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INHIBITION OF DORSAL SPINOCEREBELLAR TRACT CELLS BY INTERNEURONES IN UPPER AND LOWER LUMBAR SEGMENTS IN THE CAT

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SUMMARY

1. The topographical distribution of interneurones mediating disynaptic inhibition of dorsal spinocerebellar tract (d.s.c.t.) cells from group I muscle afferents in the cat was investigated using both physiological and morphological techniques.

2. Lesions of either the dorsal funiculi or of the lateral and ventral funiculi were made between L4 and L5 segments in two groups of cats. I.p.s.p.s. evoked from group I afferents were seen after both these lesions, showing that the i.p.s.p.s were evoked by interneurones located more caudally as well as by interneurones in the same segments as Clarke's column.

3. Distribution of the caudally located interneurones in the lower lumbar segments was investigated after marking these interneurones with horseradish peroxidase retrogradely transported from Clarke's column. The horseradish peroxidase was injected along L3–L4 segments of Clarke's column in two cats with transected dorsal funiculi. The marked cells were found in L5, L6, L7 and S1 segments, with a highest density in L6 and L7. They were seen in laminae V, VI and VII.

4. A search was made for interneurones which could be antidromically invaded following stimuli applied in Clarke's column and were monosynaptically excited by group I afferents. Such interneurones were found at locations corresponding to laminae V–VI of Rexed. The latencies of antidromic and orthodromic responses were within ranges allowing them to mediate disynaptic inhibition of d.s.c.t. cells.

INTRODUCTION

Neurones of the dorsal spino-cerebellar tract (d.s.c.t.) are not only excited but also inhibited from group I muscle afferents. The inhibition is evoked disynaptically and is of both group Ia muscle spindle afferent and group Ib tendon organ afferent origin (Laporte & Lundberg, 1956; Laporte, Lundberg & Oscarsson, 1956; Lundberg & Oscarsson, 1960; Curtis, Eccles & Lundberg, 1958; Holmqvist, Lundberg & Oscarsson,

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1956; Eccles, Oscarsson & Willis, 1961; Jansen, Nicolaysen & Walloe, 1967, 1969; Jansen & Rudjord, 1965; Hongo & Okada, 1967; Eide, Fedina, Jansen, Lundberg & Vyklický, 1969). Inhibition of d.s.c.t. cells may also be evoked via polysynaptic pathways from other groups of afferents. These facts were established long ago, but the interpretation of their functional meaning was difficult as long as the interneurones mediating inhibition of d.s.c.t. cells had not been identified. One of the main aims of the present and of the following study (Hongo, Jankowska, Ohno, Sasaki, Yamashita & Yoshida, 1983) was, therefore, to identify these interneurones.

To this end we have first investigated whether interneurones mediating inhibition of d.s.c.t. cells, in particular inhibition from group I afferents, are located at the same segmental levels as Clarke's column (local or upper lumbar interneurones) or in more caudal segments (caudal or lower lumbar interneurones). The strongest indications that they are among local interneurones, as is usually assumed, came from the morphological observation that the pericellular network of terminals with flattened vesicles, assumed to be the structural substrate of post-synaptic inhibition (Uchizono, 1965; Bodian, 1966), appears to be unaltered by section of the spinal cord just caudal to Clarke's column (Réthelyi, 1970). However, it will be shown that such local interneurones may be only partly responsible for inhibition of d.s.c.t. cells and that interneurones of the lumbar enlargement are likewise involved. Preliminary observations of this study have been published as an abstract (Hongo, Jankowska, Ohno, Sasaki, Yamashita & Yoshida, 1981).

METHODS

Preparation. The experiments were performed on twelve cats under sodium pentobarbitone anaesthesia (Nembutal, Abbott; 30–40 mg/kg I.P. initial dose, supplemented two to three times by 10 mg/kg I.V.) and on three cats under chloralose anaesthesia (60–80 mg/kg I.V.) after initial dissection done under ether. Nembutal anaesthesia was used primarily when records were taken from dorsal spinocerebellar tract neurones and chloralose anaesthesia in experiments involving recording from interneurones. All cats were paralysed with gallamine triethiodide (Gallamine, Teisan, or Flaxedil, May & Baker) and artificially ventilated. The blood pressure was 100–150 mmHg, both during dissection and recording, and the CO_2 level in the expired air was kept close to 4%.

The following left hind-limb nerves were usually dissected, cut distally and mounted for stimulation: quadriceps (q.), posterior biceps and semitendinosus (p.b.s.t.), anterior biceps and semimembranosus (a.b.s.m.), hamstring (ham.), sural (sur.), gastrocnemius and soleus (g.s.), plantaris (pl.), flexor digitorum and hallucis longus (f.d.l., with or without the interosseous nerve or branches to popliteus and tibialis posterior), posterior knee joint nerve (joint), the remaining part of tibial nerve (tib.), tibialis anterior and extensor digitorum longus (d.p.), and superficial peroneal (s.p.). In two cats in which muscle stretches were used to excite group Ia muscle spindle afferents of triceps surae and plantaris (see Fetz, Jankowska, Johannisson & Lipski, 1979; Jankowska, McCrea & Mackel, 1981*b*) the nerves of these muscles were dissected in continuity. A laminectomy exposed the spinal cord from L2 to sacral segments as well as Th12–Th13 segments. The dorsal funiculi and the right lateral and ventral funiculi, contralateral to the recording side, were sectioned at the Th13 level and bipolar stimulating electrodes were put rostrally to the transection in contact with the left lateral funiculus. The electrodes were used to stimulate axons of d.s.c.t. cells, for their identification by antidromic activation, and rubrospinal tract fibres, for adjusting location of an electrode in the red nucleus according to antidromic field potentials. In the cats in which no lesions were made at the level of the lumbar cord (referred to as intact preparations), small holes were made in the dura over areas of the ipsilateral dorsal funiculus which were free from blood vessels (Eide *et al.* 1969). Through these holes pia was torn away before micro-electrodes were inserted. In the remaining cats the dura was cut longitudinally over the whole

exposed part of the lumbar cord, and either the dorsal funiculus or the lateral and ventral funiculi were transected between L4 and L5 or in the caudal part of L4 segments. The spinal lesions were made with watchmakers' forceps under a dissecting microscope. In some experiments craniotomy was in addition performed over the right occipital lobe and an electrode was inserted into the red nucleus.

Recording was carried out, on the left side, from d.s.c.t. cells in L3 and L4 segments and from interneurones in Rexed's (1952, 1954) laminae V–VI in L6–S1 segments, with simultaneous records of peripheral or descending volleys from the surface of the spinal cord. Micro-electrodes filled with 2 M-potassium citrate, with tips broken to about 1·5 μm, were used for intracellular recording from d.s.c.t. cells. Similar electrodes filled with 2 M-sodium chloride were used for extracellular recording from interneurones. The recorded post-synaptic potentials were displayed on the oscilloscope and photographed, either directly (single superimposed records) or after they had been averaged (in an ATAC 350 or Nicolet 1170 averager).

