td>305 ± 15</td>
<td>214 ± 11</td>
</tr>
<tr>
<td>Central latency, ms</td>
<td>4.9 ± 0.05</td>
<td>9.2 ± 0.2</td>
<td>21.2 ± 0.6</td>
<td>126.6 ± 3.8</td>
</tr>
<tr>
<td>Threshold, mA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse 500 μs</td>
<td>1.50 ± 0.07</td>
<td>1.63 ± 0.13</td>
<td>1.83 ± 0.14</td>
<td>23.20 ± 1.57</td>
</tr>
<tr>
<td>Pulse 200 μs</td>
<td>2.17 ± 0.12</td>
<td>2.36 ± 0.24</td>
<td>2.38 ± 0.21</td>
<td>40.14 ± 2.73</td>
</tr>
<tr>
<td>Pulse 100 μs</td>
<td>3.01 ± 0.13</td>
<td>3.21 ± 0.29</td>
<td>3.28 ± 0.23</td>
<td>66.40 ± 3.99</td>
</tr>
<tr>
<td>Pulse 50 μs</td>
<td>4.88 ± 0.23</td>
<td>5.70 ± 0.46</td>
<td>5.81 ± 0.42</td>
<td>86.50 ± 6.50</td>
</tr>
</tbody>
</table>

The crossed extensor reflex was recorded from the left tibial anterior muscle after stimulation of the right tibial nerve, and the H wave reflex recorded from the right tibialis anterior muscle after stimulation of the right sciatic nerve in a series of 205 control rats. Values are means ± SE.

sensory and efferent motor volleys, increased for all groups and reflex components (C1: 105–130%, C2: 104–144%, C3: 120–150%, H: 104–131%) after injury but tended to normalize over time. However, for C1 (105–113%), C3 (106–110%), and H responses (105–113%), the final values remained significantly longer than preoperative values in all groups, whereas for C2 significant differences were only found for groups SIL4 (104%) and SIL8 (108%).

Spinal cord reflex facilitation

To analyze the functional performance of the crossed extensor reflex arch independently on the success of reinnervation of the injured nerve, the amplitude of C1–C3 was normalized by the amplitude of the tibial CNAP, whose regenerated sensory afferents served to induce the reflex (Table 3). The reflex H wave was also normalized by dividing its amplitude by that of the M wave of the tibialis anterior CMAP. In group CNT, all reflex responses remained stable over the 90 dpo. However, in all injured groups, C1 reached values between 3.5 and 4.5 times its original values by 30 dpo (group CRH) or 60 dpo (groups CS, SIL4 and SIL8; Fig. 5). Thereafter there was a tendency to decline; final values were close to preoperative in group CRH but remained higher than normal in groups CS, SIL4, and SIL8. Similarly, the H/M ratio was largely increased (2–7 times) 1 mo after injury and tended to return to near normal values at the end of follow-up (Fig. 5). On the contrary, for C2 and C3 components group CRH had only slight early facilitation followed by return to preoperative levels. In groups CS and SIL4, statistically significant increases were found for C2 and C3 (2–3 times) with respect to preoperative levels. Such facilitation was maintained for C2 in both groups still by 90 dpo, whereas final values of C3 were not different from preoperation. In group SIL8, C2 increased 2.5 times and remained higher than normal over time, and C3 showed a mild, not significant increase above controls (Fig. 5, Table 3).

DISCUSSION

Our results show that after peripheral nerve injuries not all motor reflex responses show the same pattern of restitution. Reflex arches dependent on myelinated afferent fibers, such as the stretch reflex (H wave) and the components C1 and C2 of the crossed extensor reflex, recovered in all the animals despite the varying severity of the nerve injury induced. In contrast, component C3 of the crossed extensor reflex, mediated by unmyelinated afferents, reappeared in a lower proportion of animals, especially if a gap was produced between the nerve stumps. Furthermore, H wave, C1, and C2, but not C3, reflex responses showed a marked increase in the amplitude of the motor response during the first stages of regeneration, and, as reinnervation progressed, tended to return down to normal values. However, at the end of the 3 mo follow-up, the reflex
amplitude remained steady at significantly higher levels than in control animals, more in groups showing a lower degree of sensory-motor peripheral reinnervation.

