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B-50 (GAP-43) in the Spinal Cord Caudal to Hemisection: Indication for Lack of Intraspinal Sprouting in Dorsal Root Axons

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Sprouting of dorsal root axons has been suggested to occur in the mature cat spinal cord caudal to a hemisection at a low thoracic level sparing the dorsal columns. The lesion interrupts supraspinal descending projections, while leaving ascending collaterals of dorsal root axons intact. This hypothesis was re-evaluated by comparing the light and electron microscopic immunoreactivity of B-50 (GAP-43) on both sides of the postulated target regions for sprouting, the intermediate gray and the dorsal horn. The neural-specific phosphoprotein B-50 is involved in regenerative and developmental axonal outgrowth and synaptic plasticity. The light microscopic distribution pattern and density of B-50 immunostaining, measured by quantitative densitometry, were bilaterally symmetrical in all segments below the hemisection 3.5, 8, 14, 21, and 56 days postoperatively, as they were in the intact animal. Ultrastructurally, growth cone-like profiles were not detectable during putative periods of sprouting in regions of interest. After removal of degenerated axon terminals, vacated postsynaptic places appeared to be covered by astrocytic processes. These results indicate that, under the present experimental conditions, sprouting of primary afferents in adult cats is unlikely to be involved in functional plasticity after removal of descending pathways.

Key words: descending tracts, sprouting, dorsal roots, plasticity, spasticity

INTRODUCTION

Reactive reinnervation by sprouting of undamaged pathways in response to partial denervation has been described in many parts of the adult central nervous system (see Raisman, 1985; Tsukahara, 1985, for reviews) and has been proposed to account for functional recovery after spinal cord injury (McCouch et al., 1957; Goldberger and Murray, 1978, 1982, 1988; Goldberger, 1988). This anatomical reorganization has been confirmed in the "spared-root" preparation in rats and cats in which transection of several consecutive dorsal roots cranial and caudal to an intact remaining lumbar root was performed. Using more recent labeling techniques, sprouting axons of the spared root were seen to reinnervate partially denervated lumbosacral spinal gray areas (Traub et al., 1989; McNeill et al., 1990, 1991). In spite of its intrinsic experimental interest, this model of spinal injury does not have a clinical counterpart. In contrast, partial spinal cord hemisection that spares the dorsal columns provides a paradigm with more clinical relevance. Here, possible structural changes induced by removal of descending tracts, and not by injury of ascending dorsal root axons, can be studied in spinal segments caudal to the lesion by comparison to the unoperated side (McCouch et al., 1957; Murray and Goldberger, 1974; Goldberger and Murray, 1988). Sprouting of dorsal root axons to reinnervate partially denervated spinal gray areas caudal to this lesion has been suggested to contribute to motor recovery (Murray and Goldberger, 1974; Goldberger and Murray, 1988) and to be involved in spasticity (McCouch et al., 1957). Whether sprouting actually occurs under this condition in the spinal cord, however, remains controversial (Rodin and Kruger, 1984). Indications for such a sprouting were derived primarily from silver degeneration studies in cats (McCouch et al., 1957; Murray and Goldberger, 1974) which however have been criticized because of technical limitations (Ro-

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din and Kruger, 1984). Negative evidence for dorsal root sprouting caudal to a hemisection resulted from horseradish peroxidase (HRP)-transport studies in rats in which unoperated animals were used as controls (Rodin and Kruger, 1984). These data have also been criticized, since the number of dorsal root ganglion neurons in a particular ganglion may vary considerably from one animal to the next but not significantly between the two sides of the same animal (Polistina et al., 1990). Thus, an intra-animal comparison is crucial for this experimental purpose. However, in an intra-animal quantitative comparison of dorsal root axons following hemisection, Hulsebosch and Coggeshall (1983) found indication for sprouting in neonate but not in adult rats. Therefore, species and age differences in plasticity within the spinal cord may be relevant. Since the hypothesis of an intraspinal sprouting in adult animals has been derived from cats (McCouch et al., 1957; Murray and Goldberger, 1974), this species should be considered for re-examination. Accordingly, we reinvestigated in this study the question of intraspinal sprouting in dorsal root axons after removal of descending tracts in cat.

In the present study, we used the nervous tissue-specific phosphoprotein B-50, revealed by immunohistochemistry, as a marker for sprouting axons. This molecule is a prominent growth-associated protein that was discovered independently by several groups and given a variety of names, including B-50, GAP-43, GAP-48, F1, pp46, and P57 (see Skene, 1989; Gispen et al., 1992, for reviews). B-50 accumulates in growth cones after fast axonal transport, and has been implicated in virtually all changes involving axonal outgrowth and synaptogenesis during both regeneration and ontogenesis (see Skene, 1989; Benowitz and Perrone-Bizzozero, 1991; Gispen et al., 1992, for reviews). Moreover, B-50 is believed to have a critical role in the regulation of neurotransmitter release (Dekker et al., 1989; Bendotti et al., 1991) and in presynaptic mechanisms of long-term potentiation (Nelson et al., 1989; Cianotti et al., 1992). Recent evidence has shown that B-50 (GAP-43) is also expressed by glial and Schwann cells under certain conditions (Curtis et al., 1992; Woolf et al., 1992b; Vitkovic, 1992), raising new questions about the role of this protein. B-50 (GAP-43) has been shown to persist at lower levels in particular regions of the mature central and peripheral nervous systems under normal conditions (Oestreicher and Gispen, 1986; Benowitz et al., 1988,1989; De la Monte et al., 1989; Stewart et al., 1992, Curtis et al., 1993a; Nacimiento et al., 1993b). It has been proposed that these B-50-positive neuronal systems retain the capacity for plasticity throughout adulthood. In the intact cat spinal cord, the distribution of B-50-IR was found to be bilaterally symmetrical (Nacimiento et al., 1993a), which is a prerequisite to the assessment of possible plasticity in experimental animals.

In our experimental model, sprouting and reactive reinnervation after partial hemisection should be represented by a transient postoperative increase in B-50 immunoreactivity (B-50-IR) in the intermediate gray and the dorsal horn. These are regions where descending pathways and dorsal root input fields overlap (Kuypers and Martin, 1981), which is a postulated precondition for sprouting (Goldberger and Murray, 1988). Since subtle axonal outgrowth might not be detectable by light microscopic B-50 immunohistochemistry, we also examined the ultrastructural immunoreactivity of B-50 in the spinal gray during putative axonal sprouting periods. Since the protein accumulates in growth cones (Gordon-Weeks, 1989; Knyihar-Csillik et al., 1992), we were particularly interested in identifying immunoreactive growth cone-like profiles. Additionally, we attempted to reveal growth cones by their typical ultrastructural features (Knyihar-Csillik et al., 1985) in unstained material.

MATERIALS AND METHODS

All experiments performed in this study were approved by the local animal care and use committee (Saarland University Medical School, D-6650 Homburg, F.R.G.).

These experiments were conducted in eight adult cats, 2.5 to 3.2 kg in body weight. B-50 immunohistochemistry was carried out in six cats after spinal cord hemisection, in one cat after sciatic nerve transection, and in an unoperated animal.

Surgical Procedures

Spinal cord hemisection. Animals were operated under deep pentobarbital anesthesia. The dorsal and lateral surfaces of the low thoracic spinal cord were exposed following laminectomy at the levels Th9 and Th10 under sterile conditions. Guided by observation through a Zeiss surgical microscope, subtotal right hemisections, sparing the dorsal funiculus, were performed with a straight-edged scalpel blade. Care was taken in each case to avoid damage to the dorsal columns and to the large blood vessels on the surface of the cord. Following the lesion, the wound was closed in anatomical layers. Postoperative survival periods were 3.5, 8, 14, 21, and 56 days, with one cat assigned to each postoperative interval, except for 14 days (two animals).

Sciatic nerve transection. The right sciatic nerve was transected in the middle one-third of the thigh under deep pentobarbital anesthesia. A length of 1 cm was resected from the distal stump and the proximal stump was ligated. Afterward, the wound was closed in ana-

tomical layers. The cat survived for 21 days following this operation.