Stimulation. Peripheral nerves were stimulated with bipolar silver electrodes using 0·1 msec pulses of up to 10 times threshold for the most sensitive fibres. Group Ia afferents of triceps surae and plantaris muscles were selectively activated by brief stretches of these muscles of 30–35 μm amplitude. Stimuli in Clarke's column were applied either via glass capillary electrodes with tip diameters of 2–4 μm, filled with 2 M-sodium chloride solution made into a stiff consistency with 1 % agar, or via tungsten needle electrodes insulated except for 5–10 μm from the tip. Usually three electrodes were inserted into Clarke's column at different rostro-caudal levels of the L3–L4 segments. The electrodes were positioned while recording antidromic field and unitary spike potentials evoked from the ipsilateral dorsolateral funiculus at a lower thoracic level.

Histological control was made of the positions of the stimulating electrodes both in the red nucleus and in the spinal cord and, in some cases, also of the electrodes used for recording from d.s.c.t. cells and interneurones. The extent of all the spinal lesions was likewise verified.

Horseradish peroxidase (HRP) was injected into Clarke's column in L3–L4 segments in two cats with the dorsal funiculi transected between L4 and L5 segments. To this aim a micropipette containing 10 % Tris-buffered HRP solution (pH 7·2) was repeatedly inserted into Clarke's column under guidance of antidromic field potentials and unitary potentials evoked from the ipsilateral dorsolateral funiculus at Th13 level. HRP injections were made at fifteen or eighteen sites, at intervals 0·8–1·5 mm rostrocaudally, over an 18 mm length of the Clarke's column. HRP was injected electrophoretically using current of 2 μA for 7–8·5 min at each site. Animals were killed about 60 hr after the last injection, perfused with saline followed by a fixative solution (1 % paraformaldehyde and 1 % glutaraldehyde in 0·1 M-sodium phosphate buffer, pH 7·2). L3–S1 spinal cord segments were cut at 100 μm. The sections were subjected to peroxidase reaction with 3,3′-diaminobenzidine (DAB); in every second section cobalt intensification (Adams, 1977) was used. All the sections were counterstained with thionine.

RESULTS

1. Inhibition of d.s.c.t. cells by both local and more caudally located interneurones demonstrated by effects of various spinal lesions

Two kinds of spinal lesions were made caudal to Clarke's column. Lesions of the dorsal funiculi were made to interrupt input from primary afferents to interneurones located at the same level as Clarke's column. Any inhibition of d.s.c.t. cells found after such a lesion was to be attributed to more caudally located interneurones with axons ascending in the lateral or ventral funiculi. Lesions of the lateral and ventral funiculi were made to elimate actions of such interneurones and to reveal the contribution of the local interneurones to the inhibition of the d.s.c.t. cells (we had no indications that cells with axons ascending in the dorsal funiculi could be responsible for this inhibition: see the next section and the Discussion).

The dorsal funiculi were transected in three cats, unilaterally or bilaterally (Fig. 1 *A–C*) and both the histological control and the physiological tests showed that

the transections were complete. The physiological tests involved stimulation of peripheral nerves and recording from the dorsal funiculi rostral to the lesion (Fig. 1*F*), or stimulation of the dorsal funiculi and recording from the peripheral nerves (Fig. 1*G*). Thirty-seven d.s.c.t. cells were recorded from after such lesions. In contrast to cells in intact preparations none showed any monosynaptic e.p.s.p.s from the dissected hind-limb nerves; additional evidence that the section of the dorsal funiculi was complete. In thirty-two of these cells i.p.s.p.s of group I origin were evoked,

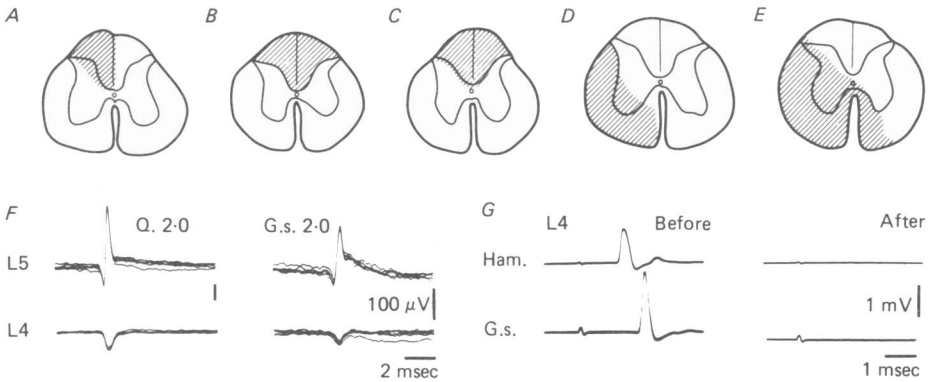

Fig. 1. Lesions of dorsal funiculi and of lateral and ventral funiculi. *A–E* histological reconstructions of the lesions. *F–G*, physiological control of the completeness of the lesions of the dorsal funiculi. *F* shows potentials monopolarly recorded from the surface of the left dorsal funiculus at L5 (upper traces) and L4 (lower traces) after stimulation of the left quadriceps (q.) and gastrocneminus–soleus (g.s.) nerves. Records, with the negativity upwards, were taken after lesions of the dorsal funiculi between L4 and L5. Note that conducted (positive-negative) spike potentials are recorded from L5 but not from L4, where only positivity is seen. *G* shows that stimulation of L4 dorsal funiculi evoked spike potentials in the hamstring (ham.) and g.s. nerves before, but not after a lesion of the dorsal funiculi.

mainly from quadriceps, gastrocnemius–soleus, plantaris, flexor digitorum longus and/or the distal part of the tibial nerves (Figs. 2*A–C*, 4 and 5). Since these i.p.s.p.s were not superimposed on monosynaptic e.p.s.p.s, as was often observed in intact preparations (Fig. 2*J–L*), they were even more distinct than those recorded before the lesions. However, the proportion of neurones in which they appeared depended to a great extent on the state of the animals and the depth of anaesthesia. For example, in one cat tested after injection of more than 50 mg of Nembutal per kilogram, instead of the usual dose of 35–40 mg/kg, the i.p.s.p.s were absent in four out of the seven first recorded neurones but appeared regularly after application of 4-aminopyridine (1 mg/kg), which was used to enhance synaptic transmission via the interposed interneurones (see Jankowska, Lundberg, Rudomin & Sykova, 1982). For this reason, a comparison of the proportion of d.s.c.t. neurones showing group I i.p.s.p.s in intact preparations (twenty-four out of twenty-nine) and after lesions of the dorsal funiculi (thirty-two out of thirty-seven) is of only limited value.

