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COMMON INTERNEURONES IN REFLEX PATHWAYS FROM GROUP Ia AND Ib AFFERENTS OF KNEE FLEXORS AND EXTENSORS IN THE CAT

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SUMMARY

1. Input from group I afferents of knee flexors and extensors to interneurones in Rexed's laminae V–VI in the cat spinal cord was analysed by use of intracellular recording and electrical stimulation of the nerves to differentiate between group Ia and and Ib synaptic actions. The aim was to find out if these interneurones may mediate synaptic actions of both group Ia and Ib afferents.

2. 28% of the forty-nine neurones analysed were excited by both group Ia and group Ib afferents; 32% were inhibited by both and 35% were excited by the one and inhibited by the other.

Taking into account all of these actions, input from both subgroups of group I afferents was found in nearly 60% of neurones. Most were also excited and/or inhibited by group I afferents of ankle and toe extensors.

3. Selective (excitatory and/or inhibitory) input from Ia afferents was found in 18% and from Ib afferents in 22% of the neurones.

4. Excitation was evoked from Ia afferents of either knee flexors or extensors but not from both. In several of the neurones Ia i.p.s.p.s were, however, evoked from both posterior biceps-semitendinosus and quadriceps.

5. Intracellular staining with horseradish peroxidase revealed axonal projections of laminae V–VI interneurones to motor nuclei as well as to the intermediate zone, ipsilateral as well as contralateral. No correlation was found between patterns of input from group I afferents and axonal projections, and interneurones co-excited by Ia and Ib afferents were among those with different axonal projections.

INTRODUCTION

Until recently it was considered that either the muscle spindles or the Golgi tendon organs are responsible for the main reflex actions of group I muscle afferents. However, the autogenetic inhibition of motoneurones is evoked not only from Ib tendon organ afferents (Granit, 1950; Laporte & Lloyd, 1952; Eccles, Eccles & Lundberg, 1957*a*, *b*) but also from Ia muscle spindle afferents (Fetz, Jankowska, Johannisson & Lipski, 1979), and the same appears to be true for the di- and

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trisynaptic inhibition of synergists generally and for the associated excitation of flexors (Jankowska, Mackel & McCrea, 1980). Both Ia and Ib origin of the presynaptic inhibition of group Ia afferents has been also evidenced (see Schmidt, 1973).

These observations prompted the present study of spinal pathways which are activated by both muscle spindles and tendon organs since they would be best suited to mediate similar actions of these different receptors. Some interneurones influenced by Ia as well as by Ib afferents have been found in Rexed's laminae V and VI (Hongo, Jankowska & Lundberg 1966, 1972; see also Eccles, Eccles & Lundberg, 1960) but neither the full pattern of convergence onto them, nor neuronal pathways in which they are interposed have been defined so far. As a first step in the analysis of their function we have now re-investigated the group I input to laminae V–VI interneurones and correlated it with axonal projections of these. The present paper reports observations on convergence of Ia and Ib afferents from knee flexors, posterior biceps and semitendinosus, and knee extensor, quadriceps, which were excited by graded electrical stimulation of the nerves of these muscles. Because of their higher sensitivity to electrical stimuli, one can activate a great proportion of group Ia afferents of these nerves before reaching threshold for Ib afferents (Bradley & Eccles, 1953; for further references see Jack, 1978). The use of electrical stimuli to activate selectively Ia afferents of other nerves is less satisfactory. Therefore observations on group I input from ankle and toe extensors to laminae V–VI interneurones are reported in the following paper (Jankowska, Johannisson & Lipski, 1981); they are based on effects of activation of Ia spindle afferents by adequate stimuli.

In both experimental series the axonal projections of the analysed interneurones were established after staining them with intracellularly applied horseradish peroxidase (Snow, Rose & Brown, 1976; Jankowska, Rastad & Westman, 1976), which allowed reconstruction of their stem axons and initial axon collaterals within about half a segment. It will be shown that interneurones with input from both Ia and Ib afferents project to motor nuclei and/or the intermediate zone and may mediate disynaptic inhibition and excitation of motoneurones, as well as some other actions of muscle spindles and tendon organs.

Some of the results of this study have been published as a preliminary communication (Czarkowska, Jankowska & Sybirska 1976*a*, *b*).

METHODS

Preparation. The experiments were performed on eight cats under chloralose (50–70 mg/kg) or under both chloralose (40–50 mg/kg) and sodium pentobarbitone (Nembutal, Abbott, 5–10 mg/kg) anaesthesia, after initial dissection under ether anaesthesia. The dissection included intubation, laminectomy (L4–L7 and Th13–L1) and dissection of a number of hind-limb nerves including those to: quadriceps, posterior biceps and semitendinosus, gastrocnemius-soleus, plantaris, and flexor digitorum and hallucis longus. All animals were spinalized at Th13–L1 level, paralysed with gallamine triethiodide (Flaxedil, Rhodia) and artificially ventilated. Otherwise the preparation was as described in the preceding paper (Fetz *et al.* 1979).