Perfusion and Immunohistochemical Procedures for Light and Electron Microscopic Demonstration of B-50

Following the postoperative intervals indicated above, the animals were reanesthetized. When anesthesia was deep, the thorax was opened and the cats were perfused through the left ventricle with 700 ml of a 0.9% saline solution. When the fluid flowing from an incision in the right auricle was free of blood, the perfusing fluid was changed to 2.5 liters of a mixture of 0.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). After perfusion was complete, the spinal cord was fully exposed. In hemisected cats, each segment from Th11 (including the lesion) to S2 was identified using the dorsal root entry zones as landmarks and separately removed. In the cat with sciatic nerve transection, the segments of the lumbosacral enlargement were removed. The tissue blocks were postfixed for 24 hours in fixative solution as used for perfusion. Part of the tissue was then cryoprotected in graded sucrose (10%, 20%, and 30% in 0.1 M phosphate-buffered saline [PBS]) for 24–48 hours, frozen in liquid nitrogen, and sectioned on a cryostat at transverse plane (14 μm) for light microscopy. The rest of the tissue was transferred to 2% paraformaldehyde in 0.1 M phosphate buffer, cut on a vibratome at transverse plane (50 μm), and further processed for electron microscopy.

Free-floating tissue sections were immunohistochemically stained for B-50 using the peroxidase-antiperoxidase method of Sternberger (1986). Prior to immunostaining, sections were incubated in a solution containing 0.3% hydrogen peroxide in PBS for 30 minutes to block endogenous peroxidase activity, followed by three 10-minute rinses in PBS. Briefly, sections were then incubated in affinity purified polyclonal rabbit antibodies to rat B-50 (anti-B-50 IgGs of rabbit 8920; 1:2,000) for 48 hours at 4°C, and in secondary antisera (goat-anti rabbit IgG, 1:100, Accurate Chemicals) and rabbit PAP (1:500, Accurate Chemicals) for 1 hour each at room temperature. Primary antisera, secondary antisera, and PAP were diluted in 0.1 M PBS containing 1% normal goat serum (NGS) and 0.75% Triton X-100. Tissue sections were rinsed in two changes of 0.1 M PBS (10 minutes each) and 3% NGS (30 minutes) before each antisera or PAP incubation. Following the PAP incubation, tissue sections were rinsed twice in PBS and reacted in 0.05% diaminobenzidine (Sigma) and 0.01% hydrogen peroxide in 0.1 M PB for 10 minutes. Cryostat sections were mounted on L-polylysine-coated slides, dehydrated in alcohol, cleared in xylene, and coverslipped with Depex.

For electron microscopic examinations, vibratome sections were immunoreacted as above with the exception that Triton X-100 was omitted in antisera solutions. These sections were then postfixed in 2.5% glutaraldehyde for 20 minutes and in Dalton's chrome-osmium tetroxide solution for 30 minutes, dehydrated, and flat embedded in Araldite between slides coated with Repelcoat (dimethyldichlorsilane). After polymerisation, regions of interest were excised from the tissue and reembedded on Araldite blocks. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined in a Zeiss EM10 electron microscope.

Control sections were incubated in PBS/NGS and PBS/NGS with preimmune-rabbit IgG instead of the primary antisera in the first incubation step and no staining of the tissue resulted. The specificity and characteristics of the rabbit polyclonal antibody against B-50 have been reported previously (Oestreicher et al., 1983; Gorgels et al., 1989; Mercken et al., 1992). Various dilutions of B-50 antibody (in the range from 1:100 to 1:10,000) were tried out in pilot experiments. Optimum conditions for immunohistochemical labeling were found in a dilution of 1:2,000.

The accuracy of hemisections was tested under microscope examination: Thirty micron transverse serial sections were cut through the spinal segment containing the hemisection and Nissl stained with cresyl violet to check the extent of the damage.

Quantitative Analysis

We used an automated image analysis system to quantify the density and spatial extent of B-50-IR.

The area occupied by B-50-IR and the average optical density of this label were measured in the dorsal horn and in the intermediate gray on both sides of the segments L1 to S2 in all hemisected animals and in the intact cat. In the cat that received sciatic nerve transection, the same measurements were carried out in the dorsal horn of the segments L5-S1. Densitometric measurements in the medial portion of the ventral funiculus were made on the control side to provide an internal control for background staining since this region is devoid of B-50-IR. Areas in which the density significantly exceeded the background were coded by colors indicating the intensity of staining. The color-enhanced regions were delineated on the video screen with a mouse-type cursor (separately in the dorsal horn and in the intermediate gray) and used for measurements. The threshold level for B-50 staining was established for sections in each animal measured to reduce the effects of variability of the staining. Measurements were made on 9–15 sections of each segment in all animals. The mean values of areas occupied by B-50-IR and of integrated optical staining densities were calculated on both sides in the

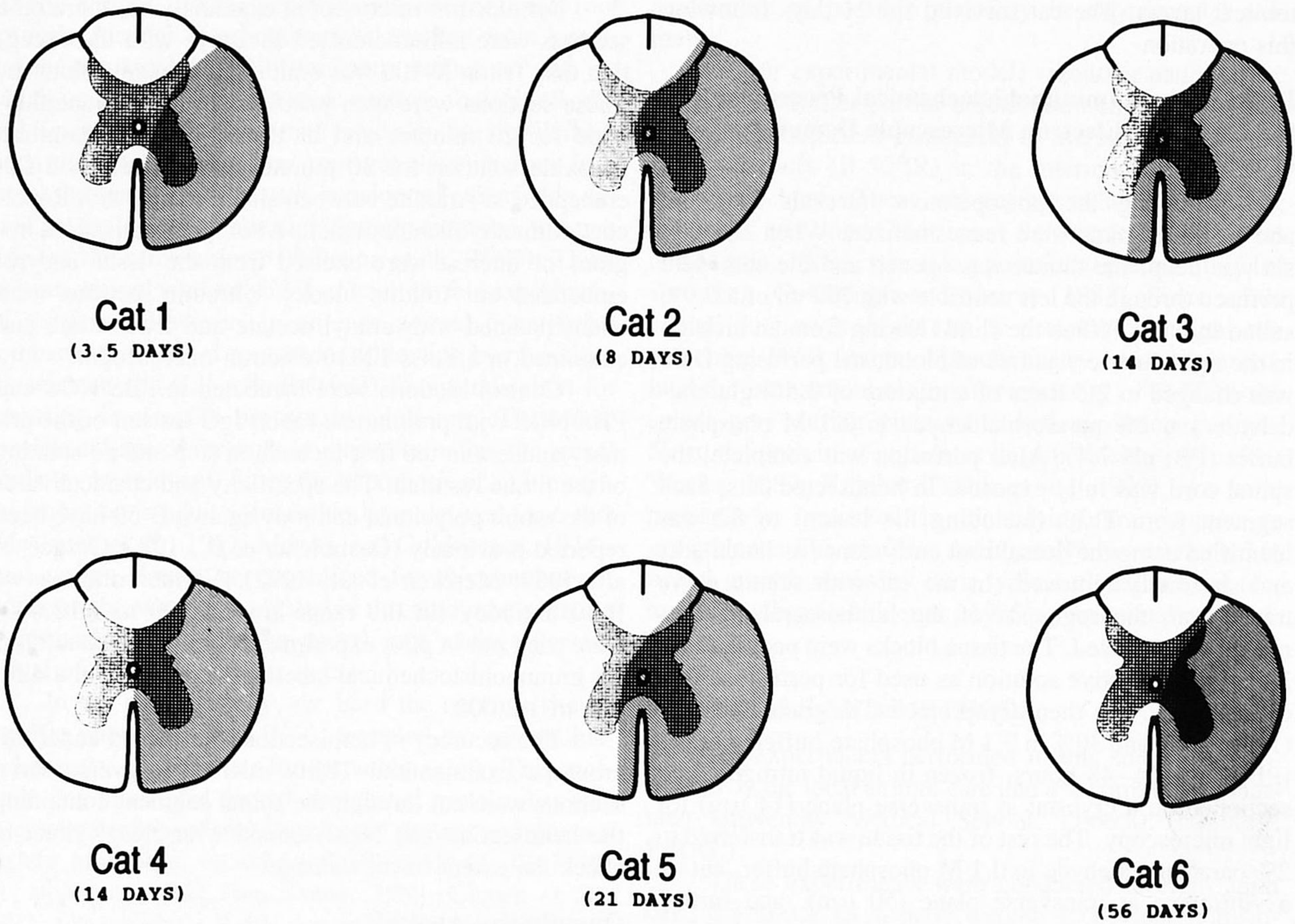


Fig. 1. Shaded areas represent schematically the extent of the lesion at the low thoracic level in each cat, as identified by gliosis and fibrosis in Nissl-stained sections. The postoperative survival stages of the animals are indicated in parentheses.

dorsal horn and in the intermediate gray. The significance of differences between both sides was tested by means of the paired Student's t test. Measurements in one unoperated animal were carried out in order to demonstrate the bilaterally symmetrical distribution pattern of B-50-IR under normal conditions.

