Inputs to group II-activated midlumbar interneurones from descending motor pathways in the cat

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1. Connections from descending motor pathways to group II-activated interneurones in the midlumbar segments of the spinal cord have been examined by intracellular recording. Interneurones, many of which had axonal projections to the hindlimb motor nuclei, were tested for inputs from rubro-, reticulo-, vestibulo- or corticospinal fibres.

2. Of 138 cells, 113 were monosynaptically excited by electrical stimulation of at least one of the descending motor pathways. Monosynaptic excitation from reticulo-, vestibulo- and rubrospinal pathways was common. Monosynaptic corticospinal EPSPs were identified in fewer neurones.

3. Convergent monosynaptic inputs from pathways which descend in the ventrolateral and ventral funiculi were common. Although few neurones with monosynaptic input from the corticospinal tract were identified, most also had monosynaptic rubrospinal input. In contrast, few neurones (4·3%) had convergent monosynaptic input both from pathways in the dorsolateral funiculus and from fibres in the ventral/ventrolateral funiculi.

4. The patterns of convergence from the different descending motor pathways differ from the patterns expected if the descending connections were distributed independently. Thus there is a significant segregation between rubrospinal and reticulo- or vestibulospinal inputs, and a significant association of reticulo- and vestibulospinal inputs.

5. Since descending motor pathways make monosynaptic connections with most group II-activated midlumbar neurones, many of which project to the hindlimb motor nuclei, some of these neurones provide a disynaptic pathway for the supraspinal control of hindlimb movements. The distribution of descending connections is consistent with the hypothesis that pathways descending in the dorsal part of the lateral funiculus and those descending in the ventrolateral or ventral funiculi contact different sets of interneurones.

Supraspinal structures control the activity of spinal motoneurones via a number of fast-conducting descending motor pathways. To a large extent the influence of these pathways is indirect, being relayed in spinal interneurones. These interneurones can be seen as nodal points where descending and afferent information are integrated at a premotoneuronal level (see Baldissera, Hultborn & Illert, 1981).

The extent and form of this integration is unclear, largely because of the lack of information on the functional connections of premotor interneurones. The best-studied group of premotor interneurones to date is short propriospinal neurones located in the midcervical (C3–C4) segments of the cat spinal cord, which mediate forelimb target-reaching movements (see Alstermark, Lundberg, Norsell & Sybirska, 1981). Individual C3–C4 short propriospinal neurones receive convergent monosynaptic excitation from many descending motor pathways, including the rubro-, cortico-, tecto- and reticulospinal tracts, but not from vestibulospinal fibres (Illert, Lundberg, Padel & Tanaka, 1978; Illert, Jankowska, Lundberg & Odutola, 1981; reviewed by Baldissera *et al.* 1981). The neurones also have inputs from peripheral afferents from the forelimb; thus they integrate various descending commands with peripheral information at a premotoneuronal level.

Another group of short propriospinal interneurones has been found in the midlumbar segments of the cat spinal cord and these project to hindlimb motoneurones (Edgley & Jankowska, 1987). These interneurones have strong input from group II muscle afferents from certain hindlimb muscle nerves, as well as multisensory inputs from other sources, including group Ia, Ib, joint and cutaneous afferents (Edgley & Jankowska, 1987*b*). Some midlumbar interneurones have been shown to evoke postsynaptic potentials in motoneurones (Cavallari,

Edgley & Jankowska, 1987), some being excitatory and others inhibitory, but it is not known at present to which muscles the different actions are directed. Supraspinal inputs to these interneurones have not been well studied: monosynaptic EPSPs from rubrospinal and corticospinal fibres have been demonstrated in some neurones and indirect evidence also suggests that some are excited by medullary reticulospinal neurones (see Edgley, Jankowska & Shefchyk, 1988). Other studies have indicated that group II-activated midlumbar interneurones may be influenced by the vestibular system (Suzuki, Timerick & Wilson, 1985; Yates, Kasper & Wilson, 1989).

The principal aim of this study was to determine the organization of descending connections to midlumbar interneurones, particularly the convergence of inputs from different descending motor pathways on individual interneurones. Some of this work has been described previously (Davies & Edgley, 1991*a*, *b*).

METHODS

Successful experiments were performed on seven healthy adult cats (2·7–3·4 kg). In all experiments anaesthesia was induced with ketamine (20–25 mg kg^{-1}, I.M.). In five experiments surgery was performed under halothane and recording under α-chloralose anaesthesia, using a regime which has been described in full in recent publications from this laboratory (see Arya, Bajwa & Edgley, 1991). In two experiments Nembutal (initially 25–30 mg kg^{-1}, I.V., supplemented with doses of 2–4 mg kg^{-1} as required) was used to maintain anaesthesia during surgery and recording. During recording the animals were paralysed with either gallamine triethiodide (16 mg initially, 8 mg subsequently) or pancuronium bromide (1–2 mg kg^{-1} initially, single doses of 1 mg subsequently). Several precautions were taken to ensure that full anaesthesia was maintained during paralysis; these included ensuring that blood pressure and heart rate did not alter in response to noxious stimulation, that the pupils remained fully constricted, and ensuring areflexia as the paralysis waned. These tests were made at 30 min intervals.

A number of nerves in the right hindlimb were ligated, transected and dissected free for stimulation. In all experiments, the nerve to quadriceps (Q) was mounted in a bipolar tunnel electrode for stimulation. Branches of the sciatic nerve were stimulated via pairs of silver wire electrodes; these included the nerve to the hamstring muscles (HAM), the nerve to gastrocnemius and soleus (GS), the nerve to the two heads of flexor digitorum longus (including branches to popliteus, tibialis posterior and the interosseus nerve; FDHL) and the branches of the deep peroneal nerve which innervate tibialis anterior and extensor digitorum longus (TA–EDL). Stimuli (0·2 ms square pulses) were delivered at a rate of 1–2 Hz. Recordings were made with glass microelectrodes filled with 1 M potassium citrate with tip impedances of 3–15 MΩ. Neurones with projections to the motor nuclei in the caudal lumbar or first sacral segment were identified by antidromic activation. The GS and HAM motor nuclei were first located using antidromic field potentials recorded via a glass electrode. Various electrode trajectories were tested to find one where large antidromic fields were recorded from both nerves. The glass electrode was then removed and a fine tungsten stimulating electrode substituted; when searching for neurones this electrode was left at a depth between the centres of the two motor nuclei. Neurones were considered to be antidromically activated when they discharged all-or-none spikes with fixed, short latency and which could follow a train of stimuli (3 shocks at 200–300 Hz). In some intracellular recordings the antidromic spike failed to invade the cell body, but a small all-or-none blocked spike could be seen.