When electrical stimuli were used to excite the afferents, the receptor origin of the i.p.s.p.s could be determined only for those evoked from posterior biceps-

semitendinosus and quadriceps. The thresholds of seventeen of the i.p.s.p.s were below threshold for the second component of incoming volleys from these nerves (Fig. 2*A*, *D*, *G* and *J*) and therefore were attributed to Ia afferents. Group Ia i.p.s.p.s evoked by stretches of triceps surae and plantaris were seen in four d.s.c.t. cells (see Fig. 9 of Hongo *et al.* 1983). We also observed i.p.s.p.s growing with stimulus intensity over

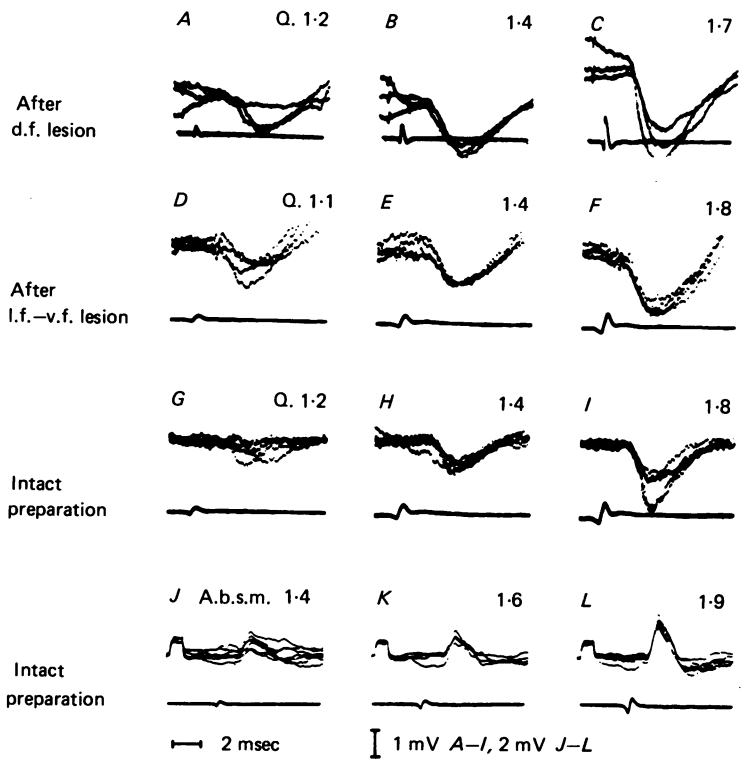

Fig. 2. Examples of disynaptic i.p.s.p.s evoked in d.s.c.t. cells after lesions of dorsal funiculi (d.f.; *A–C*) or lateral and ventral funiculi (l.f. and v.f.; *D–F*), and in an intact preparation (*G–L*). The i.p.s.p.s in *A* and *B* were evoked by Ia afferents while those in *B*, *C*, *E* and *F* most likely came from both Ia and Ib afferents. Records in *G–I* show i.p.s.p.s recorded in an intact preparation, similarly evoked by both Ia and Ib afferents of quadriceps. *J–L* show that monosynaptic e.p.s.p.s (*J*) are curtailed by following disynaptic i.p.s.p.s (*K* and *L*) when stimuli were increased to excite Ib afferents. In this Figure, as well as in Figs. 4 and 5, upper traces are intracellular records from d.s.c.t. cells while lower traces are records from the surface of the spinal cord at L6. Extracellular field potentials were negligible and therefore are not shown. Stimulus intensities (in multiples of threshold for the most sensitive fibres) are given above the records.

the whole group I range, and most likely due to both Ia and Ib afferents (Fig. 2*A–C*), or i.p.s.p.s appearing only at group Ib range, but possibly dependent on subthreshold actions of Ia afferents.

The latencies of i.p.s.p.s recorded after dorsal funiculi lesions were $2{\cdot}2 \pm 0{\cdot}6$ msec (mean and S.D., $n = 60$), and were similar to those obtained in 'intact preparations', $2{\cdot}1 \pm 0{\cdot}5$ msec ($n = 66$), as shown in Fig. 3*A* and *B*. These values were about 1 msec

longer than latencies of monosynaptic e.p.s.p.s in 'intact preparations', $1{\cdot}1 \pm 0{\cdot}4$ msec ($n = 46$) (Fig. 3*A*, blank columns; see also Eccles *et al.* 1961). These latencies were measured in relation to the time of arrival of the peripheral impulses to the spinal cord (in relation to incoming volleys recorded at L6 or rostral L7 level) and include conduction time along axons of the interposed interneurones. Since both the location and conduction velocity of the interneurones involved remained unknown, the latencies alone were not sufficient to define the synaptic linkage in the pathways.

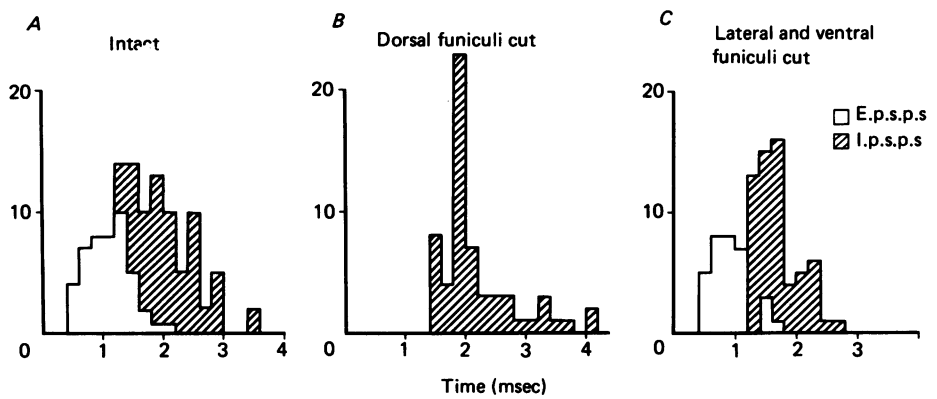

Fig. 3. Latencies of e.p.s.p.s and of i.p.s.p.s evoked from group I afferents. *A*, *B* and *C*, distribution of the latencies in intact preparations, after lesions of the dorsal funiculi and after lesions of the lateral and ventral funiculi, respectively. The latencies were measured with respect to incoming afferent volleys recorded at L6 level. See text for further details.

However, as will be described in section 3, observations on interneurones projecting to Clarke's column are fully compatible with the disynaptic nature of i.p.s.p.s, at least for those with latencies up to about 3 msec. Since a capability to follow repetitive stimuli is one of the characteristic features of disynaptically (in contrast to polysynaptically) evoked responses this test was used with some of the i.p.s.p.s appearing with longest latencies. For example, an i.p.s.p. with 3·8 msec latency (Fig. 4*A*) could follow repetitive stimuli, as did i.p.s.p.s with shorter latencies (Fig. 4*C* and *D*).

The number of interneurones contributing to i.p.s.p.s recorded in individual d.s.c.t. cells appeared to be rather small. With graded stimulus intensities only a few, in extreme cases one or two, unitary i.p.s.p.s were often seen to compose them, as illustrated in Fig. 5 (see also Fig. 2 of the companion paper, Hongo *et al.* 1983).