RESULTS

Extent of the Lesions

The lesion configuration in each animal, identified by gliosis and fibrosis in Nissl-stained sections, is schematically illustrated in Figure 1. The partial hemisections were intended to spare the dorsal columns. In four cases (cats 1, 3, 4, and 6) the latter appeared to be intact, whereas in two cases (cats 2 and 5) the lesion extended into the lateral edge of the dorsal funiculus. In only two animals (cats 3 and 5), the injury encroached slightly upon the contralateral half in the ventral white matter. In two cats (1 and 6), the lesion spared the dorsal border of the lateral funiculus; the ventral and lateral funiculi were completely destroyed in all other animals.

Light Microscopy in the Unoperated and Hemisected Cats

In both the unoperated and hemisected cats, the distribution pattern and intensity of B-50 staining in the lumbrosacral spinal cord was always bilaterally symmetrical. Moreover, the B-50 labeling did not differ between hemisected animals and the unoperated cat. In the spinal cord, a granular pattern of B-50 staining was confined to the neuropil, whereas the neuronal cell bodies were unreactive. The labeling varied greatly in intensity among different Rexed's laminae, and slightly in distribution pattern between different segments. Typical sections immunostained for B-50 of different segments of a hemisected cat are shown in Figure 2.

In lumbar segments, B-50-IR was conspicuous in

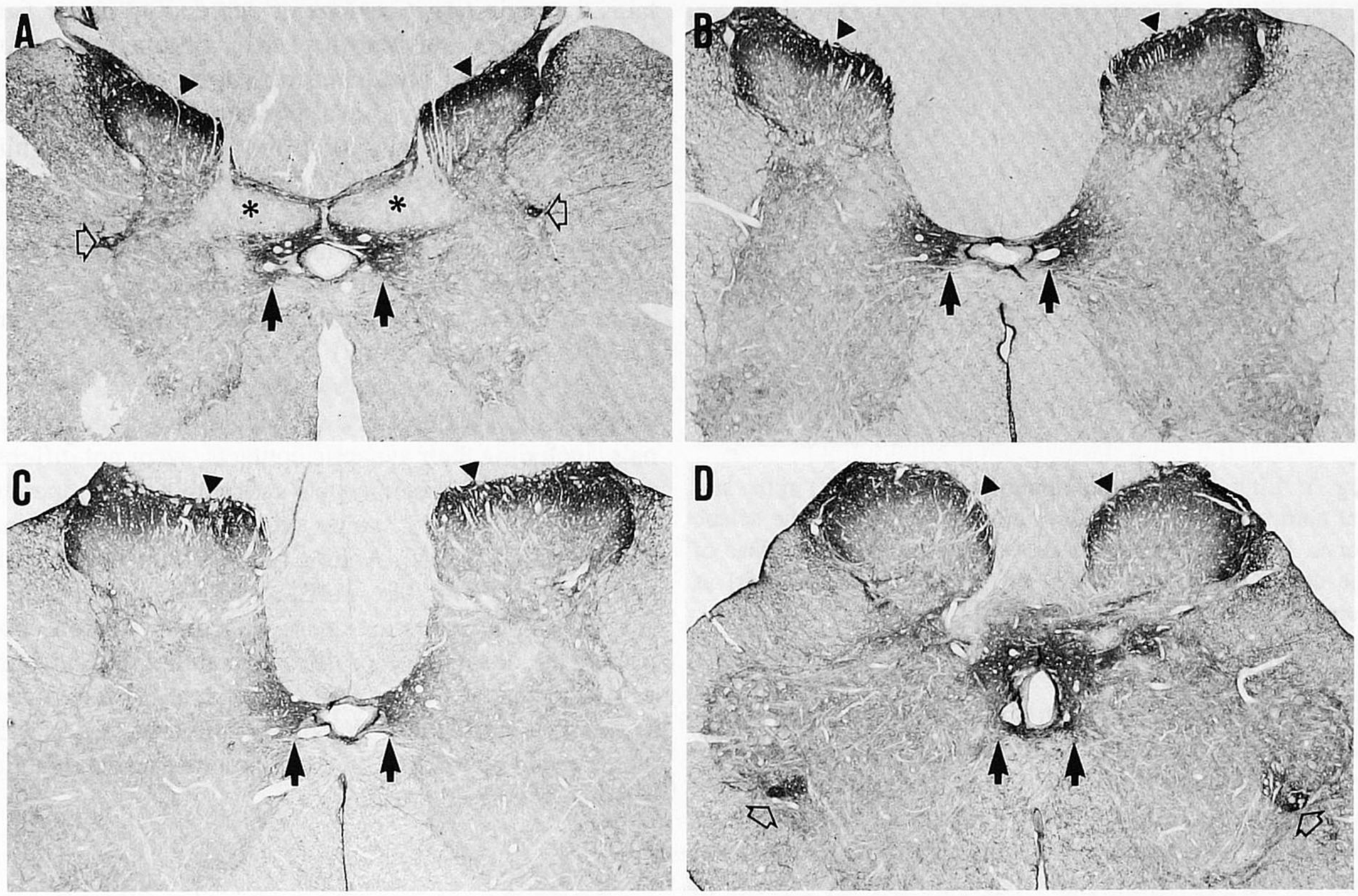


Fig. 2. Light microscopic immunohistochemistry of B-50 in the cat spinal cord below the hemisection, 14 days postoperatively. Transverse sections through spinal segments L3 **(A)**, L5 **(B)**, L7 **(C)**, and S2 **(D)**. Operated side on the left, control side on the right. Note the bilaterally symmetrical immunoreactivity in superficial laminae of the dorsal horn (arrowheads) and in the central portion of the intermediate gray (arrows). In the lateral portion of the intermediate gray, B50-IR is virtually absent. Faint labeling in the ventral horn, except for a well-circumscribed dense immunoreactivity in Onuf's nucleus (D, open arrows). Intense staining in the intermediolateral nucleus (A, open arrows). Clarke's nucleus is unstained (A, asterisks).

superficial Rexed's laminae (I-II) of the dorsal horn. Immunostaining was also present in the central portion of the intermediate zone (mainly lamina X), and in nerve fiber bundles crossing the dorsal gray commissure. In contrast, the lateral portion of the intermediate zone (lamina VI-VIII) was nearly devoid of immunostaining. In cranial lumbar segments, immunoreactivity was found in the neuropil of the intermediolateral columns, whereas the Clarke's nuclei were unstained. Only low levels of B-50 labeling were observed in the ventral horn around lumbar motoneurons (lamina IX).

In the sacral segments examined, the superficial laminae of the dorsal horn and the medial part of the intermediate gray were stained; this was also the case in the lumbar region. Dense B-50-IR was detectable in the neuropil around motoneurons of Onuf's nucleus (Nacimiento et al., 1993b) which innervate striated sphincter muscles (Holstege and Griffith, 1990), whereas only slight labeling was present around the other sacral motoneurons.

Light Microscopy Following Sciatic Nerve Transection

In laminae I-III of those segments in which projections of the sciatic nerve terminate (L5-S1), there was a slight increase in B-50 labeling on the operated side as compared to the control side (Fig. 3). The B-50-IR was otherwise identical with the above mentioned staining pattern.

