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Morphology of Interneurones in Pathways From Group II Muscle Afferents in Sacral Segments of the Cat Spinal Cord

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ABSTRACT

The morphology of 12 sacral interneurones with peripheral input from group II muscle afferents was analyzed after intracellular injection of horseradish peroxidase (HRP). The neurones were located in Rexed's laminae III–V overlying the pudendal (Onuf's) motor nucleus. The interneurones had medium sized elongated somata and dendrites projecting radially. All of the interneurones were funicular neurones and fell into two categories depending on whether their axons ran within the dorsal part of the lateral funiculus (DLF; n = 7) or within the ventral funiculus, or the ventral part of the lateral funiculus (VF or VLF; n = 4). The latter were located more rostrally. Within the DLF similar proportions of stem axons and secondary axonal branches descended and ascended. Within the VF and VLF all of the axons ascended. Collaterals of axons running in the DLF arborized primarily within the dorsal horn and the intermediate zone; none were found to approach the motor nuclei. In contrast, collaterals of axons running in the VF/VLF arborized in both the intermediate zone and the ventral horn and passed close to the motor nuclei. We conclude that sacral interneurones with group II input are morphologically nonhomogenous and that only those located most rostrally might have direct actions upon motoneurones. Both the axonal projections and the input (from group II but not from group I muscle afferents and from skin afferents) of sacral interneurones indicate that they are homologous to dorsal horn group II interneurones in the midlumbar segments. They appear, however, to form part of more local neuronal networks than their midlumbar counterparts.

Key words: spinal interneurones, reflex pathways, HRP, intracellular labelling

A population of interneurones relaying information from group II muscle afferents and skin afferents has recently been found in the sacral segments of the cat spinal cord (Jankowska and Riddell, '93b). These neurones lie in the dorsal horn at the level of the pudendal (Onuf's) motor nucleus, i.e., at the same level as motoneurones innervating the external urethral and anal sphincter muscles, and caudal to most of the motoneurones innervating hindlimb muscles. The interneurones differ from previously studied interneurones with input from muscle group II and skin afferents in the midlumbar segments (Edgley and Jankowska, '87; Cavallari, et al., '87; Bras et al., '89) in that they process information from different muscles and from different areas of skin. The sacral interneurones appear in addition to be homologues of only one of the two populations of midlumbar interneurones with group II input: they are excited by group II muscle afferents, like the midlumbar dorsal horn interneurones, and not by both group I and group II muscle afferents, like the intermediate zone midlumbar interneurones. Furthermore, while the intermediate zone midlumbar interneurones project to lower lumbar and sacral segments, where they have direct actions upon motoneurones, there is as yet no evidence that the sacral interneurones contact motoneurones (Jankowska and Riddell, '93b). In the present study intracellular labeling of electrophysiologically characterized sacral interneurones with input from group II afferents has been used to investigate the morphology of this population of neurones and to compare it with the morphology of the midlumbar interneurones. One of the main aims was to define the areas of terminal axonal arborizations of sacral interneurones and thereby to determine the location and likely identity of their target neurones. Another aim was to investigate the degree of morphological heterogeneity within the population of sacral interneurones with group II input. The results of previous studies suggest that these interneurones

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include at least three functional subpopulations: interneurones with excitatory or inhibitory postsynaptic actions (on other interneurones and/or ascending tract neurones) and interneurones mediating presynaptic inhibition of primary afferents (see Discussion of Jankowska and Riddell, '93b). It was therefore of interest to find out whether the sacral interneurones fall into morphological groups corresponding to the subpopulations suggested by their functional properties.

MATERIALS AND METHODS

Preparation

Twelve sacral interneurones with input from group II muscle afferents were successfully labelled with horseradish peroxidase (HRP) in three deeply anaesthetized cats. The anesthesia was induced with one dose of pentobarbital (40 mg/kg intraperitoneally) and was maintained with several doses of chloralose (up to 50 mg/kg). The depth of anesthesia was verified by monitoring withdrawal and pupillary reflexes. The preparation, the main experimental procedures, and the animal care were as described in the preceding papers of this series (Jankowska and Riddell, '93a,b).

Selection of interneurones to be labeled

Interneurones were searched for in the area of the sacral spinal cord in which large monosynaptic focal field potentials were evoked by group II muscle afferents of the posterior biceps-semitendinosus (PBST) and/or gastrocnemius-soleus (GS) nerves, that is, in the dorsal horn area overlying and just rostral and/or caudal to the pudendal motor nucleus (as described by Jankowska and Riddell, '93b; see Fig. 1). Interneurones were searched for and injected within regions that were 0.5–1.0 mm apart to avoid overlap of labeled structures. In two experiments neurones were labelled bilaterally. The neurones were usually first recorded from extracellularly, before their spike generating mechanism was damaged by penetration, to select a neurone with input from group II muscle afferents that did not project rostral to the lumbosacral enlargement. Any neurones antidromically activated by stimuli applied to the left or right lateral funiculi at the Th13 level were eliminated from further investigation. These tests having been completed, an attempt was made to penetrate the neurone, either in the same electrode track or 20–100 μm away. After a successful penetration the absence of antidromic activation from beyond the lumbosacral enlargement was confirmed, postsynaptic potentials (PSP)s evoked from peripheral nerves were recorded, and HRP was injected.

HRP application and histological procedures

HRP was ejected iontophoretically from microelectrodes filled with a 7% solution of HRP (Toyobo, grade 1C) in Tris buffer containing 0.3 M KCl (pH 8.6); the electrodes had a tip diameter of about 1.5 μm and a resistance of 7–15 MΩ. HRP was iontophoresed by passing a constant positive current of 5–15 nA for 1–28 minutes, giving 10–190 nA × minutes (Table 1, column 3). Ejection of HRP was continued as long as simultaneously recorded PSPs showed that the electrode remained in the cell. The cells were injected 2–10 hours before perfusion.

The cats were perfused through the thoracic aorta, first with 1 liter of phosphate buffer (0.15 M at pH 7.4, with 2,500 IE heparin and 40 mg xylocaine) and then with 2 liters of a 2% glutaraldehyde–1% paraformaldehyde solution [with 5% dimethyl sulfoxide (DMSO)] at room temperature. The spinal cord was then removed and kept in 5% sucrose solution in phosphate buffer overnight. Serial 60 μm sections were cut either in the transverse plane (two cats) or in the sagittal plane (one cat; cells no. 1, 2, 7, and 8) on a vibratome. HRP was revealed according to the procedure of Hanker et al. ('77) with cobalt intensification (Adams, '77), after which the sections were counterstained with toluidine blue.

