Reorganization of Reflex Responses Mediated by Different Afferent Sensory Fibers After Spinal Cord Transection

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Valero-Cabrè, Antoni, Joaquim Forés, and Xavier Navarro. Reorganization of reflex responses mediated by different afferent sensory fibers after spinal cord transection. J Neurophysiol 91: 2838–2848, 2004. First published February 4, 2004; 10.1152/jn.01177.2003. Adult rats were submitted to a complete spinal cord transection at T9 level to address peripheral and spinal reflex changes in the caudal lumbar segments. Compound muscle and nerve action potentials decreased in amplitude and increased their duration between 14 and 30 days but recovered to near to normal values thereafter. The H wave amplitude increased during follow-up, resulting in significantly higher H/M ratio in tibialis anterior (223%), gastrocnemius (160%), and plantar (304%) muscles with respect to preoperative values (P < 0.01). Sixty minutes after spinal cord transection, component C1 (conveyed by Aβ afferents) disappeared in the crossed but not in the ipsilateral withdrawal reflex. Components C2 (Aδ) and C3 (C afferents) were abolished on both. C1 and C3 reappeared for both reflexes in all injured animals, while C2 reappeared in a few cases. C1 ipsilateral component became highly facilitated (209% of presurgery values, P < 0.01), whereas C3 (82%) and C2 (24%) recovered partially. Crossed reflex component C1 attained in all animals similar to normal values (85%) but with longer duration. C3 increased with time although it remained significantly lower than the original (67%) whereas C2 reappeared in only 2/8 animals. In conclusion, spinal cord injury induces a transient disability of caudal spinal cord segments that progressively reverts along time. Ipsilateral reflex components mediated by thick Aβ fibers (H reflex and C1) but not those mediated by thin fibers (C2 and C3) remained present after injury showing long-lasting facilitation whereas contralateral reflex components were abolished after injury and showed limited recovery.

INTRODUCTION

Spinal cord injuries (SCI) result in loss of control mechanisms of motor function conveyed by the interrupted pathways to the spinal cord. The prognosis, particularly after complete transection or severe contusion, is extremely bad and chances for functional recovery rather scarce. Nevertheless, spinal segments that are not directly injured show potential for developing functional activity mediated by reflex pathways. SCI in humans and animals are followed by a “spinal shock” period with muscle paralysis, flaccid muscle tone, and loss of tendon reflexes below the level of injury. Weeks or months after injury, a spastic syndrome develops exaggerated tendon jerks, increased muscle tone, and muscle spasms (Hiersemenzel et al. 2000). This second phase has been explained as the result of cortical and spinal release of spinal cord reflex circuits connecting muscle and skin sensory afferents with intact spinal interneurons and motoneurons (Engberg et al. 1968). Corticospinal projections from primary, premotor, and supplementary motor areas have been implicated in the modulation of spinal reflexes by mechanisms of presynaptic inhibition onto afferent sensory projections and their synapses with interneurons and motoneurons (Petersen et al. 1998). The cortically driven regulatory activity ceases when descending pathways are interrupted by the injury, thus giving rise to enhanced activity of spinal segmental reflexes under the lesion level. Different studies have shown an increase of spinal excitability after partial or complete SCI in animals (Hultborn and Malmsten 1983; Malmsten 1983; Thomson et al. 1992) and humans (Hiersemenzel et al. 2000). They focused their attention in reflex pathways mediated by thick-myelinated sensory afferents mediating pauci-synaptic H responses or the fast component of withdrawal ipsilateral responses.

Nevertheless, in intact animals, ipsilateral and crossed spinal reflexes conveyed by polysynaptic circuits do play an important role during walking (Schouenborg 2002). In this study, we address the impact of spinal cord section on spinal reflex circuits mediated by different populations of sensory afferents. Electrical stimulation of the tibial nerve in the rat evokes in ipsilateral or contralateral muscles three bursts of EMG activity at different latencies, which are compatible with the characteristics of different sensory afferents (Aβ, Aδ, and C) (Clare and Landau 1975; Cook and Woolf 1985; Meyerson et al. 1995; Valero-Cabrè and Navarro 2002; Woolf and Sweet 1984). Those signals combined with the recording of H reflexes in hindlimb muscles allow evaluating specific reflex pathways in the same group of animals after neural injuries (Navarro et al. 1999; Valero-Cabrè and Navarro 2001, 2002).
Surgical procedures