Quantitative Results

Concerning the extent and density of the B-50 label, no significant difference ($P > .05$) could be established between both sides in the unoperated cat and in all hemisected animals in the measured spinal gray areas of all segments below the lesion. In the cat with sciatic nerve transection, the density of immunolabeling ($P < .05$) was significantly higher in laminae I-III of segments L5-S1 on the operated side as compared to the control side. Furthermore, the area occupied by B-50-IR was

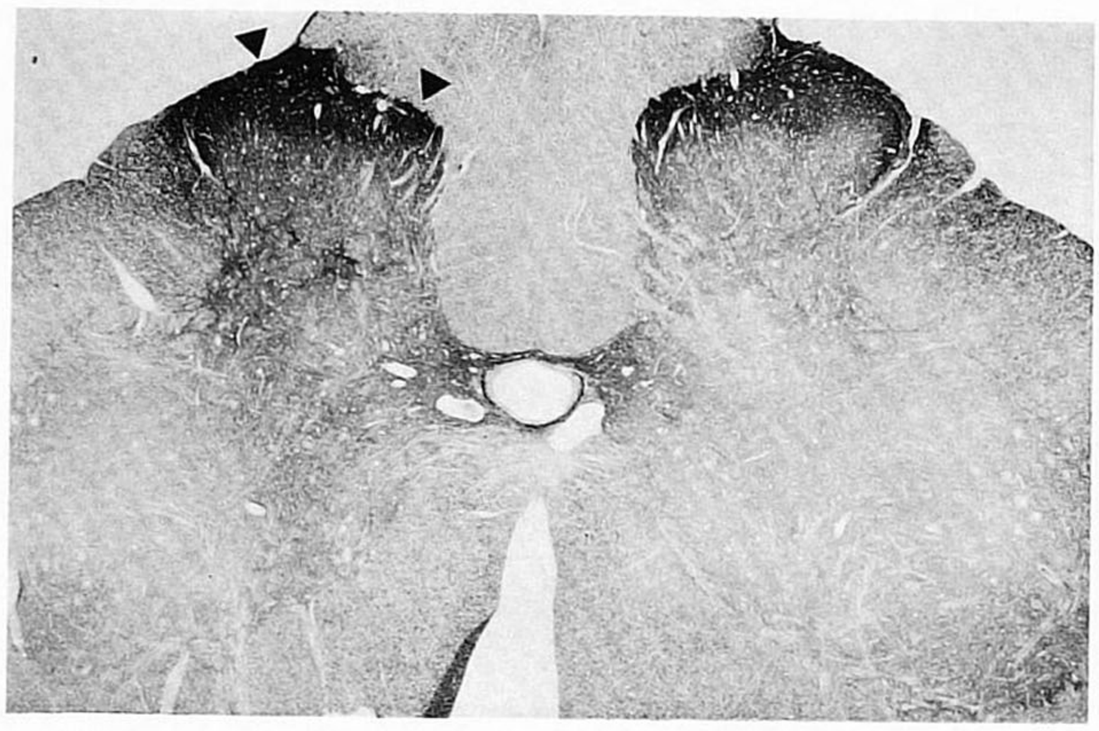


Fig. 3. Light microscopic immunostaining of B-50 in the spinal cord segment L7, 21 days after transection of the sciatic nerve. Note the increase in B-50-IR in superficial laminae of the dorsal horn (arrowheads) on the operated side **(left)** as compared to the control side **(right).**

increased in the dorsal horn on the operated side (except for L7). Examples of the quantitative results for hemisected cats are illustrated in the histograms of Figure 4. These data reflect some interanimal variability of staining. The quantitative results of the unoperated cat and of the cat with sciatic nerve lesion are shown in Figure 5.

Electron Microscopy

The ultrastructural distribution pattern of B-50 immunolabeling in the dorsal horn and the intermediate gray was essentially the same on both sides in all hemisected animals and in the intact cat. No significant differences in staining could be observed with respect to the segmental level below the lesion. Electron microscopy of the cat with sciatic nerve transection is not included in this paper.

In the dorsal horn of hemisected cats (Fig. 6), B-50-IR was mainly found within unmyelinated small diameter nerve fibres. Additionally, some axon terminals were labeled. Myelinated axons, dendritic and somatic profiles of neurons, as well as glial and vascular cells were devoid of immunoreactivity. In agreement with the light microscopic staining pattern, most of the labeled structures were found in superficial laminae (I and II) of the dorsal horn (Fig. 6), indicating that they represent primary afferents (C-fibres) and some of their terminals.

In the intermediate gray, conspicuous B-50-IR occurred in unmyelinated small diameter axons and in axon terminals of the central portion, predominantly in lamina X (Fig. 7A). Immunolabeling was absent in the lateral portion of the intermediate gray (lamina VI–VIII), (Fig. 7B,C, 8A,B). In this part of the spinal gray, which contains the main target regions of descending motor pathways (Kuypers and Martin, 1981), degenerating axons are present at all postoperative stages on the operated side (Fig. 7B). The removal of axon terminals is almost completed a few days after the lesion (McLaughlin, 1972). After phagocytosis of degenerated boutons by microglial cells (Fig. 7C), somatic and dendritic surfaces of neurons were covered by intact synapses and by astrocytic processes (Figs. 7B, 8A). The latter appeared to be more extensive on the operated side (Fig. 8A) as compared to the control side (Fig. 8B).

In the intermediate gray and in the dorsal horn, the ultrastructural features and the size of intact axon terminals, including their synaptic contacts, were not different on the two sides in hemisected cats and in the unoperated cat. On both sides, some axon terminals contained B-50-IR (figs. 6, 7A). Among the immunoreactive structures, growth cone-like profiles were not detectable in any case. The examination of unstained sections confirmed the absence of profiles containing typical ultrastructural features of growth cones, such as cisternae of axoplasmic reticulum (Knyihar-Csillik et al., 1985), which could be masked by the diaminobenzidine reaction product in stained samples.

DISCUSSION

The considerable degree of functional recovery which follows the initial severe deficits resulting from spinal lesions to descending motor pathways in cats has been in part ascribed to an anatomical reorganization of intact remaining systems (McCouch et al., 1957; Murray and Goldberger, 1974). The present study considers the question of intraspinal axonal sprouting and reactive reinnervation by undamaged systems in response to removal of descending pathways in cats, which has been suggested as a mechanism of structural reorganization (McCouch et al., 1957, Murray and Goldberger, 1974). In this experimental paradigm, sprouting could arise from dorsal root axons and from axons of intraspinal neurons. Both systems partially overlap in terminal fields with the degenerated descending tracts in this preparation (Kuypers and Martin, 1981), a postulated precondition for sprouting (Goldberger and Murray, 1978, 1988).

We chose cats for these studies because 1) this species has been used in previous silver impregnation studies, in which sprouting of dorsal root axons has been proposed to contribute to the recovery in locomotion (McCouch et al., 1957: Murray and Goldberger, 1974), and 2) the functional alterations following the lesion, in particular the initial paresis of the affected hindlimb and the following increase in intrinsic reflexes mediated by ipsilateral dorsal root input (McCouch et al., 1957), are