Recording. Glass micropipettes filled with a solution of horseradish peroxidase in NaCl (see below), with tips broken to 1·5–2·0 μm and resistance of 20–30 MΩ were used throughout. A preliminary series of records from each interneurone was taken without passing any polarizing current through the electrode. The penetrated neurones were then identified as interneurones if they were not

antidromically activated by stimulation of ventral roots or of either half of the spinal cord at Th13 level. This test excluded motoneurones and cells of origin of long ascending tracts with axons in the ipsilateral, or contralateral, lateral or ventral funiculi. In these preliminary records we also verified that the neurones were excited or inhibited by group I afferents from the posterior biceps semitendinosus and/or quadriceps, and the I a or I b origin of these synaptic actions was determined. Subsequent records were made during passage of depolarizing current applied both to eject horseradish peroxidase and to increase amplitude of i.p.s.p.s.

Stimulation. Electrical stimulation of the posterior biceps semitendinosus and quadriceps nerves was graded from the threshold for group I a afferents to maximum for all group I afferents; the effects of higher threshold afferents were tested only at 5 and 10 or 20 times threshold stimulus intensities, to differentiate between group I and group II effects. E.p.s.p.s and i.p.s.p.s were attributed to I a afferents if they were evoked by stimuli near threshold for the first component of the afferent volleys recorded from the entry zone of L6 or L7 dorsal roots (Bradley & Eccles, 1953; Eccles, Eccles & Lundberg, 1957*a*). To I b afferents were attributed synaptic actions evoked by higher stimulus intensities, appearing in parallel with the second components of the afferent volleys. However, if e.p.s.p.s or i.p.s.p.s appeared with stimuli near maximum for the first component and increased with stimulus intensities along with the second component (as in Fig. 2*F–H*), they were also classified as of I b origin. In view of a certain overlap between thresholds of I a and I b afferents of posterior biceps and semintendinosus (Laporte & Bessou, 1957; Coppin, Jack & McIntyre, 1969) and probably also of quadriceps, a selective activation of I a afferents of these nerves may be expected only with stimulus intensities within lower ranges for the first component of the incoming volleys or up to about 1·2–1·3 times threshold (see Jack, 1978; Coppin *et al.* 1969). No attempts were made to determine whether synaptic actions from other nerves originated from I a or I b afferents.

Staining. Horseradish peroxidase (Sigma, type VI) was electrophoretically ejected from micropipettes filled with its 10–15% solution in 0·5 or 0·3 M-NaCl (with pH set to 9·5–10·5). Constant current of 2–15 nA (usually 5–10 nA), with electrode tip positive was used for this purpose. The amount of the ejected enzyme was estimated by multiplying the intensity of the applied current (in nA) by the time of its application (in min). In well stained cells this ranged from 50 to 200 nA × min. After allowing about 2 h for diffusion of horseradish peroxidase in the last injected cell (corresponding to 8–12 h diffusion time for the first injected cells) the cats were perfused through the thoracic aorta with 3 l. 2·5% glutaraldehyde and 1% paraformaldehyde in 0·1 M-phosphate buffer. After post-fixation for 6–8 h and washing in phosphate buffer (with or without 30% sucrose) for the next 24 h, the spinal cords were cut at 50 μm and incubated in 0·05% solution of diaminobenzidine tetrahydrochloride and 0·05% hydrogen peroxide in Tris buffer (pH 7·6). The sections were counterstained in 0·05% aqueous solution of toluidine blue, dehydrated in alcohols and cleared in xylol.

RESULTS

Observations were made on forty-nine interneurones that were held sufficiently long to allow analysis of their responses to graded electrical stimulation of the nerves and injection of horseradish peroxidase. The sample includes five interneurones recorded by E. Jankowska, T. Johannisson and J. Lipski.

Selective and combined synaptic actions of Ia and Ib afferents

In agreement with previous reports (Eccles *et al.* 1960; Hongo *et al.* 1966, 1972) we have found that laminae V–VI interneurones may be (i) selectively excited by either group I a or group I b afferents of posterior biceps-semitendinosus and quadriceps, (ii) co-excited or co-inhibited by both these subgroups of group I afferents or (iii) excited by one and inhibited by the other. The synaptic actions evoked in individual interneurones fell into one of the thirteen patterns of input indicated by the thirteen rows that make up Fig. 1. The first column shows the response of the group of neurones to afferent stimulation. The open squares indicate e.p.s.p.s and the

