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THE POSTSYNAPTIC DORSAL COLUMN PATHWAY MEDIATES CUTANEOUS NOCICEPTIVE INFORMATION TO CEREBELLAR CLIMBING FIBRES IN THE CAT

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SUMMARY

1. The location in the spinal cord of the pathway mediating cutaneous nociceptive C fibre input to climbing fibres projecting to the forelimb area of the C3 zone in the cerebellar anterior lobe was investigated in pentobarbitone-anaesthetized cats. Lesions of the spinal cord at the segmental level of C3 sparing the dorsal funiculi (DF preparation) or lesions of the ipsilateral and part of the contralateral dorsal funiculi were made.

2. In the DF preparation, the cutaneous input to climbing fibres projecting to the C3 zone was the same as in cats with an intact spinal cord. Also, the topography of tactile and nociceptive receptive fields and the distribution of A- and C fibre-evoked climbing fibre field potentials was similar to that in cats with an intact spinal cord.

3. In cats with an initially intact spinal cord the cutaneous nociceptive C fibre input and the topographically well organized tactile input to the C3 climbing fibres disappeared following a lesion of the ipsilateral and part of the contralateral dorsal funiculi. Following this lesion the receptive fields of the climbing fibres became indistinct and only irregular responses were evoked on skin stimulation.

4. It is concluded that the cutaneous nociceptive C fibre input from the forelimb to climbing fibres projecting to the C3 zone is mediated by the ipsilateral dorsal funiculus. Since cutaneous C fibres terminate exclusively in the spinal cord close to their entrance zone the postsynaptic dorsal column pathway must be part of this spino-olivocerebellar pathway.

INTRODUCTION

Climbing fibres projecting to the different sagittal zones in the cerebellar anterior lobe are usually activated by two or three ascending spinal pathways (Andersson, Ekerot, Oscarsson & Schouenborg, 1987). The spino-olivocerebellar pathways (SOCPs) ascending in the dorsal funiculi (DF) have the most extensive projections and innervate all identified sagittal zones in the anterior lobe. The DF-SOCPs have

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different peripheral receptive fields, olivary relays and termination areas in the cerebellar cortex (Oscarsson, 1969; Ekerot & Larson, 1979). It has been assumed that the DF-SOCPs originate from ascending primary afferent fibres which relay in the dorsal column nuclei (Oscarsson, 1969; Ekerot & Larson, 1979).

Information from the forelimb to climbing fibres projecting to the C3 zone in the anterior lobe is also mediated by a pathway ascending in the ipsilateral dorsolateral funiculus (DLF-SOCP) (Larson, Miller & Oscarsson, 1969; Oscarsson, 1969).

As yet, most studies of the peripheral input to the climbing fibres have been limited to the input mediated by myelinated afferent fibres. Recently, a powerful input from cutaneous nociceptive unmyelinated (C) fibres to climbing fibres projecting to the forelimb area of the C3 zone in the anterior lobe was demonstrated (Ekerot, Gustavsson, Oscarsson & Schouenborg, 1987*a*; Ekerot, Oscarsson & Schouenborg, 1987*b*; Ekerot, Garwicz & Schouenborg, 1991). Since climbing fibres to the lateral and the medial C3 zone give off collaterals to the Y zone in the lateralmost part of the anterior lobe and to the C1 zone, respectively (Ekerot & Larson, 1982), these two zones also receive a nociceptive input.

The aim of the present investigation was to identify the ascending spinal pathways(s) mediating the cutaneous nociceptive input to the forelimb areas of the Y, C3 and C1 zones. Some of the results have been presented in a preliminary form (Garwicz, Ekerot & Schouenborg, 1989).

METHODS

The experiments were performed on six cats (weight 2·6–4·5 kg) anaesthetized with pentobarbitone (40 mg kg^{-1} I.P.). Supplementary doses of the anaesthetic (3–4 mg kg^{-1} I.V.) were given to keep a level of anaesthesia characterized by constricted pupils and a blood pressure which remained stable during noxious stimulation of the skin. A brief description of the experiments is given below (for additional information on the preparation see Ekerot *et al.* 1987*a*, *b*, 1991).

A laminectomy was made of the C2 vertebra and lesions of defined spinal cord funiculi were made at the segmental level of C3. In four cats an initial lesion was made sparing the dorsal funiculi (DF preparation) (Ekerot & Larson, 1979). In the two remaining cats, following control recordings of climbing fibre responses, a lesion was made of the ipsilateral and part of the contralateral dorsal funiculi.