Stimulation of descending pathways

Descending motor pathways were stimulated by means of tungsten electrodes (250 μm shank diameter, insulated with varnish except for approximately 10 μm at the tip). After initial positioning using stereotaxic co-ordinates, appropriate placement of these electrodes was achieved by monitoring the descending volleys evoked from them in recordings taken from the surface of the upper lumbar cord (L3). Reticulospinal and rubrospinal fibres were stimulated in all experiments, the lateral vestibulospinal pathway in four experiments and the corticospinal tract in three experiments.

Selective activation of the rubrospinal and corticospinal tracts was achieved by delivering stimuli in the posterior part of the contralateral red nucleus (RN) and the contralateral medullary pyramid (PYR), respectively, using an approach based on that described by Harrison & Jankowska (1985*a*). The red nucleus was first located by recording antidromic field potentials after stimulating the contralateral dorsolateral funiculus of the spinal cord at L3, the electrode being positioned in the posterior part of the nucleus where the rubrospinal fibres exit to maximize the likelihood of activating these fibres directly. Subsequently spinal volleys evoked by stimulation in the red nucleus were used to optimize the position of the electrode. In practice segmented volleys were evoked from the red nucleus, presumably reflecting both direct and trans-synaptic activation of rubrospinal neurones. Latencies were measured from the onset of the first component of these volleys. Effective stimulation of the medullary pyramid was verified by monitoring volleys evoked in the spinal cord from the electrode. In positioning this electrode, great care was taken to ensure that reticulospinal pathways were not activated, even with stimuli of 400 μA. This was especially important in one of the experiments where the threshold for corticospinal volleys was 80 μA. In the other experiments corticospinal volleys were present at 50 μA. When recording from interneurones the response thresholds were determined, taking care to ensure that they could not be attributed to reticulospinal fibres. It was also the case that the cells which did have corticospinal input never had inputs from reticulospinal fibres stimulated with another electrode positioned 2 mm rostral and 2–4 mm dorsal to the pyramid electrode in the medullary reticular formation.

Vestibulospinal fibres were activated via an electrode positioned stereotaxically in the ipsilateral lateral vestibular nucleus (LVN), the position being verified by recording antidromic activation of vestibulospinal neurones from the spinal cord (see Hongo, Kudo & Tanaka, 1975). Reticulospinal fibres were activated via an electrode placed in the medullary reticular formation (MRF) at the level of the inferior olive (AP, −8·0; ML, 1·5; V, −5·0 to −7·0; co-ordinates according to Berman's (1968) atlas, coronal sections). Very large volleys in fast-conducting descending fibres were evoked in the spinal cord from these electrodes. In one experiment electrodes were placed bilaterally in the reticular formation. In the others a

single electrode was used, contralateral to the recording site; this was done to avoid spurious activation of the lateral vestibulospinal tract (which descends ipsilaterally). In practice the EPSPs evoked from the MRF had different latencies, sizes and shapes from those evoked from the LVN. All electrodes were positioned such that stimuli of 50 μA or less evoked visible volleys (with the exception of the electrodes in the pyramid in one experiment, as described above). Square pulses (0·2 ms duration) were used, both as single stimuli and in brief trains (2–4 stimuli at 2–4 ms intervals). For each cell the threshold of the EPSPs from descending inputs was determined to ensure that the responses appeared before the descending volleys were maximal. To further ensure that it was appropriate to attribute effects to a specific pathway, stimuli of 400–500 μA were delivered to the ineffective electrodes to ensure that supramaximal stimuli did not evoke additional responses.

In each experiment the timings and thresholds of the volleys evoked by descending systems were recorded at the L3 segment via a silver ball electrode on the ipsilateral lateral funiculus. Electrodes in the RN, LVN and MRF could all activate descending fibres both directly and trans-synaptically and clear waves in the descending volleys verified that this occurred. Latencies were measured from the earliest component of the volleys in each case, as illustrated in Figs 1–4. The latencies of EPSPs and IPSPs in midlumbar neurones were taken from a different ball electrode placed on the L7 dorsal root entry zone.

At the end of each experiment, the animal was killed by overdose with sodium pentobarbitone and perfused with 10% formaldehyde saline. The stimulating electrodes were then removed, and the spinal cord and brainstem were sectioned and stained with Cresyl Violet to allow reconstruction of the stimulating sites.

RESULTS

Identification of interneurones

This report is based on intracellular recordings from neurones from the midlumbar region of the spinal cord (mainly L4 and rostral L5), all of which were excited by group II afferents in the quadriceps nerve (Figs 1–4). These neurones were located in the intermediate zone of the grey matter, 1·8–3·5 mm deep to the cord dorsum. In the midlumbar segments of the spinal cord a characteristic pattern of focal synaptic potentials is evoked by quadriceps afferents: in the dorsal horn very large field potentials are evoked by group II afferents in laminae IV and V generated by inputs to interneurones and ascending spinocerebellar tract cells. In the intermediate zone (lamina VII) longer latency field potentials were found, in combination with group I field potentials (Edgley & Jankowska, 1987*a*). In this region many cells with group I and II inputs and descending axonal projections are found, as well as many interneurones. None of the intermediate zone neurones with group II excitation have ascending axons (Edgley & Jankowska, 1987*b*). Many of these neurones and other neurones in the same region were not excited but were inhibited by group II afferents and some of these had projections to the motor nuclei. We include here observations only on the neurones which were not excited by quadriceps group II afferents.

Intracellular recordings were obtained from 138 neurones which were excited by group II afferents. The EPSPs were usually large (Figs 1–4). The onset latencies of the EPSPs from quadriceps group II afferents ranged from 2·3 to 4·2 ms (mean $\pm$ S.D., 3·05 $\pm$ 0·41 ms). Based on previous studies (Edgley & Jankowska, 1987*b*), these latencies are those expected for monosynaptic activation from the slow-conducting intraspinal branches of group II afferents. In support of this, all of the EPSPs had fixed sizes, shapes and latencies.

As previously described, many of these neurones also had group I inputs. Group I EPSPs were found in sixty-five of the cells (47%) at latencies ranging from 0·6 to 1·5 ms (mean $\pm$ S.D., 1·04 $\pm$ 0·19 ms), indicating that most must have been monosynaptic. Inhibition from group I afferents was seen in fifty-eight neurones (42%); the latencies of the IPSPs ranged from 1·34 to 2·6 ms (mean $\pm$ S.D., 1·85 $\pm$ 0·25 ms). In all, ninety-three cells (67%) had either EPSPs or IPSPs (or both) from group I afferents of the nerves tested.

All of the neurones were tested for projections to the gastrocnemius or hamstring motor nuclei in L7–S1 and antidromic activation could be demonstrated for forty-nine of the neurones (35·5%) with stimuli of 100 μA or less. Other cells may have had descending projections which were not activated by the electrode used.