Group I input					Axonal projection to:					
Patterns of input from PBSt and Q		Contribution from			Motor nuclei		Interm. zone			Crossed
	n	PBSt	Q	G-S, Pl, FDL	1	2	3	4	5	6
	2	2/		2/2	1					
	4	/4	2/	3/4			3			
	3	2/2	1/3	2/2	1					1
	6	/4	/3	4/3		1		1		
	1	/1	/1	1/1					1	
	4	4/3	1/1	2/1	1		1	1		
	8	3/3	5/6	4/6	3		1	3		
	1	/1	1/	1/1						
	3	2/3	1/1	1/3		1				
	2		2/2	1/2		1				
	4	2/3	2/3	3/3		1	1			
	6†	2/1	4/2	5/5	1		1			
	5	/5	/1	4/5	1	1			1	
Total 49		11/15	11/19	33/38	8	5	5	5	2	1

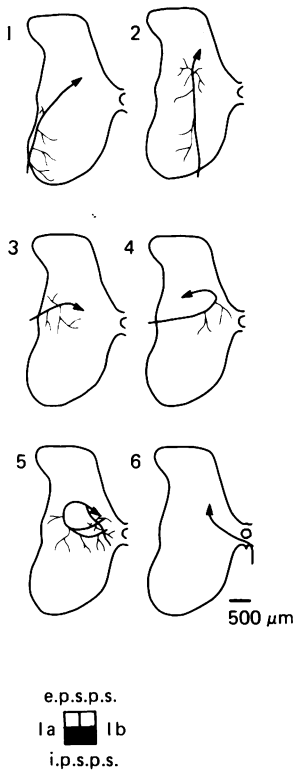

Fig. 1. Correlation between input from group I afferents in posterior biceps-semitendinosus (PBSt) and quadriceps (Q) and axonal projections of the stained interneurones.

In the first column are patterns of excitatory and inhibitory actions of Ia and Ib afferents, and the numbers of neurones in which they were found. As indicated by key in bottom right, input from Ia afferents is on left and from Ib afferents on right, and e.p.s.p.s and i.p.s.p.s are represented by open and filled squares, respectively. Columns 2 and 3 show the contribution of afferents in PBSt and Q. Numbers of neurones with e.p.s.p.s (numerator) or i.p.s.p.s (denominator) from gastrocnemius-soleus (G-S), plantaris (Pl) or flexor digitorum longus (FDL) are in column 4. Six columns to the right show the six patterns of axonal projections of interneurones according to Czarkowska *et al.* (1976): (1) to motor nuclei with stem axon in the lateral funiculus, (2) to motor nuclei with stem axon in the ventral funiculus, (3) to the lateral part of the intermediate zone with stem axon in the lateral funiculus, (4) to medial part of the intermediate zone with stem axon in the lateral funiculus, (5) to intermediate zone in Golgi type II cells and (6) with contralateral projections as indicated in the diagrams to the right. † Contribution of Ib afferents to the inhibition evoked from Ia afferents was uncertain in some of these interneurones but could not be excluded.

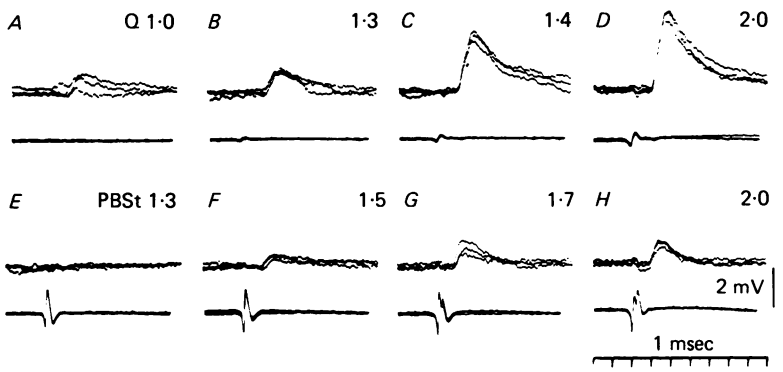

Fig. 2. Co-excitation by Ia afferents of quadriceps (Q) and Ib afferents of posterior biceps-semitendinosus (PBSt) and probably also of quadriceps. Upper traces, intracellular records. Lower traces, afferent volleys recorded from the surface of the spinal cord close to L6 (*A–D*) or L7 (*E–H*) dorsal roots entry zone. The separation between Ia and Ib afferents of Q was poor. However, since the e.p.s.p.s were evoked already by near-threshold stimuli, and grew over the whole range of stimulus intensities up to 2·0 times threshold they were certainly evoked by Ia and probably also by Ib afferents. Note that the maximal stimuli for the whole of the group I afferents were 2·0–2·25 times threshold. The separation between Ia and Ib afferents of PBSt was good. The threshold for Ib afferents was about 1·4–1·5 times threshold for Ia afferents and the e.p.s.p.s were first evoked at this stimulus strength, growing with the second component of the incoming volley. In this and the following figures, the stimulus intensities, expressed in multiples of threshold for the lowest threshold afferents, are given above the corresponding records.