Under deep pentobarbital anesthesia (50 mg/kg ip), the dorsum of the animals was shaved and disinfected (povidone iodine). A longitudinal midline incision was made through the skin and muscle, and paravertebral muscle insertions were gently removed along T9–T11 vertebral bodies. A selective laminectomy was then practiced to expose the spinal cord. By means of a thin scalpel, the spinal cord was completely sectioned at T9 vertebral level. To ensure that the injury transected the whole spinal cord both stumps were gently lifted away and repositioned back into the vertebral channel. A measured 1.5- to 2-mm-long gap was generated by stump self-retraction. Muscle fascia and skin were sutured. Animals were rehydrated with a bolus of saline (10 ml ip) and preventively treated with wide spectrum antibiotic. Finally, they were placed in individual cages for 3–4 days and observed daily during the next 8 wk. Manual evacuation of the bladder was provided three times per day for 5–8 days after injury, until rats gained reflex micturition.

Electrophysiological evaluation

Electrophysiological tests, including peripheral nerve conduction, motor- and somatosensory-evoked potentials, and ipsilateral and contralateral reflexes, were performed on both hindlimbs of each animal. In the SCT group, all the rats were tested prior to surgery to obtain baseline values, and 1 h, 14, 30, 45, and 60 days postoperation (dpo). The intact control rats (group CNT) were tested in parallel at baseline (day 0) and 30, 45, and 60 days thereafter to assess the potential impact of repeated anesthesia and variability of repeated electrophysiological testing. During the tests, the rat body temperature was maintained by means of a thermostated flat coil, and anesthesia was maintained steady after the initial induction (40 mg/kg) by injection of additional pentobarbital bolus (10 mg/kg) every 60 min. For all electrophysiological tests, values from both hindlimbs of each animal were averaged.

The right sciatic nerve was stimulated with single electrical pulses (100 μs duration and up to supramaximal intensity) delivered by monopolar needles percutaneously placed at the sciatic notch. Compound muscle action potentials (CMAPs), including the direct muscle response (M wave) and the monosynaptic reflex response (H wave), of the tibialis anterior, gastrocnemius, and plantar muscles were recorded by means of needle electrodes and displayed on an oscilloscope (Saphyre 4M, Vickers). Likewise, compound nerve action potentials (CNAPs) were recorded by needle electrodes inserted near the tibial nerve at the ankle and near the fourth digital nerve. To ensure reproducibility, the recording needles were placed using a surgery microscope to secure the same placement on all animals guided by anatomical landmarks (see Navarro et al. 1999; Valero-Cabrè and Navarro 2001, 2002 for further details). For CNAP recordings, the active electrode was placed under the Achilles tendon near the tibial nerve and then at the lateral side of the base of the fourth toe near the digital nerve. For CMAP recordings, the active electrode was inserted subcutaneously on the middle of the medial gastrocnemius belly, on the proximal third of the tibialis anterior muscle belly, on the midpoint of the biceps femoris muscle between knee and hip insertions, and, for the plantar muscle, at the third metatarsal space. A reference electrode was placed at the tip of the fourth toe, and a ground electrode was inserted at the tip of the hindpaw. A measured 1.5- to 2-mm-long gap was generated by stump self-retraction. Muscle fascia and skin were sutured. Animals were rehydrated with a bolus of saline (10 ml ip) and preventively treated with a wide spectrum antibiotic. Finally, they were placed in individual cages for 3–4 days and observed daily during the next 8 wk. Manual evacuation of the bladder was provided three times per day for 5–8 days after injury, until rats gained reflex micturition.

The reflex responses conveyed by large myelinated (Aα) afferent fibers were evaluated by means of the late H response recorded in the motor nerve conduction tests described in the preceding text (see Valero-Cabrè and Navarro 2001). The intensity of electrical stimulation was progressively increased toward the optimal level to ensure that a maximal H reflex response was recorded. The minimal stimulus threshold to elicit 50-μV amplitude H and M waves in ≥5 of 10 responses was determined in all animals. In two animals, recruitment of plantar muscle M and H responses at increasing levels of stimulation was carried out prior to surgery and at 60 dpo.

