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REVIEW
Functional subdivision of feline spinal interneurons in reflex pathways from group Ib and II muscle afferents; an update

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Abstract

A first step towards understanding the operation of a neural network is identification of the populations of neurons that contribute to it. Our aim here is to reassess the basis for subdivision of adult mammalian spinal interneurons that mediate reflex actions from tendon organs (group Ib afferents) and muscle spindle secondary endings (group II afferents) into separate populations. Re-examining the existing experimental data, we find no compelling reasons to consider intermediate zone interneurons with input from group Ib afferents to be distinct from those co-excited by group II afferents. Similar patterns of distributed input have been found in subpopulations that project ipsilaterally, contralaterally or bilaterally, and in both excitatory and inhibitory interneurons; differences in input from group I and II afferents to individual interneurons showed intra- rather than inter-population variation. Patterns of reflex actions evoked from group Ib and II afferents and task-dependent changes in these actions, e.g. during locomotion, may likewise be compatible with mediation by premotor interneurons integrating information from both group I and II afferents. Pathological changes after injuries of the central nervous system in humans and the lineage of different subclasses of embryonic interneurons may therefore be analyzed without need to consider subdivision of adult intermediate zone interneurons into subpopulations with group Ib or group II input. We propose renaming these neurons 'group I/II interneurons'.

Introduction

Spinal interneural networks are exceptionally complex, but play a pivotal role in determining motor output. In a network of many elements, the obvious first approach is to seek to identify groups of neurons with common properties as its building blocks. In many neural networks, such as the retina or cerebellar cortex, distinct populations of neurons with specific functions can be identified based on morphology, connectivity, membrane properties, transmitter and receptor expression patterns and, increasingly, in terms of embryonic transcription factor expression patterns. Despite very substantial effort, mammalian spinal cord circuitry has not as yet revealed a basis for categorization that can be applied to more than a fraction of its neurons, and this continues to hamper progress in understanding how spinal neuronal networks operate. This situation may change in the near future. A recent review on the organization of spinal interneuronal networks led to the conclusion that '... the advent of novel molecular and genetic techniques coupled with recent advances in our knowledge of spinal cord development means that a comprehensive understanding of how the motor circuitry is organized and operates may be within our grasp (Goulding, 2009).' This conclusion refers principally to the neuronal networks that underlie spinally generated rhythmic locomotor movements, but the recent advances in developmental biology combined with those on adult interneuronal networks might allow its extension to other forms of behaviour.

However, two major intermediate stages might be needed before we take full advantage of these advances. One would be to define which adult spinal interneurons are essential elements of the networks generating rhythmic locomotor movements, and which are not. This question is less important for species in which practically all forms of motor behaviour might boil down to being variants of locomotion, such as frog embryos, lampreys or zebra fish, but is important for mammals, especially humans, where spinal neurons contribute to a great variety of phasic as well as rhythmic movements, from the simplest to the most skilled voluntary movements. Depending on the proportions of mammalian neurons that mediate both phasic and rhythmic movements, conclusions based on studies of rhythmically active interneurons may or may not generalize to other interneurons.

A second intermediate stage would involve defining relationships between various classes of embryonic neurons (defined by transcription factor expression patterns during development) and the various populations of adult neurons that develop from them (for recent reviews see Goulding, 2009; Grillner & Jessell, 2009; Garcia-Campmany *et al.*, 2010). In addition to motoneurons, relationships have so far been firmly established between mammalian embryonic spinal neurons of class V1 and two classes of adult interneurons: Renshaw cells and Ia inhibitory interneurons (see e.g. Moran-Rivard *et al.*, 2001; Pierani *et al.*, 2001; Sapir *et al.*, 2004; Alvarez *et al.*, 2005; Gosgnach *et al.*, 2006). Steps have been made to relate embryonic neurons of classes dl6, V0 and some V3 embryonic commissural interneurons to adult commissural interneurons (Moran-Rivard *et al.*, 2001; Pierani *et al.*, 2001; Lanuza *et al.*, 2004; Zhang *et al.*, 2008), although which specific adult commissural interneurons (e.g. dorsal horn, intermediate zone or lamina VIII; excitatory or inhibitory) are derived from subclasses of these embryonic interneurons has not yet been fully established. Steps have been also made to

