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GROUP II-ACTIVATED LUMBOSACRAL INTERNEURONES WITH AN ASCENDING PROJECTION TO MIDLUMBAR SEGMENTS OF THE CAT SPINAL CORD

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SUMMARY

1. In anaesthetized cats, single-unit microelectrode recordings were made in the lateral funiculus at L6, from the axons of lumbosacral interneurones discharged by hindlimb group II muscle afferents.

2. The level of the ascending projection of these interneurones was investigated by antidromic activation of their axons in the lateral funiculus from different spinal levels. The majority of units encountered were found to have an ascending projection to at least the L4 level and, of these, most (85%) did not project beyond the L4 or L3 segments of the cord.

3. The axons studied were discharged by group II afferents primarily from knee extensor muscles. Some units were discharged in addition by cutaneous and/or joint afferents.

4. The implications of this ascending projection are discussed.

INTRODUCTION

Until recently it had been assumed that the reflex actions of hindlimb group II muscle afferents were mediated by interneurones in the lumbosacral enlargement (L6–S1) and indeed some interneurones with the appropriate properties are located there (Fukushima & Kato, 1975; Lundberg, Malmgren & Schomburg, 1987*b*). However, more recent work has focused attention on another group of interneurones, located in the middle lumbar segments (L3–L5), which are, at least in part, monosynaptically activated by group II afferents and which project to motoneurones (Edgley & Jankowska, 1987*b*; Cavallari, Edgley & Jankowska, 1987*a*).

In order to further our understanding of the respective roles that the interneurones in these two regions play in the control of movement, we have investigated the extent to which the lumbosacral group of interneurones project rostrally. This is of interest for two main reasons. Firstly, a projection from the lumbosacral interneurones to the location of the group II-activated neurones in the middle lumbar segments might imply that the two groups of interneurones are functionally integrated. Secondly, defining the pattern of projection of group II lumbosacral interneurones could provide a means of elucidating further details of group II reflex pathways. A

projection to Clarke's column in L3 and L4 is one characteristic feature of lumbosacral interneurones mediating group I non-reciprocal inhibition (Brink, Harrison, Jankowska, McCrea & Skoog, 1983*a*; Hongo, Jankowska, Ohno, Sasaki, Yamashita & Yoshida, 1983*a*, *b*). By analogy, the characterization of a similar projection from group II-activated interneurones might likewise allow a population of last-order lumbosacral interneurones activated by group II afferents to be identified.

In the present report, we provide evidence that some lumbosacral interneurones, discharged by group II afferents, indeed project to the middle lumbar segments. Moreover, the levels of projection of these group II interneurones are similar to those of lumbosacral interneurones interposed in group I reflex pathways (Fern, Harrison & Riddell, 1988*a*). An abstract of some of these results has been published (Fern, Harrison & Riddell, 1988*b*).

METHODS

Preparation

The observations described in this report were made during experiments on nine cats drawn from a larger series of experiments, some of which also provided data published in a previous article (Fern, Harrison & Riddell, 1988*a*) to which the reader is referred for additional experimental details. The cats were anaesthetized with chloralose (70 mg/kg I.V. initial dose, supplemented with two or three additional doses of 10–20 mg/kg as required). Anaesthetic level was assessed by inspection of a continuous blood pressure recording and the diameters of the pupils of the eyes. The animals were intermittently paralysed with gallamine triethiodide and artificially respired. End-tidal CO_2 and body temperature were continuously monitored and maintained within physiological limits.

The following nerves were transected, dissected free and mounted for stimulation on pairs of bipolar silver wire electrodes: quadriceps, sartorius, hamstring, gastrocnemius–soleus, plantaris, flexor digitorum longus (which also included the nerves to tibialis posterior, popliteus and the nerve to the interosseous membrane), sural, tibial, common peroneal and the posterior nerve to the knee joint. The spinal cord was exposed from Th13 to sacral segments, and at Th10. The dura was opened and six pairs of bipolar silver ball electrodes placed at various levels on the lateral funiculus for stimulation: typically, at L4, L3, L2, L1, Th13 and Th10. The dorsal columns were sectioned at caudal L5 in order to reduce the possibility of orthodromic activation of interneurones in L6–S1 as a result of stimulus spread to the dorsal columns. In addition, the dorsal column lesion together with sectioning of the L5 dorsal roots avoided the possibility of recording from the descending axons of interneurones located rostral of the lesion.