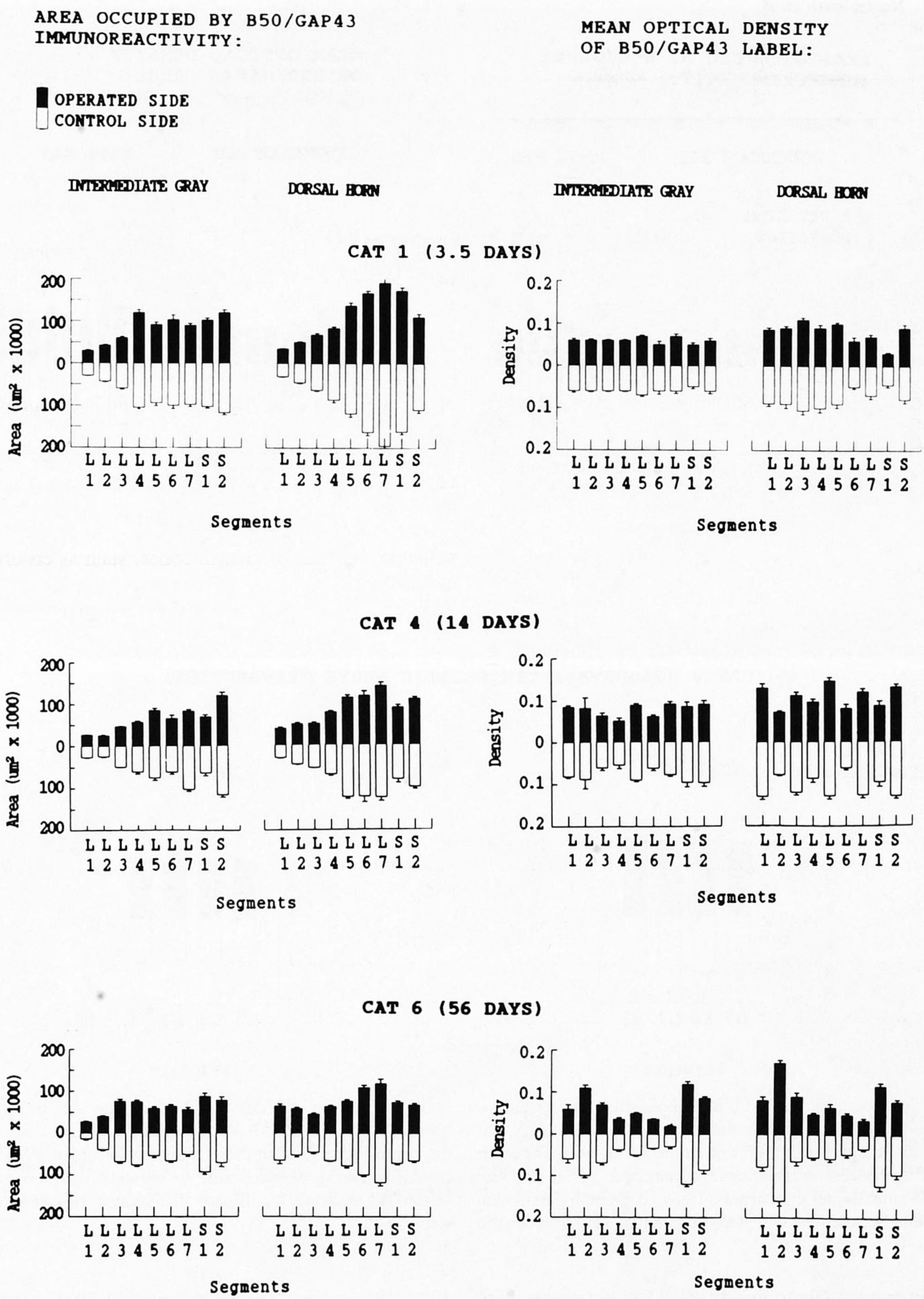


Fig. 4. Quantitative results of cats 1, 4, and 6; the postoperative stages are indicated in parentheses. The histograms show the area occupied by B-50 immunoreactivity and the mean optical density of the label in putative target regions for sprouting, the intermediate gray and the dorsal horn, in segments L1–S2. Measurements were made with an automated image analysis system. Note that no significant differences in both parameters were found between the two sides in any of the hemisected animals.

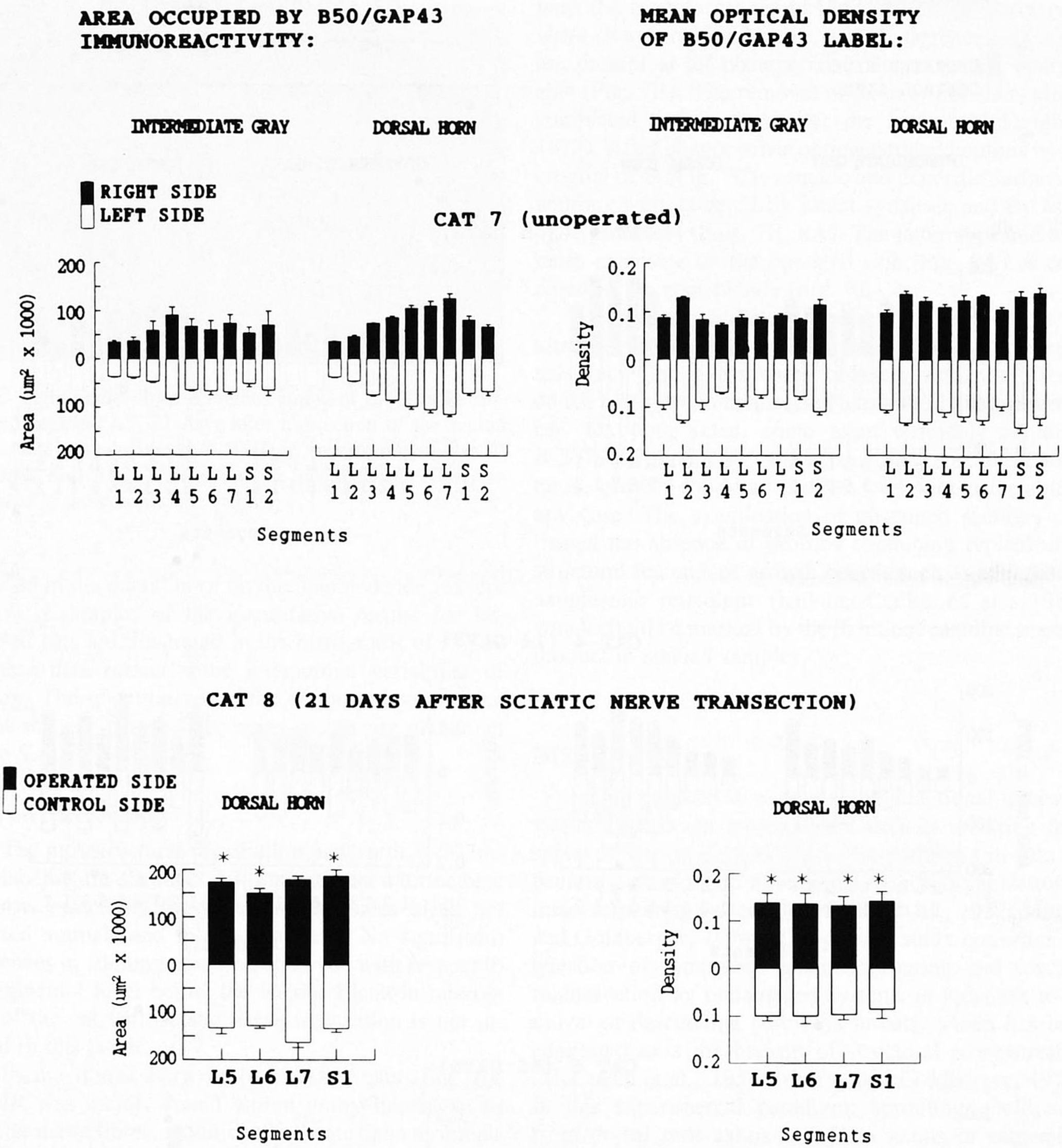
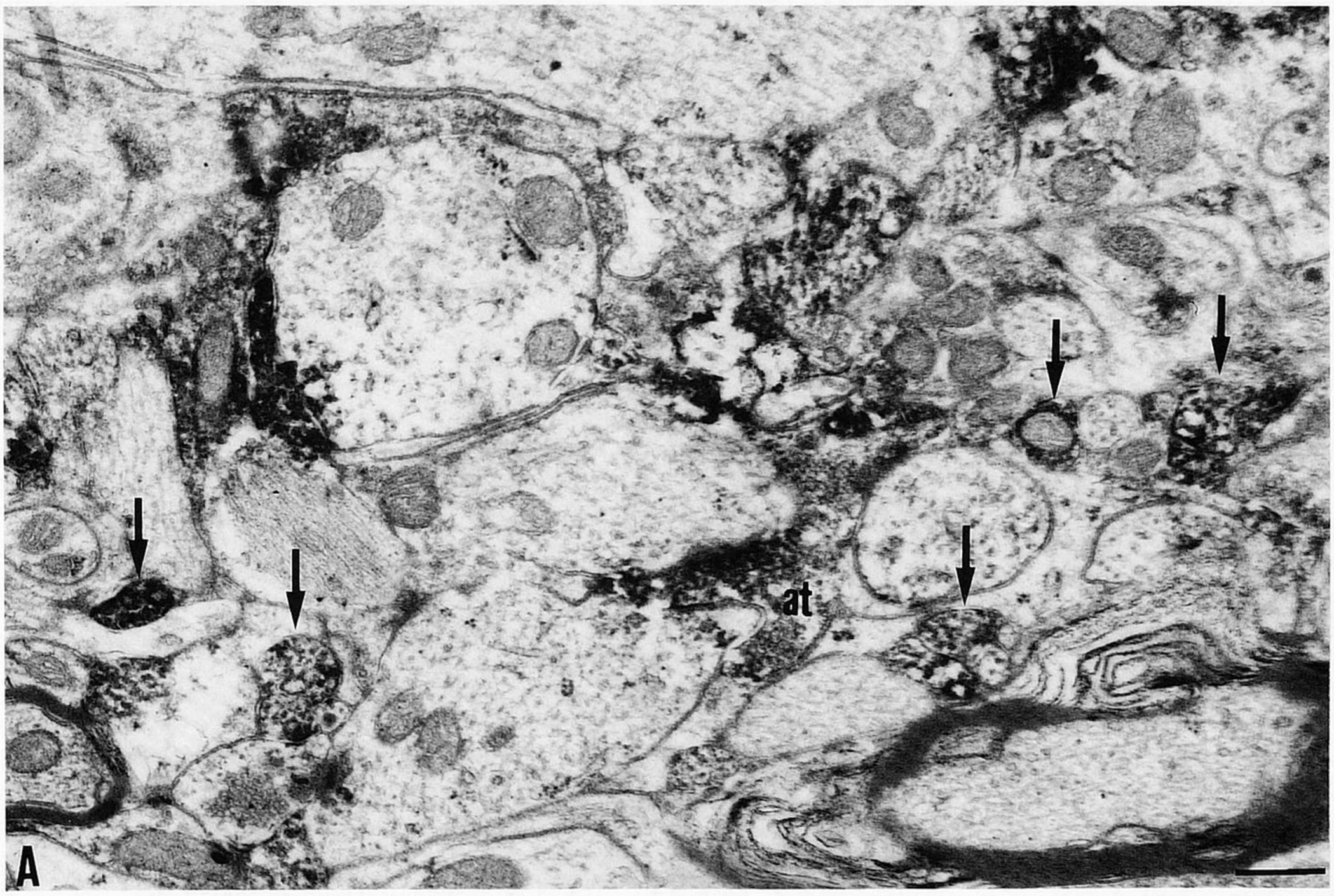