In four experiments a branch of the left superficial radial nerve was prepared for electrical stimulation and for recording of the afferent nerve volley. The nerve was left in continuity with the skin. In two of the DF preparations the left forelimb was intact to permit a comprehensive topographical mapping of the cutaneous receptive fields of the climbing fibres.

The left cerebellar anterior lobe was exposed by craniotomy and ablation of the occipital lobe.

Manual mechanical stimulation of the skin included light tapping, maintained firm innocuous pressure and noxious pinch with a pair of forceps (Ekerot *et al.* 1991). Care was taken to avoid pinch of the subcutaneous tissue.

Climbing fibre responses were recorded with glass-covered tungsten electrodes from single Purkinje cells and with ball-tipped electrodes (tip diameter, 0·2 mm) as climbing fibre field potentials from the cerebellar surface. At the end of the experiments the cats were killed by an overdose of pentobarbitone and perfused with 10% formalin in saline. Histological verifications of the extent of the lesions and identification of the cerebellar lobules recorded from were made.

RESULTS

Preparations sparing the dorsal funiculi

Responses to nerve stimulation

As in the intact cat, graded nerve stimulation revealed a convergence of input from cutaneous Aβ, Aδ and C fibres onto the climbing fibres tested ($n = 14$). Climbing fibre

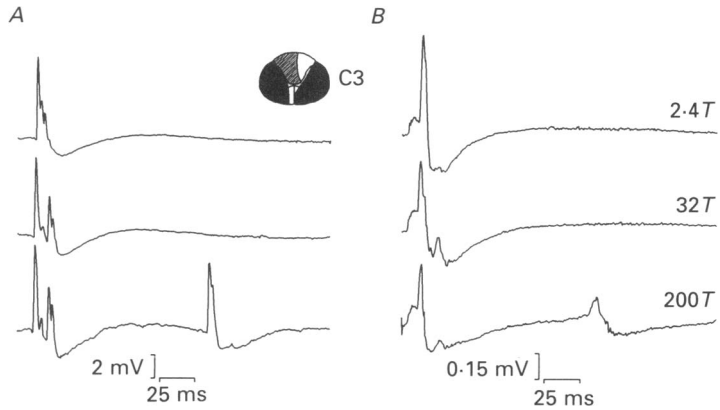

Fig. 1. Climbing fibre responses recorded in a DF preparation. The ipsilateral superficial radial nerve was stimulated at three different intensities (frequency 1 Hz): maximal for Aβ fibres, but subthreshold for Aδ fibres (2·4T); maximal for Aδ fibres, but subthreshold for C fibres (32T); and maximal for C fibre activation (200T). *A*, samples of climbing fibre responses recorded from a single Purkinje cell. Onset of stimulation at the start of the sweeps. Calibrations are shown below. Positivity upwards in all figures. *B*, averaged ($n =$ 20) climbing fibre field potentials recorded from the cerebellar surface. (The decrease of the Aβ fibre-evoked field potential on stimulation of C fibres in these records was not a regular finding.) Inset, lesions at the C3 segment of the spinal cord. Blackened areas mark the initial lesion, shaded area marks the second lesion.

responses recorded from a Purkinje cell and as surface field potentials elicited by stimulation of the superficial radial nerve at three different intensities are shown in Fig. 1. Stimulation at Aβ fibre strength (2·4 times nerve threshold (T)) elicited a short-latency (11–12 ms) climbing fibre response. On recruiting Aδ fibres (32T) a second climbing fibre potential occurred (latency 20–24 ms). Stimulation of C fibres (200T) evoked a long-latency (125–150 ms) climbing fibre response.

The distribution of Aβ and C fibre-elicited climbing fibre field potentials was compared in two cats. In Fig. 2 the amplitudes of the Aβ and C fibre-evoked potentials recorded in the two folia exhibiting maximal responses on stimulation of the superficial radial nerve are plotted. There was an approximately congruent distribution of these potentials indicating a projection from Aβ and C fibres similar to that in cats with an intact spinal cord (Ekerot *et al.* 1987*a*).

Following an additional lesion of the ipsilateral dorsal funiculus (Fig. 1, inset) the responses to nerve stimulation disappeared. Control stimulation of the DF rostral to the lesions still evoked normal climbing fibre responses.