Search stimuli (0·1 ms duration) were applied to each of the muscle nerves in turn whilst tracking in the lateral funiculus at L6 with glass micropipettes filled with 3 M-KCl or NaCl. Recordings were made of action potentials, evoked by stimulation of peripheral nerves, in the ascending collateral of single interneurones. Simultaneous recordings were made from a ball electrode placed on the dorsal columns near the dorsal root entry zone at L6. This was used to determine the time of arrival of afferent volleys at L6 and to determine the thresholds (T) of afferent fibres effective in discharging interneurones in relation to the threshold of the most excitable fibres in peripheral nerves. Stimuli of above 1·5 T are required to activate group II fibres in muscle nerves (Eccles & Lundberg, 1959; Jack, 1978; Ellaway, Murphy & Tripathi, 1982; Lundberg, Malmgren & Schomburg, 1987*a*). However, stimuli of 1·5 T are not sufficient to activate all group I fibres. Consequently, action potentials were judged to be evoked by afferents of group II origin only when evoked by stimuli supramaximal for group I afferents as determined from the incoming group I volley. In addition, a stimulus strength of 5 T was considered to be the upper limit above which actions could no longer be safely attributable to group II afferent fibres (see Eccles & Lundberg, 1959; Ellaway *et al.* 1982; Lundberg *et al.* 1987*a*).

Criteria for antidromic activation

An attempt was made to antidromically activate interneurones by stimulation of the lateral funiculus at various spinal levels. The stimulus intensity was gradually increased until threshold for an antidromic response was reached. Antidromic impulses evoked by stimulation of the lateral

funiculus were identified either on the basis of their latency or by frequency-following and collision tests. Orthodromic activation requires a certain minimum latency, below which any impulse can clearly be attributed to antidromic activation. This orthodromic latency consists of the time required for spike generation, for orthodromic conduction and for a synaptic delay. In the present experiments, taking into account these various factors (see Fern *et al.* 1988*a* for details), spikes were considered to be antidromic when evoked from L4 (conduction distance, 20 mm) at latencies of 0·7 ms or less, or from L3 (conduction distance, 35 mm) at 0·85 ms or less. When spikes were evoked with longer latencies, the ability of impulses to follow trains of high-frequency stimuli (500/s) and to collide with orthodromically evoked activity were employed as tests of antidromic activation. Since the aim of the test was to establish the level of projection of the axon, these tests were always performed at the most rostral level from which the presumed antidromic responses were obtained.

Thus, given the above requirements for ascertaining that a given neurone was antidromically activated, the most caudal level at which the response failed or the threshold increased substantially was considered to be the level of termination of the fibre. Axons antidromically activated from the Th10 segment were presumed to belong to ascending tract neurones rather than to interneurones.

RESULTS

Single-unit recordings were made from axons in the lateral funiculus in the rostral half of L6, 1–2 mm lateral of the dorsal root entry zone. Useful data were recorded from forty axons which were selected for study on the basis of a group II input from afferents in one or more muscle nerves. The depths of the axons within the lateral funiculus were obtained directly from the micromanipulator reading. The axons were recorded throughout the lateral funiculus, though were most frequently encountered between 1 and 2 mm from the surface (mean depth, 1·7 mm).

Activation of interneurones from different rostrocaudal levels

Axons activated by group II muscle afferents were investigated for their level of projection in the lateral funiculus by electrical stimulation at different rostrocaudal levels. Stimulation of the lateral funiculus often evoked both antidromic and orthodromic impulses. However, for most axons, antidromic impulses could be distinguished on the basis of their latencies, since these were too short to allow for orthodromic conduction and a synaptic delay (see Methods and Fig. 1*A* and *B*). For the remaining units, activated at longer latencies, the ability of impulses to follow trains of high-frequency stimuli and to collide with orthodromic activity were used to identify those levels from which antidromic impulses could be evoked.