The occurrence of disynaptic e.p.s.p.s of group I origin (usually difficult to ascertain in cells with monosynaptic e.p.s.p.s) has not been previously reported. For this reason the following observations might be of value, although seen in only two cells. The cells were recorded from a cat with an incomplete lesion of dorsal funiculi (not included in the main part of this study). They did not show any monosynaptic e.p.s.p.s but e.p.s.p.s with latencies compatible with a disynaptic coupling. When repetitive stimuli were used, the amplitude of the e.p.s.p.s evoked by successive stimuli was increased and the latency decreased, or they appeared only in response to the second stimulus. No disynaptic or polysynaptic e.p.s.p.s of group I origin were seen after a complete dorsal funiculus lesion.

The lateral and ventral funiculi were transected in two cats; in one the lesion spared the most medial and dorsal part of the ventral funiculus (Fig. 1*D*) but in the other it was complete (Fig. 1*E*).

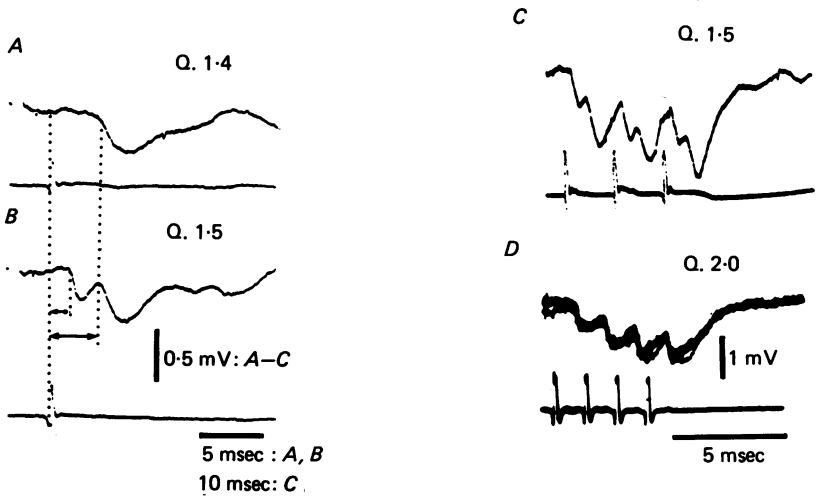

Fig. 4. An example of disynaptic i.p.s.p.s with long latencies. *A–C*, averaged records of i.p.s.p.s evoked from quadriceps (q.) in a d.s.c.t. cell located in the L3 segment after lesion of the dorsal funiculi. The i.p.s.p.s evoked by weaker stimuli (*A*) appeared with a latency of 3·8 msec while those following stronger stimuli (*B*) had a latency of 1·7 msec. Both the early and later i.p.s.p.s could follow repetitive stimuli (130 Hz) of this intensity (*C*). *D*, short latency i.p.s.p.s evoked by a train of stimuli in another preparation with lesion of the dorsal funiculi.

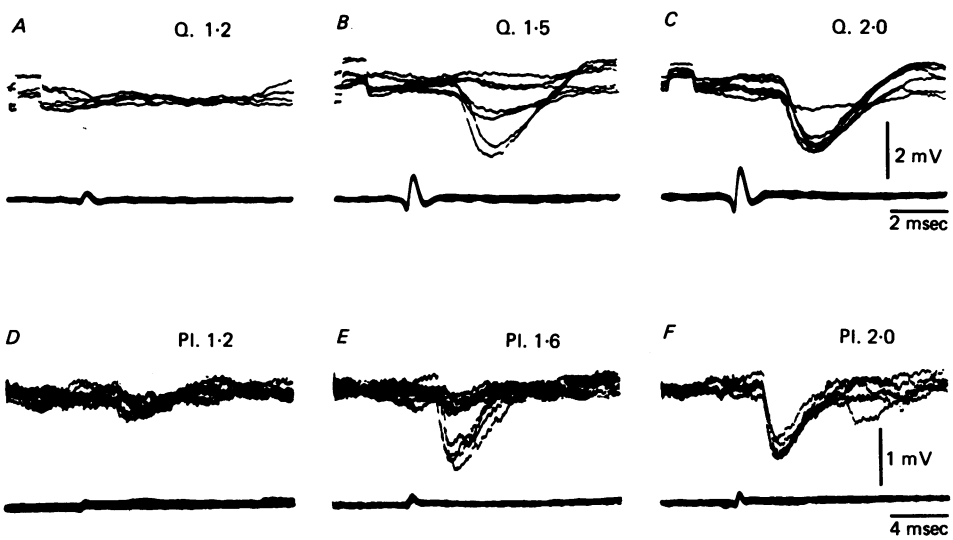

Fig. 5. Examples of unitary i.p.s.p.s in two d.s.c.t. cells (*A–C* and *D–F*, respectively) after lesion of the dorsal funiculi. The i.p.s.p.s were evoked by graded stimulation of quadriceps (q.) and plantaris (pl.) nerves. Note that apparently only two or three interneurones, as judged from the number of unitary i.p.s.p.s of different shapes, mediated disynaptic inhibition of these cells from group I afferents.

Recordings were obtained from twenty-three d.s.c.t. neurones after these lesions, nineteen of these in the cat in which the lesion was complete. All but one cell showed distinct i.p.s.p.s evoked from group I afferents, with examples in Fig. 2. In three cells the i.p.s.p.s were evoked by stimuli subthreshold for the second component of the incoming volleys (Fig. 2*D*) and grew with increased stimulus intensity (Fig. 2*E* and *F*) which indicates that both Ia and Ib afferents might have contributed to them. Other i.p.s.p.s required stronger stimuli; consequently they were likely to be due to I b afferents. The latencies of these i.p.s.p.s were $1{\cdot}7 \pm 0{\cdot}4$ msec (mean and S.D., $n = 57$) and they were distributed within the same ranges (Fig. 3*C*) as the latencies of i.p.s.p.s in intact preparations (Fig. 3*A*) and after lesions of the dorsal funiculi (Fig. 3*B*) but with a shift towards shorter latencies.

To ascertain that the i.p.s.p.s evoked after transection of the lateral and ventral funiculi are being mediated by interneurones located rostral to the lesions, even though neurones with axons in the dorsal funiculi (Tower, Bodian & Howe, 1941; Uddenberg, 1968; Angaut-Petit, 1975*a*, *b*) might add to them (see Discussion), a separate test was used. Since rubrospinal volleys could influence only those neurones which were located rostral to the lesion of the lateral funiculus, we used facilitation of the i.p.s.p.s by stimulation in the red nucleus as an indication that they were mediated by local interneurones. As described in the following paper (Hongo *et al.* 1983) the effects of such a stimulation were tested in ten d.s.c.t. cells and clear facilitation was found in five of them. In one cat an additional transection of the lateral and ventral funiculi was made between L3 and L4 segments. Disynaptic i.p.s.p.s of group I origin were then observed in the two d.s.c.t. cells recorded in L3.