Effects from descending pathways

Stimulation of descending pathways influenced a high proportion of the neurones; in the majority of cells EPSPs were evoked at short latency from at least one of the descending pathways (Figs 1–4). In many cases longer latency actions were also seen (e.g. Fig. 1), but these usually appeared only in response to trains of stimuli, often at strengths sufficient to produce a near-maximal volley from the particular descending pathway. Given their properties, these actions were attributed to oligosynaptic pathways relaying in the brain or spinal cord and were not investigated further.

A number of properties indicate that many of the short-latency EPSPs were evoked monosynaptically. Segmental latency was an important criterion: EPSPs with segmental latencies of less than 1·0 ms from the descending volleys at the level of recording were taken to be monosynaptic. Segmental latencies of greater than 1·0 ms might involve two synapses or be monosynaptically evoked by more slowly conducting fibres. A fixed latency, size and shape and the ability of the EPSPs to follow faithfully trains of stimuli at 300 Hz were also taken to indicate a monosynaptic connection in cases where segmental latencies were greater than 1·0 ms. The latter were particularly important in defining monosynaptic

effects from the corticospinal tract, since the volley is highly dispersed in the lumbar segments. Histograms of the segmental latencies of the effects from rubrospinal, reticulospinal and vestibulospinal pathways are illustrated in Fig. 5.

Using the criteria described above, most of the cells (113/138, 82%) were considered to have monosynaptic input from at least one of the descending pathways. Inputs from rubrospinal (Fig. 1) and reticulospinal fibres (Fig. 2) were most common, being found in 61 and 59 of the 138 tested neurones (44 and 43%), respectively. Monosynaptic vestibulospinal input (Fig. 2) was also common, being found in 16 out of 60 neurones tested (27%).

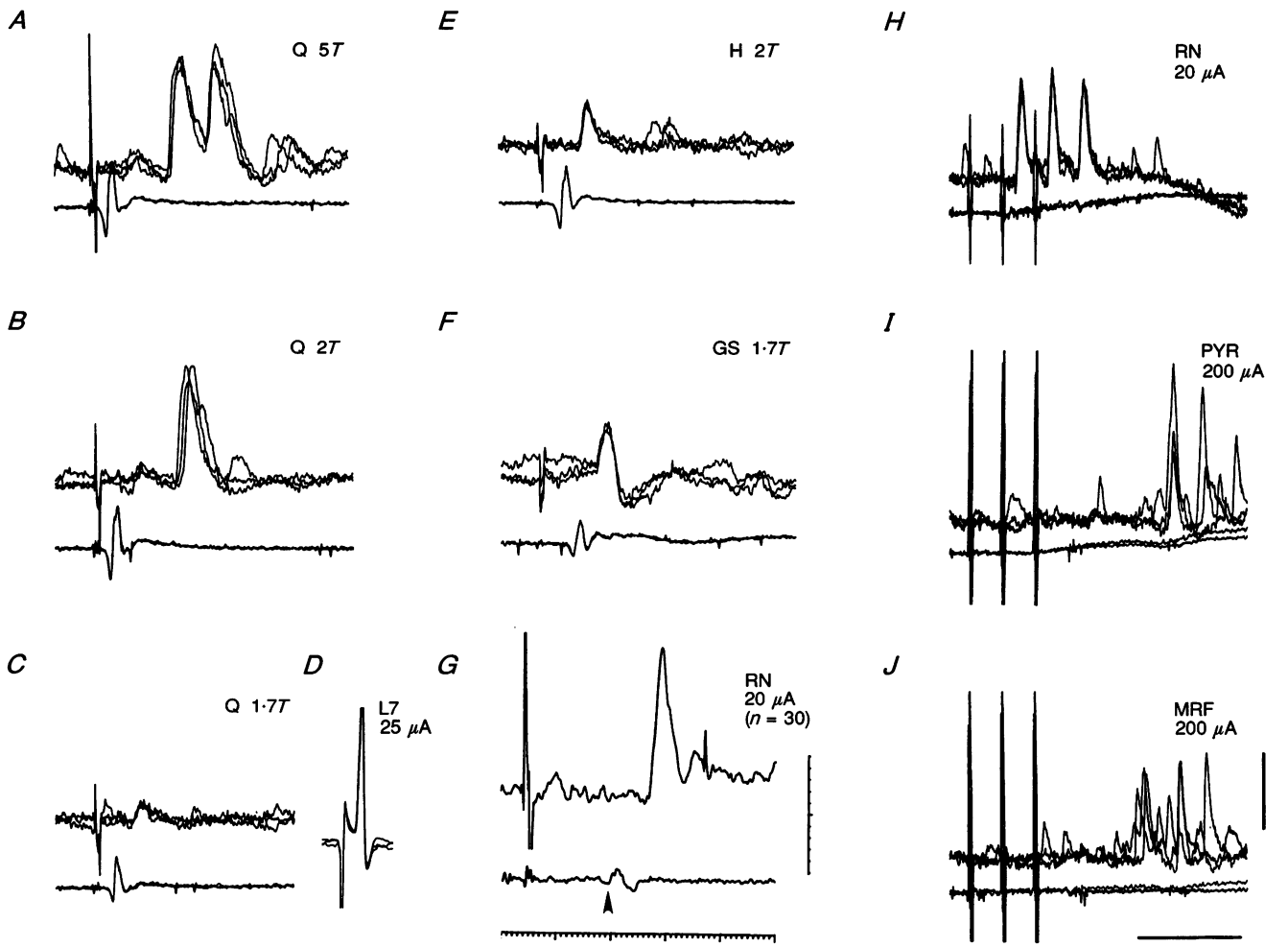

Figure 1. Intracellular recordings from an interneurone which was monosynaptically excited from the rubrospinal tract

In this and Figs 2–4 the upper traces are intracellular recordings and the lower traces are volleys recorded from the cord dorsum. The latter were taken from L6–L7 except in *G*, where they were recorded at L3. Stimulus intensities for nerves are given in terms of multiples of threshold (*T*) for the most excitable fibres and for the descending pathways in microamps. *A–C*, small EPSPs were evoked in this neurone by stimuli which activated quadriceps group I afferents (Q 1·7 *T*) with much larger EPSPs if the stimulus also activated group II afferents (2*T* and 5*T*). *E–F*, monosynaptic group I EPSPs were also evoked from the hamstring and gastrocnemius nerves, in the latter case with later, probably disynaptic group I IPSPs (H 2*T*; GS 1·7 *T*). *G–J*, weak stimuli (20 μA) delivered in the red nucleus (RN) evoked monosynaptic EPSPs in this neurone, whereas stronger trains (200 μA) of stimuli to the pyramid (PYR) or the medullary reticular formation (MRF) evoked oligosynaptic EPSPs. *G*, an averaged record (30 sweeps) showing the EPSP evoked from the RN on a faster timebase; the arrowhead marks the onset of the descending volley. Calibrations in *G* represent 2 mV and 10 ms. *D*, antidromic activation of the neurone from the motor nuclei at L7 (25 μA). Time calibration, 2·5 ms for *A–F*, 5 ms for *H–J*; voltage calibration, 2 mV for all traces except for *D* (where it represents 20 mV) and *G*.