The spinal polysynaptic reflexes were elicited by stimulating the tibial nerve at the ankle with single electrical pulses. Ipsilateral reflex responses were recorded from the biceps femoris muscle (Cook and Woolf 1985; Meyerson et al. 1995; Woolf and Sweet 1984). Crossed reflex responses were recorded from the contralateral tibialis anterior muscle; this ankle dorsiflexor muscle cooperates with other plantar flexors muscles in the fixation of the contralateral joint when the ipsilateral flexor reflex is elicited (Clare and Landau 1975). Electrodes were placed at the optimal landmarks for tibialis anterior and biceps femoris muscles (see preceding text). Ipsilateral and contralateral reflex components were initially recruited at increasing intensities of stimulation (steps of 0.1 mA at 200-μs duration stimuli) to determine the threshold of each of the three distinct reflex components (named as C1–C3). We considered as a positive response any evoked activity of ≥50-μV amplitude. Once the threshold was determined, the stimulator was set up to deliver 500-μs duration stimuli and supramaximal intensity (3–3.5 mA for C1 and C2 and 17–25 mA for C3 component). A window of onset-offset latencies was used to classify the responses into three different components: ipsilateral, C1: 6–12 ms, C2: 11–30, C3: 100–2,600; crossed C1: 10–14 ms, C2: 18–45 ms, C3: 110–400 ms (data from Valero-Cabrè and Navarro 2002).

The responses were rectified on-line and displayed on the oscilloscope at a voltage scale between 20 and 200 μV per division, and a time scale of 5–30 ms/division for C1 and C2 and of 100–500 ms/division for C3 component. The latency to the onset of each burst of activity, the amplitude of the maximal peak, the duration, and the area under the recorded burst of activity were measured on-line. At supramaximal levels of stimulation, reflexes were evoked at least eight times (with varying intervals of ≥30 s in between), and the highest amplitude recorded for each reflex component was considered. For all reflex responses, central-peripheral (sciatic notch to sciatic notch) latency was approximated by subtracting to their measured latency the latency of the efferent (onset latency of the orthodromic M wave) and afferent (onset latency of the antidromic tibial CNAP) pathways (see Valero-Cabrè and Navarro 2002 for further details).

Motor-evoked potentials (MEPs) and somatosensory-evoked potentials (SSEPs) were used to evaluate the spinal cord descending and ascending tracts and their potential regeneration (García-Álvaro et al. 2003). MEPs were evoked by electrical supramaximal stimulation (single rectangular pulses, 0.1-ms duration) of the sensorimotor cortex projected through needle electrodes inserted subcutaneously, the cathode over the skull overlying the sensorimotor area and the anode at the nose, and recorded in the contralateral tibialis anterior muscle. A single wave was elicited in control rats. SSEPs were evoked by repetitive stimulation at 3 Hz of the tibial nerve at the ankle and recorded by needle electrodes placed subcutaneously on the skull (same sites as stimulation needles for MEPs). Up to 256 responses were averaged on-line; the peak latency and the peak-to-peak amplitude of N15, N20, and N30 peaks were measured. In parallel, locomotor behavior was evaluated in an open-field test according to the BBB rating scale (see Basso et al. 1995).

Histological evaluation

At the end of the study, the peroneal branch of the sciatic nerve projecting to tibialis anterior muscle, the tibial branches entering both bodies of the gastrocnemius muscle and the tibial nerve below the
ankle providing innervation to the plantar muscles were carefully dissected and cut. Crystals of DiI (Molecular Probes), FluoroGold (Fluorochrom, Denver CO), and Fast Blue (EMS Chemie GmbH, Gross-Umstadt, Germany) were applied to their proximal stumps. Animals were allowed to survive for 8 days to allow accumulation of tracers in the soma of spinal motoneurons. Rats were then pericardially perfused with saline solution for 60 s followed by 4% paraformaldehyde in phosphate buffer solution for 15 min under deep anesthesia. The spinal cord was removed, and the lumbar segment (L1–L6) was cut longitudinally in 50-μm-thick sections on a vibratom (FTB-vibracut; Plano, Marburg, Germany). Sections were observed under an Olympus BX-40 microscope equipped with appropriate filter sets (see Valero-Cabrè et al. 2001 for further details). Images were captured by means of a digital camera (Olympus DP-20). Employing the fractionator principle (Gundersen 1986), all retrogradely labeled motoneurons with a visible cell nucleus in the 50-μm-thick sections (to avoid considering the same neuron twice) were counted in every third section through the cord and the final number multiplied by three (see Valero-Cabrè et al. 2001). The number of motoneurons labeled with DiI, Fluorogold, and Fast Blue was counted in both sides of the spinal cord in injured and control rats.