Inhibition of d.s.c.t. cells after lesions of the dorsal funiculi, or of the lateral and ventral funiculi, was tested primarily for effects evoked from group I afferents. However, stimulation of other afferents, cutaneous, joint or high threshold muscle afferents, gave rise to inhibition of d.s.c.t. cells both before and after either of the lesions. The conclusion that interneurones mediating group I inhibition are located both at the same and at more caudal levels may thus be extended to inhibition evoked via other groups of afferents and via polysynaptic pathways.

2. Demonstration of projection of L5–S1 interneurones to Clarke's column and/or its neighbourhood, as revealed by retrograde transport of HRP

Horseradish peroxidase was injected into Clarke's column in two cats with the dorsal funiculi transected. Location of cells labelled by its retrograde transport in one of these cats is shown in Fig. 6. The cells were distributed throughout L5–S1 segments, the majority (79 % in L5, 60 % in L6 and 64 % in L7 segments) being located in Rexed's laminae V–VI and in the dorsal part of lamina VII. Cobalt intensification (see Methods) revealed systematically greater numbers of cells than reaction with only DAB: the numbers were 1·5 times greater in L5 and about 3 times greater in the remaining segments. The densities of the labelled cells (per millimetre of the length of the spinal cord, with cobalt intensification) were 7·8 in L5, 22·0 in L6, 14·1 in L7 and 4·6 in S1 segments. The total numbers of cells with axons projecting to the injection area are estimated as 75 in L5, 176 in L6, 110 in L7 and 21 in S1 segments. The results obtained in the second cat were essentially the same, although the total number of cells found to be labelled was smaller.

The injection sites in L3–L4 segments were reconstructed taking into account the

centres of the densest HRP-stained areas. All were verified to be located within Clarke's column. The HRP dense areas usually had diameters of about 500 μm and covered most of Clarke's column. However, they did sometimes also extend outside the nucleus. Thus the majority of the labelled neurones probably terminated in Clarke's column, but they could also have included neurones terminating outside Clarke's column, (see Ahlsén, 1981). HRP may have also been taken up by damaged axons in the dorsal funiculus through which the HRP electrode was inserted to Clarke's column. However, it could not have been transported caudal to L4 segment since the dorsal funiculi had previously been transected between L4 and L5 segments.

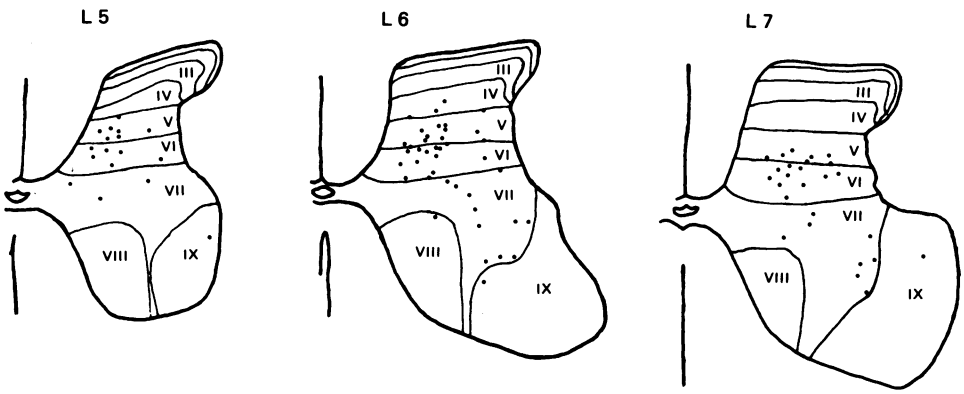

Fig. 6. Location of neurones retrogradely labelled after injection of HRP into Clarke's column. The three diagrams show the distribution of HRP-labelled neurones found in twenty sections (100 μm thick) in L5, L6 and L7 segments, respectively. The location of the cells is indicated on a representative section of the segment.

3. Ascending projections of group I excited laminae V–VI interneurones revealed by their antidromic activation by stimuli applied in Clarke's column

Observations reported in the preceding section have been supplemented by a demonstration that axons of Rexed's laminae V–VI interneurones with monosynaptic input from group I afferents may indeed be excited by stimuli applied in Clarke's column. Such interneurones were searched for in the rostral part of L7 and the caudal part of L6, at depths where maximal field potentials from group I afferents were evoked (Eccles, Fatt, Landgren & Winsbury, 1954) in cats with the dorsal funiculi transected. A second electrode or a set of electrodes was inserted into Clarke's column in L4, L3 or L2. Stimulus pulses were applied via these electrodes to test if they are followed by antidromic invasion of any interneurones. Records in Fig. 7*A* show antidromic invasion of an interneurone following stimuli applied in rostral L3 and the collision between the orthodromically and antidromically conducted impulses at a sufficiently short interval between the stimuli. The antidromic invasion from Clarke's column in L3–L4 was seen in a total of nineteen interneurones, eleven of which were fired by volleys in group I afferents. The recording sites for seven interneurones at the border between L6 and L7 segments are shown in Fig. 7*B*. The latencies of the antidromically evoked spike potentials ranged between 0·8 and

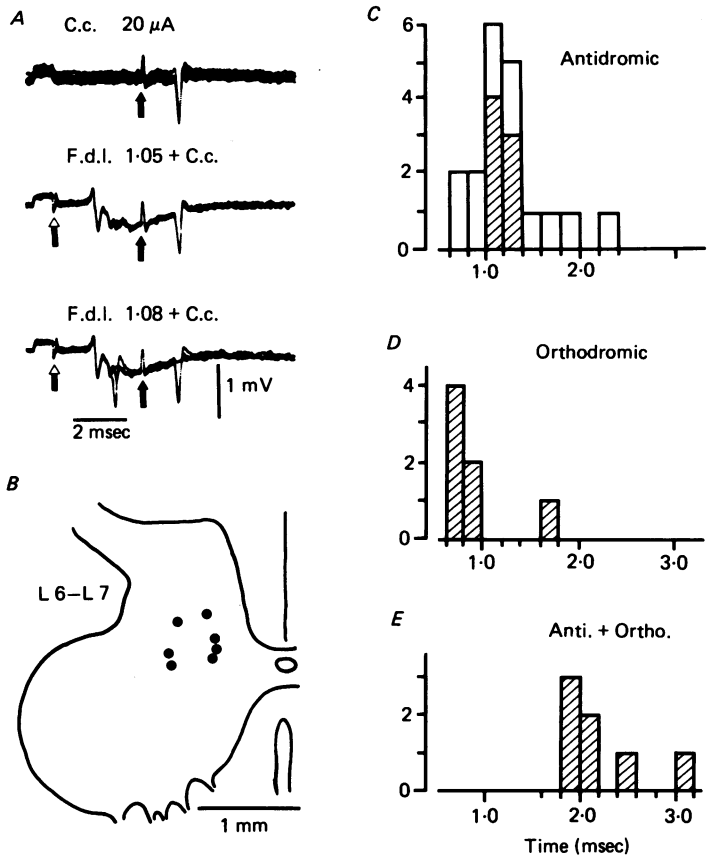

Fig. 7. Responses and location of interneurones in L6–L7 segments which were antidromically activated following stimuli applied in Clarke's column. *A*, sample records from an interneurone in L7 with input from group I afferents of flexor digitorum longus (f.d.l.); top record shows antidromic activation by stimuli applied in Clarke's column (C.c.) in rostral L3, about 40 mm rostral to the recording electrode. The other two records show antidromic activation preceded by nerve stimulation, subthreshold and at threshold for activating the cell in half of the trials; note the resulting collision between the orthodromically and antidromically conducted impulses in the bottom record. *B*, location of seven of the group I excited interneurones activated from Clarke's column in L6 and L7 segments. The histograms show distribution of latencies of antidromic activation (*C*), orthodromic activation (*D*) and sums of the two (*E*). Data for nineteen interneurones. Hatched columns are for interneurones illustrated in *B*.