The latencies of the EPSPs evoked from the rubrospinal tract and classified as monosynaptic are illustrated in Fig. 5*A*. The EPSPs classed as monosynaptic had segmental latencies ranging from 0·02 to 2·2 ms (mean ± S.D., 0·81 ± 0·41 ms). These can be compared with the latencies of IPSPs and EPSPs which have varying latencies, shapes and sizes to different stimuli and/or which could not follow a train of stimuli in the lower histogram of Fig. 5*A*. Similarly in Fig. 5*B*, oligosynaptic reticulospinal EPSPs and IPSPs overlap with the latencies of some which are classified as monosynaptic. The latencies of monosynaptic EPSPs from the reticulospinal tract ranged from 0·14 to 2·06 ms (mean ± S.D., 0·80 ± 0·43 ms). Vestibulospinal tract stimulation did not evoke short-latency oligosynaptic IPSPs or EPSPs in many neurones; Fig. 5*C* shows the latencies of monosynaptic EPSPs which are similar to those from the reticulo- and rubrospinal pathways (0·27 to 1·45 ms; mean ± S.D., 0·94 ± 0·37 ms).

Corticospinal inputs were adequately tested in fewer cases and the long latency and dispersed nature of the descending volleys made examination of descending inputs difficult; EPSPs which were accepted as being monosynaptic were seen in four out of fifty-two tested neurones (7·7%). The latencies of these were between 4·7 and 6·6 ms from the stimulus (mean ± S.D., 5·4 ± 0·72 ms). The latencies of the onset of the corticospinal volleys at L3

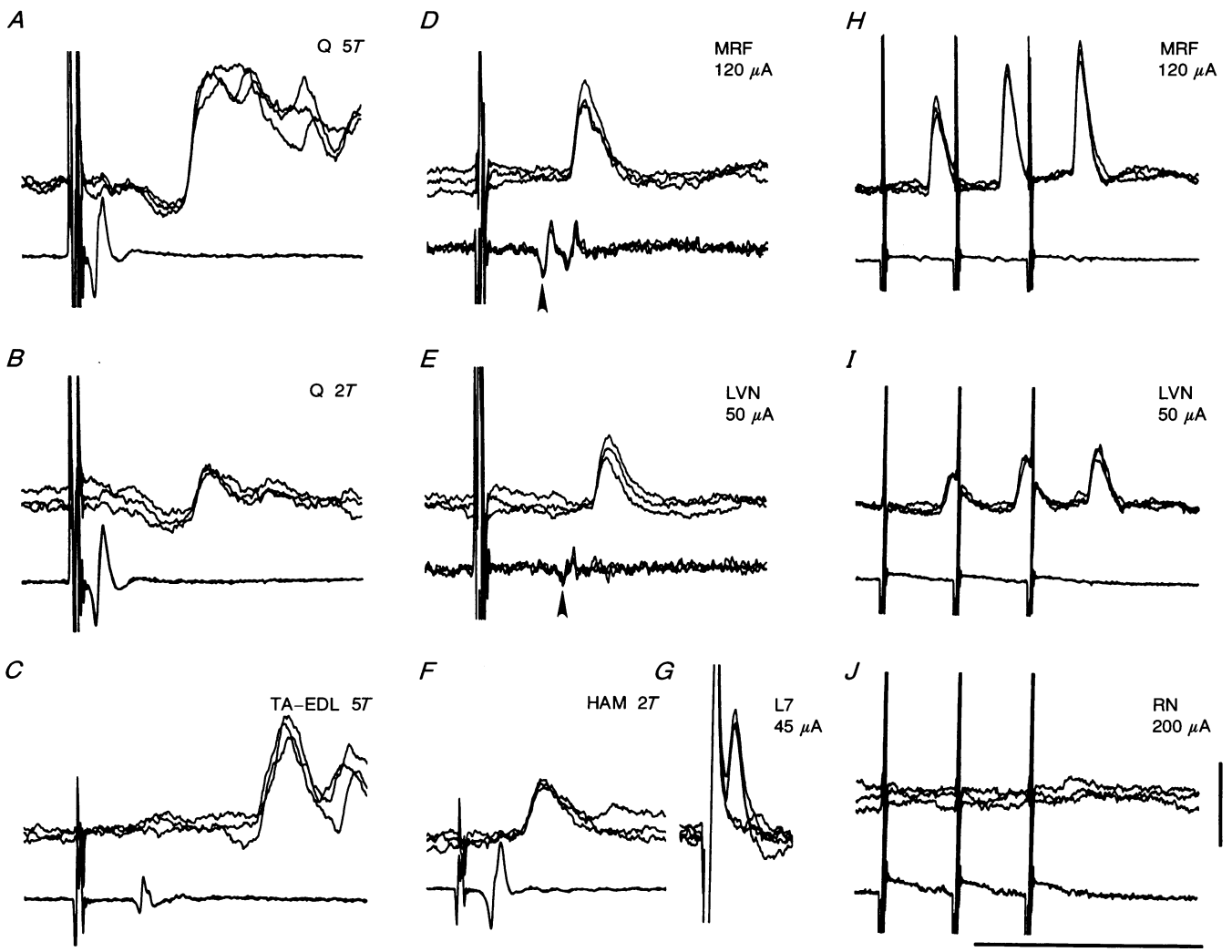

Figure 2. Intracellular recordings from a neurone with monosynaptic excitation from the reticulospinal and vestibulospinal tracts

Format as for Fig. 1. This neurone was powerfully excited by group II afferents from Q and TA–EDL (*A–C*) and monosynaptically excited by H group I afferents (*F*). Stimulation of the MRF and LVN evoked large monosynaptic EPSPs (*D* and *E*), which followed a train of stimuli (*H* and *I*). Descending volleys (from L3) are shown in *D* and *E*; the onsets of the volleys are marked with arrowheads. Supramaximal stimulation of the RN was ineffective (*J*). Antidromic activation (an all-or-none blocked spike) from the motor nuclei at L7 is also illustrated in *G* (45 μA). Time calibration, 5 ms for *A–G*, 10 ms for *H–J*; voltage calibration, 1 mV.

were 3·5 ms, giving segmental latencies greater than 1·0 ms. The difficulty of identifying clearly monosynaptic EPSPs from the corticospinal pathway means that their frequency may have been underestimated.

Connections from the reticulo-, rubro- and vestibulospinal tracts often produced large EPSPs; trains of stimuli to these pathways commonly discharged the neurones at monosynaptic latency from the effective stimulus. In addition to being less common, the EPSPs evoked from the corticospinal pathway were also generally smaller and had slower rise times than those evoked from the other pathways.