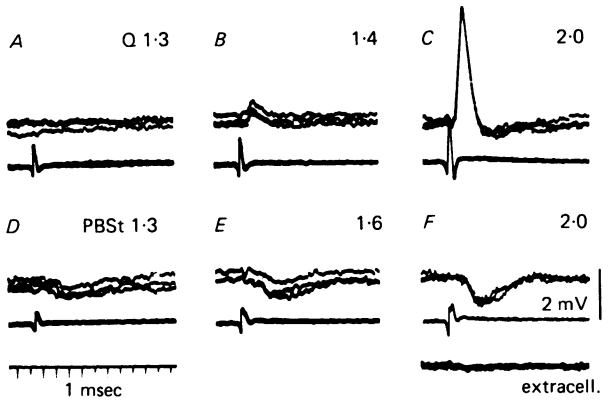

Fig. 3. Inhibition from Ia and Ib afferents combined with excitation by Ib afferents. Upper traces, intracellular records from an interneurone. Lower traces, records of afferent volleys. Lowermost trace in *F*, records taken just after withdrawing the electrode from the cell. E.p.s.p.s from Q were evoked by stimulus intensities near threshold for the second component of the afferent volley (*B*) and are attributed to Ib afferents. I.p.s.p.s from PBSt were evoked in parallel with the first component of the incoming volley (*D*) and grew with the second component (*E* and *F*). They are therefore attributed to both Ia and Ib afferents. An i.p.s.p. likewise followed e.p.s.p.s evoked from Q by stimuli near maximum for group I afferents (*C*).

filled squares i.p.s.p.s; squares to the left indicate responses evoked by Ia afferents while squares to the right indicate responses of Ib origin. The second and third columns show the numbers of neurones that were excited or inhibited by stimulation of the hamstring nerves and the quadriceps nerves. The fourth column shows the numbers of neurones that were excited or inhibited by stimulation of the nerves to triceps surae, plantaris and flexor digitorum longus.

Many interneurones in the present sample were influenced by *either* Ia (18%) or Ib (22%) afferents from knee flexors and/or extensors (rows 1–6 in Fig. 1). A larger proportion of the neurones (60%) were, however, affected by *both* Ia and Ib afferents (rows 7–13). 28% of the neurones were excited by both Ia and Ib afferents and 32% were inhibited by both; 35% showed opposite effects from the two fibre groups (see also Fig. 5 right). Fig. 5, left and middle, shows combinations of Ia and Ib actions evoked from knee flexors or knee extensors, respectively. The synaptic actions listed in Figs. 1 and 5 include both monosynaptic and disynaptic e.p.s.p.s and disynaptic and most likely trisynaptic i.p.s.p.s. The synaptic linkages could, however, be estimated only for the earliest components of the recorded post-synaptic potentials; segmental latencies of 0·9 and 1·5 msec were taken as upper values for mono- and disynaptic coupling.

Co-excitation by Ia and Ib afferents. This is illustrated in Fig. 2.The Ia origin of the e.p.s.p.s evoked from quadriceps is evidenced by their appearance with threshold stimuli (Fig. 2*A*). A Ib contribution to these e.p.s.p.s is indicated by an increase in their amplitude with stimuli within the higher range of intensities for group I afferents in this nerve (Fig. 2*C*, and *D*). The Ib origin of e.p.s.p.s. evoked from posterior biceps semitendinosus is indicated by their absence when the stimulus intensity was nearly maximal for the first (Ia) component of the afferent volley (Fig. 2*E*) and their appearance with higher stimulus intensities (Fig. 2*F–H*).

Opposite actions of Ia and Ib afferents. Excitation by Ib afferents and inhibition by Ia afferents are illustrated in Fig. 3*C* and *D*, respectively. The origin of the e.p.s.p.s. and of the i.p.s.p.s was established using the same criteria as for records in Fig. 2.

Co-inhibition of the same neurone by Ia and Ib afferents. This is exemplified in Fig. 3. Inhibition by the Ia afferents (Fig. 3*D*) was greatly enhanced when the stimulus intensity was increased above threshold for Ib afferents (Fig. 3*F*). The Ib inhibition was also evoked from quadriceps, as evidenced by the rapid decay of the e.p.s.p. (cf. Figs. 2*D* and 3*C*; see also Fig. 3 in Jankowska *et al.* 1980*a*).

Fig. 1 shows that group I afferents from knee flexors and extensors supplied only a fraction of the total group I input to our sample of interneurones. More than 90% of them were excited and/or inhibited by other muscle nerves as well, most often from nerves of triceps surae, plantaris and flexor digitorum longus. The Ia or Ib origin of e.p.s.p.s and i.p.s.p.s evoked from these nerves could not be established with electrical stimuli but, in light of results of the subsequent study (Jankowska *et al.* 1981), Ib afferents from gastrocnemius, soleus and plantaris should have contributed to all the e.p.s.p.s and to a great proportion of the i.p.s.p.s. Taking into account group I input from both the knee flexors and extensors and from the ankle and toe extensors, the proportion of neurones influenced by both Ia and Ib afferents would be even higher than that indicated in Fig. 1.