Reconstructions

Dendritic arborization, axons, and axon collaterals were reconstructed with the aid of a Leitz Dialux microscope fitted with a drawing tube attachment (at a magnification of ×300 or ×480; oil-immersion) and projected onto an outline of the spinal cord drawn from a section containing the soma of the stained neurone. When dendrites and initial axon collaterals overlapped, the dendritic trees and the axon-collaterals were redrawn separately. Dendrites seen on neighboring sections were usually easy to match except for the smallest distal dendritic branches; any profiles of uncertain origin were excluded. Reconstructions of stem axons met with another problem since the intensity of axonal labeling often decreased with increasing distance from the nodes of Ranvier, most likely because of poor accessibility of the chromogen to myelinated segments of axons deep in the section (Light and Kavookjian, '88). In several cases 100–200 μm long sections of the axons were hardly visible or not visible; these sections are indicated by dashed lines in Figures 2, 4, 5, 6, and 7. Axon lengths were measured by tracing the trajectory of the axons within the plane of the sections by using a digitizing tablet and adding the distance over which they ran perpendicularly to the plane of the sections. The same procedure was used to measure the length of individual dendrites and axon collaterals. These measurements were supplemented with measurements of straight line distances between the distal ends of dendrites or axon collaterals and the soma. As an indication of the size of the somata, measurements were made of their maximum cross-sectional areas and of their long and short axes, both in the plane of sectioning. The cells were photographed with a Leitz Vario Orthomat 2 automatic camera and Agfa Copex PAN A.H.U. Tri 13 film (16 ASA). The reconstructions were reproduced by a scanner (HP Scanjet IIc) and Image In software.

RESULTS

Functional characteristics of the labeled interneurones

In all of the labeled interneurones short latency, most likely monosynaptic EPSPs, were evoked by *group II muscle afferents* of the PBST and/or the GS nerves. In half of these neurones monosynaptic excitatory postsynaptic potentials (EPSPs) were also evoked by *cutaneous afferents* of the sural (Sur) nerve and/or fast conducting afferents, most likely of cutaneous origin, in the pudendal (Pud) nerve (Jankowska and Riddell, '93b). The latencies of these EPSPs were $\leq$2.2 ms from group I volleys and $\leq$0.9 ms from cutaneous volleys. EPSPs from *group I muscle afferents* were found only in one of the most rostrally located neurones (no. 2). Examples of EPSPs are shown in Figures 2, 4, 5, 6, and 7. These data show that the input to the labeled neurones was similar to that of the larger sample of

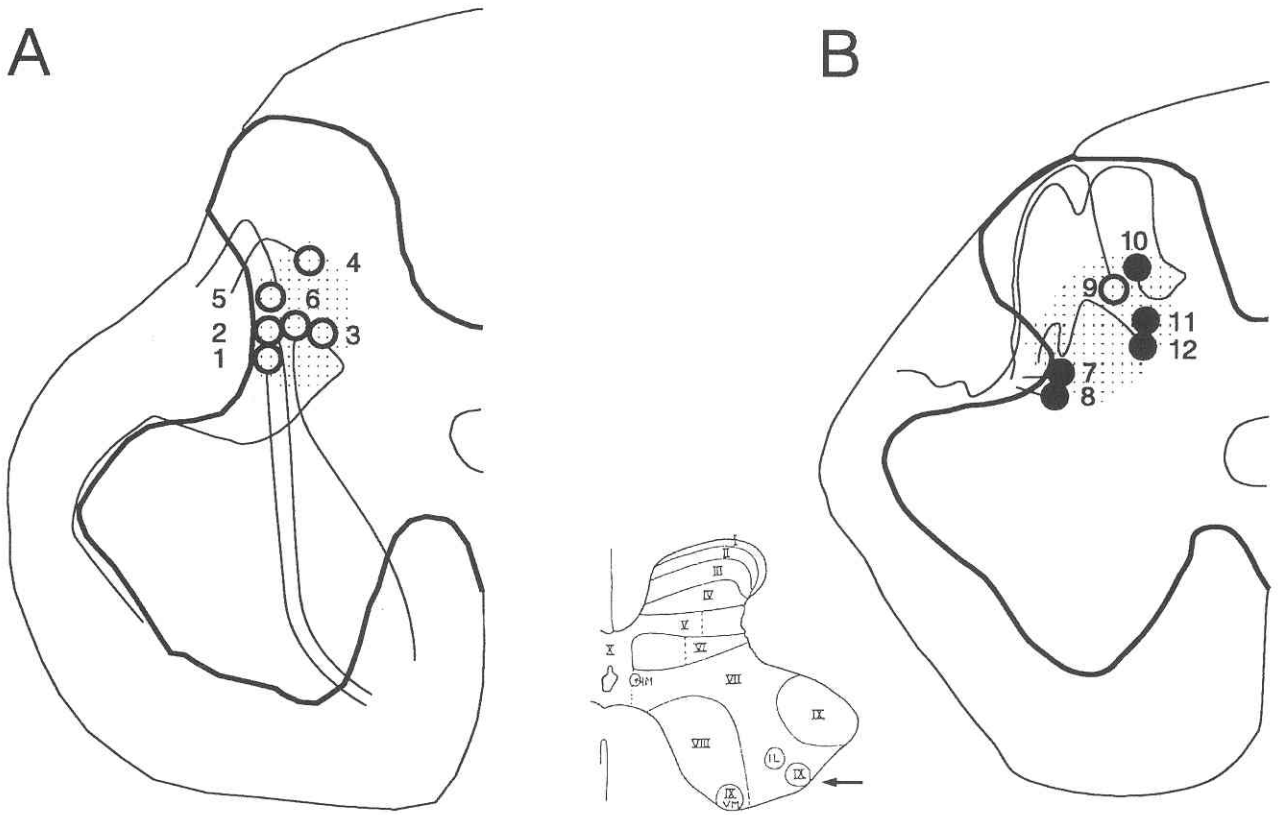

Fig. 1. Location of cell bodies and trajectories of stem axons of the labeled interneurones. **A,B:** Location of neurones within the gray matter overlying the rostral and caudal part of Onuf's nucleus, respectively: 0.5 mm rostral to 1.4 mm caudal (A) and 1.5 mm to 3.4 mm caudal (B) of its rostral border. The numbers correspond to the cell numbers in Table 1. Open circles are for neurones projecting rostrally. Filled circles are for neurones projecting caudally, or with bifurcating axons that were directed both rostrally and caudally. The shading indicates the area in which neurones investigated by Jankowska and Riddell ('93b) were located. Rexed's laminae at the level of Onuf's nucleus are indicated in the **inset** (reproduced from Rexed, '52); they are marked by I–X. Onuf's nucleus is indicated by the arrow. IM, nucleus intermedio-medialis; IL, nucleus intermedio-lateralis; VM, nucleus ventro-medialis.

sacral interneurones described by Jankowska and Riddell ('93b). The cell bodies of the two samples were also similarly located: within the dorsal horn or the dorsal part of the intermediate zone overlying, or just rostral to the pudendal (Onuf's) motor nucleus (Fig. 1; Table 1, column 2). The sample of labeled interneurones is thus considered representative of sacral interneurones with group II input.

General morphological characteristics of the labeled interneurones

The degree of labelling of the 12 interneurones injected with HRP varied greatly. The soma was visualized in ten neurones, while in two neurones the HRP appeared to have leaked from the soma. The dendritic trees were seen in all neurones, the stem axon in all but one, and axon-collaterals in nine neurones. However, in only five of the neurones were stem axons labeled over distances exceeding 3 mm (Table 1, columns 5–8) and in only four neurones could axon collaterals be followed to their terminals (Table 1, column 12).

All of the neurones with labeled axons were funicular neurones. All these neurones projected ipsilaterally but they fell into two categories depending on the trajectory of the axons; axons of seven neurones ran in the dorsal part of the lateral funiculus (DLF) and of four neurones in the ventral funiculus (VF) or the ventral part of the lateral funiculus (VLF). Since neurones with different axonal trajectories might represent distinct functional populations, these two groups of neurones will be described separately.