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relate V2a, V2b and some V3 embryonic interneurons to ipsilaterally projecting adult interneurons (Al-Mosawie *et al.*, 2007; Lundfald *et al.*, 2007; Crone *et al.*, 2008; Zhang *et al.*, 2008), dl1, dl2 and dl3 neurons to as-yet-unspecified adult ascending tract neurons (Gross *et al.*, 2002) and dl4 neurons to interneurons mediating presynaptic inhibition (Betley *et al.*, 2009). One of the reasons why these relationships have been difficult to establish is that very few classes of adult spinal interneurons are well defined. Groups of adult interneurons can be defined based on a variety of properties, some of which are not unique to one group, so borderlines between various interneuronal populations are not always sharp. In addition several criteria are needed to identify individual interneurons in functional terms, such as transmitter phenotype, target cells, patterns of input and modes of modulation, and not all of these can be examined under specific experimental conditions.

This review considers the subdivision of spinal interneurons that process information on three major parameters of muscle states: the tension developed during muscle contractions, muscle length and the dynamics of changes in the length. This information is provided by tendon organs (via group Ib afferents), muscle spindle secondary endings (via group II afferents) and muscle spindle primary endings (via group Ia afferents) respectively (Matthews, 1972). Initial expectations were that such specific signals would be processed by different interneurons. Electrophysiological analyses, however, provided evidence that many spinal interneurons are co-excited by several types of primary afferents (see below). Consistent with this, it was demonstrated that interneurons processing information from group Ib afferents are co-excited by group Ia afferents (Fetz *et al.*, 1979; Czarkowska *et al.*, 1981; Jankowska *et al.*, 1981a,b,c; Harrison & Jankowska, 1985b). Considering the distinctive response properties of group Ia and Ib afferents, integration of information from these afferents by the same interneurons was a surprising finding but since then has been generally accepted. This review raises the question whether the same interneurons integrate information from group II afferents as well, and reconsiders the subdivision of spinal interneurons in reflex pathways from group Ib tendon organ afferents and group II muscle spindle afferents into separate populations of 'Ib interneurons' and 'group II interneurons'. Interneurons mediating actions evoked by stimulation of group Ib and II afferents have traditionally been considered to be distinct functional populations, characterized by selective, or at least dominant, input from the categories of afferents after which they were named (for review see Jankowska, 1992). However, on re-examination of the properties of these interneurons we find that the reflex actions of group Ib afferents are mediated by premotor interneurons (i.e. interneurons directly exciting or inhibiting alpha-motoneurons) alternatively termed last-order interneurons, that integrate information from group II as well as group Ib afferents, whereas the actions of group II afferents are relayed both by these interneurons and by other interneurons that have more selective group II input. Neurons integrating inputs from group Ib and group II afferents are located within Rexed's laminae V-VII of the spinal cord (Rexed, 1954; see also Fig. 2), the region of the grey matter often referred to as the intermediate zone. Neurons with more selective input from group II afferents are located primarily in laminae IV and VIII (Edgley & Jankowska, 1987b). The population of intermediate zone interneurons includes subpopulations of neurons that project ipsilaterally and/or contralaterally and both excitatory and inhibitory interneurons (Bannatyne *et al.*, 2009). However, the evidence presented in this review shows that in all of these subpopulation differences between individual interneurons mediating reflex actions from group Ib afferents and/or from group II afferents are only minor, not distinctive, and they may therefore operate as one functional population.

Defining functional populations of interneurons

The characteristics of functional interneuronal populations may not be as obvious as for populations defined by their transmitter phenotype, axonal projections or embryonic origin. The main feature of functional populations is that they subserve a particular motor synergy or a particular kind of reaction. Examples of motor synergies are inhibition of flexor motoneurons associated with monosynaptically evoked stretch reflex of extensor muscles operating across the same joint, or excitation of flexors associated with inhibition of extensors throughout a limb during active flexion of a limb. Each of these synergies requires the concerted action of a variety of interneurons. The first synergy would require Ia inhibitory interneurons that mediate reciprocal inhibition between flexors and extensors but also between extensors and flexors to ensure a proper balance between the degree of activation of antagonists. The second synergy will depend on interneurons that mediate appropriately distributed and timed excitation of flexor and the intimately associated inhibition of extensor motoneurons, so will depend on interneurons with different transmitter phenotypes and axonal projections. Examples of interneurons that subserve particular reactions are interneurons that mediate limb withdrawal from painful stimuli, or interneurons that ensure co-ordinated rhythmic activation of muscles on both sides of the body during locomotion, or on one side of the body during scratching. These are likely to include interneurons that subserve various motor synergies, but also other categories of interneurons.