Data analysis

Data are presented as the group means ± SE and, for normalization, expressed in percentages with respect to baseline values. Two statistical comparisons are made. Values obtained in group SCT at postle-sion intervals are compared with the presurgery baseline values of each rat by means of paired nonparametric Wilcoxon rank test. On the other hand, values from groups SCT and CNT at the same time each rat by means of unpaired nonparametric Kruskall-Wallis test followed by Mann-Whitney U test. Significance was set at $P < 0.05$.

RESULTS

Motor- and somatosensory-evoked responses

Cortically evoked motor responses with a mean latency of ~6–7 ms and amplitude of ~6–10 mV were present in all animals before operation (see Fig. 1). Similarly, control SSEPs showed a series of negative peaks at latencies of ~15, 20, and 30 ms, with mean maximal amplitudes of 5, 14, and 10 μV, respectively. Both MEPs and SSEPs disappeared 60 min after spinal cord section and did not recover during the following 2 mo (Fig. 1), indicating complete interruption of spinal pathways along the 60 days follow-up.

Locomotor evaluation

All the animals showed a normal locomotor behavior before operation, indicated by a score of 21 in the BBB test. At 7 dpo, injured rats had a BBB score of 0 points without movements of the hip, knee, and ankle joints in both hindlimbs. Injured animals were unable to sustain their body weight and walked with their belly in contact with the floor. Hindpaws were supinated with the dorsum in contact with the floor. Thirty days after injury, three of eight animals showed slight reflex movements in one limb at the level of the ankle when the paw was in contact with the ground. However, they were still completely unable to lift their own weight (BBB score: 0.25 ± 0.13). At the end of follow-up, six animals showed only occasional, involuntary movements at the ankle, knee, and hip levels at least in one limb when walking, whereas the other two remained without joint movements. The BBB score remained very low (1.25 ± 0.41).

Innervation of peripheral targets

One hour after spinal cord section, we found a slight decrease of the amplitude of the M wave and of CNAPs combined with delayed latencies and increased signal duration. The lowest amplitude (M waves: 75–89%, CNAPs: 70–83%) and longest duration (M wave: 110–132%, CNAPs: 121%) were found by 14–30 dpo in all targets (see Figs. 2 and 3). Afterward, they tended to slowly recover back to preoperative values. However, at the end of the study, the M wave of tibialis anterior and gastrocnemius muscles remained in significantly lower amplitude (83 and 88%) and longer duration (120 and 125%) than their original presurgery values and also than values of the group CNT followed in parallel ($P < 0.05$; Fig. 4). The onset latency of the M wave and nerve potentials significantly slowed after injury and returned to normal levels in all tested targets, except for the digital nerve (112%) and the gastrocnemius muscle (109%) in which latencies remained significantly longer than in the control group ($P < 0.05$) (Fig. 4). In the control group followed in parallel, no significant changes with respect to baseline values in the amplitudes and the latencies of M waves and of CNAPs were seen in any of the studied targets at any time interval.

No significant differences were found in the mean number of labeled spinal motoneurons for any of the three spinal motor nuclei when comparing control and SCT animals (lateral and
medial gastrocnemius: 539 ± 46 vs. 532 ± 47; tibialis anterior: 523 ± 43 vs. 489 ± 28; plantar: 392 ± 19 vs. 381 ± 48; Fig. 5).