Synaptic actions from knee flexors versus synaptic actions from knee extensors

Since posterior biceps-semitendinosus and quadriceps are antagonists, it was of interest to compare synaptic actions of afferents of these muscles, especially those of Ia afferents, to see if they were opposite, as observed for motoneurones (Eccles, Eccles & Lundberg 1957*a*; Eccles & Lundberg, 1958) and Ia inhibitory interneurones (Hultborn, Illert & Santini, 1976), or similar. As shown in Fig. 5 (on the right), in

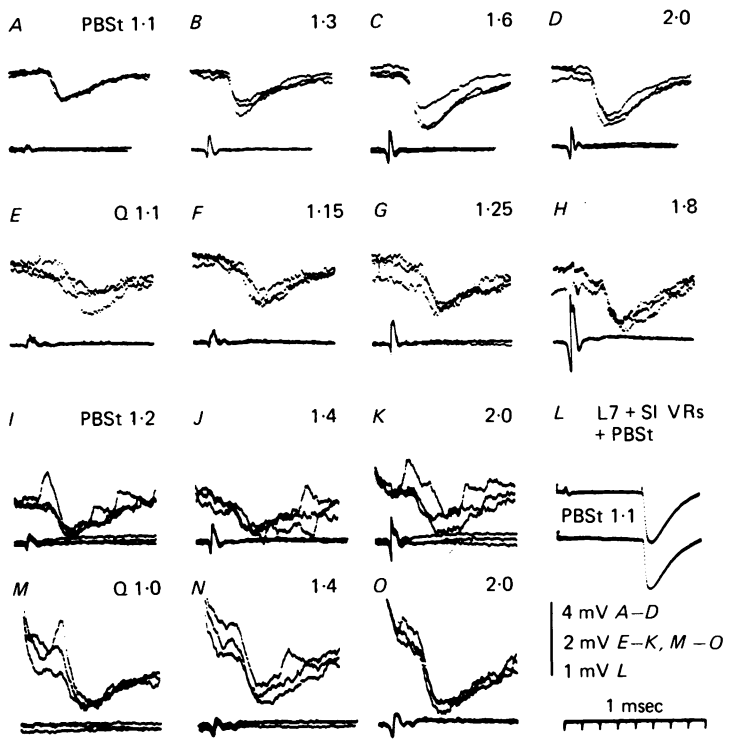

Fig. 4. Co-inhibition by Ia afferents from knee flexors and knee extensors. Intracellular records (upper traces) from two interneurones (*A–H*, *L* and *I–K*, *M–O*, respectively). Note that in both neurones i.p.s.p.s from PBSt as well as those from Q appeared to near threshold stimuli, whether or not they grew with stronger stimuli. Note also longer latency of i.p.s.p.s in *E–H*, indicating a trisynaptic coupling. Averaged records in *L* show an i.p.s.p. as in *A* (lower trace) and one preceded by a conditioning stimulation of L7+S1 ventral roots (upper trace), to illustrate absence of recurrent suppression of Ia i.p.s.p.s in laminae V–VI interneurones.

all the laminae V–VI interneurones excited by the lowest threshold (Ia) afferents, monosynaptic e.p.s.p.s were evoked from either knee flexors or extensors. There were no indications that any were co-excited by Ia afferents of antagonist muscles. On the other hand, nine interneurones were found to be co-inhibited by Ia afferents of posterior biceps-semitendinosus and of quadriceps, as illustrated in Fig. 4. In some interneurones the Ia i.p.s.p.s from these nerves were evoked disynaptically (Fig. 4*I–O*). In other interneurones disynaptic i.p.s.p.s were evoked from one (Fig. 4*A–D*) and trisynaptic i.p.s.p.s from another (Fig. 4*E–H*).

Ia i.p.s.p.s in laminae V–VI interneurones do not show depression following antidromic volleys in the ventral roots, as first observed by S. Lindström (personal communication) and now confirmed for i.p.s.p.s evoked by electrical stimulation of posterior biceps-semitendinosus and quadriceps nerves (see Fig. 4*L*), as well as by stretches of triceps surae and plantaris (cf. Jankowska *et al.* 1980*a*). Therefore they cannot be attributed to interneurones mediating Ia reciprocal inhibition of motoneurones (Hultborn, Jankowska & Lindström, 1971) and must be evoked by other neurones, e.g. some Ia excited interneurones in laminae V–VI, which are not inhibited by Renshaw cells (Hultborn *et al.* 1971; see also Lucas & Willis, 1974 and Jankowska *et al.* 1981).