Interneurones with axons in the dorsolateral funiculus

Cell bodies and dendritic trees. The cell bodies of neurones of this group (nos. 4, 5, 7, 8, 9, 10, and 12) were located in Rexed's laminae III–V, overlying Onuf's nucleus between 0.8 and 3.4 mm caudal to its rostral border (see Fig. 1 and Table 1, column 2). The maximal cross-sectional areas of the somata were 29–85 μm^2 and their short and long axes 18–20 μm and 20–62 μm, respectively (Table 1, columns 13 and 14; mean 32.2 μm). The cells had an average of 6.4 primary dendrites that formed variously shaped dendritic trees. Reconstructions of some of these are shown in Figures 2A,C, 4, and 5, and a photomontage of one of them is shown in Figure 3B. The dendrites extended over longer distances dorsally and ventrally (on average 390 and 430 μm from the soma) than medially (100 μm), laterally (220 μm), rostrally (150 μm), or caudally (150 μm). The dendrites of two of the neurones (nos. 7 and 12) displayed dendritic spines (illustrated in Fig. 3F).

TABLE 1. Location and Morphological Features of 12 Interneurones Successfully Labeled With Horseradish Peroxidase (HRP)[1]

1 No.	2 Rostro-caudal level (mm)	3 HRP nA x min	4 Axon diam. (μm)	5 Axon gm (mm)	6 Axon DLF desc. (mm)	7 Axon DLF asc. (mm)	8 Axon VF/VLF asc. (mm)	9 Coll. no. gm	10 Coll. no. wm	11 Coll. length (mm)	12 Term.	13 Soma area (μm²)	14 Soma axes (μm)
1	r0.5	75	6.7	1.48			0.31	2		0.44–0.81	No	84	18–67
2	r0.1	130	8.2	1.31			0.42	4		0.05–0.53	No	119	30–35
3	0.3	35	7.0	1.31			3.37	3	2	0.01–0.32	No	136	28–55
4	0.8	10	6.0	0.49		0.95							
5	0.9	25	5.5	0.62		0.08						65	18–55
6	1.3	110	6.8	1.31			2.05	2		0.06–0.06	Yes	96	18–43
7	1.5	30	8.0	0.53	0.92			1	3	0.21–0.91	Yes	58	20–33
8	1.7	190	8.0	0.61	3.49			1	7	0.01–0.93	Yes	85	20–53
9	2.3	35	7.0	1.09		2.61		1		0.16	No	77	20–50
10	2.6	100	8.0	2.01	2.11	2.08		2	3	0.36–0.53	No	77	18–62
11	2.8	10											
12	3.4	22	4.5	0.78	0.55	0.21			4	0.55–1.21	Yes	29	18–20

[1]The interneurones are ranked according to their rostrocaudal location with respect to Onuf's nucleus; the most rostral are at the top and the most caudal at the bottom. Columns 1–14 list: 1) the neurone number; 2) the distance of the soma, in mm, rostral (r) or caudal to the rostral border of Onuf's nucleus; 3) the current used to inject HRP, expressed as the product of the applied current in nanoAmperes and the duration of the injection in minutes; 4) the mean diameter of the axon; 5–8) the length over which the axon was followed within the gray matter (gm) and within one of the funiculi [the dorsal part of the lateral funiculus (DLF), the ventral funiculus or the ventral part of the lateral funiculus (VF/VLF)] in the descending (desc) or ascending (asc) directions; 9–11) the number of collaterals given off within the gray matter (gm) or within the white matter (wm) and their lengths in mm; 12) presence (yes) or absence (no) of labelled terminal swellings; 13) maximum cross-sectional area of the soma; and 14) dimensions of the short and long axes of the soma.

Axons. The axons of all of the DLF projecting neurones took origin from the base of one of the dendrites, as illustrated in Figures 2A, 3B, 4, and 5. Judging by the lighter region surrounding them (see Fig. 3E), the axons appeared to acquire a myelin sheath within about 0.2 mm from the soma. Their external diameters (including the myelin sheath) within the gray matter and just after they entered the white matter were estimated to range between 4.5 and 8.2 μm (Table 1, column 4). The axons of the most laterally located neurones headed directly towards the lateral funiculus while those of the more medially located neurones looped dorsomedially over the cell body, as illustrated in Figures 1A,B, 2B, and 4. In both cases they traversed the gray matter within 200 μm rostral or caudal of the soma and entered the lateral funiculus from the dorsal horn. Within the lateral funiculus they ascended (n = 3), descended (n = 2), or bifurcated to give rise to both ascending and descending axon branches (n = 2). The axons usually ran immediately next to, or within about 200 μm of the border of the gray matter, as previously reported for axons with collaterals reentering the gray matter a short distance from the soma (Czarkowska et al., '81; Jankowska et al., '81; Bras et al., '89). The secondary axonal branches of two neurones (nos. 10 and 12) appeared in fact to reenter the gray matter within a few millimeters of the soma and seemed not to pass beyond the level of Onuf's nucleus. This was the case for both the ascending and descending axonal branches of cell no. 12 (Fig. 2B) and the caudal axonal branch of cell no. 10 (Fig. 4). A number of axon collaterals of these and other neurones were also seen to reenter the gray matter (see below). Only one axon penetrated into the more superficial layers of the lateral funiculus.

Axon collaterals. Stained collaterals (one to eight per neurone; Table 1, columns 9 and 10) could be seen to originate from axons of five of the seven DLF projecting neurones. The collaterals arose both before (one to four per neurone) and after (three to seven per neurone) the axons entered the white matter. The first collaterals were given off at distances of 0.21, 0.29, 0.37, 0.49, and 2.25 mm from the soma and the following ones at distances of 0.10–0.63 mm (mean, 0.30 mm). Ten of the 22 collaterals were stained only over their more proximal parts and their terminal branching could not be visualized. Twelve more completely labelled collaterals (of four neurones) could, however, be reconstructed down to their likely terminal branches and some of the latter to terminal axonal swellings (illustrated in Fig. 3G–I). These structures are considered to define at least some of the projection areas of the interneurones.

Seven of the more completely labeled axon-collaterals of three neurones (no. 7: three collaterals; no. 8: one collateral; no. 12: three collaterals) covered a fairly restricted part of the dorsal horn (primarily laminae V–VI; Fig. 2A,B). In contrast, four collaterals of two cells (no. 8: two collaterals; no. 10: two collaterals) covered larger parts of the gray matter in the dorsal as well as in the ventral horn and intermediate zone (primarily laminae VI–VII; Figs. 4, 5), and one collateral (of cell no. 10) extended into the contralateral intermediate zone (Fig. 4). The restricted and the wider axonal projection areas might thus be considered as representative for different subgroups of group II sacral interneurones despite the fact that their extent must have been underestimated.

Interneurones with axons in the ventral and ventrolateral funiculi

Cell bodies and dendritic trees. Interneurones with axons in the ventral funiculus (nos. 1–3 and 6) were located in a region of the spinal cord either overlying the rostral one-third of Onuf's nucleus or within 1 mm of its rostral border (Fig. 1A and Table 1, column 2). The region overlapped with the ventral part of the region in which interneurones with axons in DLF were located. Reconstructions of the VF/VLF projecting neurones are shown in Figures 6 and 7. Table 1 (columns 13 and 14) shows that the cell bodies of neurones of this group tended to be somewhat larger than those of neurones with axons in the lateral funiculus (soma cross-sectional area 84–136 μm², short axis 18–30 μm, long axis 35–67 μm, with a mean axis of 37.8 μm). The dendritic trees of these neurones also tended to extend over somewhat longer distances although they had on the average the same number of primary dendrites. The dendrites extended on average 355 μm dorsally, 520 μm ventrally, 350 μm medially, 330 μm laterally, 410 μm rostrally, and 310 μm caudally from the soma.