H reflex responses

Stimulation of the sciatic nerve after SCI elicited H waves of higher amplitude than preoperative values in all injured animals in spite of the decrease in amplitude detected in M waves. This facilitation was observed in plantar muscles from 60 min postinjury and throughout the 2 mo follow-up (Figs. 2 and 6), whereas it was less marked in tibialis anterior and gastrocnemius muscles. For all three muscles, the H wave amplitude was significantly higher than preoperative values and than those of control rats ($P < 0.05$). The increase of the H/M ratio was

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**FIG. 2.** Representative recordings of M and H waves of the plantar and tibialis anterior muscles before (Preop) and 30 and 60 days after spinal cord transection (dpo). Note the slight decrease in amplitude of the M wave and the shortening in latency and increase in amplitude of the H wave.

**FIG. 3.** Representative recordings of compound nerve action potentials (CNAPs; see *) of the 4th digital and tibial nerves before (Preop) and 30 days after spinal cord transection (dpo). Vertical and horizontal scales are indicated at the right of each recording.
comparatively more marked in the distal plantar muscle (>300%) than in more proximal tibialis anterior (>200%) and gastrocnemius (160%) muscles (Fig. 6). Recruitment curves of M and H waves recorded in the plantar muscle at 60 dpo showed near to normal M amplitude but clearly facilitated H responses with decreased threshold (see examples of 2 animals in Fig. 7). In addition, a significant reduction of the peripheral-central conduction latency was found at 45 and 60 dpo in comparison with presurgery values (85%. P < 0.05). Also, a significant decrease (P < 0.05) of the H/M threshold ratio was found for the three muscles at 30, 45, and 60 dpo (plantar: 84 ± 5%, tibialis anterior: 89 ± 6%, gastrocnemius: 75 ± 4%) (Fig. 6). In the control group of rats, the H reflex amplitude, estimated central latency and threshold did not experience any significant change along follow-up.

Polysynaptic reflexes

In intact rats stimulation of the tibial nerve at the ankle consistently yielded ipsilateral (recorded in the biceps femoris muscle) and crossed (in the tibialis anterior muscle) withdrawal reflex responses as bursts of motor-unit action potentials grouped in three components. The threshold (ipsilateral C1: 1.86 ± 0.05, C2: 2.61 ± 0.04, C3: 36.9 ± 1.9 mA; crossed C1: 2.44 ± 0.13, C2: 2.62 ± 0.16, C3: 40 ± 2 mA) and onset latencies (ipsilateral C1: 7.22 ± 0.09, C2: 16.34 ± 0.65, C3: 115 ± 2 ms; crossed C1: 11.10 ± 14, C2: 23.51 ± 0.38 and C3: 122 ± 2 ms) confirmed that each of the three components is likely mediated by different types of peripheral afferent fibers (Aβ, Aδ, and C fibers for C1–C3, respectively; Fig. 8) (see Valero-Cabré and Navarro 2002 for a more detailed report).

One hour after injury, the ipsilateral component C1 (induced by stimulation of Aβ afferents) was still present in all animals (Fig. 9A). On the contrary, components C2 (Aδ fibers) and C3 (C fibers) were abolished in all animals. Component C2 reappeared at 14 dpo in five of eight rats, while so did C3 in all animals. The maximal amplitude and the area under the rectified recording of C1 were markedly increased, being significantly higher (190 ± 19 and 209 ± 26%) than the preoperative baseline values (P < 0.05). At 60 dpo, C3 was present in all injured rats while C2 was absent in two. The ipsilateral C3 recovered 82 ± 14%, whereas C2 averaged only 24 ± 8% of preoperative values of area. The peripheral-central latency was significantly reduced in ipsilateral C1 component (88 ± 3%)
but increased in the other two components (C2 158 ± 11%, C3 137 ± 10%) at 60 dpo (Figs. 9A and 10, Table 1).

All three components of the crossed spinal reflex were abolished when tested 60 min after injury (Fig. 9B). C1 and C3 recovered in all rats, whereas C2 reappeared in only two of the eight rats at the end of the follow-up. Component C1 recovered over time reaching values that were close to those presurgery (85 ± 33%), whereas C3 remained at lower levels (Table 1). The duration of components C1 and C3 was largely increased (C1: 10 times, C3: 2 times) with respect to controls. On the contrary, in the two animals in which C2 was present, its area was only 7 ± 5% of baseline value. Central-peripheral latency was longer than normal for all three components during follow-up (Fig. 10, Table 1). In the group CNT studied in parallel, the area, amplitude, duration, and latency of ipsilateral and contralateral reflex components remained essentially unchanged across a similar follow-up period, showing final values not statistically different from those recorded as baseline at the beginning of the study (Table 1). This proves that neither repeated testing and anesthesia nor normal variability of the results across time account for changes found in group SCT.