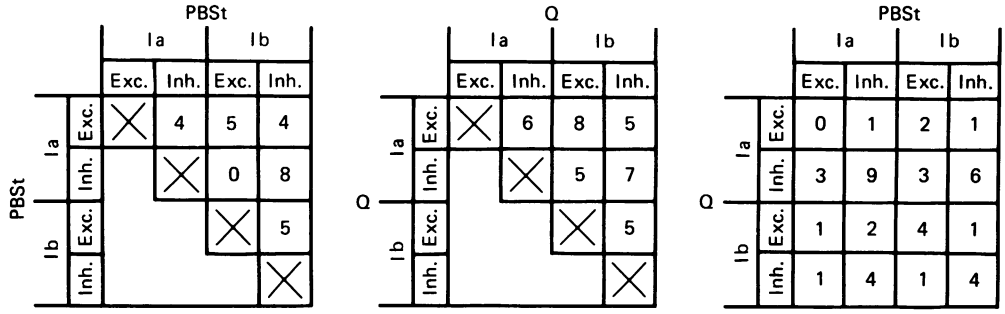

Fig. 5. Combined excitatory (exc.) and inhibitory (inh.) actions of Ia and Ib afferents of posterior biceps-semitendinosus (PBSt) and quadriceps (Q). Data for thirty-five interneurones with the input from PBSt (left), thirty-two interneurones with the input from Q (middle) and for the entire sample of the interneurones (right).

Opposite synaptic actions of Ia afferents of posterior biceps-semitendinosus and quadriceps. These were seen in four interneurones. Thus our observations show that muscle spindle afferents of these antagonists may influence laminae V–VI interneurones in the same direction (by inhibiting them) or in a reciprocal way. It should be added in this context that Ia afferents of the same muscle or of close synergists may likewise have opposite effects on individual neurones (see Fig. 5). The opposite effects of Ia afferents activated by stretch of ankle extensors are reported in the following paper (Jankowska *et al.* 1981).

Synaptic actions of Ib afferents of posterior biceps-semitendinosus and of quadriceps. These appeared to be either the same, as illustrated in Fig. 2, or opposite, as indicated by records of Fig. 3 (see also Fig. 5).

Correlation between the group I input and axonal projections

Horseradish peroxidase was injected into thirty six of the interneurones described above. Twenty-six were stained well enough to allow reconstruction of the initial part of their stem axons and of a number of axon collaterals. On the basis of these reconstructions the neurons were classified into one of the six previously described patterns of axonal projections of laminae V–VI interneurones (Czarkowska *et al.* 1976*a*), which are schematically indicated in the right part of Fig. 1. As shown in this Figure all the six patterns of projections were represented in the present sample of neurones, the most numerous being interneurones with the stem axon projecting to the lateral funiculus and displaying terminal branching within both the motor nuclei and the intermediate zone, or only in the intermediate zone (projection patterns 1–4).

No correlation has been found between patterns of group I synaptic actions and axonal projections. It should be noticed, however, that nine of thirteen neurones projecting to motor nuclei showed input from both Ia and Ib afferents

Projections to the motor nuclei covered a large part of lamina IX. In L7 and in caudal L6 the terminal branching of the stained collaterals was densest in the central part of lamina IX (Fig. 6*A*) and in rostral L6 also in its more lateral part. The area of the densest terminal branching of the present sample of neurones overlapped with, but was generally more ventral than the L7 area of branching of other interneurones which had input from ankle and toe extensors but not from knee flexors or extensors (cf. Fig. 8 of Jankowska *et al.* 1981). Since only some of the collaterals were stained to their terminal knobs, the actual area of their terminations could not be established. The terminal branches were considered to be those branches with diameters of about 1 μm which showed multiple subdivisions within an area.

Interneurones sending stem axons to the ventral funiculus (second pattern of axonal projections of Czarkowska *et al.* 1976*a*) appeared to branch more ventrally than those with their axon running in the lateral funiculus near the lateral border of the ventral horn (first pattern).

A common feature of virtually all the interneurones projecting to lamina IX was that they also sent axon collaterals to the intermediate zone. The terminal branching of the latter was densest within the central parts of laminae V–VI, where the majority of interneurones with group I input were located. Interneurones projecting to motoneurones could thus influence in parallel some of their fellow interneurones as well.

The intermediate zone was also the terminal branching area of interneurones with the third and fourth projection patterns (Fig. 6*B*). Whether these interneurones sent some axon collaterals to motor nuclei at more rostral or caudal levels could not be established. However, in most cases, the trajectory of their stem axons was in the middle part of the lateral funiculus, at the level of the dorsal, rather than the ventral horn, while axons of interneurones projecting to motor nuclei usually ran more ventrally. It will be also noticed that axonal branches in the lateral funiculus at the level of the base of the dorsal horn were usually directed rostrally (in nine of twelve cells). Descending branches were similarly infrequent among those running around the ventral horn. Of the thirteen neurones projecting to the motor nuclei nine had only ascending axonal branches, two only descending and two both ascending and descending branches.