The choice of criteria allowing classification of a group of interneurons as of one functional population may thus depend on the basis of the subdivision. However, interneurons within a functional population should share the essential features and show only minor differences.

Minor differences between members of a population are easiest to illustrate for unequivocally identified functional classes of neurons, e.g. Renshaw cells. The population of Renshaw cells includes neurons with somewhat differing somatic locations, extents of dendritic arborisation, directions and extents of axonal projections, and distributions of terminal axonal branches within and outside the motor nuclei: these reflect intra-population variability. In contrast, there are characteristic properties specific to Renshaw cells that are not shared by other inhibitory interneurons located nearby (such as Ia inhibitory interneurons), showing that Renshaw cells are a clearly distinct functional interneuronal population. These differences include in particular the origin of the input and the target cells. In adult animals Renshaw cells are directly excited by motoneuron axon collaterals but not by muscle spindle group Ia afferents, while the converse is true for Ia inhibitory interneurons (for references see Eccles *et al.*, 1961a; Windhorst, 1990; Jankowska, 1992; Alvarez & Fyffe, 2007). There are also essential differences in their output connections: Renshaw cells inhibit other Renshaw cells as well as Ia interneurons but the converse is not true. They also target different alpha-motoneurons; Renshaw cells target motoneurons that are synergistic to those providing input to them while motoneurons of antagonist muscles are targeted by Ia interneurons. These differences were originally found in adult animals (cat, rat and primates) but have recently also been demonstrated in neonatal mice (Wang *et al.*, 2008). At some stages of development these two populations of interneurons share input from group Ia afferents which are subsequently withdrawn from Renshaw cells (Mentis *et al.*, 2006) and express gamma-aminobutyric acid (GABA) as well as glycine; glycine continues to act as transmitter together with GABA in adult Renshaw cells (Schneider & Fyffe, 1992) but is the only transmitter of Ia inhibitory interneurons (Wang *et al.*, 2008). This is in keeping with the

origin of these two populations of interneurons from the same embryonic progenitor cells, concluded to be class V1 ventral interneurons, with precursors expressing the transcription factors Pax6 and En1 (Burrill *et al.*, 1997; Ericson *et al.*, 1997; Sapir *et al.*, 2004; Alvarez *et al.*, 2005; Goulding, 2009). Nevertheless, Wang *et al.* (2008) recently raised the question whether all Ia interneurons and Renshaw cells originate from precursors expressing the transcription factor Pax6 as they found that Ia reciprocal inhibition is present in Pax6-mutant mice in which Renshaw cells fail to develop. It is thus possible that reciprocal inhibition might be at least partially mediated by some as-yet-undefined embryonic class as well as V1 interneurons (Goulding, 2009).

Furthermore, differences in input from peripheral afferents found in individual interneurons do not necessarily imply that these interneurons belong to different functional populations because input to neurons within one population may vary. Variations in input have been reported for Ia interneurons (Hultborn & Udo, 1972) and for Renshaw cells (Ryall & Piercey, 1971; Ryall *et al.*, 1972) as well as for interneurons with dominant group Ib (Harrison & Jankowska, 1985b) or group II (Edgley & Jankowska, 1987b; Edgley, 2001) input. Conversely, interneurons with similar inputs need not necessarily belong to the same functional population. Examples are motoneurons and Ia inhibitory interneurons (both of which are monosynaptically excited by the same Ia afferents and inhibited by the same groups of Renshaw cells), Ia interneurons and Renshaw cells (both inhibited by the same groups of Renshaw cells), Ia inhibitory interneurons, Ib interneurons and dorsal spinocerebellar tract neurons (all three with monosynaptic input from the same groups of Ia afferents; for references see Jankowska 1992).