The levels of projection of the forty units investigated in this manner are summarized in the histograms of Fig. 2. As can be seen, the majority were found to project to the L3 or L4 segments, although a small number of fibres terminated more rostrally in L2 or even L1.

Orthodromic activity evoked by stimulation of the lateral funiculus frequently consisted of repetitive firing as in the example shown in Fig. 1*A–C*. Since the dorsal columns were sectioned at L5, this orthodromic activity was most probably due to the action of descending fibres. In recordings from some group II-activated axons, this repetitive orthodromic activity prevented unequivocal identification of antidromic impulses and such units were therefore excluded from the sample. Because of these difficulties, an accurate assessment of the proportion of fibres failing to discharge antidromically at any segmental level has not been possible.

Conduction velocity of axons in the lateral funiculus

The data obtained from group II-activated axons ascending the lateral funiculus were analysed graphically by plotting the latencies of antidromic impulses against the conduction distance for each of the stimulation sites. Plots for a number of group

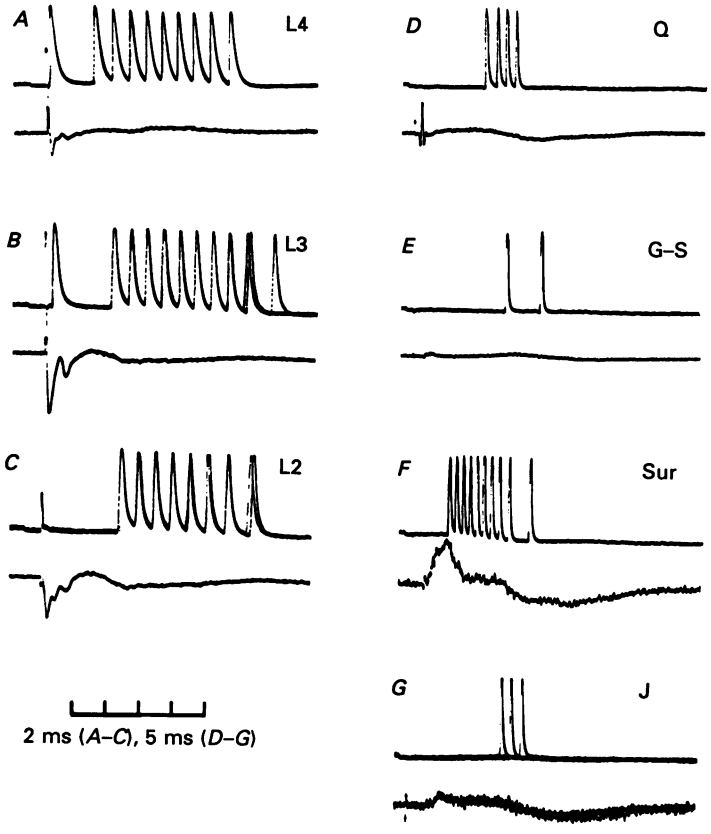

Fig. 1. Examples of antidromic and orthodromic responses in a single group II-activated interneurone. *A*, stimulation of the lateral funiculus at L4 evoked a short-latency antidromic spike followed by a longer-latency, orthodromic, repetitive discharge. *B* shows a similar response, though at longer latency from L3. *C*, the response from L2 consists only of the orthodromic discharge. Hence, this neurone was antidromically activated from L4 and L3 but not from L2. Records on the right (*D–G*) illustrate sources of input from different peripheral nerves to this interneurone. The records show an axon discharged by afferents in the quadriceps (Q), gastrocnemius–soleus (G–S), sural (Sur) and joint (J) nerves. The upper traces are axonal recordings (negativity downwards) and the lower records afferent volleys recorded from the cord dorsum (negativity upwards). Some of the records are composed of several superimposed sweeps.