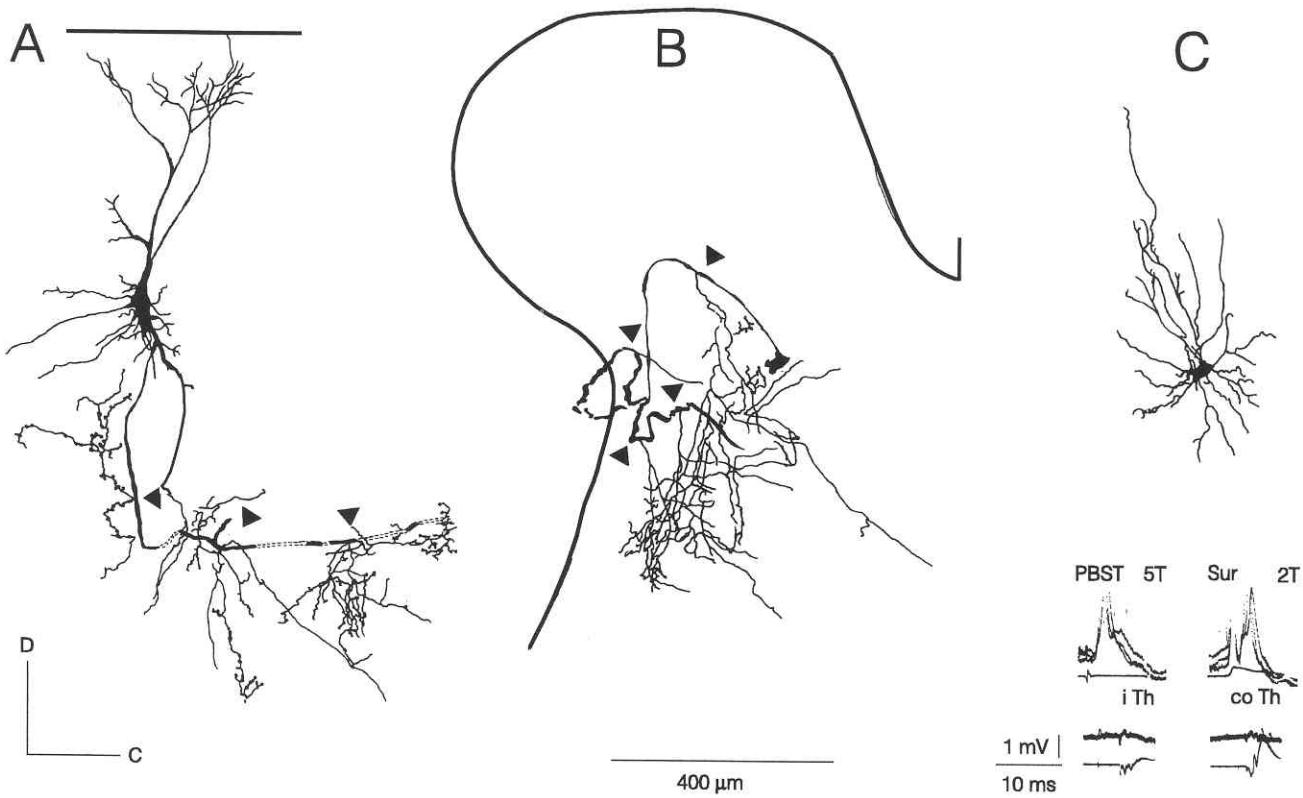

Fig. 2. Reconstruction of two neurones (cells 7 and 12) with axons running in the lateral funiculus and terminal branching areas in the intermediate zone. **A:** Cell body, dendrites, and the proximal part of the axon of cell 7 in the sagittal plane (see also Fig. 3B). Arrowheads indicate the first three axon collaterals. Dashed lines indicate the missing parts of the axon. Note extensive branching of the two dorsally directed dendrites close to the dorsal border of the dorsal horn (indicated by the horizontal line). **B:** Soma, stem axon, and axon collaterals of cell 12 (in the transverse plane; the first four collaterals are marked by arrowheads). Note that the stem axon bifurcated before entering the lateral funiculus; one secondary branch turned medially and rostrally within the gray matter while the other made a loop first dorsally then caudally and reentered the gray matter. Note also that some terminal branches of three collaterals covered the same area. **C:** The dendritic tree of cell 12 and records obtained from it. The upper traces of each pair are intra- or extracellular recordings from the interneurone and the lower traces are from the surface of the spinal cord. Intracellular recordings (upper row) illustrate monosynaptically evoked excitatory postsynaptic potentials (EPSP)s following stimulation of group II afferents of the PBST nerve and of low threshold afferents of the sural nerve. Extracellular recordings (lower row) show that the cell did not respond to stimulation of either the ipsilateral or the contralateral lateral funiculus at the Th13 level. Abbreviations used in Figures 2–7: GS, gastrocnemius-soleus; PBST, posterior biceps-semitendinosus; Pud, pudendal; Q, quadriceps; Sur, sural; iTh, ipsilateral thoracic; co Th, contralateral thoracic; 2T, two times threshold; 5T, five times threshold.

Axons. Axons of this group of interneurones took origin from the base of a dendrite as for axons of the laterally projecting neurones. The axons of three of these neurones headed almost directly towards the ventral funiculus and entered the white matter medial to the motor nuclei (within 400 μm of the soma; Figs. 6B, 7). The axon of the fourth neurone entered the ventral part of the lateral funiculus, looped around the ventral horn, and ran within the vetrolateral funiculus parallel to the pudendal nucleus (Fig. 6A). All of these axons were directed rostrally and all ran within 100 μm of the border of the gray matter. They appeared to aquire a myelin sheath within 200 μm of the soma, and their external diameters were estimated to be 7–8.2 μm (Table 1, column 4).

Axon collaterals. Stained collaterals (two to five per neurone) originated from the axons of all four of the neurones in this group. All of the axons gave rise to more than one collateral (two to four) during their course through the gray matter, and two axon collaterals could in addition be seen to be given off within the white matter (see Table 1 and Figs. 6, 7). The first collaterals arose at distances of 0.11, 0.14, 0.14, and 0.44 mm from the soma and the following ones at distances of 0.10–0.99 mm (mean, 0.35 mm). These mean intercollateral distances are similar to those within the initial parts of the axons of interneurones projecting in the DLF and are also within the range previously reported (0.09–0.56 mm) for axons of another population of spinal interneurones (Czarkowska et al., '76).

Most of the collaterals could be followed only over the initial part of their trajectory; only five collaterals were labeled over more than 300 μm and none to their terminal swellings. However, the five most extensively labeled collaterals extended much more ventrally than collaterals of the most completely labeled neurones with axons in the lateral funiculus, and reached not only the intermediate zone but also the ventral horn. The axon collaterals of the two most rostrally located interneurones (nos. 1 and 2, located just rostral of Onuf's nucleus) would in fact have been likely to traverse the caudal parts of the PBST and GS motor nuclei since these nuclei extend as far caudally as the rostral border of Onuf's nucleus (Romanes, '51, see also Jankowska and Riddell, '93a).