2·4 msec (Fig. 7*C*). The conduction time was estimated to be 0·1–0·2 msec shorter (see Jankowska & Roberts, 1972) and the calculated conduction velocities were 20–50 m/sec. None of these neurones projected rostral to the L1 segment as evidenced by their lack of response to stimulation of the lateral funiculus in Th13.

The histogram in Fig. 7*E* gives the estimated latencies with which the seven analysed interneurones could evoke p.s.p.s in d.s.c.t. cells. They represent sums of the latencies of the antidromic activation of these interneurones by weak (< 40 μA) stimuli applied in Clarke's column (hatched columns in Fig. 7*C*) and of segmental

latencies of their synaptic activation by afferent volleys (Fig. 7*D*). An additional 0·3 msec should be added to these values to account for synaptic delays of transmission between the interneurones and the d.s.c.t. cells. However, since the activation time (0·1– 0·2 msec) should be subtracted from the latencies of the antidromic invasion to give the conduction time, these two corrections might cancel each other. The range of the expected latencies of i.p.s.p.s mediated by these interneurones in d.s.c.t. cells is in fair agreement with those observed (Fig. 3*B*).

DISCUSSION

The reported physiological observations show that the disynaptic inhibition of d.s.c.t. cells from group I muscle afferents is mediated by interneurones located caudal to Clarke's column as well as by interneurones located at its level. Theoretically, four classes of interneurones can be considered to mediate inhibition of these cells: (1) interneurones located caudally to L4, which have axon collaterals ascending in the lateral and/or ventral funiculus, (2) interneurones similarly located in lower lumbar segments, but with axon collaterals ascending in the dorsal funiculus, (3) interneurones located within Clarke's column (see Réthelyi, 1968, 1970) and (4) interneurones located rostral to L5 but outside Clarke's column.

The first class of interneurones have now been shown to exist and they have been the main subject of the present study. The majority of cells marked following injection of HRP to Clarke's column in the lower lumbar and sacral segments, and all cells activated antidromically from Clarke's column, and orthodromically from group I afferents, were found in laminae V–VI of Rexed (1952, 1954). This is the same region in which a previous study has demonstrated a number of group I excited interneurones with axon collaterals ascending in the lateral funiculus (Czarkowska, Jankowska & Sybirska, 1981; Jankowska, Johanisson & Lipski, 1981*a*; Jankowska *et al.* 1981*b*). Termination in Clarke's column of fibres from the lateral funiculus has been shown by Golgi and degeneration studies (Szentágothai & Albert, 1955; Boehme, 1968). These separate groups of observations are thus in good agreement in showing that these neurones should be responsible for inhibition of d.s.c.t. cells from group I afferents (see also Hongo *et al.* 1983).

Réthelyi (1970) considered rostrally rather than caudally located interneurones as inhibiting d.s.c.t. cells in view of his finding that terminals with flattened vesicles appeared unaltered after transection of the spinal cord, while the number of terminals with spherical vesicles greatly decreased. However, if the vesicles in terminals of the caudally located inhibitory interneurones are not as flattened as in some other inhibitory synapses (see, e.g. Uchizono, 1965; Bodian, 1966) this might give one explanation of the apparent discrepancy between Réthelyi's and the present results. Rastad (1981) found only a very moderate degree of flatness of vesicles of another group of spinal inhibitory interneurones, those which mediate the Ia reciprocal inhibition. The possibility that the caudally located inhibitory interneurones have terminals with spherical rather than flattened vesicles in Clarke's column is, on the other hand, difficult to reconcile with the location of such terminals. They were found to make synaptic contacts with smaller cells or with thinner dendrites, while they

would be expected to terminate on proximal dendrites of the largest cells if they mediated inhibition of group I origin (see the recent evidence of Randić, Miletić & Loewy, 1981, that group I inhibition is evoked in the largest d.s.c.t. cells). Thus it may well be that the caudal inhibitory interneurones have terminals with flattened vesicles, but that their disappearance was not noticed in Réthelyi's (1970) study; perhaps they could not be identified among the freshly degenerated terminals and their disappearance might have been masked by abundance of terminals of local inhibitory interneurones.

The number of interneurones labelled with HRP most likely gives an underestimate of the total number of laminae V–VI interneurones projecting to Clarke's column. As mentioned under Results, the dense HRP area covered most but not the whole of Clarke's column in L3–L4 and no attempt was made to inject its more rostral regions. Furthermore, the weakest labelled neurones might have been overlooked. For these reasons the reported observations should be considered as showing the relative density of interneurones projecting to the upper lumbar segments in L5–S1 segments and not their total numbers. Most of the labelled interneurones probably terminated on d.s.c.t. cells but projections to areas outside Clarke's column cannot be excluded; some of these interneurones might well have been marked by HRP diffusing from Clarke's column to the neighbouring regions and even low HRP concentrations must be considered as effective (see Ahlsen, 1981). The identity of the additionally labelled ventral horn neurones remains to be established. They were found in an area wider than the area of location of interneurones with group I input and might represent interneurones in flexor reflex afferents (Eccles & Lundberg, 1959) pathways to d.s.c.t. cells (Lundberg & Oscarsson, 1960; Lundberg, Norrsell & Voorhoeve, 1963; Hongo & Okada, 1967). Their target cells might also be outside Clarke's column.

Preliminary observations of subsequent studies are well in keeping with these conjectures. According to these observations, lower lumbar interneurones with input from group I muscle afferents and with axon collaterals ascending to L4 and L3 segments, inhibit motoneurones and some interneurones in the intermediate zone in addition to d.s.c.t. cells (E. Brink & B. Skoog, unpublished); the inhibition has been found in preparations in which the dorsal funiculi were transected. Another group of observations has shown that interneurones in lower lumbar segments which are located in laminae VII and project to L3–L4 segments have input from high threshold muscle, skin or joint afferents (flexor reflex afferents) but not from group I afferents (P. J. Harrison & E. Jankowska, unpublished).

Conduction velocities along axons of laminae V–VI interneurones which were antidromically activated by stimuli applied in Clarke's column were estimated to be between 20 and 50 m/sec. These values are very close to the expected conduction velocities of laminae V–VI interneurones calculated from the diameters of the proximal parts of their axons stained with HRP (between 30 and 75 m/sec, Czarkowska, Jankowska & Sybirska, 1976), and are as required for evoking i.p.s.p.s with the observed latencies, as described under Results.