Fig. 5. Area occupied by B-50 immunolabeling and mean optical density of the staining in the intermediate gray and dorsal horn of the unoperated cat (7) and in the dorsal horn of the cat (8) that underwent sciatic nerve transection. As in the hemisected animals, no significant differences in both parameters were found between the two sides in the intact cat. After sciatic nerve transection, the density of label increases in the dorsal horn of segments L5-S1 on the operated side as compared to the control side. Furthermore, the area occupied by this label increases on the operated side in segments L5, L6, and S1. (Asterisks indicate significant differences between the two sides.)

partially comparable to the situation in human spasticity (Noth, 1991).

The phosphoprotein B-50 (GAP-43) has been functionally implicated in regenerative and developmental axonal outgrowth and synaptic plasticity (see Skene, 1989, for review). The reliability of B-50 (GAP-43) immunohistochemistry for demonstrating neuronal sprouting and subsequent synaptogenesis in response to partial denervation has been recently confirmed in the rat hippocampus following removal of specific afferents (Be-



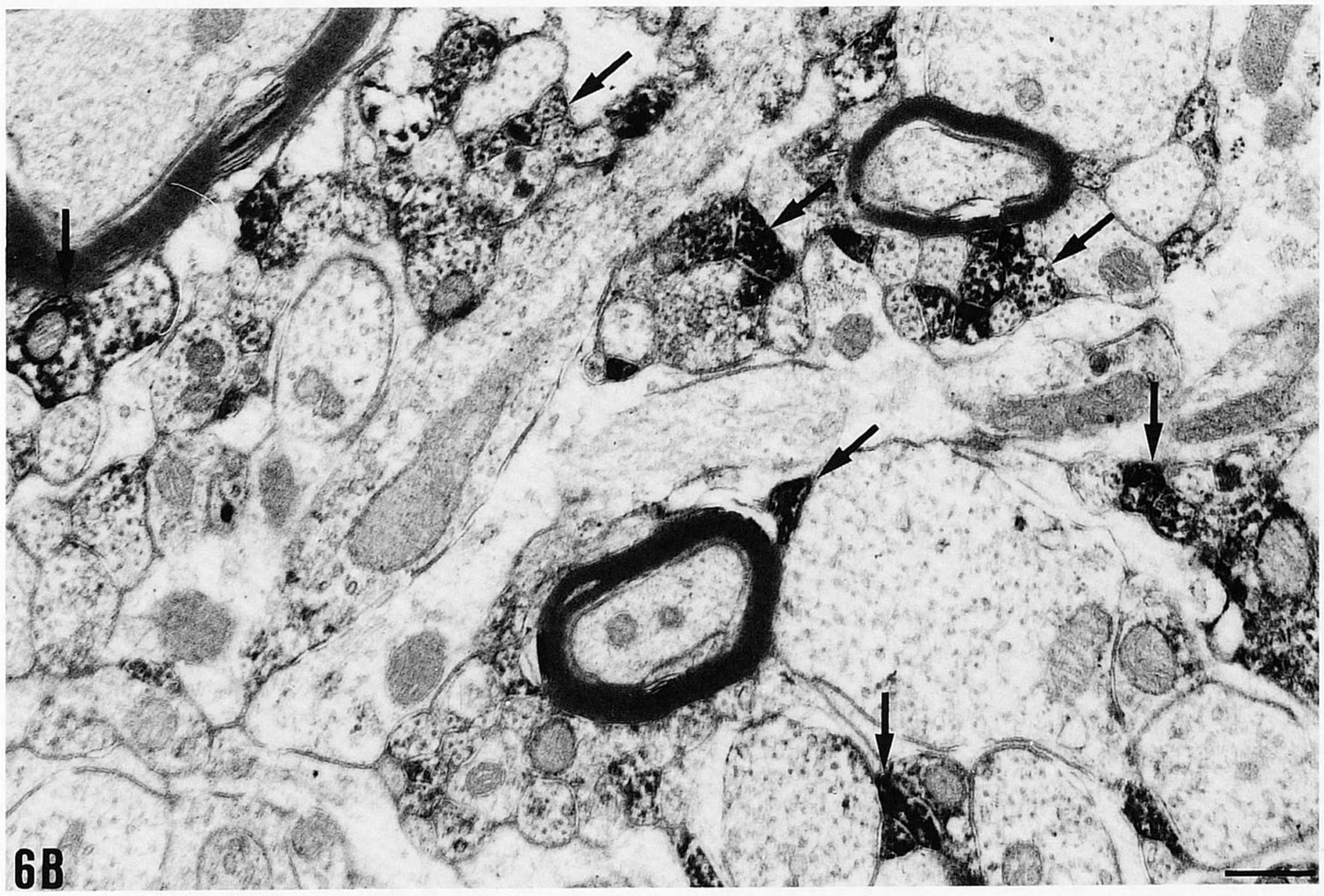


Fig. 6. Electron micrographs showing B-50-IR in superficial laminae of the dorsal horn, caudal part of L4 segment, 14 days after hemisection, (**A:** operated side, **B:** control side). On the two sides, the labeling predominates in small diameter unmyelinated fibres (arrows). Additionally, some axon terminals (at) are stained. (Scale bar: 0.4 μm.)