Convergence from different descending pathways

Although monosynaptic inputs from descending pathways were common, it was apparent that particular patterns of input occurred much more frequently than others. Convergence from different pathways in the ventrolateral or ventral funiculus, or from different pathways in the dorsolateral funiculus were quite common; convergence from dorsolateral *and* ventrolateral or ventral descending fibres was rare.

Monosynaptic connections from rubrospinal and reticulospinal fibres were both common (44 and 43% of neurones, respectively), but very few neurones had convergent monosynaptic input from both of these pathways (6 neurones; 4·3%). In the latter the rubrospinal EPSPs tended to be small (e.g. Fig. 4). Some of these also had long central latencies, but all had fixed sizes, shapes and latencies and followed a train of stimuli. Fewer neurones were tested for inputs from either vestibulo- or corticospinal fibres, but while thirteen of the sixteen cells (81%) with monosynaptic vestibulospinal input also had monosynaptic reticulospinal input, only one (6·2%) had monosynaptic rubrospinal input (the cell illustrated in Fig. 4). Similarly, three of the four cells with monosynaptic corticospinal EPSPs also had monosynaptic rubrospinal EPSPs, but none had reticulospinal EPSPs.

The relationship between inputs from the different descending pathways in these neurones has been further analysed by comparing the observed frequencies of

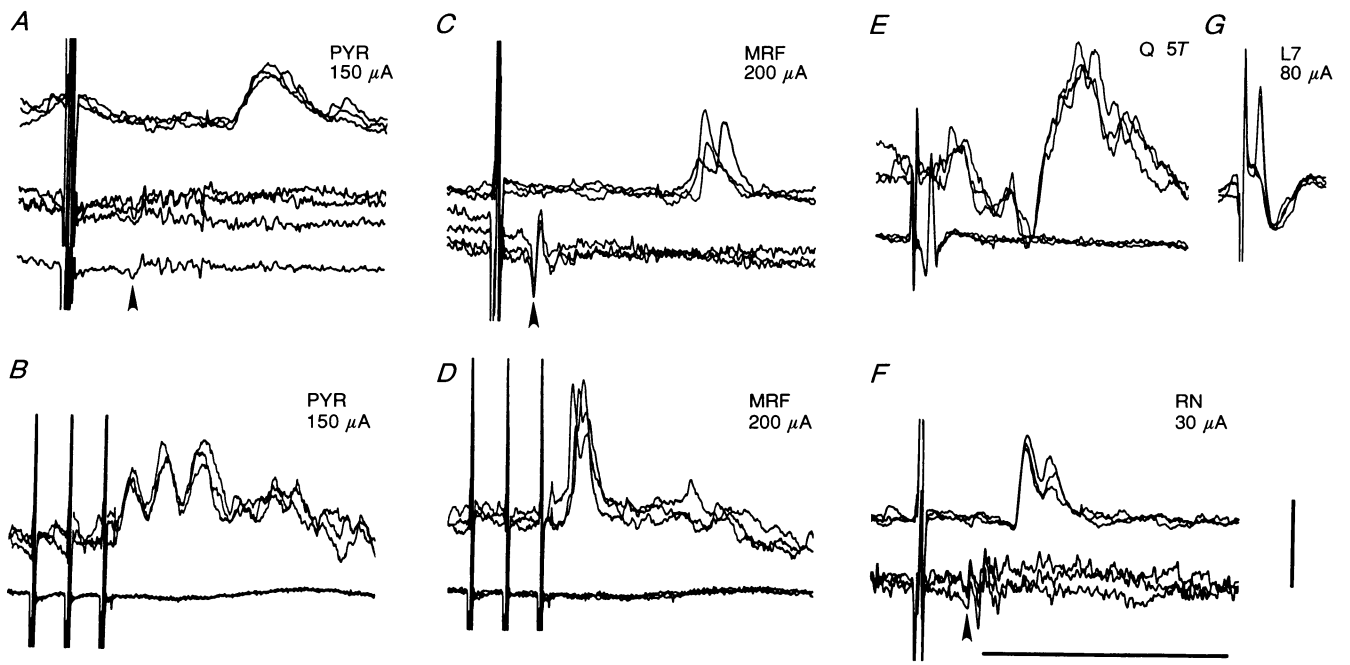

Figure 3. Intracellular recording from a neurone monosynaptically excited from the pyramid
A shows responses of the neurone to stimulation of the medullary pyramid at 150 μA. Single shocks evoke an EPSP with slow rise time but of fixed size, shape and latency with a long central latency (the arrowhead marks the onset of the descending volley). The EPSP follows a train of stimuli at 333 Hz (*B*). In *C*, a single stimulus to the MRF evokes a clearly oligosynaptic EPSP which does not follow repetitive stimulation (*D*); the onset of the much larger volley from the MRF is indicated by the arrowhead. The neurone was inhibited by group I afferents and excited by group II afferents from the quadriceps nerve (*E*), and monosynaptically excited from the red nucleus (*F*). Antidromic activation from the motor nuclei at L7 is illustrated in *G*. The volleys in *A*, *C* and *F* were recorded from the L3 segment; in *B*, *D* and *E* they were recorded from the L7–L6 border. Time calibration, 5 ms, except for *B* and *D* (10 ms); voltage calibration, 2 mV.

occurrence of particular patterns of connectivity with those which would be expected under different circumstances (based on an approach used by Harrison & Jankowska, 1985*b*). The analysis compares the proportions of neurones found with particular patterns of input connectivity with the proportions predicted if the two inputs were independent, were preferentially associated, or were preferentially segregated. For example in a population of neurones with two inputs, A and B, each of which occur in 50% of the neurones, if inputs A and B tend to be associated, then we may expect the proportion of neurones with both inputs to approach 50%, of those with input A alone or input B alone to be small and of those with neither input to approach 50%. If A and B were distributed independently then we would expect the proportions of neurones with both inputs, A alone, B alone or neither all to approach 25%. Finally, if the inputs were preferentially segregated then none of the neurones should have convergent input from A and B and the proportions with input A alone and input B alone should approach 50%.

Each histogram of Fig. 6*A–C* compares the observed proportions of neurones with inputs from different pairs of descending pathways (open columns) with the proportions which would be expected if the inputs were distributed independently (cross-hatched columns).