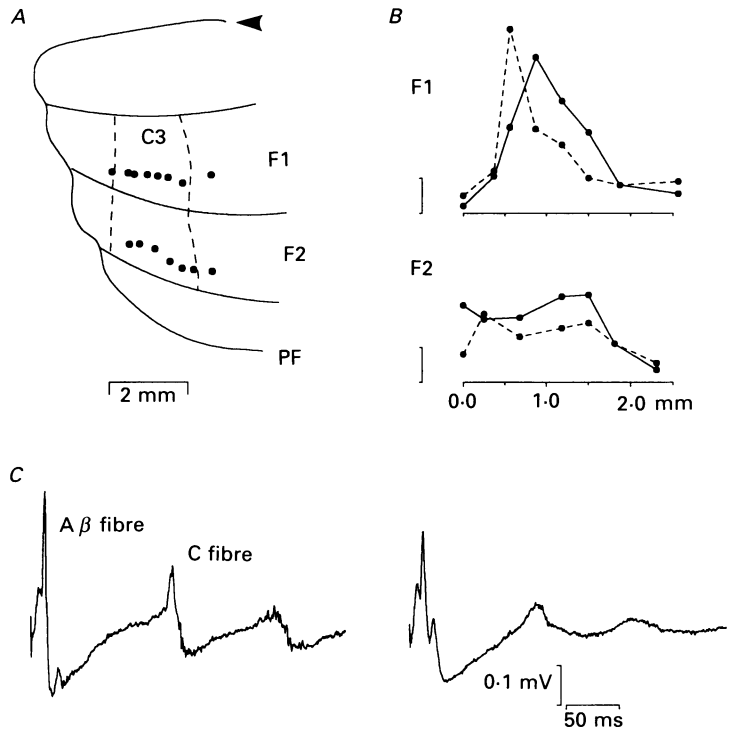

Fig. 2. Recordings of climbing fibre field potentials in two folia (F1 and F2) on stimulation of the ipsilateral superficial radial nerve. Same experiment as in Fig. 1. *A*, recording sites on the surface indicated by ●. Dashed lines indicate borders of the C3 zone. PF, primary fissure; arrow-head, border between lobules IV and V. *B*, the corresponding amplitudes of the evoked field potentials, as measured from the peak of the positive potential to the peak of the negative potential, are plotted. Continuous and dashed lines indicate amplitudes of Aβ and C fibre-evoked field potentials, respectively. (Calibration, 0·2 mV for Aβ and 0·04 mV for C fibre-evoked field potentials.) Each value is the average value of twenty responses. Medio-lateral distance between recording sites is indicated below the graphs. *C*, the maximal C fibre-evoked climbing fibre responses in folia F1 (left trace) and F2 (right trace). Each trace is the average of twenty responses.

Responses to skin stimulation

As in intact cats, most (sixty-two of sixty-seven) climbing fibres studied in the DF preparation received both tactile and nociceptive input. The remaining five climbing fibres received a tactile input only. Tactile stimulation, such as light tapping or maintained innocuous pressure, usually provoked one or two climbing fibre responses. On increasing the strength of the pressure to noxious intensities the climbing fibre activity lasted for the duration of the stimulation (5–10 s). The responses elicited from the centre of the receptive field were synchronous in many climbing fibres, producing large field potentials (Fig. 3, upper traces) and the response frequency often exceeded 5 Hz in single Purkinje cells. The receptive fields for tactile and nociceptive inputs were the same. Typically, the receptive fields were distinct and

exhibited an eccentrically located focus, from which maximal responses were evoked, and a proximal border close to a joint (Figs 3 and 4).

In order to map the topographical organization of the nociceptive input to the C3 zone the receptive fields of climbing fibres ($n = 52$) were systematically investigated with noxious pinch in three folia in one of the DF preparations (Fig. 4). An orderly sequence of receptive fields across the C3 zone was found, which was the same as that in cats with an intact spinal cord (cf. Figs 2 and 5 in Ekerot *et al.* 1991).