A contribution of the *second class of interneurones* to inhibition of d.s.c.t. cells is only hypothetical. The majority of neurones with axons in the dorsal funiculi project to the level of the dorsal column nuclei (see Nathan & Smith, 1959; Angaut-Petit, 1975*a*) but they give off axon collaterals, at least in the same segment (Brown & Fyffe, 1981). Intracellular records from these neurones have shown that their main

monosynaptic input is from low threshold cutaneous afferents (Uddenberg, 1968; Angaut-Petit, 1975*b*; Jankowska, Rastad & Zarzecki, 1979) but in nearly half of them weak monosynaptic input from group I afferents was found (Jankowska *et al.* 1979) even if their location is outside the main areas of termination of group I afferents (Brown & Fyffe, 1978, 1979; Ishizuka, Mannen, Hongo & Sasaki, 1979; Hongo, Ishizuka, Kudo, Mannen & Sasaki, 1980) and coincides only with the most dorsal terminal branching area of Ib afferents (Hongo *et al.* 1980). With respect to neurones with axon collaterals ascending in the dorsal funiculi over a distance of only a few segments (Nathan & Smith, 1959; Angaut-Petit, 1975*a*, *b*) not much more is known than that such neurones do exist. In particular it is totally unknown whether they have any group I input and on which neurones they terminate. Since there is no positive evidence for either of these neurones to be involved in inhibition of d.s.c.t. cells from group I afferents we list them as a possibility but, until any evidence to the contrary is obtained, we consider the disynaptic effects of group I origin found after lesions of the lateral and ventral funiculi to be due to local interneurones.

With respect to *the third class of interneurones*, Golgi studies have shown the existence of interneurones in Clarke's column (border cells) which may be contacted by collaterals from the dorsal funiculi and project to the d.s.c.t. (focal) cells (Réthelyi, 1968; see Introduction) but no physiological evidence is as yet available as to what are their synaptic actions. They might, for instance, mediate inhibition from other kinds of afferents or from descending systems (Lundberg *et al.* 1963; Hongo & Okada, 1967; Jansen *et al.* 1969).

Data on *the fourth class of interneurones* are also scarce. No attempts have been made to locate such interneurones since the available methods (by labelling them with retrogradely transported HRP or by looking for cells activated antidromically from Clarke's column) are not reliable for interneurones at the same levels as the explored region of Clarke's column. However, since HRP-labelled laminae V–VI interneurones were found in the most rostral part of L5 segment, one could expect such cells to be distributed also rostral to this segment, the choice of the border between L4 and L5 segments as separating 'lower' and 'upper' lumbar interneurones being quite arbitrary. In fact, axon collaterals of group I afferents from hind-limb muscles have been shown to reach laminae V–VI of L3–L4 segments (N. Ishizuka, H. Mannen, T. Hongo, N. Kudo, S. Sasaki, M. Yamashita & K. Yoshida, unpublished observation; see also Hogg, 1944; Réthelyi, 1968). Boehme (1968) reported that some cells in the base of the dorsal horn send axon collaterals to terminate in Clarke's column. Some of them might thus mediate inhibition from group I afferents to d.s.c.t. cells, and belong to the same functional population as the first class of interneurones.

For the assessment of the relative contribution of the third and fourth class of interneurones it is worth noting that inhibition of L3 d.s.c.t. cells has also been seen after transection of the lateral and ventral funiculi between L3 and L4 segments.

Observations reported in the following paper (Hongo *et al.* 1983) will further define the identity of interneurones mediating inhibition of d.s.c.t. cells. They will show that at least some of them, the laminae V–VI interneurones with axon collaterals ascending in the lateral and ventral funiculi, are among interneurones which mediate the non-reciprocal inhibition of motoneurones from group I afferents and that the inhibition of d.s.c.t. cells is collateral to the inhibition of motoneurones.

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REFERENCES

ADAMS, J. C. (1977). Technical considerations on the use of horseradish peroxidase as a neuronal marker. *Neuroscience* **2**, 141–145.

AHLSÉN, G. (1981). Retrograde labelling of retinogeniculate neurones in the cat by HRP uptake from the diffuse injection zone. *Brain Res.* **223**, 374–380.

ANGAUT-PETIT, D. (1975*a*). The dorsal column system. I. Existence of long ascending postsynaptic fibres in the cat's fasciculus gracilis. *Exp. Brain Res.* **22**, 457–470.

ANGAUT-PETIT, D. (1975*b*). The dorsal column system. II. Functional properties and bulbar relay of the postsynaptic fibres of the cat's fasciculus gracilis. *Exp. Brain Res.* **22**, 471–493.

BODIAN, D. (1966). Electronmicroscopy: Two major synaptic types of spinal motoneurons. *Science, N.Y.* **151**, 1093–1094.

BOEHME, C. C. (1968). The neural structure of Clarke's nucleus of the spinal cord. *J. comp. Neurol.* **132**, 445–462.

BROWN, A. G. & FYFFE, R. E. W. (1978). The morphology of group Ia afferent fibre collaterals in the spinal cord of the cat. *J. Physiol.* **274**, 111–127.

BROWN, A. G. & FYFFE, R. E. W. (1979). The morphology of group Ib afferent fibre collaterals in the spinal cord of the cat. *J. Physiol.* **296**, 215–228.

BROWN, A. G. & FYFFE, R. E. W. (1981). Form and function of dorsal horn neurones with axons ascending the dorsal columns in cat. *J. Physiol.* **321**, 31–47.

CURTIS, D. R., ECCLES, J. C. & LUNDBERG, A. (1958). Intracellular recording from cells in Clarke's column. *Acta physiol. scand.* **43**, 303–314.

CZARKOWSKA, J., JANKOWSKA, E. & SYBIRSKA, E. (1976). Diameter and internodal length of axons of spinal interneurones excited by group I afferents in the cat. *Brain Res.* **118**, 119–122.

CZARKOWSKA, J., JANKOWSKA, E. & SYBIRSKA, E. (1981). Common interneurones in reflex pathways from group Ia and Ib afferents of knee flexor and extensor in the cat. *J. Physiol.* **310**, 367–380.

ECCLES, J. C., FATT, P., LANDGREN, S. & WINSBURY, G. J. (1954). Spinal cord potentials generated by volleys in the large muscle afferents. *J. Physiol.* **125**, 590–606.

ECCLES, J. C., OSCARSSON, O. & WILLIS, W. D. (1961). Synaptic action of group I and II afferent fibres of muscle on the cells of dorsal spinocerebellar tract. *J. Physiol.* **158**, 517–543.

ECCLES, R. M. & LUNDBERG, A. (1959). Synaptic actions in motoneurones by afferents which may evoke the flexion reflex. *Archs ital. Biol.* **97**, 199–221.