Many interneurons have multisensory input, from both several types of afferent and from many different muscles. In classifying interneurons it is easy to subdivide them based on the combinations of inputs found in individual interneurons. A problem with this approach is the generation of very large numbers of different classes of interneurons based on what might be minor differences. Given a substantial number of neurons, a more appropriate way to envisage a population might be to consider the distribution of inputs among the population, for example connections from one type of afferent or a specific muscle might occur in a certain proportion of individual neurons in a population, but not all of them. Taking this approach, one of the hallmarks of a specific population might be a given probability of finding a particular input; in one population the combinations of inputs should thus be found in proportions predicted if the inputs were distributed independently; in different populations (with different distributions of inputs) the probabilities of finding given inputs would be different. This approach has been taken for afferent inputs to samples of interneurons with inputs from Ib and group II afferents, in which the probability distribution suggests a single functional population (Harrison & Jankowska, 1985a; Edgley, 2001) and for descending inputs to interneurons with group II input where distinctly different populations could be identified (Davies & Edgley, 1994).

Intermediate zone premotor interneurons with input from group I and/or group II afferents operate as one functional population despite differences in input to individual interneurons

Subdivision of intermediate zone adult interneurons into those with dominant input from group I or II muscle afferents is often easily done when they are sampled using extracellular recording and when electrical stimulation of muscle nerves is used to activate them. Most neurons are discharged by stimuli that are either below or well above threshold for group II afferents (Fig. 1A and C respectively) and fewer respond to stimulation of both group I and group II afferents (Fig. 1B). This is possible because electrical stimulation very conveniently activates group I and group II afferents in different intensity ranges. Group I afferents are activated at intensities generally less than twice the threshold of the most excitable fibers in a muscle nerve while group II afferents are activated generally at 2–5 times this threshold (Matthews, 1972; Jack, 1978). Selective activation of group Ia or group Ib afferents is less easily achieved, usually requiring a combination of electrical and natural stimuli, and was not attempted in most experiments comparing input from group I and II afferents to intermediate zone interneurons. However, in previous experiments dedicated to this issue, group Ia and Ib afferents were demonstrated to co-excite these interneurons (Fetz *et al.*, 1979; Jankowska *et al.*, 1981a,b,c; Harrison & Jankowska, 1985b).

The subdivision based on input from group I or II muscle afferents is much less sharp when the interneurons are examined intracellularly, because excitatory postsynaptic potentials (EPSPs) from group I afferents frequently precede those from group II afferents in interneurons in which extracellular spike potentials are only induced by group II afferents (Edgley & Jankowska, 1987b). Conversely