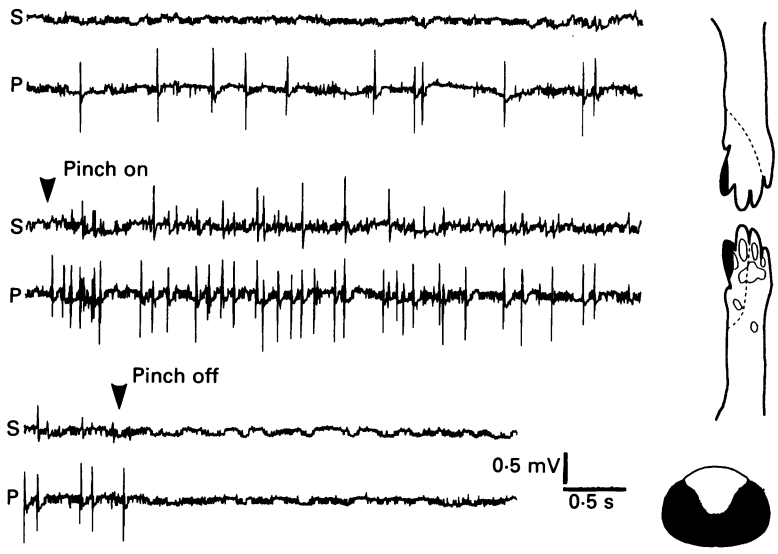

Fig. 3. Climbing fibre responses simultaneously recorded from the surface (S) and from an adjacent Purkinje cell (P) on noxious stimulation of the most sensitive area of the receptive field of the climbing fibre. Simple spikes were absent during this recording. The traces are continuous from top to bottom. Note that the climbing fibre responses in the Purkinje cell often coincide with the large field potentials recorded on the surface. The receptive field of the climbing fibre is indicated on the right with a dashed line. The most sensitive area of the receptive field is blackened. The extent of the lesion of the spinal cord is indicated below.

In cats with an intact spinal cord, the cutaneous receptive fields of the C3 climbing fibres can be divided into eight classes containing a total of thirty subclasses on the basis of the location of their proximal border and area of maximal responses (Ekerot *et al.* 1991). The receptive fields found in the DF preparation fit well into this classification.

In summary, the characteristics of the nociceptive climbing fibre input to the C3 zone in the DF preparation appear to be the same as those in cats with an intact spinal cord.

Preparations with lesions of the dorsal funiculi

At the start of these experiments the spinal cord was intact. The typical multireceptive input to climbing fibres projecting to the C3 zone was first confirmed by recording climbing fibre field potentials (Fig. 5*A*) and climbing fibre responses