Axons of neurones of the fifth type did not leave the grey matter; after multiple subdivisions they terminated within the intermediate zone, either ipsilaterally as in the previous types, or with one or two thin collaterals crossing to the other side. Axons of neurones of the sixth type coursed ventro-medially and crossed the mid line through either the ventral or the dorsal commissures. These neurones gave off very few, if any, initial axon collaterals ipsilaterally.

Interneurones with different patterns of axonal projections did not differ clearly in their location in the transverse plane except that interneurones projecting to motor nuclei might have been located somewhat more ventrally than those found to terminate only in the intermediate zone.

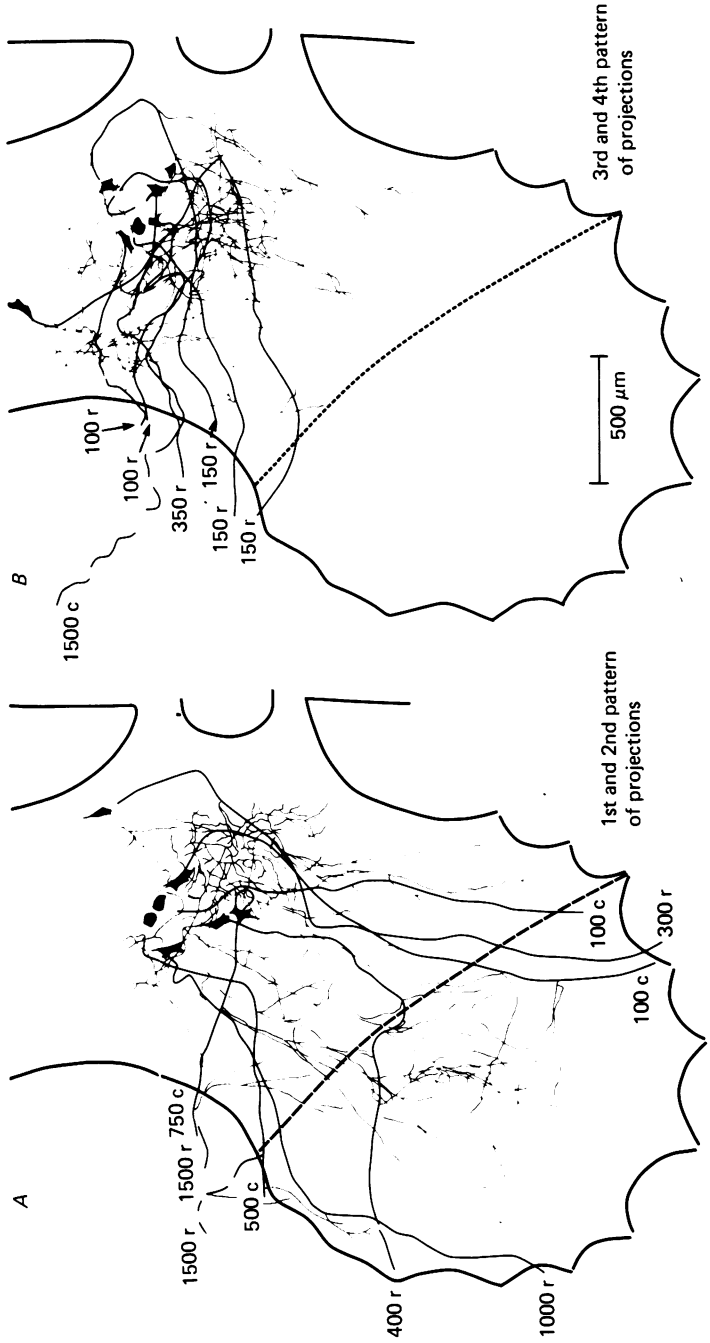

Fig. 6. Reconstruction of axonal projections of thirteen interneurones in caudal L6 and L7 with the first and second (*A*) and with the third and fourth (*B*) patterns of projections. Thick continuous lines indicate the contours of the ventral horn, the base of the dorsal horn, the central canal and the mid line. Dashed lines indicate the borders of lamina IX. Shaded are somata of the interneurones with dendrites truncated. Their stem axons and axon collaterals are indicated by thicker and thinner lines respectively. Numbers at the ends of the stem axons indicate distances from the soma (in μm) to which they were traced (r, rostrally; c, caudally).

DISCUSSION

In the results reported in the present and in the following paper (Jankowska *et al.* 1981) the stress was put on input from, respectively, knee and ankle muscle afferents to interneurones of laminae V–VI of Rexed (1954), although many of the interneurones were influenced from both these groups of muscle. The reason for dividing the material in this way was that different methods were used to activate afferents of posterior biceps, semitendinosus and quadriceps (by electrical stimulation of the nerves) and of triceps surae and plantaris (by muscle stretches). The methods were chosen as optimal for selective activation of Ia afferents of these muscles. However, the inherent differences in the approach to the evaluation of the results obtained with them precluded pooling these results. In addition the following paper further extends the analysis of observations reported now.