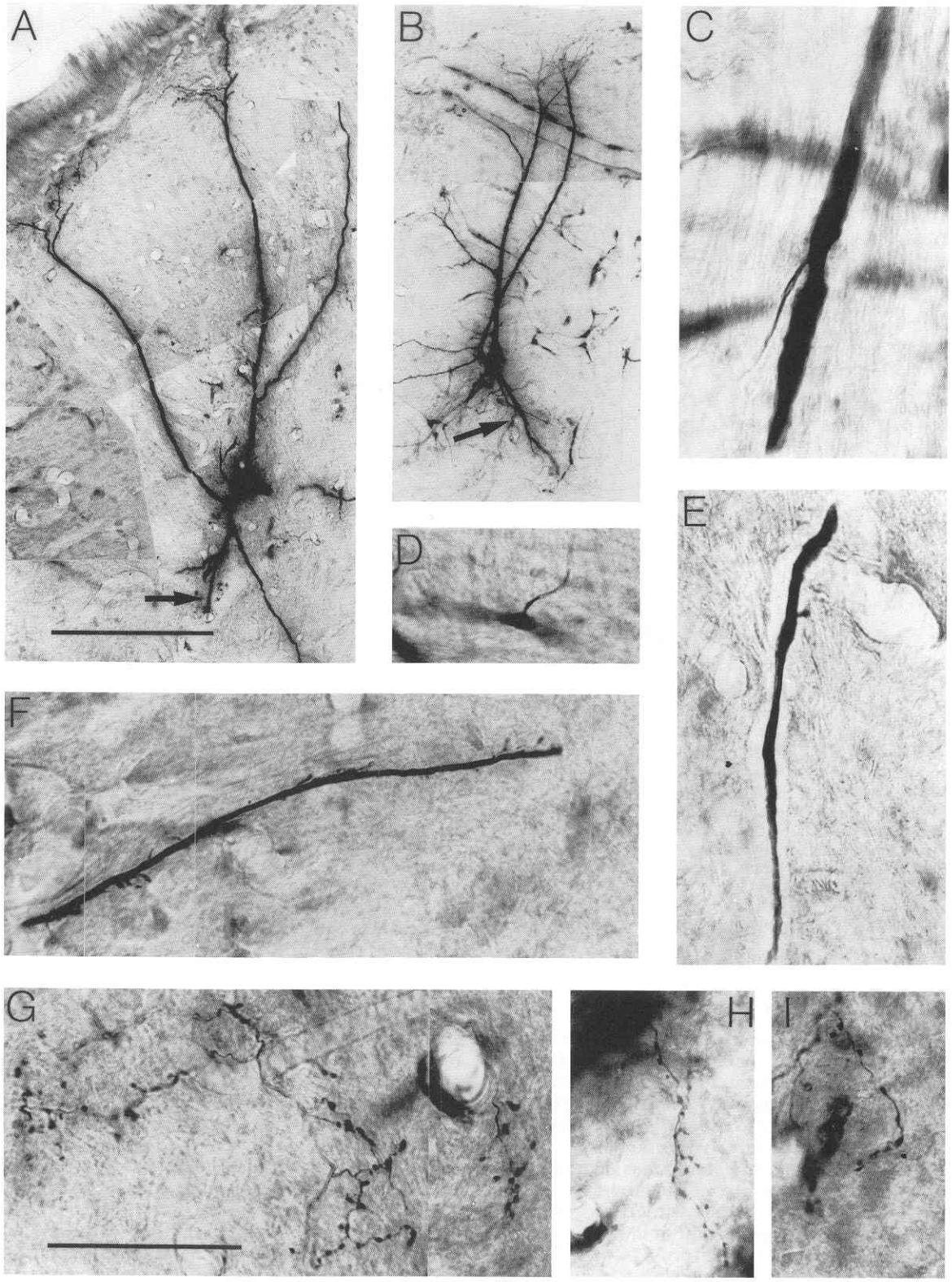

Fig. 3. **A,B:** Photomicrographs of the cell bodies and dorsally directed dendrites of neurones 6 and 7; see Figures 2A and 6B for reconstructions. The arrows indicate the initial segment of the axon. **C–E:** Stem axons of neurones 7, 8, and 3; C and D show nodes of Ranvier from which axon collaterals arise, while E illustrates the appearance of axons where they have acquired a myelin sheath (the clear areas on both sides of the axon). **F:** Section of dendrite from neurone 12 that displayed dendritic spines. **G–I:** Examples of terminal branches from neuron 10; note axonal swellings corresponding to boutons en passage as well as boutons terminaux. Scale bar in A = 200 μm and applies to A and B; bar in G = 50 μm and applies to C–I.