Peripheral reinnervation after injuries

After nerve injuries, the projection pathways of the spinal reflex arches become disrupted and rewire with target receptors and effectors only if axonal regeneration and target reinnervation take place. The amount of sensory and motor reinnervation and the degree of accuracy of reinnervation of the originally innervated dermatomes and myotomes are essential to achieve the restitution of functional reflexes after nerve injuries. To assess the effect of these factors on spinal reflex recovery, four types of injury and repair, resulting in varying levels of reinnervation and of misdirected reinnervation (Evans et al. 1991; Valero-Cabré et al. 2001), were applied to the rat sciatic nerve. As expected, animals submitted to a crush injury showed the fastest and highest recovery. A crush causes complete axotomy, but the endoneurial tubes remain intact, thus providing the proximal axonal ends with immediate neurotrophic support as well as a physical guide to their original targets (De Medinaceli 1988; Gutmann and Guttmann 1942). After nerve transaction, epineurial suture repair allows the apposition of the nerve stumps, with access of the transected axons to the degenerating distal stump, as a source of neurotrophic factors. However, disruption of nerve continuity and misalignment of endoneurial and perineurial sheaths lead some axons to misguidance toward territories different from the originals, resulting in poorer reinnervation (Brushart 1993; Navarro et al. 1994; Valero-Cabré et al. 2001). Tubulization of a gap between nerve stumps allows for slower reinnervation timing because extra time is needed to form an intratubular connective matrix able to support the elongation of regenerating axons across the tube (Butí et al. 1996; Williams et al. 1983). With a short interstump gap, just produced by self-retraction of the nerve stumps accounting for a 3–4 mm gap, no significant differences in the speed and in the levels of reinnervation was found with respect to a direct suture (Bodine-Fowler et al. 1997; Navarro et al. 1994; Valero-Cabré and Navarro 2001). However, when a segment of the nerve is resected, resulting in a longer gap, reinnervation is slower and achieves significantly lower values at the end of the follow-up than with less severe injuries (Butí et al. 1996; Valero-Cabré et al. 2001).

Our results also show that CMAPs and CNAPs recorded in proximal targets recovered in all the animals used for the study, whereas reinnervation of distal targets occurred in lower pro-

### Table 3

<table>
<thead>
<tr>
<th>Percent amplitude</th>
<th>CNT</th>
<th>CRH</th>
<th>CS</th>
<th>SIL4</th>
<th>SIL8</th>
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<tr>
<td>Percent latency</td>
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<td>Percent normalized amplitude</td>
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**FIG. 5.** Percentage with respect to preoperative values of the normalized amplitude of C1–C3 components of the crossed extensor reflex and of the H/M amplitude ratio during 3 mo postoperation in the groups of rats evaluated after sciatic nerve crush (A), section and suture repair (B), or tube repair (C). Values are the mean for each group at each interval.
portion and to lower levels at the same time points. Unpublished observations from our laboratory in rats subjected to similar nerve lesions showed no significant changes of CMAPs and CNAPs from 3 to 6 mo postoperation, assuring that in 90 days a steady state of reinnervation was achieved.