II-activated units projecting to different segmental levels are shown in Fig. 3. The slopes of the lines drawn between the points give the approximate conduction velocities of the axons over various parts of the conduction path. Conduction velocities between the recording electrode (L6) and L4 ranged widely from 19 to 95 m s^{-1}, with a mean of 51 m s^{-1}. These conduction velocity data are similar to

those for the ascending collaterals of group I-activated interneurones of the lumbosacral cord (Fern *et al.* 1988*a*).

As is evident from the plots in Fig. 3, some of the variability in conduction velocity was related to the level of projection of individual interneurones. Thus, the mean conduction velocity of those fibres which projected only as far as L4 was 37 m s^{-1},

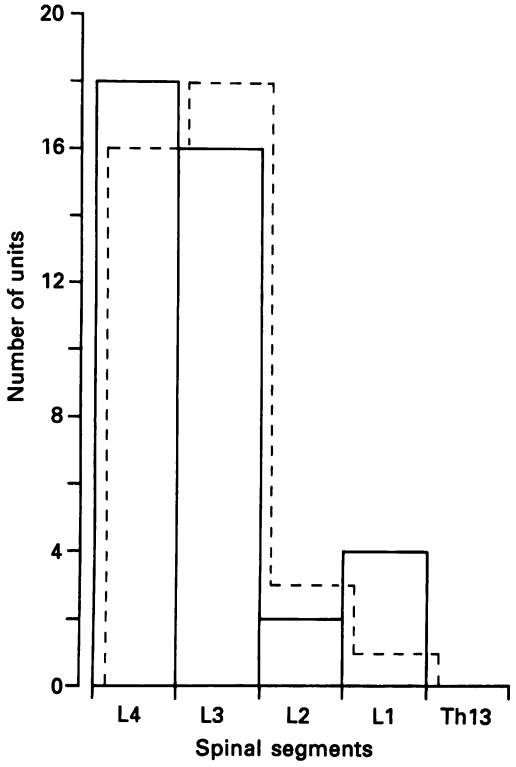

Fig. 2. Histogram summarizing the most rostral level of projection of group II-activated axons. The histogram shows the level of projection of all forty group II-activated interneurones, the majority of which projected to the L4 or L3 segments. For comparison, the dashed line is a histogram of the projections of forty group I interneurones (from Fern *et al.* 1988; with four additional units).

while the mean conduction velocity of those that projected to L3 or further, over the same conduction path (i.e. L6–L4), was 66 m s^{-1}. There was a correlation therefore between the conduction velocities of group II-activated axons and their levels of projection in the lateral funiculus, with a clear tendency for the faster-conducting axons to project to more rostral segments. All axons projecting to L3, and the small number of axons which reached the L2 and L1 segments, exhibited reductions in conduction velocity as they travelled rostrally.

Sources of primary afferent activation of the interneurones

The sources of the primary afferent input to the sample of axons are summarized in Fig. 4. In general, group II afferents of quadriceps muscles were the most effective

source of excitation of these interneurones, evoking discharges in almost 80% of the sample of axons. Some units (50% of those tested) could be discharged by stimulation of more than one muscle nerve, an example being shown in Fig. 1*D* and *E*. However, thresholds of fibres discharging interneurones were generally determined only for those muscle nerves evoking the shortest-latency response. We therefore