**DISCUSSION**

The study of spinal reorganization and plasticity after SCI could be of great interest for the development of rehabilitation therapies. The present report is a longitudinal study comparing changes in spinal cord reflexes mediated by several populations of sensory afferents after spinal transection. Using a combination of electrophysiological and tracing techniques, we found that thoracic spinal cord transection induced a decrease in the amplitude of muscle and nerve compound action potentials in targets innervated by lumbar spinal cord segments. This transient impairment is not likely to be caused by neuronal loss but by a reversible alteration in axonal excitability and conduction properties. Ipsilateral reflex components mediated by thick sensory fibers, Aα (H) and Aα/Aβ (C1), remained functional...
after injury and showed marked facilitation. In contrast, reflex components conveyed by Aβ (C2) or C (C3) fibers were abolished after cord transection and recovered only partially. Crossed reflex components behaved in a different manner. All three components disappeared immediately after injury and tended to recover in amplitude and duration. Components C1 and C3 achieved near to normal values, but C2 recovered incompletely. Different patterns of recovery and reorganization of spinal reflexes were found depending on the type of afferent and spinal circuitry.

Peripheral nerve and muscle activity after spinal cord injuries

Our animals were submitted to spinal cord transection at T9. The expected hindlimb paralysis was evident by the poor walking skills in the open field task. Furthermore, the absence of MEPs and SSEPs proved that the transection was complete and that no regeneration of descending or ascending pathways took place at least during the 2 mo postinjury. Acute implantation of needle electrodes was used at every test time during follow-up. Our design allows for longitudinal assessment of the same animals over time thus reducing interindividual variability. To minimize variability due to electrode location, we followed a careful, standardized procedure to record from the same region defined for each target the action potentials of maximal amplitude (see METHODS) (see also Navarro et al. 1999; Valero-Cabrè and Navarro 2001, 2002). A control group of intact animals was followed at similar testing intervals to ensure that changes over time were not attributable to methodological variations. Complete thoracic cord section resulted

FIG. 9. Representative recordings of withdrawal ipsilateral reflex responses (A) and crossed reflex responses (B), elicited by stimulation of the tibial nerve. Recordings correspond to the same animal before (Preop) and 60 min, 45 and 60 days after spinal cord transection (dpo). Preoperative recordings in A and B show components C1–C3. Note in A the persistence and facilitation of C1, absence of C2, and initial absence followed by recovery of C3 after injury. Note in B the abolition of all 3 components and progressive but incomplete recovery of C1 and C3 but not of C2. Vertical and horizontal scales are indicated at the right of each recording. The time axis is interrupted between C2 and C3; both the voltage (vertical) and time scale (horizontal) were adapted in the recording to the optimal setting for each component. The 1st 100 ms of the C3 recordings (showing C1 and C2 in a compressed scale) are eliminated to avoid overlapping when displayed in continuity with those of C1–C2. In the preoperative condition, C3 component showed an onset time window of 100–130 ms (ipsilateral, A) and 120–130 ms (crossed, B).
Changes of H reflex activity

The H reflex amplitude and the H/M amplitude ratio have been traditionally used to evaluate spinal excitability. In our study, the H response increased in amplitude after spinal cord transection ~1.5–3.5 times depending on the muscle. Simultaneously, the H/M threshold ratio and the estimated latency across the spinal cord decreased significantly in all muscles. These three findings strongly suggest that the paucisynaptic H reflex pathway became facilitated by the withdrawal of supraspinal control. Thomson et al. (1992) failed to find differences in the H/M ratio after spinal cord contusion. Notwithstanding, other excitability-related parameters such as H wave threshold and sensitivity to high-frequency depression proved an overall facilitation of the stretch reflex circuit. In agreement with our observations, an early increase in hindlimb muscle H/M ratio and H amplitude has been consistently found after high thoracic SCI in cats (Hultborn and Malmsten 1983), rats (Malmsten 1983) and humans (Hiersemensel et al. 2000; Leis et al. 1996; MacDonnell et al. 1989; Shemesh et al. 1977),

![FIG. 10. Changes with respect to preoperative values of components C1 (Aβ afferents), C2 (Aδ afferents), and C3 (C afferents) amplitude and central latency of the ipsilateral withdrawal reflex recorded in the biceps femoris muscle and of the crossed reflex recorded in the contralateral tibialis anterior muscle. * P < 0.05 vs. preoperative values of the injured group and versus values of the control group (not shown) at the end of follow-up.