Comparison of group I input from knee flexors and extensors and from ankle and toe extensors

In spite of different techniques used to activate Ia and Ib afferents of knee flexors and extensors and of ankle and toe extensors, the features of the input from these two subgroups of group I afferents of all three groups of muscles to the laminae V–VI interneurones appeared to be very similar. Most of the interneurones were influenced in common by Ia and Ib afferents whether they were from knee flexors and extensors (60 %) or from ankle and toe extensors (64 %). The co-excitation, co-inhibition and opposite actions of Ia and Ib afferents were found in 28, 32 and 35 % of the interneurones with input from knee flexors and extensors and in 41, 33 and 50 % of interneurones with input from ankle and toe extensors respectively. A selective input from Ib afferents of knee flexors and extensors was found in 22 % of interneurones and from Ib afferents of ankle and toe extensors in 36 % of interneurones. The only difference found so far is the occurrence of a selective input from the Ia muscle spindle afferents of knee flexors and extensors but not from ankle and toe extensors (Jankowska *et al.* 1981).

It is of interest that excitatory and inhibitory actions of Ia afferents of antagonistic groups of muscles, knee flexors and knee extensors were differently distributed. Laminae V–VI interneurones co-excited by Ia afferents from these antagonists were never found, although many were co-inhibited. In contrast, tendon organ afferents of knee flexors and extensors co-excited, as well as co-inhibited these interneurones.

The intermediate zone interneurones influenced by group I afferents of the three analysed groups of muscles, knee flexors, knee extensors, and ankle and toe extensors, may to a great extent belong to the same population. Since more than 90 % of the interneurones with input from knee flexors and extensors were also excited and/or inhibited by afferents from ankle and toe extensors, and only about 50 % with input from the latter were affected by the former, the group I afferents from ankle and toe extensors may be considered the major input to these interneurones. Possible functional subdivisions of these interneurones are discussed in the following paper; we now comment only on some features of their morphological differentiation.

Subdivision of laminae V–VI interneurones on the basis of their intracellular staining with horseradish peroxidase

Staining of vertebrate neurones with horseradish peroxidase clearly gives much better results than obtained with Procion Yellow or cobalt (for references see Nicholson & Kater, 1973). Introduced intracellularly, however, horseradish peroxidase only fills cell processes within a limited distance of its injection site. Consequently only Golgi type II cells may be expected to be stained completely and cells with longer axonal processes only partially. Recent evidence shows that axons of some laminae V–VI interneurones with group I input may descend or ascend over a distance of two to three segments (E. Jankowska, T. Johannisson & J. Lipski, unpublished; T. Hongo, E. Jankowska, T. Ohno & S. Sasaki, in preparation). Since stem axons of the interneurones stained in this study could be followed only up to about 2 mm from the cell body and their axon collaterals to about 1 mm, we have no information about the destination of their distal axon collaterals. This raises a question concerning the sharpness of the differentiation between the six patterns of projections of these interneurones as described previously (Czarkowska *et al.* 1976*a*; and see diagrams to the right of Fig. 1), in particular whether interneurones found to project only to the intermediate zone might also send axon collaterals to the motor nuclei as well. The best stained neurones of the latter type, those whose axon collaterals given off at successive nodes of Ranvier were followed until they subdivided to a diameter of about 1 μm (see Czarkowska *et al.* 1976*b*), would hardly reach motor nuclei within a short distance of their cell bodies. They could make synaptic contacts only with the distal dendrites of the motoneurones. Axonal trajectories in the white matter of practically all funicular cells with patterns 3 and 4 likewise made them more likely to re-enter the intermediate zone than to project to the motor nuclei, and thus differed markedly from the trajectories of stem axons of neurones with the first two patterns of axonal projections. However, it might be best to leave the question open until more is known about the functional subdivision of the laminae V–VI interneurones. For the same reasons we would not put much weight on relative predominance of interneurones with a selective or convergent input from group Ia and group Ib afferents among those with different patterns of axonal projection. The fact that interneurones with all the positively established projections may be influenced from both the muscle spindles and tendon organs (see also Fig. 9 in Jankowska *et al.* 1981) shows that a number of spinal reflexes would be evoked by combined actions of Ia and Ib afferents. This conclusion will not be changed by a certain redistribution of interneurones between those classified as projecting only to the intermediate zone or both to the intermediate zone and the motor nuclei.

For a future analysis of the function of laminae V–VI interneurones with different inputs it might be of interest that interneurones with the second and fourth patterns of axonal projections (to motor nuclei with the stem axon in the ventral funiculus and primarily to the medial part of the intermediate zone) were found only among interneurones with the input from knee flexors and extensors (cf. Fig. 9 in Jankowska *et al.* 1981). This feature, together with the somewhat more ventral terminal projection area of these interneurones, indicate that they may have different target cells from interneurones influenced primarily by afferents from ankle and toe extensors, although the latter also contributed to excitation and inhibition from knee flexors and extensors in the present sample of neurones.

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