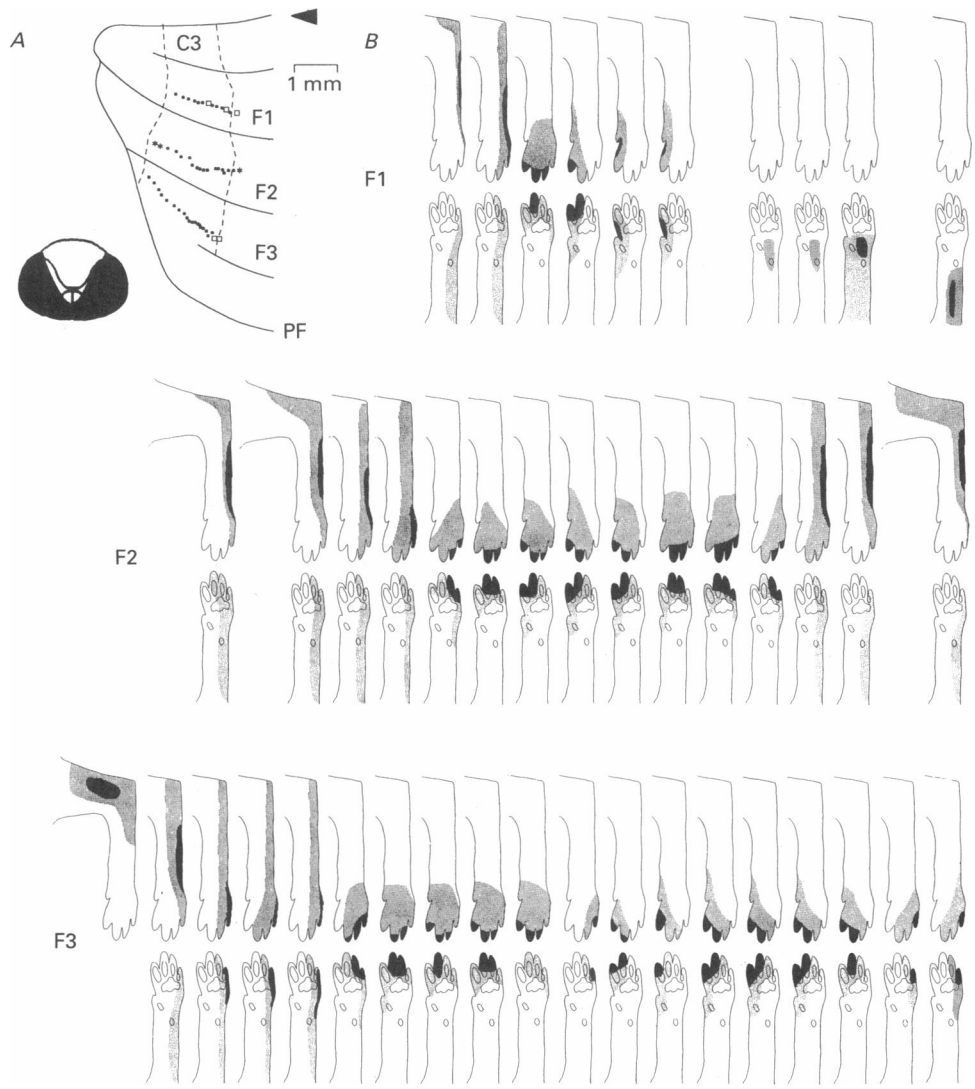

Fig. 4. Topographical organization and cutaneous receptive fields of the climbing fibres projecting to the forelimb area of the C3 zone in a DF preparation. *A*, the recording sites ($n = 52$) in three folia (F1–3) are given. Dashed lines indicate the borders of the C3 zone. ●, climbing fibres with a receptive field on the forelimb; ★, receptive fields on the thorax; □, lack of a cutaneous receptive field; PF, primary fissure; arrow-head, border between lobules IV and V; inset, lesioned part of the spinal cord at the C3 segment is shown by the blackened area. *B*, the cutaneous receptive fields of forty-four climbing fibres in the depicted cerebellar folia. The shaded area marks the extent of the receptive field, the blackened area marks the most sensitive area within the receptive field.

from single Purkinje cells. Then a lesion was made in the C3 segment of the ipsilateral and part of the contralateral dorsal funiculi (Fig. 5*B*, inset). The climbing fibre responses recorded from single Purkinje cells ($n = 9$) and as field potentials (Fig. 5*B*)

following this lesion were compared to those recorded prior to the lesion. The following differences were observed: (1) the C fibre-evoked climbing fibre responses were absent and the latency of the A fibre-evoked responses following the lesion was longer (latency about 25 ms) as compared to the situation with an intact spinal cord

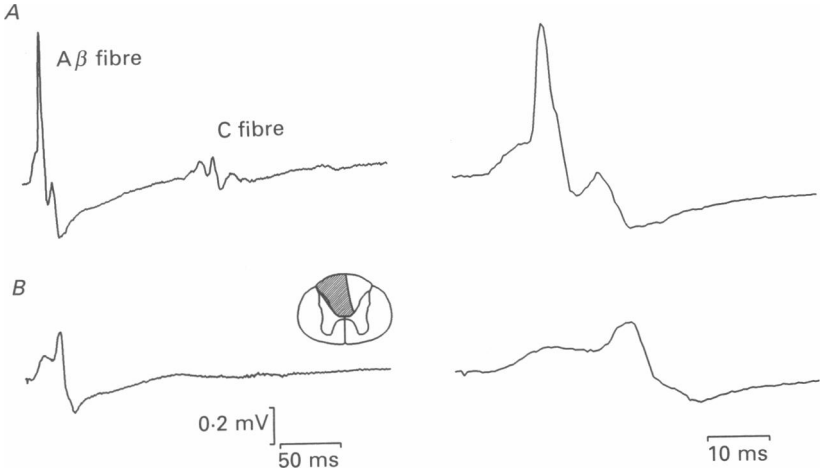

Fig. 5. Climbing fibre responses recorded as surface potentials before (*A*) and after (*B*) a lesion of the ipsilateral and part of the contralateral DF (see inset) on stimulation of the ipsilateral superficial radial nerve at a strength supramaximal for activation of C fibres. Each trace is the average of ten responses. The right panels show the traces with an expanded time scale.

(11–12 ms); (2) tactile stimulation of the skin provoked unreliable responses and noxious stimulation did not produce any additional responses. The extent of the receptive fields was impossible to determine accurately.