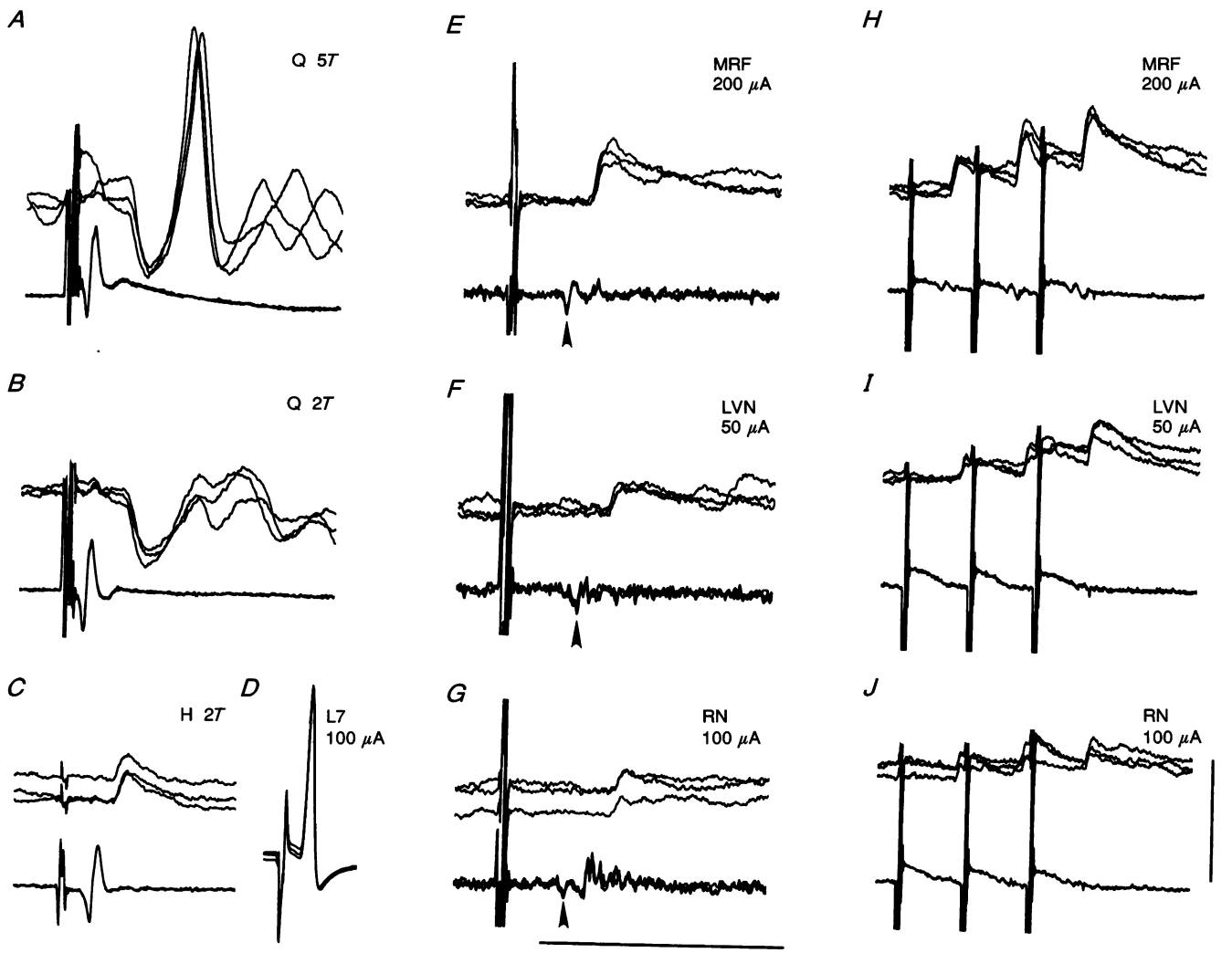

Figure 4. Intracellular recording from an interneurone with convergent monosynaptic EPSPs from rubro-, reticulo- and vestibulospinal fibres

This neurone was excited by quadriceps group II afferents (*A*) and inhibited by quadriceps group I afferents (*B*). It was also excited by hamstring group I afferents (*C*) and antidromically activated from the motor nuclei at L7 (*D*). Small monosynaptic EPSPs were evoked by reticulospinal, vestibulospinal and rubrospinal fibres (*E–G*), all of which followed a train of stimuli faithfully (*H–J*). Descending volleys were from L6–7 in *A–C* and *H–J*, and from L3 in *E–G*. Time calibration, 5 ms for *A–G*, 10 ms for *H–J*; voltage calibration, 2 mV for all traces except *D* (25 mV).

Consider, for example, rubrospinal and reticulospinal inputs (Fig. 6*A*). Rubrospinal inputs were found in 44% of the total sample and reticulospinal inputs in 43%. If these were distributed independently then convergent inputs would be expected in about 18·9% of the neurones (44% of 43%). A much lower proportion (4·3%) was observed (first column in Fig. 6*A*). Accordingly, rubrospinal or reticulospinal inputs alone were observed in a greater proportion of neurones than would be expected if the inputs were distributed independently (the second and third columns of Fig. 6*A*, respectively). A similar pattern is seen for combinations of rubrospinal and vestibulospinal inputs (Fig. 6 *B*); fewer neurones have convergent inputs from both and more have just one of the inputs. These data suggest a segregation of rubrospinal inputs and reticulo- or vestibulospinal inputs.

Convergence from reticulospinal and vestibulospinal fibres is examined in Fig. 6*C*, which reveals that convergent inputs from both pathways was found in a greater proportion of neurones than would be expected if the inputs were distributed independently (first column). In line with this the proportion of neurones observed with either reticulo- or vestibulospinal inputs alone (columns 2 and 3) is lower than expected. These data imply a preferential association of reticulo- and vestibulospinal inputs in midlumbar neurones.

Since rubrospinal and reticulo- or vestibulospinal inputs appear to be segregated we have compared the observed proportions of neurones with different combinations of these inputs with those which would be expected if the inputs were strictly segregated (Fig. 6*D* and *E*, respectively). In both cases the observed and expected proportions are closely similar. Conversely, Fig. 6*F* compares the observed convergence patterns of reticulo- and vestibulospinal inputs with those expected if the inputs were strictly associated. The histograms are similar implying an association of these inputs.

A statistical analysis of the histograms of Fig. 6*A–C* can be made, the most appropriate test being a *G* test for independence (Sokal & Rohlf, 1981). For convergence of rubrospinal and reticulospinal inputs the observed proportions of neurones with convergent or independent inputs differ significantly from those expected if the inputs were distributed independently ($P < 0{\cdot}001$). Thus there is a significant negative association or segregation of rubrospinal and reticulospinal inputs. The same is true for convergence of rubrospinal and vestibulospinal inputs ($P < 0{\cdot}001$). Application of these tests to the data from reticulospinal and vestibulospinal inputs (Fig. 6*C*) reveals that the observed data again differ significantly from the expected values for independently distributed inputs ($P < 0{\cdot}001$), but that these inputs have a significant degree of association.