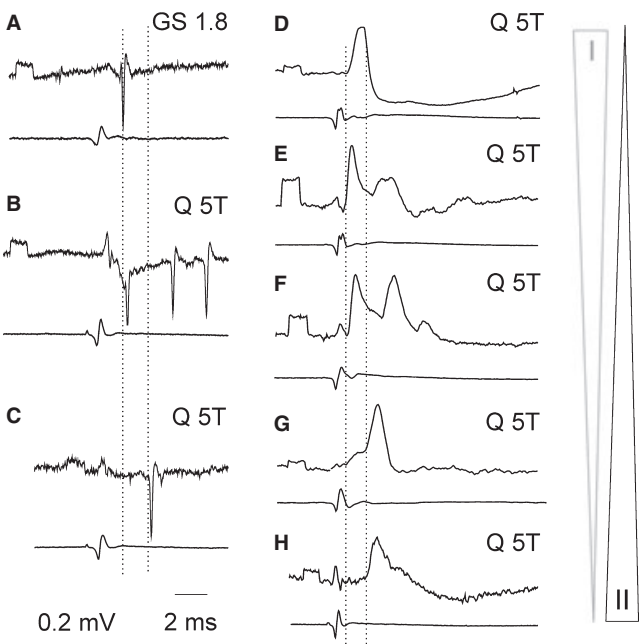

FIG. 1. Examples of different degrees of contribution of group I and group II afferents to excitation of intermediate zone interneurons following stimulation of muscle nerves. Each panel shows recordings from a different interneuron (upper traces) and from the surface of the spinal cord close to the dorsal root entry zone (lower traces). (A–C) Extracellular recordings from three neurons activated by stimulation of (A) group I afferents, (B) both group I and II afferents and (C) only group II afferents. They were activated by stimulation of the gastrocnemius–soleus (GS) nerve at 1.8× threshold and stimulation of the quadriceps (Q) nerve at 5× threshold as indicated. (D–H) Intracellular recordings from five interneurons. Monosynaptic EPSPs evoked in these interneurons from the quadriceps nerve ranged from (D) group I alone to (H) group II alone, but with different combinations in between, as schematically indicated to the right. Dotted lines indicate onset times of the spikes and of the earliest EPSPs from group I and group II afferents, at latencies of 0.7–0.9 and 1.7–1.9 ms from the afferent volleys respectively. Negativity is downwards in intracellular records and upwards in extracellular records. Rectangular pulses at the beginning of the records are calibration pulses (0.2 mV).

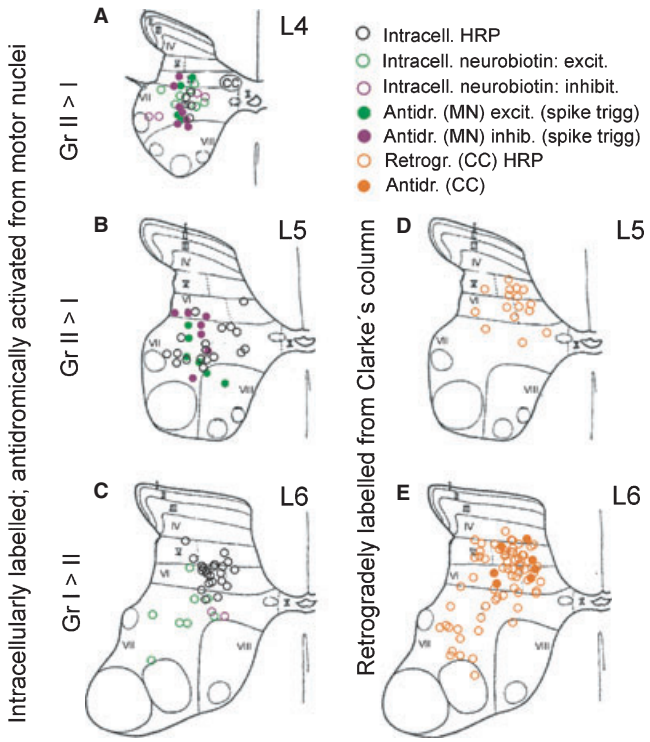

FIG. 2. Locations of intermediate zone interneurons with input from group I and/or group II afferents. Location of the different samples of intermediate zone interneurons indicated on Rexed's diagrams of the L4, L5 and L6 segments. (A–C) Open circles, locations of interneurons labelled intracellularly with horseradish peroxidase (HRP; A, data from fig. 1 in Bras *et al.*, 1989a and from fig. 11 in Bannatyne *et al.*, 2009; B and C, data from fig. 10 in Jankowska *et al.*, 1981a and figs 1 and 2 in Czarkowska *et al.*, 1981) or a mixture of rhodamine dextran and neurobiotin (data from fig. 5 in Bannatyne *et al.*, 2009). Green, glutamatergic (excitatory) interneurons; red, glycinergic (inhibitory) interneurons; black, interneurons with undefined transmitter phenotype. Most of these neurons were antidromically activated by stimuli applied in ipsilateral gastrocnemius–soleus or hamstring motor nuclei (MN) in the L7 segment. Filled circles, antidromically activated but extracellularly recorded interneurons that evoked population EPSPs (green) or IPSPs (red) in hindlimb motorneurons as found by spike-triggered averaging (from fig. 5 in Cavallari *et al.*, 1987). (D and E) Open circles, location of interneurons labelled by retrograde transport of HRP from CC (from fig. 6 in Hongo *et al.*, 1983a); filled circles, location of interneurons antidromically activated from Clarke's column (CC; from fig. 7 in Hongo *et al.*, 1983a).