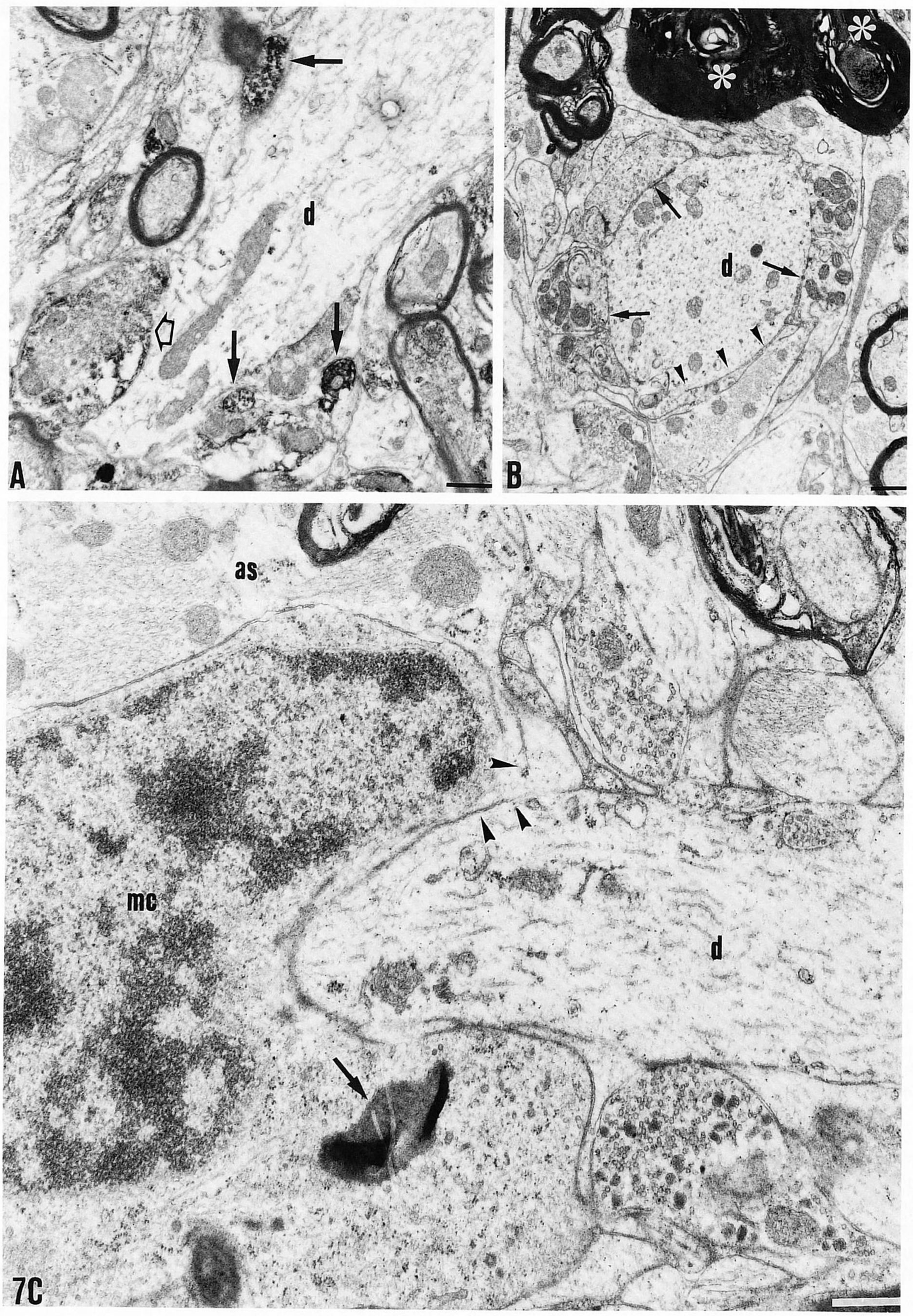


Fig. 7.

nowitz et al., 1990; Mashliah et al., 1991). The suitability of this method for our purpose has been further confirmed in a very recent light microscopic study by Curtis and coworkers (1993b). These authors found an up-regulation of GAP-43 synthesis in some axotomized intraspinal neurons after compression injury of the rat spinal cord. GAP-43 immunoreactivity was detected in both cell bodies and outgrowing axons adjacent to the lesion-induced gray matter cavities and also in axons within these cavities.

In the mature intact nervous system, B-50 (GAP-43) has been shown to persist in specific regions which presumably maintain their capacity to engage in structural remodeling and functional plasticity in adulthood (Benowitz et al., 1988, 1989; De la Monte et al., 1989; Stewart et al., 1992). In the uninjured adult spinal cord of rat and cat, the intensity of B-50 (GAP-43) immunolabeling varies in different studies (Woolf et al., 1990; Schreyer and Skene, 1991; Arvidsson et al., 1992; Knyihar-Csillik et al., 1992; Curtis et al., 1993a; Nacimiento et al., 1993a) which may be related to differences in antibodies recognizing different epitopes of the protein (Schreyer and Skene, 1991). Moreover, these discrepancies may be explained by variations in the titers of antibodies used, differential detoriation of the antigen by the different types of fixation, and the occurrence of different contents in B-50 within neuronal systems of different species or of different strains within the same species. In the intact spinal cord, the protein is predominantly localized in unmyelinated primary afferent fibres in the dorsal horn (Knyihar-Csillik et al., 1992; Curtis et al., 1993a; Nacimiento et al., 1993a). Thus, the B-50 positive dorsal root axons seem to be particularly capable of participating in posttraumatic structural rearrangement (e.g., sprouting) which then shows an increase in B-50-IR (Knyihar-Csillik et al., 1992). In our model, plasticity may also derive from primary afferent systems with no constitutive B-50 expression in which the protein is newly produced in response to injury (Van der Zee et al., 1989; Verge et al., 1990; Sommervaille et al., 1991).

We found a bilaterally symmetrical immunohistochemical staining pattern of B-50 in the spinal cord of the unoperated cat, a prerequisite for studying possible plasticity post-hemisection. Furthermore, the immunohistochemical staining pattern was identical in hemisected and unoperated animals. This is of major relevance for the assessment of possible posttraumatic alterations in B-50-IR since in hemisected animals the control side is also affected. The lesion includes ascending axons which arise from sensory neurons on the control side and cross at the segmental level. Thus, the B-50-IR is not influenced by the axotomy of these sensory neurons on the control side. Moreover, in the cat that underwent sciatic nerve transection, we found an increase in B-50-IR in the ipsilateral dorsal horn of spinal segments in which projections from this nerve terminate (Tessler et al., 1985). This control experiment confirms that our antibody is capable of revealing plasticity in the cat spinal cord with the same reliability as previously shown in the rat spinal cord (Knyihar-Csillik et al., 1992; Woolf et al., 1990, 1992a). Hence, this method would allow the detection of intraspinal axonal sprouting after hemisection in the cat, independent of the source of sprouting.

In the present work, the removal of descending pathways did not elicit a detectable increase in B-50-IR in putative target regions for sprouting which would be compatible with plasticity of intact remaining systems, particularly of dorsal root axons. In the lumbosacral cord of all cats, B-50-IR was nearly absent in laminae VI–VIII. This is an important region in terms of motor recovery with maximum convergence of descending motor pathways and primary afferents (Kuypers and Martin, 1981), and therefore a presumptive main target for sprouting (Goldberger and Murray, 1978). Furthermore, a bilaterally symmetrical B-50 staining was observed in the superficial laminae of the dorsal horn. Here, descending serotonergic projections from the raphe nuclei, which are presumably important in the regulation of locomotion (Rossignol et al., 1986), overlap with terminals of primary afferents (Ruda et al., 1982; Rossignol et al., 1986; Pubols et al., 1990). In these relevant regions, we could not detect any growth cone-like profiles at the ultrastructural level which should be readily revealed by B-50 immunolabeling (Coggeshall et al., 1991; Knyihar-Csillik et al., 1992) or by typical subcellular features (Knyihar-Csillik et al., 1985) in unstained material. Interestingly, in partially denervated areas of the spinal gray, dendritic and somatic profiles of neurons appear to be more extensively covered by astrocytic processes on

Fig. 7. Electron micrographs of the intermediate gray, caudal part of L4 segment, tissue reacted for B-50. (**A:** Operated side, medial portion, 56 days postoperatively; **B,C:** operated side, lateral portion, 14 days postoperatively.) **A:** Intense labeling within many unmyelinated small diameter axons (arrows); faint staining within an axon terminal (open arrow) on a dendrite (d). (Scale bar: 0.4 μm.) **B:** Degenerated large myelinated axons of descending pathways (asterisks) in close proximity to a dendrite (d) covered by an astrocytic process (arrowheads) and by intact remaining axon terminals (arrows). Immunoreactive structures are absent. (Scale bar: 0.4 μm.) **C:** Microglial cell (mc) apparently after phagocytosis of degenerated axon terminals on the surface of a dendrite (d). Cytoplasmatic inclusion of phagocytosed material (arrow) within the microglial cell. Note that a process (arrowhead) of an astrocyte (as) appears to invade between the dendrite and the microglial cell. Labeled profiles are lacking. (Scale bar: 0.4 μm.)