Segregation of rubro- and vestibulo- or reticulospinal inputs has been proposed previously on the basis of cell location. A general organization of this type may exist but it is not strict. Interneurones with rubrospinal inputs *tended* to be superficial to those with reticulospinal or vestibulospinal inputs, as might be expected from the terminations of the pathways. However, there was a good deal of overlap and in some electrode penetrations neurones with monosynaptic reticulospinal input were

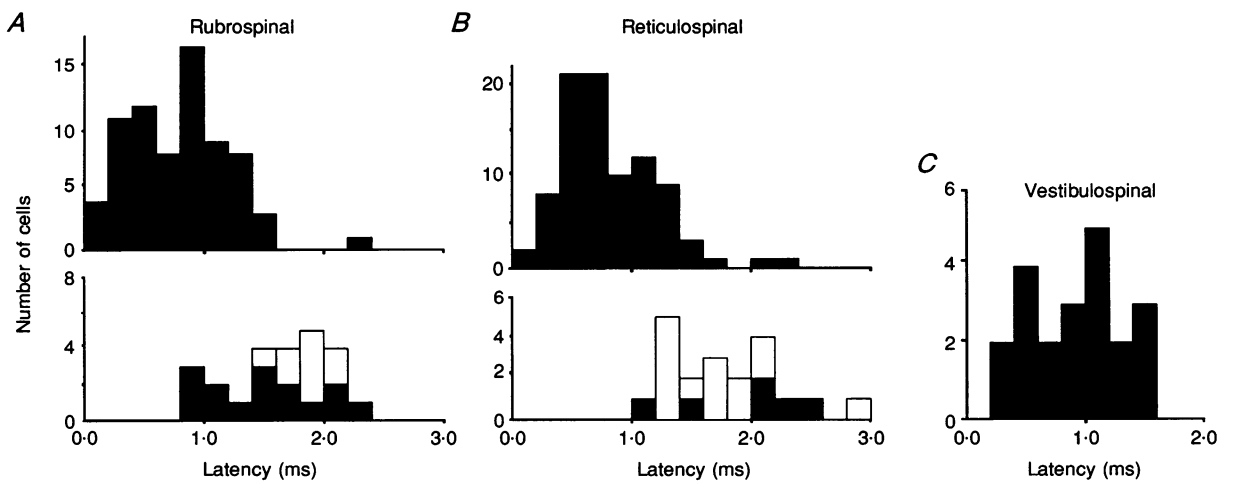

Figure 5. Segmental latencies of actions evoked from descending pathways
A, segmental latencies of monosynaptic EPSPs (upper histogram), oligosynaptic EPSPs and IPSPs (lower histogram, filled and open bars, respectively) evoked by stimulation of the rubrospinal tract. *B*, segmental latencies of monosynaptic EPSPs (upper histogram), oligosynaptic EPSPs and IPSPs (lower histogram, filled and open bars, respectively) evoked by stimulation of the medullary reticular formation (reticulospinal). *C*, histogram of segmental latencies of monosynaptic EPSPs evoked from the lateral vestibular nucleus (vestibulospinal).

encountered superficial to neurones with monosynaptic rubrospinal input. This suggests that although there is some specificity in the pattern of connectivity from different descending pathways to different neurones, it is not determined entirely by cell location. The neurones with convergent inputs from rubrospinal and reticulo- or vestibulospinal neurones were distributed throughout the intermediate zone.

DISCUSSION

These experiments reveal that convergent monosynaptic input from descending pathways and group II input is very common in midlumbar neurones (82%) and that all four of the sources of descending fibres examined in these experiments contribute monosynaptic excitation to some neurones. Furthermore, in the majority of neurones the

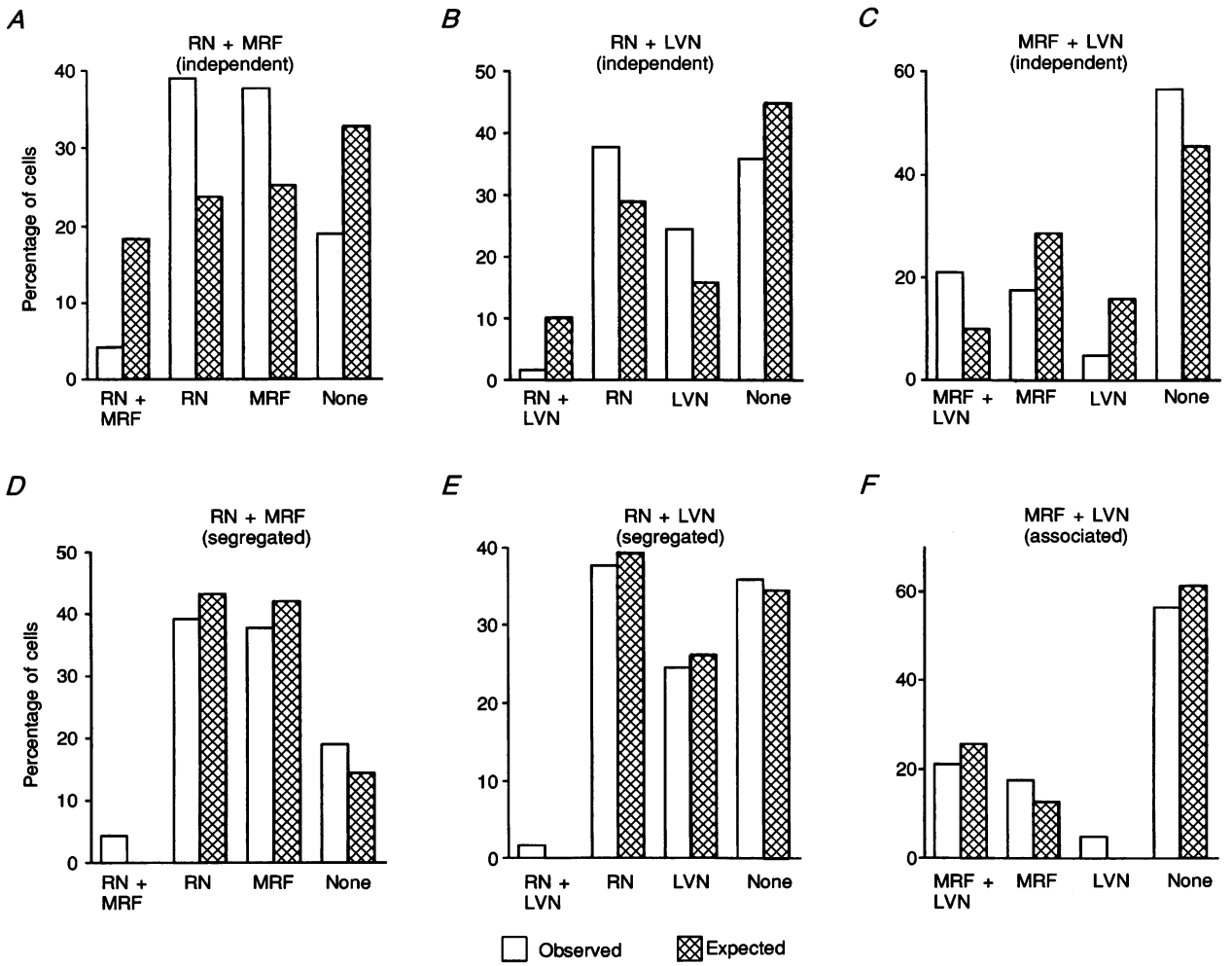

Figure 6. Comparison of observed proportions of neurones with particular patterns of input with the proportions expected