EPSPs evoked by group II afferents are often superimposed on those from group I afferents in interneurons activated by group I afferents (Harrison & Jankowska, 1985b). Only in a fraction of these interneurons are EPSPs from one source substantially larger that those from the other, as illustrated in Fig. 1D, G and H, and in many other interneurons they are of similar amplitude (Fig. 1E and F).

In view of the absence of sharp dividing lines between intracellularly recorded intermediate zone interneurons with input from groups I, I and II or II afferents the question arises whether these afferents contact individual interneurons more or less randomly or in a specifically segregated manner. The question of distributed input from afferents in different nerves was analyzed in samples of intermediate zone interneurons found when searching for last-order neurons with input from groups Ia and Ib afferents (Harrison &

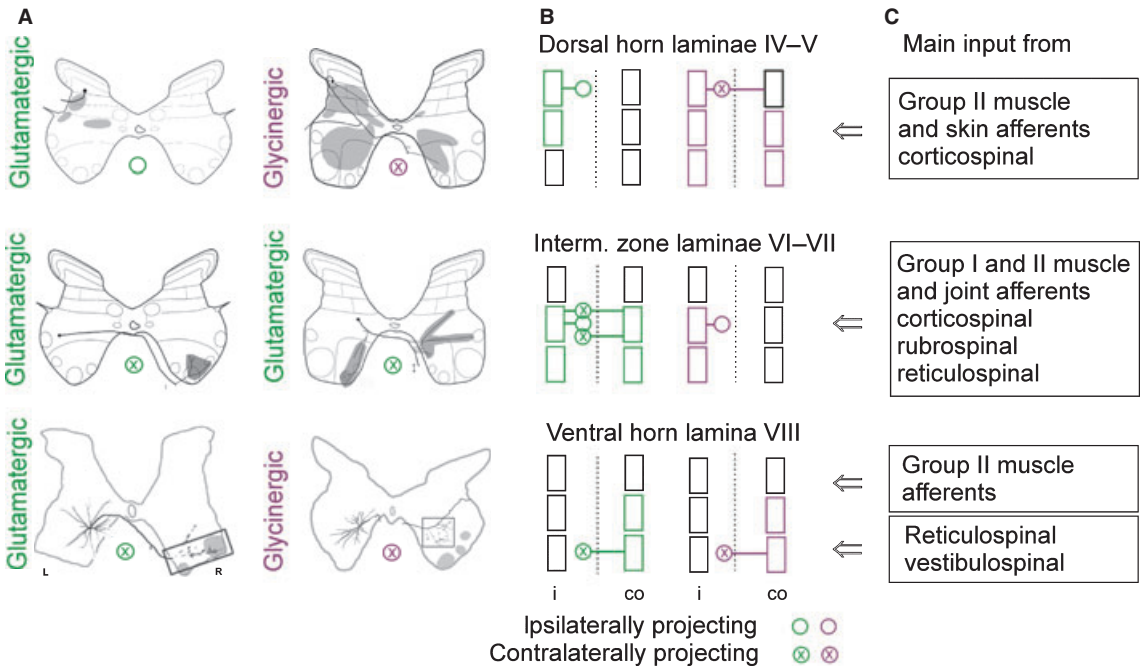

FIG. 3. Relationships between the transmitter phenotype, location, projection areas and input to interneurons activated by muscle afferents. (A) Examples of excitatory and inhibitory interneurons located in the dorsal horn (top), the intermediate zone (middle) and lamina VIII (bottom), with their typical terminal projection areas (shaded). (B) Diagrams summarizing the axonal projections for the groups of neurons at these locations that we have studied: glutamatergic and glycinergic. The circles represent cell bodies of the interneurons (all located to the left of the midline indicated by the dotted line; crosses in the circles represent contralaterally projecting neurons) while rectangular boxes to the left and right of these circles represent ipsilateral (i) and contralateral (co) projection areas within the dorsal horn, intermediate zone and the ventral horn (including motor nuclei) to which they project. Green and red boxes denote regions of axonal projections of excitatory and inhibitory interneurons respectively. Black boxes denote regions in which no projections from these interneurons were found. Note that all intermediate zone interneurons projecting to the motor nuclei also had terminal projection areas that were outside motor nuclei, showing that they target other neurons as well as motoneurons. (C) The main sources of input to these interneurons. Modified from figs 6, 7 and 11 in Bannatyne *et al.* (2009), figs 7 and 8 in Bannatyne *et al.* (2006) and figs 5 and 9 in Bannatyne *et al.* (2003).