Spinal cord reflex recovery

The stimulation of sciatic nerve sensory afferents in control animals yield to ipsilateral flexion and crossed extension responses recordable at the biceps femoris (Navarro et al. 1996; Woolf and Sweet 1984) and at the tibialis anterior muscle respectively (Clare and Landau 1975). Stimulation of a limb induces an ipsilateral flexor reflex yielding to the withdrawal of the stimulated limb by inducing a flexion. Simultaneously, activation of several contralateral muscles is induced by spinal crossed pathways to keep the whole limb in an extended position with the aim to prevent loss of balance. This crossed extensor reflex involves co-activation of extensor muscles of the knee (quadriceps muscle) to keep the limb extended but also of dorsiflexors of the paw (tibialis anterior and peroneal muscles) and plantarflexors muscles (gastrocnemius and soleus muscles) (Clare and Landau 1975), both contributing to the fixation of the ankle joint. Although the contralateral response results in a general extension of the limb, we have recorded it in a muscle (tibialis anterior) whose function is a dorsal flexion of the ankle, contributing to the fixation of the ankle to prevent loss of balance when the flexor withdrawal reflex is produced. By stimulating with supramaximal pulses the sensory fibers of the tibial nerve at the ankle, we recorded a multiburst reflex signal with three differentiated components of activity. Differences of latency and stimulus threshold indicated that they were compatible with being conveyed by Aβ, Aδ, and C afferent fibers, respectively (Clare and Landau 1975; Meyerson et al. 1995; Navarro et al. 1999; Woolf and Sweet 1984). We have previously proved that selective lumbar rhizotomy abolished the three components in ipsilateral and contralateral reflex responses and that their recovery was correlated with regeneration of sensory afferents onto spinal cord dorsal laminae (Navarro et al. 1999).

The algesimetry test proved that thin nociceptive fibers reinnervated the plantar skin in all the rats and reached higher levels of recovery than that of CNAPs evoked by thick myelinated afferent fibers at the paw, thus confirming the higher capabilities for sprouting and achieving target reinnervation of thin than thick nerve fibers (Gutmann and Gutmann 1942; Navarro et al. 1994). In contrast with these results, reflex components mediated by thick sensory fibers recovered to higher amplitude than those mediated by C fibers. Axotomized sensory neurons are more likely to atrophy and die by apoptosis when access to the distal stump as a source of trophic factors is not provided, whereas surgical guidance of axons to the distal stump, by interposing a nerve graft or an impermeable tube, prevents neuronal death (Melville et al. 1989). However, according to the literature, not all sensory neurons are equally affected by ineffective or delayed regeneration (Fu and Gordon 1997; Groves et al. 1997; Himes and Tessler 1989). Small sensory neurons, such as nociceptive neurons projecting C fibers, seem to be more sensitive than larger diameter neurons, such as mechanoreceptive and proprioceptive neurons, to cell death (Tandrup et al. 2000). In our study, regeneration was delayed after nerve resection followed by short and long gap tubulization in comparison with crush and direct suture repair, and although no histological confirmation is provided, a comparatively higher loss of small sensory neurons than of large sensory neurons could partly explain the lower recovery of the C3 component of the crossed extensor reflex with respect to reflex components mediated by myelinated fibers. After unpaired or repaired peripheral nerve injuries, myelinated A primary afferents, shown by others to be less susceptible to nerve injury-induced cell death, are able to extensively sprout in the dorsal horn gray matter projecting to Rexed’s laminae I and II previously occupied by unmyelinated nociceptive projections. In contrast, unmyelinated spinal projections are reduced, yielding to an empty space in the most superficial laminae of dorsal horn that explains the extension of myelinated collaterals into these regions (Ma et al. 2000; Woolf et al. 1992, 1995). The decreased spinal projections of surviving nociceptive cells would reduce reconnection with enough number of interneurons, thus diminishing the amplitude of reflex responses evoked by peripheral afferent stimulation. On the other hand, surviving nociceptive cells, probably selected for their high resistance to neurotrophic factors deprivation, show a high capability for peripheral sprouting and extensive reinnervation into a wider distal territory (Devor et al. 1979; Navarro et al. 1994), thus compensating the loss of regenerative neurons for peripheral target innervation.

Spinal cord reflex facilitation

It is worthwhile to note that reflex components mediated by thick myelinated afferent fibers showed higher amplitudes during the follow-up after the nerve injury than before the injury. Despite the reduced number of regenerated myelinated fibers, as indicated by the subnormal recovery of CNAP amplitudes, components C1 and C2, although not C3, had similar to normal latency and number of peaks but significantly higher levels of maximal amplitude. Similarly, the ratio of H/M waves amplitude of ipsilateral muscles increases after repaired section and resection injuries (Valero-Cabré and Navarro 2001). The facilitation of spinal reflexes was highly significant during the initial stages of reinnervation and tended to decrease toward preoperative values in a reinnervation-dependent manner.