Each histogram has 4 major columns and compares inputs from 2 sources, as indicated. The 4 columns represent (from left to right) the proportion of neurones with both inputs, the proportion with the first input alone, the proportion with the second input alone and the proportion with neither input. The columns of each histogram are further divided to show observed and expected proportions (open and cross-hatched half-columns, respectively). *A–C* compare observed patterns of convergence with those expected if the inputs were distributed independently. *A*, for inputs from RN and MRF, fewer neurones had convergent input (RN + MRF), and more had only one of these inputs (RN or MRF) than were expected. *B*, a similar distribution is seen for inputs from RN and LVN. *C*, convergence from MRF and LVN (MRF + LVN) was found in a greater proportion of neurones than was expected. *D* and *E* compare the observed convergence from RN with MRF and LVN, respectively, with that expected if the inputs were strictly segregated in different populations of neurones (expected RN + MRF and RN + LVN are zero). *F* compares the observed convergence from MRF and LVN with the proportions expected if these inputs were strictly associated (if all cells with MRF inputs also have LVN inputs). For further explanations see text.

descending input was either from pathways descending in the dorsolateral funiculus (most commonly rubrospinal) or from pathways descending in the ventral or ventrolateral funiculus (reticulo- and vestibulospinal); only a small proportion of neurones had monosynaptic input from both.

In general, convergence of descending and group II input to midlumbar interneurones implies that the reflex actions mediated by these neurones can be modulated from the brain by means of the descending pathways or, alternatively, that specific descending commands for movement are integrated with information on limb position and movement at a premotoneuronal level.

Convergent inputs from reticulo- and vestibulospinal pathways or rubrospinal and corticospinal fibres have been suggested previously, partly on the basis of the anatomical observations that these groups of pathways terminate in separate and largely non-overlapping regions of the spinal grey matter (Petras, 1967; Kuypers, 1981, 1982). However, morphological studies of intracellularly stained midlumbar neurones has revealed that most neurones have dendritic arborizations which extend throughout most of the spinal grey matter (Bras, Cavallari, Jankowska & Kubin, 1989). This suggests that it is not only the locations of neurones which determine the descending connections to these neurones. Our observation that some deeply located neurones were monosynaptically activated by rubrospinal fibres, and some superficial neurones by reticulospinal fibres, further supports this notion. Segregated descending inputs of this type have been suggested previously for propriospinal neurones in lumbar segments (Vasilenko, Kostyukov, Pilyavskij, 1972; Kozhanov & Shapovalov, 1977; see Edgley & Jankowska, 1987*b* for discussion), but peripheral inputs were not found or not determined in these studies.

Based on the consequences of lesions to the various descending pathways, it has been proposed that rubro- and corticospinal fibres largely control the distal musculature, whereas reticulospinal and vestibulospinal fibres principally control the axial musculature. Little is known of the output connections of midlumbar neurones, but a prediction from the above would be that different midlumbar neurones with very similar peripheral inputs should make connections with proximal and distal motoneurones, the former via interneurones with reticulo- and vestibulospinal inputs and the latter via interneurones with rubro- or corticospinal inputs. Some midlumbar neurones excite motoneurones and others are inhibitory (Cavallari *et al.* 1987); the descending pathways can evoke disynaptic excitation or inhibition of motoneurones of different types, so it is not possible to deduce with certainty the probable outputs of midlumbar neurones to different types of motoneurones from these indirect observations.

The pattern of descending inputs to midlumbar neurones contrasts with the convergent input from cortico-, rubro-, reticulo- and tectospinal pathways found in C3–4 short propriospinal interneurones (Illert, Lundberg & Tanaka, 1976). In the latter neurones corticospinal inputs are also much more frequent and very powerful. This different organization presumably reflects the differing functions of the fore- and hindlimbs in the cat, the actions of the hindlimbs being mainly in postural regulation and stereotyped movements of locomotion and scratching, while the forelimbs can be used for visually guided reaching and manipulative tasks and under these circumstances would need a more integrated descending control.

Corticospinal EPSPs in midlumbar neurones were generally small and rare. Previous observations had indicated that corticospinal fibres evoked small monosynaptic EPSPs in some group II-activated midlumbar interneurones (Edgley *et al.* 1988), but not that they were so infrequent. However, the frequency of such actions is difficult to estimate from these experiments given the long segmental latencies of the EPSPs: some monosynaptic connections may have been missed. In contrast, rubrospinal actions were generally much more potent; often trains of stimuli would discharge neurones in extracellular recordings.

As to the functional role of midlumbar interneurones, it has been suggested, based on their peripheral input, that they might be activated when the limb extends and thus mediate the switch from the stance to the swing phase of the step cycle (Edgley & Jankowska, 1987*a*) although not all of the inputs to the neurones would be appropriate to this role. If this hypothesis is correct, the descending inputs to these neurones might allow adjustment of the timing of the switch from stance to swing, as a route for supraspinal modification of gait. Inputs to many of the neurones from vestibulo- and reticulospinal pathways also indicate that the neurones might be involved in postural adjustment. Previous studies have implicated these neurones in vestibular and neck reflexes (Suzuki *et al.* 1985; Yates *et al.* 1989) and the crossed interconnections between midlumbar neurones might also imply a role in interlimb co-ordination (Bajwa, Edgley & Harrison, 1992). Convergent reticulo- and vestibulospinal connections to these neurones are also consistent with such a role.

In relation to a possible role in locomotion, it has been demonstrated that some group II-activated midlumbar neurones discharge phasically during fictive locomotion evoked by stimulation of the mesencephalic locomotor region (MLR) in decerebrate cats (Shefchyk, McCrea, Kriellaars, Fortier & Jordan, 1990). The powerful monosynaptic excitation of many midlumbar neurones by medullary reticulospinal neurones provides a potential pathway for the generation of these phasic discharges: the MLR induces locomotion via a pathway involving medullary reticulospinal neurones (see Jordan, 1986), many of which are phasically active during MLR-evoked locomotion in both decerebrate (Orlovsky, 1970) and intact

animals (Drew, Dubuc & Rossignol, 1986). Thus the phasic discharges of the midlumbar neurones might have a supraspinal origin rather than a more direct origin from a spinal pattern generating network. Rubro- and vestibulospinal neurones might also be involved in driving the neurones, since they also discharge phasically during MLR-evoked treadmill locomotion (Orlovsky, 1972*a*, *b*). The fact that only a minority of midlumbar neurones were phasically active during MLR-evoked fictive locomotion (Shefchyk *et al.* 1990) may indicate that the descending inputs to many of the neurones were, under those experimental circumstances (fictive locomotion), not sufficiently potent to drive the neurones.

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