DISCUSSION

The present results demonstrate that the dorsal columns mediate cutaneous nociceptive C fibre input to climbing fibres projecting to the forelimb areas of the Y, C3 and C1 zones in the cerebellar anterior lobe. Since it is well known that cutaneous nociceptive C fibres terminate exclusively in the spinal cord close to their entrance zone the ascending spinal tract must be postsynaptic to these afferents (Molander & Grant, 1986; Sugiura, Lee & Perl, 1986). Hence, the ascending neurones belong to the postsynaptic dorsal column (PSDC) pathway (Uddenberg, 1968; Rustioni, 1974; Angaut-Petit, 1975; Brown, Brown, Fyffe & Pubols, 1983; Lu, Bennett, Nishikawa, Hoffert & Dubner, 1983; Noble & Riddell, 1988; Enevoldson & Gordon, 1989). PSDC neurones are known to originate mainly in laminae III–IV and to some extent in medial laminae V–VII of the spinal cord (Rustioni & Kaufman, 1977; Enevoldson & Gordon, 1989). The PSDC neurones project to the dorsal column nuclei (DCN) and nucleus Z (Rustioni, 1974). Some of the neurones located in the area of DCN

receiving terminals from PSDC neurones project to the rostral dorsal accessory olive (Ebbesson, 1968; Boesten & Voogd, 1975; Berkley, Budell, Blomqvist & Bull, 1986). This part of the inferior olive is the source of climbing fibres to the C3 zone (Groenewegen, Voogd & Freedman, 1979).

Like climbing fibres projecting to the C3 zone, most PSDC neurones receive a convergent input from tactile and nociceptive cutaneous fibres (Uddenberg, 1968; Angaut-Petit, 1975; Brown *et al.* 1983; Lu *et al.* 1983) and from muscle and joint afferent fibres (Uddenberg, 1968; Jankowska, Rastad & Zarzecki, 1979). In most reports on PSDC neurones, notably on pentobarbitone-anaesthetized cats, co-extensive cutaneous receptive fields for tactile and nociceptive stimuli have been described (Uddenberg, 1968; Angaut-Petit, 1975; Lu *et al.* 1983). It is thus likely that much of the multimodal convergence onto the climbing fibres projecting to the Y, C3 and C1 zones occurs already in the spinal cord.

The significance of the information signalled by the PSDC neurones in the pathways to the Y, C3 and C1 zones is not yet known. Clearly, these neurones neither code the exact type or precise location of the peripheral stimulus nor its exact timing (since there is convergence of impulses from both A and C fibres) (see also Ekerot *et al.* 1987*a*, *b*, 1991). An interesting feature of the cutaneous receptive fields of the C3 climbing fibres is that their distal and/or proximal borders are located close to joints (Ekerot *et al.* 1991). This may suggest that these fibres carry multimodal information related to movements. The fact that at least some of the PSDC neurones issue axon collaterals to ventral areas of the spinal cord may suggest that they are reflex interneurones (Bennett, Nishikawa, Lu, Hoffert & Dubner, 1984). Hence, these PSDC neurones and thus the C3 climbing fibres may signal the activity in multimodal spinal reflex circuits (Oscarsson, 1973; Schouenborg & Kalliomäki, 1990; see also discussion in Ekerot *et al.* 1991).

The present findings show that the claim by Lu *et al.* (1983) that the PSDC pathway does not receive a C fibre input must be wrong. In their study *subcutaneous* electrical stimulation was used, but no evidence that the stimulation intensity used was sufficient to activate cutaneous C fibres was provided.

It appears that the PSDC pathway should not be regarded as one pathway but as several different pathways. The PSDC pathways originate from several laminae of the spinal cord and contain neurones with different response properties. Furthermore, the PSDC pathways project to the large area surrounding the cluster regions in the DCN (Rustioni, 1974). This area projects to numerous nuclei in the brain, including different parts of the inferior olivary nuclei (Berkley *et al.* 1986), which have different cerebellar projections and functional properties (see Andersson *et al.* 1987). However, it is not yet known if axons of PSDC neurones are part of the DF-SOCPs to zones other than Y, C3 and C1 in the anterior lobe.

The present study shows that the nociceptive input and the topographically well organized tactile input to the C3 zone was abolished following a lesion of the ipsilateral dorsal funiculus. In such a preparation the C3 zone receives climbing fibre input only through the dorsolateral funiculus (Larson *et al.* 1969). Hence, the DLF-SOCP does not appear to mediate a cutaneous nociceptive input nor a topographically organized tactile input to the C3 zone.

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