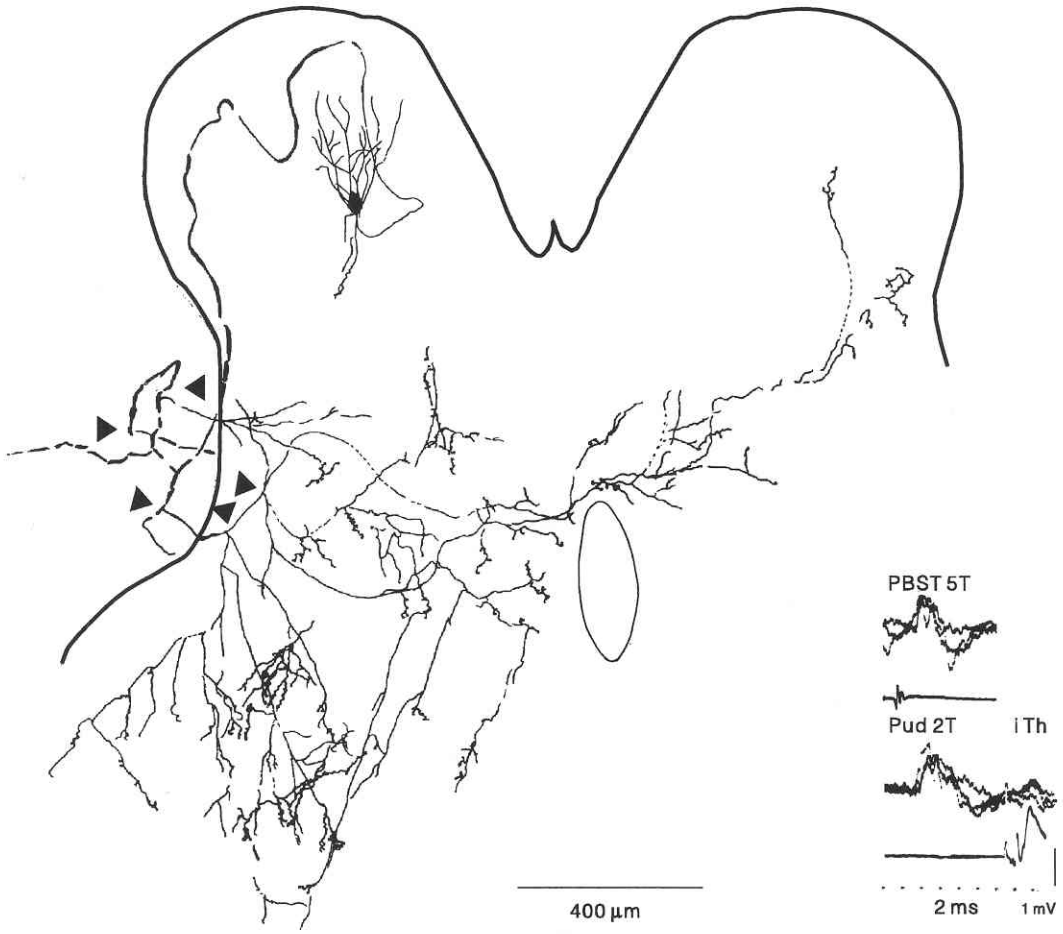

Fig. 4. Reconstruction in the transverse plane of the cell (no. 10) in which the labeled terminal axonal branching was the most extensive. Note several axon collaterals (arrowheads) leaving the stem axon in the white matter and entering, or approaching, the gray matter. Some of the terminal branches of this neurone are illustrated in Figure 3G–I. The unstained parts of the larger axon collaterals in the gray matter and their likely trajectory are indicated by dashed lines. The recordings illustrate EPSPs evoked in the cell from the PBST and Sur nerves and lack of antidromic activation following stimulation of the ipsilateral lateral funiculus at the Th level.

DISCUSSION

Indications for morphological heterogeneity of interneurones with input from group II muscle afferents

On the basis of their axonal projections the labeled interneurones fell into two main groups: those with axons in the dorsal part of the lateral funiculus (DLF) and those with axons in the ventral funiculus (VF) or the ventral part of the lateral funiculus (VLF). The two groups differed also with respect to their location, the direction in which their stem axons projected, and the main areas of terminal or near-terminal axonal branching in the gray matter. Only interneurones located close to the rostral border of Onuf's nucleus had axons that projected within the VF or VLF. All of the axons that entered the VF or VLF were found to ascend, run close to the border of the ventral horn, and have axon collaterals reaching the ventral part of the ventral horn. In contrast, the stem axons and secondary axonal branches of DLF projecting neurones were directed either rostrally or caudally and at least some of these did not appear to project beyond the level of Onuf's nucleus. Most of the stem axons ran close to the lateral border of the intermediate zone; their axon collaterals traversed the base of the dorsal horn, the intermediate zone, and the dorsal but not the ventral part of the ventral horn.

Two additional morphological features that were not general to the sample of stained interneurones as a whole are worthy of comment. Firstly, two DLF projecting neurones possessed numerous dendritic spines. This feature appeared to be related to particularly localized actions since these two neurones were those with axons arborizing in the most restricted regions of the gray matter. Furthermore, both appeared to make synaptic contacts only within the sacral segments; interneurone no. 7 projected only caudally (see Fig. 2A), and both ascending and descending secondary axonal branches of interneurone no. 12 reentered the gray matter less than 1 mm from the soma (see Fig. 2B). Secondly, two neurones had dorsally projecting dendrites that branched profusely within the most superficial parts of the dorsal horn. This kind of dendrite could not be related to other morphological features since one of the two neurones had an axon that descended in the lateral funiculus (no. 7; Fig. 2A) and the other an axon that ascended in the ventral funiculus (no. 6; Fig. 6B), and only the former displayed dendritic spines.

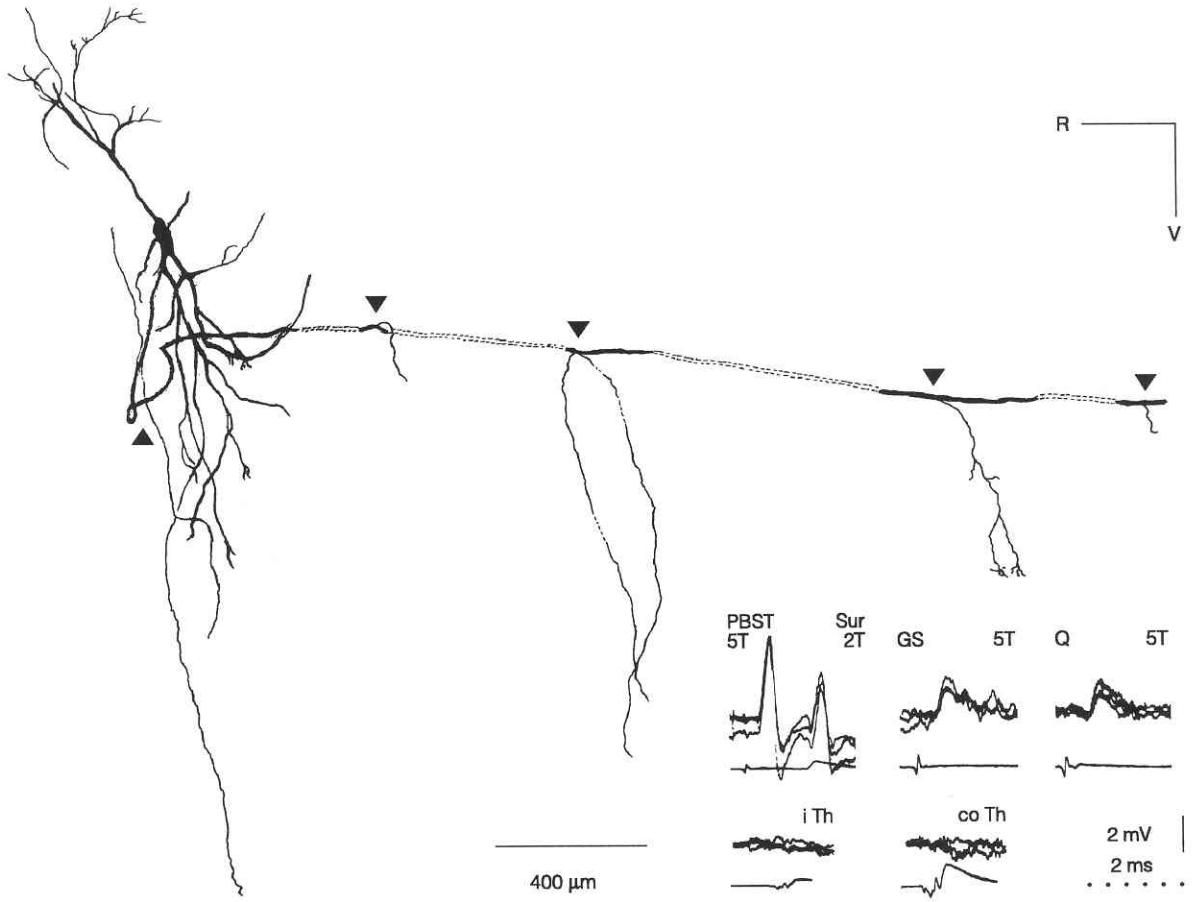

Fig. 5. Reconstruction in the sagittal plane of another interneurone (cell 8) with an axon running in the lateral funiculus and axon collaterals extending into the ventral horn. The first five axon collaterals are indicated by arrowheads. Note the decrease in the length of the labeled parts of successive collaterals. Intracellular recordings (top traces) and recordings from the surface of the spinal cord (bottom traces) illustrate monosynaptic input to this cell from group II afferents of the PBST, GS, and Q muscle nerves and from low threshold afferents in the sural nerve. The bottom two recordings illustrate the lack of responses to stimuli applied at the thoracic level.