EIDE, E., FEDINA, L., JANSEN, J., LUNDBERG, A. & VYKLICKÝ, L. (1969). Properties of Clarke's column neurones. *Acta physiol. scand.* **77**, 125–144.

FETZ, E., JANKOWSKA, E., JOHANNISSON, T. & LIPSKI, J. (1979). Autogenetic inhibition of motoneurones by impulses in group I muscle spindle afferents. *J. Physiol.* **293**, 173–195.

HOGG, I. D. (1944). The development of the nucleus dorsalis (Clarke's column). *J. comp. Neurol.* **81**, 69–95.

HOLMQVIST, B., LUNDBERG, A. & OSCARSSON, O. (1956). Functional organization of the dorsal spinocerebellar tract in the cat. V. Further experiments on convergence of excitatory and inhibitory actions. *Acta physiol. scand.* **38**, 76–90.

HONGO, T., ISHIZUKA, N., KUDO, N., MANNEN, H. & SASAKI, S. (1980). Quantitative analysis of the morphology of group Ib fibres in the spinal cord of the cat. *Neurosci. Lett.*, Suppl. 4, 26.

HONGO, T., JANKOWSKA, E., OHNO, T., SASAKI, S., YAMASHITA, M. & YOSHIDA, K. (1981). Neural pathways mediating disynaptic inhibition of dorsal spinocerebellar tract neurones from group I muscle afferents in cats. *J. physiol. Soc. Japan* **43**, 303.

HONGO, T., JANKOWSKA, E., OHNO, T., SASAKI, S., YAMASHITA, M. & YOSHIDA, K. (1983). The same interneurones mediate inhibition of dorsal spinocerebellar tract cells and lumbar motoneurones in the cat. *J. Physiol.* **342**, 161–180.

HONGO, T. & OKADA, Y. (1967). Cortically evoked pre- and post-synaptic inhibition of impulse transmission to the dorsal spinocerebellar tract. *Exp. Brain Res.* **3**, 163–177.

ISHIZUKA, N., MANNEN, H., HONGO, T. & SASAKI, S. (1979). Trajectory of group Ia afferent fibres stained with horseradish peroxidase in the lumbo-sacral spinal cord of the cat: three dimensional reconstructions from serial sections. *J. comp. Neurol.* **186**, 189–212.

JANKOWSKA, E., JOHANNISSON, T. & LIPSKI, J. (1981*a*). Common interneurones in reflex pathways from group Ia and Ib afferents of ankle extensors in the cat. *J. Physiol.* **310**, 381–402.

JANKOWSKA, E., LUNDBERG, A., RUDOMIN, P. & SYKÓVA, E. (1982). Effects of 4-aminopyridine on synaptic transmission in the cat spinal cord. *Brain Res.* **240**, 117–129.

JANKOWSKA, E., MCCREA, D. & MACKEL, R. (1981*b*). Pattern of 'non-reciprocal' inhibition of motoneurones in spinal cats by impulses in group Ia muscle spindle afferents. *J. Physiol.* **316**, 393–409.

JANKOWSKA, E., RASTAD, J. & ZARZECKI, P. (1979). Segmental and supraspinal input to cells of origin of non-primary fibres in the feline dorsal columns. *J. Physiol.* **290**, 185–200.

JANKOWSKA, E. & ROBERTS, W. J. (1972). Án electrophysiological demonstration of the axonal projections of single spinal interneurones in the cat. *J. Physiol.* **222**, 597–622.

JANSEN, J. K. S., NICOLAYSEN, K. & WALLOE, L. (1967). On the inhibition of transmission to the dorsal spinocerebellar tract by stretch of various ankle muscles of the cat. *Acta physiol. scand.* **70**, 362–368.

JANSEN, J. K. S., NICOLAYSEN, K. & WALLOE, L. (1969). The firing pattern of dorsal spinocerebellar tract neurones during inhibition. *Acta physiol. scand.* **77**, 68–84.

JANSEN, J. K. S. & RUDJORD, T. (1965). Dorsal spinocerebellar tract: response pattern of nerve fibers to muscle stretch. *Science, N.Y.* **149**, 1109–1111.

LAPORTE, Y. & LUNDBERG, A. (1956). Functional organization of the dorsal spino-cerebellar tract in the cat. III. Single fibre recording in Flechsig's fasciculus on adequate stimulation of primary afferent neurones. *Acta physiol. scand.* **36**, 204–218.

LAPORTE, Y., LUNDBERG, A. & OSCARSSON, O. (1956). Functional organization of the dorsal spino-cerebellar tract in the cat. II. Single fibre recording in Flechsig's fasciculus on electrical stimulation of various peripheral nerves. *Acta physiol. scand.* **36**, 188–203.

LUNDBERG, A., NORRSELL, U. & VOORHOEVE, P. (1963). Effects from the sensorimotor cortex on ascending spinal pathways. *Acta physiol. scand.* **59**, 462–473.

LUNDBERG, A. & OSCARSSON, O. (1956). Functional organization of the dorsal spino-cerebellar tract in the cat. IV. Synaptic connections of afferents from Golgi tendon organs and muscle spindles. *Acta physiol. scand.* **38**, 53–75.

LUNDBERG, A. & OSCARSSON, O. (1960). Functional organization of the dorsal spino-cerebellar tract in the cat. VII. Identification of units by antidromic activation from the cerebellar cortex with recognition of five functional subdivisions. *Acta physiol. scand.* **50**, 356–374.

NATHAN, P. W. & SMITH, M. (1959). Fasciculi proprii of the spinal cord in man. *Brain* **82**, 610–668.

RANDIĆ, M., MILETIĆ, V. & LOEWY, A. D. (1981). A morphological study of cat DSCT neurons after intracellular injection of HRP. *J. comp. Neurol.* **198**, 453–466.

RASTAD, J. (1981). Ultrastructural morphology of axon terminals of an inhibitory spinal interneurone in the cat. *Brain Res.* **223**, 397–401.

RÉTHELYI, M. (1968). The Golgi architecture of Clarke's column. *Acta morph. hung.* **16**, 311–330.

RÉTHELYI, M. (1970). Ultrastructural synaptology of Clarke's column. *Exp. Brain Res.* **11**, 159–174.

REXED, B. (1952). The cytoarchitectonic organization of the spinal cord in the cat. *J. comp. Neurol.* **96**, 415–496.

REXED, B. (1954). A cytoarchitectonic atlas of the spinal cord in the cat. *J. comp. Neurol.* **100**, 297–379.

SZENTÁGOTHAI, J. & ALBERT, A. (1955). The synaptology of Clarke's column. *Acta morph. hung.* **5**, 43–51.

TOWER, S. S., BODIAN, D. & HOWE, H. (1941). Isolation of intrinsic and motor mechanisms of the monkey's spinal cord. *J. Neurophysiol.* **4**, 388–397.

UCHIZONO, K. (1965). Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. *Nature, Lond.* **207**, 642–643.

UDDENBERG, N. (1968). Functional organization of long, second-order afferents in the dorsal funiculus. *Exp. Brain Res.* **4**, 377–382.