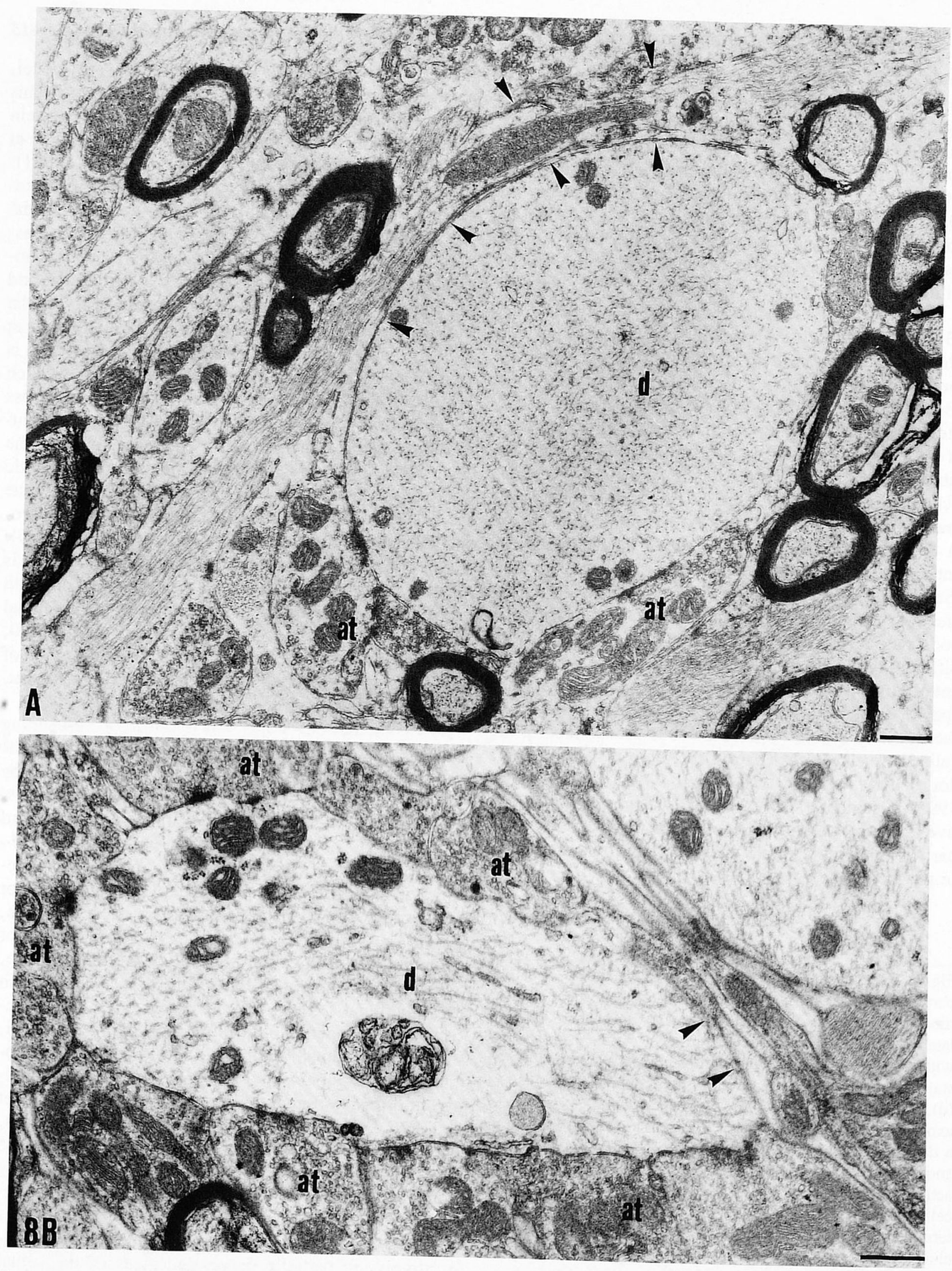


Fig. 8. Electron micrographs showing the lateral portion of the intermediate gray, caudal part of L4 segment, 14 days postoperatively, tissue reacted for B-50. Immunolabeled structures are absent in this region. **A:** Dendrite (d) which is extensively covered by astrocytic processes (arrowheads). Intact axon terminals (at). (Scale bar: 0.4 μm.) **B:** Typical dendrite (d) on the control side which is predominantly covered by axon terminals (at). Only a small part of the dendritic surface is covered by astrocytic processes (arrowheads). (Scale bar: 0.4 μm.)

the operated side as compared to the control side. This indicates that, following the removal of degenerated terminals, the vacated postsynaptic places are occupied by glial extensions rather than by new terminals.

Taken together, these results do not support the idea that in the cat primary afferents sprout in response to injury of descending tracts into spinal regions with significant convergence of both systems. Our data are in apparent contrast to the interpretation of previous silver impregnation studies in which an increased density of dorsal root projections has been most clearly revealed in the intermediate spinal gray caudal to the hemisection (McCouch et al., 1957; Murray and Goldberger, 1974). Possibly, changes in staining properties of chronically denervated tissue leading to silver deposition in non-axonal profiles may account for these findings (Matthews and Kruger, 1973; Rodin and Kruger, 1984). For instance, lysosomes of glial cells in chronically denervated spinal tissue are likely to be argyrophilic (Maxwell and Kruger, 1973). Another explanation for these conflicting results is that the impregnation of degenerating axons may be more resistant to suppression in chronically denervated than in normal tissue (Rodin et al., 1983; Rodin and Kruger, 1984).

Our results do not call into question the fact that in experimental conditions other than hemisection, sprouting of dorsal root axons and intraspinal synaptic remodeling occur in response to denervation. For instance, these mechanisms of plasticity have been clearly demonstrated by means of more recent anatomical techniques after complete or partial unilateral spinal deafferentation by injury of primary afferents and following peripheral nerve transection (Hulsebosch and Coggeshall, 1981; Tessler et al., 1984, 1985; Murray and Goldberger, 1986; LaMotte et al., 1989; McNeill et al., 1990, 1991; Polistina et al., 1990; McMahon and Kett-White, 1991; Knyihar-Csillik et al., 1992; Woolf et al., 1992a). Thus, the occurrence of sprouting and reactive reinnervation in the spinal cord may depend on the type of denervation.

There are several electrophysiological studies that suggest increased motoneuronal excitability following chronic spinal transection or hemisection in cat (see Mendell, 1984, for review) and this is in keeping with lesion-induced functional alterations (e.g., increase in intrinsic reflexes). Accordingly, EPSP amplitude recorded from motoneurons is enhanced after chronic spinal transection (Mendell, 1984). Our results are not compatible with the hypothesis that collateral sprouting of dorsal root axons and subsequent reinnervation of interneurons projecting to motoneurons might account for these physiological alterations. The latter may be explained by other mechanisms, such as denervation supersensitivity or non-neuronal (e.g., glial) factors. An attractive hypothesis is that a strengthening of latent or silent synapses on motoneurons, normally present but ineffective, may mediate the functional changes (Merrill and Wall, 1973). A possible morphological substrate for the unmasking of functionally ineffective synapses has been recently described in the rat phrenic nucleus after an ipsilateral hemisection rostral to the nucleus (Goshgarian et al., 1989, 1991). Here, a significant increase in the number of double synapses on motoneurons and in the length of dendrodendritic membrane appositions was noted as early as 4 hours post-hemisection. This phenomenon is most likely due to an active retraction of astroglial processes from their normal position on the neuronal surface. Current investigations are under way in order to study whether comparable changes occur in the lumbar ventral horn of cat spinal cord following low thoracic spinal hemisection.

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