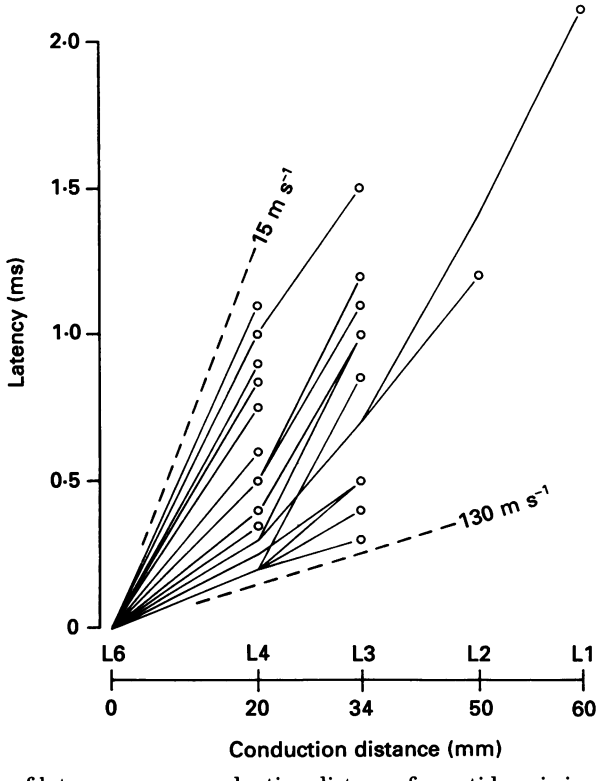

Fig. 3. Plots of latency *versus* conduction distance for antidromic impulses recorded from fibres ascending to different segmental levels. The axons were recorded in several experiments and so the average conduction distances between the spinal segments of different animals have been used. Because of this, the slopes of the lines only indicate the approximate conduction velocities of the axons; slopes corresponding to conduction velocities of 15 and 130 m s^{-1} are indicated by the dashed lines.

have little information concerning convergence from group II afferents of different muscle nerves. In addition to input from muscle afferents, stimulation of several non-muscular nerves (at stimulus strengths up to five times threshold), including cutaneous nerves and the posterior nerve to the knee joint, were found to discharge a proportion of axons (see Fig. 1*F* and *G*).

DISCUSSION

The results of the present study reveal a population of lumbosacral interneurones, activated by group II muscle afferents, with axons in the lateral funiculus at L6, that project rostrally to the L4 or L3 segments of the spinal cord. There are two likely

groups of neurones in the L3/L4 segments to which these interneurones may conceivably project: these are (i) neurones of Clarke's column and (ii) interneurones and ascending tract neurones outside Clarke's column.

Clarke's column

The most striking feature of the results of this study is that the levels of projection of these group II interneurones are similar to those of lumbosacral interneurones mediating group I non-reciprocal inhibition (Fern *et al.* 1988*a*), the great majority of

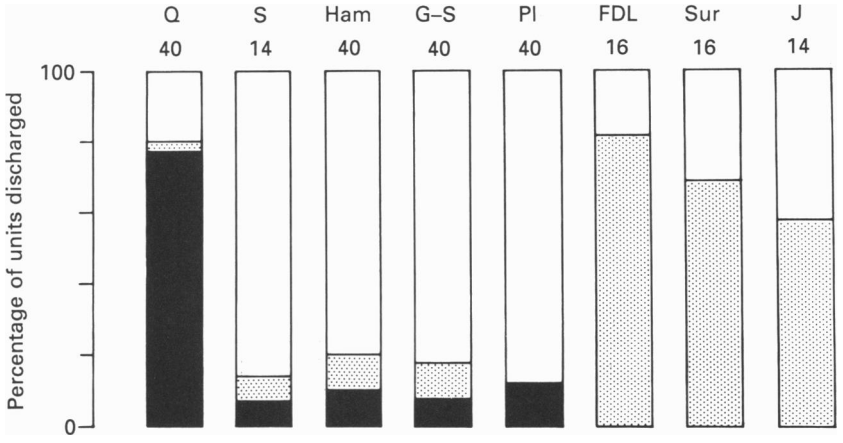

Fig. 4. Sources of peripheral input to the sample of interneurones. The bar chart shows the proportions of axons discharged by electrical stimulation of peripheral nerves. Abbreviations: Q, quadriceps; S, sartorius, Ham, hamstring; G–S, gastrocnemius–soleus; P, plantaris; FDL, flexor digitorum longus; Sur, sural; J, joint. Filled bars indicate those units for which discharges were confirmed as evoked by group II fibres. The numbers of axons tested from each nerve are indicated above each column. Some units received a convergence of input from several nerves and so are represented in more than one of the bar graphs.