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<th>60 dpo</th>
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<th>Crossed Reflex</th>
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<tr>
<td>CNT</td>
<td>99 ± 16</td>
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<td>SCT</td>
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<td>Percent duration</td>
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<td>CNT</td>
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<td>Percent central latency</td>
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<tr>
<td>CNT</td>
<td>98 ± 2</td>
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<td>SCT</td>
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<td>Percent threshold</td>
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<td>SCT</td>
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The table contains changes in values of area under the recorded burst of activity, duration, central-peripheral latency, and threshold of the ipsilateral and contralateral withdrawal reflexes C1–C3 components at the end of the 60 days follow-up after spinal cord transection. Data correspond to the control (CNT, n = 8) and the spinal cord transection (SCT, n = 8) groups. Values are means ± SE. * P < 0.05 vs. group CNT at the end of the 60 day follow-up; † P < 0.05 vs. preoperative baseline values of the same group of animals.
Changes in withdrawal reflex responses

The fast component (C1) of the ipsilateral flexor reflex mediated by Aαβ afferents was the only polysynaptic response that remained present and facilitated after the lesion. Its amplitude increased significantly between 14 and 45 dpo, reaching a maximal value of 300% of its original level as did the H reflex response. Similar results were found after spinal cord hemisection in the rat and the cat (Hultborn and Malmsten 1983; Malmsten 1983). Collateral sprouting of Aα and Aβ afferents onto an increased number of motoneurons or interneurons has been described after peripheral or spinal cord injuries (Krenz and Weaver 1998). However, the rapid onset of the facilitation and the decrease of reflex threshold observed in our data rather suggest as a mechanism the unmasking of preexisting collaterals initially inhibited by descending corticospinal and rubrospinal projections (Engberg et al. 1968; Thomson et al. 1992). We also explored the mid (C2) and late (C3) components of the ipsilateral reflex that are conveyed by Aδ and C fibers. In contrast with C1, both components were abolished 1 h after transection and did not recover until the spastic state was established 2 wk after lesion. Moreover, instead of being facilitated, these components remained in lower than normal levels at the end of the follow-up. The hypothesis that ipsilateral C2 and C3 depression is caused by a decrease in the excitability of the output motoneuronal pool is difficult to sustain. Studies in anesthetized cats found a significant decrease of spinal motoneuron excitability after thoracic spinal cord transection (Baker and Chandler 1987; Buller et al. 1960; Cope et al. 1986), but controversy exists about if the anesthetized state induced that decrease of the motoneuronal system. More recent work on unanesthetized animals and humans has shown that motoneurons become hyperexcitable after long-term injury (Kiehn and Eken 1997; Li and Bennett 2003). However, the F wave, which is an antidromic volley evoked by electrical stimulation of motor axons, appears also to be consistently decreased in SCI human patients (Curt et al. 1997; Hiersemenzel et al. 2000). In any case, changes in the excitability of the output motoneurons cannot simultaneously account for the facilitation of components mediated by thick sensory afferents (Aαβ).