Comparison of the morphology of sacral and midlumbar interneurones with input from group II muscle afferents

The results of this study are in keeping with previous indications that group II interneurones overlying Onuf's nucleus are homologues of the dorsal horn rather than of the intermediate zone midlumbar group II interneurones. These previous indications were based primarily on the findings that most group II activated sacral interneurones are located in the dorsal horn and have input from group II (but not from group I) muscle afferents and cutaneous afferents (Jankowska and Riddell, '93b), as is the case for the dorsal horn midlumbar interneurones. Further observations revealed that the cutaneous afferents that synapse on sacral interneurones affect motoneurones with latencies too long to be compatible with a direct action of these interneurones upon motoneurones (Jankowska and Riddell, '93b), a feature of the intermediate zone but not of the dorsal horn midlumbar interneurones. The results of the present study also lead to the conclusion that sacral interneurones, in particular those overlying the caudal two-thirds of Onuf's nucleus, are unlikely to have direct actions upon motoneurones. They failed to reveal any terminal branching of these interneurones in the vicinity of the motor nuclei. Furthermore, all of the stained sacral interneurones (except one in which the axon was not labeled) had axons that entered the lateral funiculus. The location of these axons within the lateral funiculus was appropriate for reentering the dorsal horn or the intermediate zone but not for approaching motoneurones. The axons of half of these neurones were directed caudally, and at least some of those directed rostrally terminated at the level of Onuf's nucleus. These projections were therefore suitable for making synaptic contacts with neurones in the same segments as Onuf's nucleus but not more rostrally, where most of the hindlimb motoneurones are located.

The situation was somewhat different with regard to interneurones located within about 1 mm of the rostral border of Onuf's nucleus. Four of the six interneurones with such a location sent their axons to the VF or VLF and the trajectory of these axons, close to the ventral border of the ventral horn and in the ascending direction, was more favorable for reaching the area in which motoneurones are located. At least some collaterals given off by these axons were found to pass close to the sacral motor nuclei, in particular Onuf's nucleus (motoneurones of the external

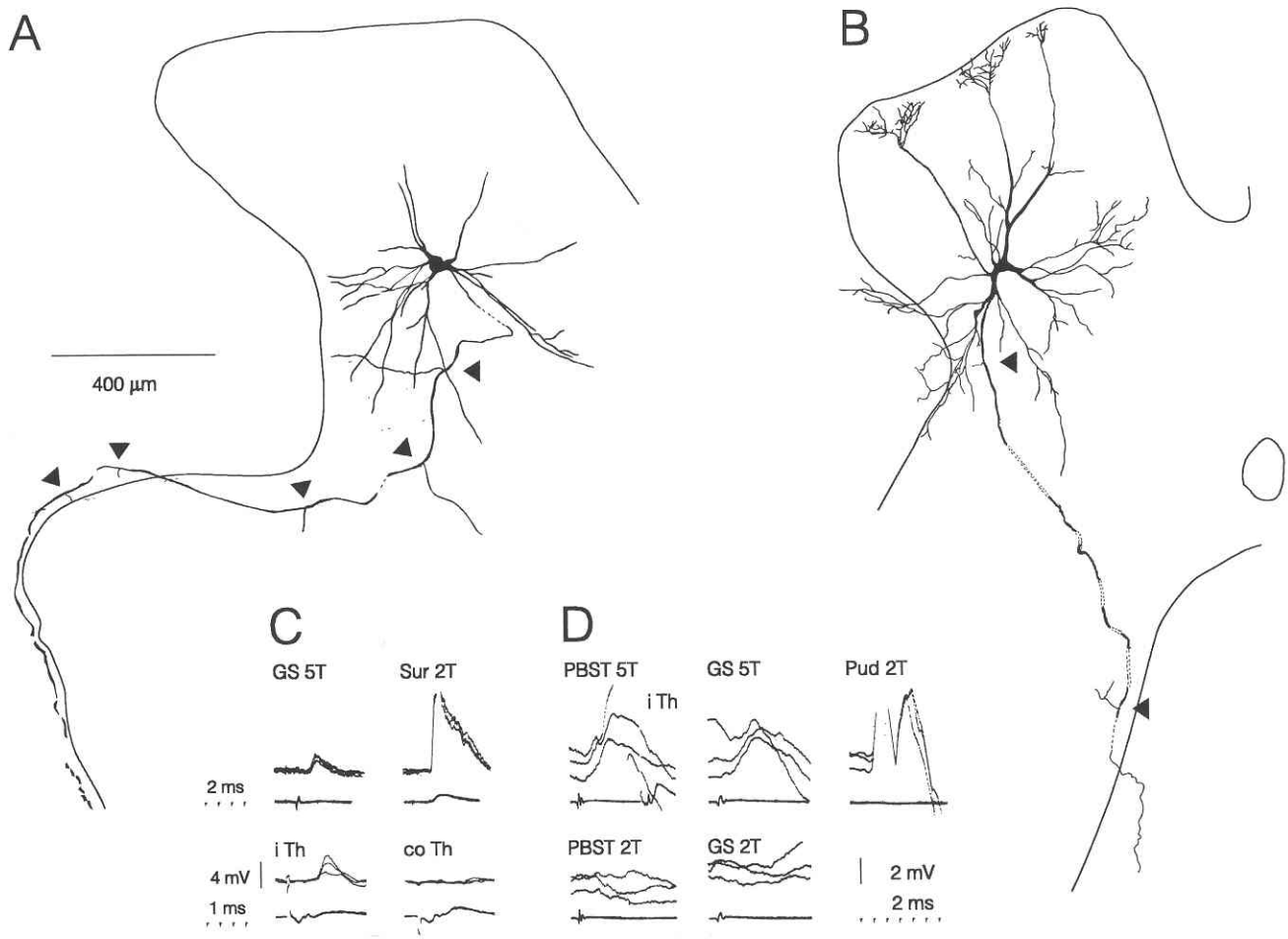

Fig. 6. Reconstruction in the transverse plane of two neurones, one with an axon in the ventral part of the lateral funiculus (**A;** cell 3) and one with an axon in the ventral funiculus (**B;** cell 6). Note that only the initial parts of axon collaterals (arrowheads) were labeled. Note also that the dorsally directed dendrites of the cell in B showed a profuse branching within the most superficial parts of the dorsal horn similar to that of the neurone illustrated in Figure 2A. The recordings in **C** and **D** illustrate input from the indicated muscle and skin nerves to the neurones shown in A and B, respectively.

urethral and anal sphincter muscles) and the PBST and GS motor nuclei located just rostral to it. So far, electrophysiological investigation of samples of motoneurones from these nuclei have failed to reveal any signs of direct actions of sacral group II interneurones (Fedirchuk et al., '92; Jankowska and Riddell, '93b), so that more caudally located tail motoneurones, ventral horn interneurones, or ascending tract cells might be considered more likely targets of these interneurones. However, interneurones only slightly more rostrally located than those stained in the present experiments are co-excited by group I and group II muscle afferents (Jankowska and Riddell, '93b; P.J. Harrison, G.P. Connolly, J.M. Guzman Villalba, personal communication), and it may be that these interneurones represent the more caudal counterparts of the intermediate group II interneurones found in midlumbar segments. If so, then the ventrally projecting neurones of the present sample, all of which were located close to the rostral border of Onuf's nucleus, and one of which (no. 2) was excited by both group I and group II muscle afferents, might be the most caudal representatives of this population of interneurones. If this were the case, then unlike in the midlumbar segments where the dorsal interneurones directly overly the intermediate interneurones, the caudal homologues of these neurones (in pathways from PBST and GS group II afferents) would be distributed over rostrocaudally separate lengths of the spinal cord.