interneurones in both groups terminating in L4 or L3 segments. It is now well established that this latter group of neurones project to Clarke's column (Hongo *et al.* 1983*a*, *b*) where they evoke inhibition in neurones of the dorsal spinocerebellar tract. The most obvious possibility to consider, therefore, is that the group II interneurones revealed in the present study form a similar group II ascending pathway to neurones of Clarke's column. Two pieces of evidence support this possibility: firstly, Hongo *et al.* (1983*a*) have reported that, with the dorsal columns cut, electrical stimulation of higher threshold, as well as group I, muscle afferents evoke inhibition in Clarke's column neurones. Secondly, the same authors injected horseradish peroxidase into Clarke's column in order to study the location of lumbosacral interneurones with a projection to this region. Labelled cells were located in the main regions of termination of group I fibres (laminae V–VI), but neurones were also found in a wider area. Some of these latter cells might be the group II-activated interneurones of the present study.

Outside Clarke's column

Recent investigations have shown that the middle lumbar segments (L3, L4 and L5) are specialized to a great extent in processing information from group II afferents. Group II afferents, including muscle spindle secondary afferents, have a substantial projection to these segments (Edgley & Jankowska, 1987*a*; Harrison, Jami & Jankowska, 1988) and activate both interneurones and ascending tract neurones in this region (Cavallari *et al.* 1987*a*; Edgley & Jankowska, 1987*b*; Edgley & Jankowska, 1988; Edgley & Gallimore, 1988). Furthermore, group II afferents terminating in this region are themselves under presynaptic control from other group II-activated pathways (Harrison & Jankowska, 1987).

Many of the group II-activated axons of the present study were found to terminate in the L4 or L3 segments of the spinal cord. This ascending group II projection provides the possibility of a link with the group II-activated neurones of middle lumbar segments, some of which might therefore be influenced by interneurones located in the lumbosacral cord. Such a possibility is strengthened by the observation that a large proportion of the present sample of interneurones were activated by quadriceps afferents. This is important because in the studies of Edgley & Jankowska (1987*a*, *b*), quadriceps was one of the nerves that consistently evoked powerful effects in the L4 and L3 segments. The only other nerve which produced powerful effects in the work of Edgley & Jankowska (1987*a*, *b*) which was also available for stimulation in the present experiments was sartorius. However, since only a small proportion of sartorius afferents enter the spinal cord caudal of our dorsal column lesion at the L5–L6 border, strong effects from sartorius would not have been expected in the present experiments.

Implications for the study of group II reflex pathways

An ascending projection from group II interneurones either to neurones of Clarke's column or to the group II-activated dorsal spinocerebellar tract neurones (Edgley & Jankowska, 1988; Edgley & Gallimore, 1988) could be of significance to further studies of group II reflex pathways. All of the presently identified interneurones interposed in spinal reflex pathways (e.g. Renshaw cells, Ia inhibitory interneurones, interneurones mediating group I non-reciprocal inhibition, C3–C4 propriospinal neurones) have a collateral projection to at least one ascending tract. Indeed, it was the demonstration that a population of group I-activated lumbosacral interneurones inhibit neurones in Clarke's column in the L3 and L4 segments, via an ascending axon collateral in the lateral funiculus (Hongo *et al.* 1983*a*, *b*), that allowed the elucidation of the characteristic features of interneurones mediating group I non-reciprocal inhibition of motoneurones. This ascending collateral provided a means by which the interneurones could be identified and their peripheral inputs and influence upon motoneurones studied directly (Brink *et al.* 1983*a*; Brink, Jankowska, McCrea & Skoog, 1983*b*; Harrison & Jankowska, 1985*a*, *b*). Given that a population of group II-activated lumbosacral interneurones do, in fact, partly mediate group II reflexes (Cavallari, Lundberg & Pettersson, 1987*b*), if it transpires that the projection of the present sample of interneurones is to neurones of the dorsal spinocerebellar tract, it may follow that these interneurones also project to lumbosacral motoneurones. The

ascending projection described in the present study could therefore provide a means of elucidating the characteristic features of last-order *lumbosacral* interneurones interposed in group II reflex pathways.

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