The presynaptic elements of reflex circuits occupy precise locations in the spinal cord. Aα and Aβ fibers project over motoneurons in Rexed’s laminae VIII–IX and interneurons of laminae IV–V, respectively. In contrast, A6 and C afferents synapse with interneurons located at laminae I–II and III–IV. Interneurons play a role as neural encoders in charge of regulating the sensory-motor transformation of the afferent input. This process takes place under regulation by supraspinal centers (Schomberg 1990). The loss of descending control distorts excitability of such systems and rapidly erodes normal reflex somatotopy and function (see Schouenborg 2002 for a review). In this context, it may be hypothesized that short-latency, low-threshold reflex component receives in normal conditions efficient and precise presynaptic inhibition by descending corticospinal and rubrospinal projections. On the other hand, late reflex components conveying slower muscle responses remain only slightly inhibited or even enhanced. As a result of the spinal transection, the former (H and C1) are released yielding the observed facilitated responses, whereas the latter (components C2 and C3) might become less excitable or even unresponsive. As time after injury progresses, spinal networks might readapt to the new situation in which no descending control is provided. Accordingly, our data showed that fast reflex responses remained facilitated but tended to decrease its amplitude, whereas late responses increased their amplitude toward normal levels across time.

The crossed spinal reflex was recorded in the contralateral tibialis anterior muscle, a muscle that provokes dorsiflexion of the ankle. During a withdrawal reflex response, the summed action of ankle dorsiflexor (tibialis anterior) and plantarflexor muscles (soleus and gastrocnemius) helps to stabilize the ankle during the ipsilateral flexor response of the stimulated limb to preserve body balance and stability (Clare and Landau 1975; Schouenborg and Kalliomaki 1990). We selected the tibialis anterior muscle because according to our previous observations, it provides a more consistent reflex response than the triceps surae muscle. During the spinal shock phase, the three components of the crossed spinal reflex were abolished and reappeared in different proportions of animals at 14 dpo. The interruption of descending projections might cause a loss of synchrony in the impulse transmission through pathways crossing the spinal cord midline. Also impaired circulation of cerebrospinal fluid in the central canal after transection might have increased pressure—inducing a posttraumatic syringomyelia—blocking crossed conduction around the spinal midline. This fact may explain the initial dispersion and low-amplitude of all three crossed components. All these changes were intense and long-lasting. In fact, only crossed components C1 and C3 recovered up to nearly normal values, whereas C2 remained absent in a majority of animals. Electrophysiological evaluations in CNT and SCT rats were done under the effects of pentobarbital anesthesia. It is well known that barbiturates decrease spinal reflexes in intact or spinalized cats (Baker and Chandler 1987) and rats (Duke and Advokat 2000; Lu and Xu 2002) as well as excitability of rat
motoneurons and interneurons in slice preparations (Guertin and Hounsgaard 1999). However, the nature and characteristics of this partially inhibitory effect seem to vary depending on the reflex pathway and the injury condition. Duke and Advokat (2000) reported that only the flexor reflex fast component but not the H reflex was significantly depressed under pentobarbital anesthesia in comparison to awake rats. Furthermore, this depressive effect was only significant in chronically spinalized animals but not after acute spinal transection. On another hand, no differences have been found between the effects of pentobarbital and those of other anesthetic agents, such as chloral hydrate, tribromomethanol, or urethane, on depression of the H reflex (Meinck 1976; Valero-Cabré, unpublished results). According to the inhibitory spinal effect of pentobarbital described in the preceding mentioned studies, we might have underestimated the degree of facilitation found for low-threshold cutaneous (C1 component) and proprioceptive (H wave)-induced reflex responses and overestimated the degree of decline and lack of recovery of late C2 and C3 components after SCI. However, these assumptions do not invalidate our findings because in the intact CNT group, evaluated under the same anesthesia, all the spinal reflex responses remained stable and without significant changes along the 2-mo follow-up. Because in all cases the dose of pentobarbital was calculated according to the animal weight, it is reasonable to think that reorganization of reflex pathways is responsible for changes in spinal reflexes after SCT.

Conclusions

After complete spinal cord transection, reflex responses mediated by thick-myelinated afferent fibers (Aα and Aβ) remain active and increase their excitability, whereas those conveying stimuli by Aδ and C fibers are abolished and recover only partially with time. These differences may arise from a particular organization of descending corticospinal and rubrospinal excitatory or inhibitory projections on spinal cord circuits conveying specific reflex responses. The precise map of descending projections, its regulatory effect on specific spinal pathways, and the detailed mechanism of spontaneous organization after SCI need to be studied in detail. Basic knowledge may improve therapeutic strategies for the modulation of spinal reflexes by means of electrical stimulation or neurorehabilitation to decrease spasticity, reduce neuropathic pain, or optimize reflex walking in SCI patients.

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References