Other features of the stained neurones such as the size of the soma, the number of primary dendrites, and the areas covered by dendritic trees were similar to those of the dorsal horn midlumbar group II interneurones described by Bras et al. ('89; see their Table 1). This was true of both sacral interneurones with axons that entered the DLF and those with axons running in the VLF or VF, even though the somata of the ventrally projecting neurones tended to be somewhat larger. The mean axes of the somata (32.2 and 37.8 μm) of the sacral interneurones and the length of their dendrites (rarely exceeding 500 μm) were on the other hand smaller than those of the intermediate zone midlumbar interneurones (42.1 μm and more than 1,000 μm, respectively). Another difference between the sacral and midlumbar interneurones may be that the stem axons of the sacral interneurones project primarily ipsilaterally while crossed projections characterize a fair proportion of midlumbar interneurones. However, this difference might only be quantitative because there is evidence that some sacral

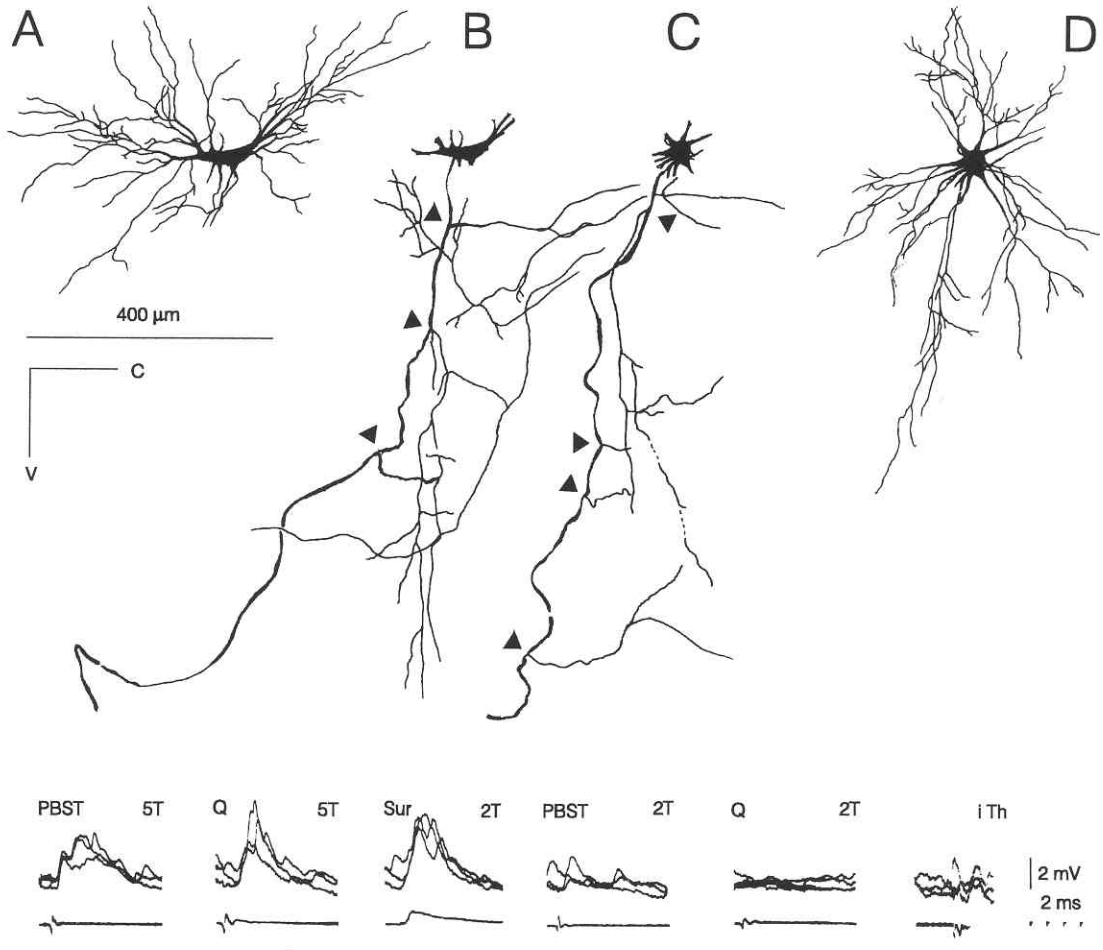

Fig. 7. Reconstructions in the sagittal plane of two neurones with axons in the ventral funiculus. **A:** The soma and dendritic tree of cell 1. **B:** The soma (with truncated dendrites) and the stem axon with three initial axon collaterals (arrowheads) of the same neurone. **C:** The soma (with truncated dendrites) and the stem axon with four initial axon collaterals (arrowheads) of cell 2. **D:** The soma and the dendritic tree of the same neurone. The recordings at the bottom are from neurone 2 and show the monosynaptic actions of muscle and cutaneous afferents and the lack of antidromic responses to stimulation at the thoracic level. Note that the shortest latency components of EPSPs evoked by afferents of PBST are attributable to group I afferents because they were evoked by stimuli of 2T as well as 5T. The longer latency components of these EPSPs and those evoked from Q had latencies and thresholds attributable to monosynaptic actions of group II muscle afferents.

interneurones have crossed actions (Jankowska and Riddell, '93b).

Concluding remarks on the likely target cells of sacral group II interneurones

The morphological features of the labeled sacral group II interneurones (in particular those with axons running in the DLF) indicate that these neurones are elements of locally operating neuronal networks. The strongest indications for this conclusion are that the axons of the stained neurones gave rise to a number of axon collaterals that branched within a short distance of the soma, that more than one-third of the interneurones had caudal projections, that some secondary axonal branches of the labeled interneurones actually terminated within a few millimeters of the soma, and that none of the primary axons reached the contralateral funiculi. Axons of some of the labeled neurones might nevertheless have ascended rostral to Onuf's nucleus and in addition contacted some more distant target neurones.

Neurones with local terminations, in particular those in which the terminal axonal branching area was limited to the dorsal horn, would appear to have a morphology particularly appropriate to interneurones mediating presynaptic inhibition of transmission from group II afferents. Such interneurones have been shown to have rostrocaudally restricted actions that would match the short distance projections of the present sample of interneurones (Riddell et al., '93b; Riddell and Jankowska, '93 and in preparation). However, since the minimal linkage in pathways of presynaptic inhibition appears to be di- rather than monosynaptic (see Jankowska, '92 for references), only the first order interneurones in pathways of presynaptic inhibition could be represented by neurones of the present sample of stained neurones.

Locally operating neurones would also be required to mediate the excitation and postsynaptic inhibition of interneurones and ascending tract neurones located in the same region that is evoked disynaptically by the same group II muscle and cutaneous afferents as synapse on sacral inter-

neurones (Jankowska and Riddell, '93b; Riddell et al., '93a). Neurones operating over longer distances as well as neurones operating locally might on the other hand be used to mediate oligo- and polysynaptic actions of these afferents upon motoneurones (Fedirchuk et al., '92; Jankowska and Riddell, '93b).

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