After nerve lesions, spinal cord synapses are progressively stripped by the action of astrocytes and microglia, thus impeding the transmission through reflex arches (Aldskogius and Kozlova 1998; Bränström et al. 1992a; Mendell 1984). This process reverses as soon as target reinnervation starts, and motoneuron dendrites reextend and reestablish functional synapses with afferent inputs (Bränström 1992b). In agreement with previous observations, our results indicate that after peripheral nerve injury functional or structural changes affect the performance of spinal cord monosynaptic (Hellgren and Kellett 1989; Valero-Cabré and Navarro 2001) and polysynaptic (Navarrete et al. 1990; Vejsada et al. 1991), as well as brain stem (Cossu et al. 1999) reflex circuits, so that they become hyperresponsive to the stimulation of sensory terminals. Furthermore, after severe injuries, spinal reflexes remained facilitated at the end of the 3 mo follow-up, thus suggesting that permanent rather than transitory new patterns of functional connectivity in the spinal cord have been established (Koerber et al. 1994).
Different mechanisms may account for the abnormalities in reflex responses following nerve injuries. Mechanisms that include cell death (Tandrup et al. 2000), sprouting of sensory afferents within the spinal cord (Shortland and Fitzgerald 1994; Woolf et al. 1992), increase of cellular (Eccles et al. 1958) and synaptic excitability (King and Thomson 1995), and decrease of the effectiveness of local (Sanna et al. 1993) and descending (Castro-Lopes et al. 1993) inhibitory systems may simultaneously account for the abnormalities in reflex responses following nerve injuries. The death of a number of small sensory cells after unrepaird nerve injuries induce sprouting of Aβ and Aδ afferents from the same spinal level as well as Aβ and C fibers from other spinal levels into the empty dorsal horn laminae I and II (Shortland and Fitzgerald 1994; Woolf et al. 1992, 1995). Even if regeneration is allowed after nerve crush, wide collateralization of Aβ fibers has been proved to occur and last for months on the spinal cord gray matter (Woolf et al. 1992). The large number of myelinated endings that sprout into laminae I and II, and secondarily also into laminae III–V (Koerber et al. 1994; Shortland and Fitzgerald 1994), would raise the number of synaptic contacts with correct and mismatched spinal interneurones and motoneurones mediating C1 and C2 crossed extensor reflex components, thus increasing the maximal amplitude and duration of these responses above normal values. The ipsilateral stretch reflex, assessed by recording the H wave, follows a similar pattern of restitution and facilitation as C1 and C2 responses (Valero-Cabré and Navarro 2001). However, Koerber et al. (1994) reported extensive sprouting of Ib and Ia afferents into laminae V–VII but not into a larger number of spinal cord motoneurones 12 mo after nerve section and repair.

Because effector motoneurones and muscles remained intact in the contralateral side, where withdrawal responses were recorded, the observed facilitation of components C1 and C2 does not depend on changes in the pattern of muscle reinnervation but is more likely to occur as a result of central sprouting or unmasking of sensory-motor connections (Koerber et al. 1994). In addition, peripheral nerve injuries alter local and corticospinal pathways that induce presynaptic inhibition on spinal cord interneurones. Reduction of GABA immunoreactive interneurones (Castro-Lopes et al. 1993) or decrease of Renshaw cells activity after peripheral nerve injury (Sanna et al. 1993) could account for the release of inhibitory influences on the excitability of spinal cord reflexes.

Reflex facilitation clearly shows a regeneration and target-reinnervation-dependent course. Increased amplitude of reflex responses was detected only 30 days after nerve crush and 60 days after complete transection and repair and tended to normalize with time. Therefore in groups achieving fast and high levels of reinnervation the size of reflex responses was close to normal at the end of the follow-up. These observations are compatible with the fact that neurotrophic factors released by Schwann cells of the distal stump and target cells are able to modulate in a complex way the electrophysiological behavior of intact or axotomized neurons (McAllister et al. 1999; Mendell and Munson 1999) through changes induced in the expression of ion channels, receptors, or neurotransmitters (Chew and Gallo 1999).

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REFERENCES
SPINAL REFLEXES AFTER PERIPHERAL NERVE INJURIES