Jankowska, 1985a) and when searching for last-order neurons with input from group II afferents (Edgley, 2001). It was analyzed by comparing proportions of interneurons co-excited by stimulation of different pairs of peripheral nerves, with the probability of co-excitation assuming that the coupling between afferents in these nerves and individual interneurons is at random (Harrison & Jankowska, 1985a; Edgley, 2001).

Using the same reasoning we have re-examined our original data to ask whether the proportions of intermediate zone interneurons co-excited by group I and II afferents deviate significantly from the proportions predicted for a random distribution. In the samples of interneurons from the L3-5 segments of Davies & Edgley (1994), Bannatyne *et al.(*2009) and Edgley & Jankowska (1987b), the proportions of interneurons projecting to motor nuclei in which EPSPs were evoked from both group I afferents (monosynaptically) and group II afferents (most likely monosynaptically) were 54, 47 and 62% respectively. These proportions deviate by < 10% from the proportions expected if inputs from group I and II afferents were randomly distributed (58, 51, and 53% for these samples, respectively). The proportions of intermediate zone interneurons co-excited by group I and II afferents are thus very similar to those predicted for a single population with distributed input from group I and II afferents. This supports the conclusion that dominant input from either group I or II afferents does not characterize distinct functional populations of intermediate zone interneurons. In two other studies neurons were sampled more caudally, in the L6-7 segments of the spinal cord, and the proportions of interneurons found to be co-excited by group I and II afferents differed, but the differences could be due to both sampling procedures and the location of the interneurons. The number of interneurons co-excited by group I and II afferents was reported to amount to 29% in the sample of Riddell & Hadian (2000) and to only 9% in the sample of Harrison & Jankowska (1985a). However, additional input from group I or II afferents of nontested nerves cannot be excluded in the interneurons that apparently had selective input from one of these sources; proportions of both the rostrally and more caudally located intermediate zone interneurons potentially co-excited by group I and II afferents might thus be larger than reported.

If intermediate zone interneurons with input from group I afferents are co-excited by group II afferents, it might be expected that input from other afferent systems and from various descending tract neurons would be associated with input from both group I and group II afferents. Such association has indeed been found for input from skin, joint and high-threshold muscle afferents (Czarkowska *et al.*, 1981; Jankowska *et al.*, 1981a; Harrison & Jankowska, 1985b; Edgley & Jankowska, 1987b; Aggelopoulos *et al.*, 1996a), as well as from the rubrospinal, corticospinal and reticulospinal neurons (Hongo *et al.*, 1972; Harrison & Jankowska, 1985b; Edgley & Jankowska, 1987b; Davies & Edgley, 1994; Takakusaki *et al.*, 2001; Cabaj *et al.*, 2006; Stecina *et al.*, 2008a). Input from vestibulospinal neurons was less widely distributed. However, it was found in interneurons co-excited by group I and II afferents as well as those with more selective input from group II afferents (Davies & Edgley, 1994). Conversely, in another study it was not found in reflex pathways from either group Ib or group II afferents (Grillner & Hongo, 1972). Major differences in vestibulospinal actions on interneurons in pathways from group Ib and II afferents would thus be unlikely.

Some differences in input to intermediate zone premotor interneurons have nevertheless been noted. For instance, EPSPs from group I afferents were most frequently evoked from gastrocnemius–soleus, plantaris and posterior biceps–semitendinosus in interneurons with dominant Ib input, but from flexor digitorum and hallucis longus, posterior biceps–semitendinosus and quadriceps in those with dominant group II input (Harrison & Jankowska, 1985b; Edgley & Jankowska, 1987b; Lundberg *et al.*, 1987b; Riddell & Hadian, 2000). The shortest latency EPSPs from cutaneous mechanoreceptors appeared to be evoked di- and trisynaptically in the former but mono- or disynaptically in the latter. Input from rubrospinal, corticospinal and reticulospinal neurons was often found in intermediate zone interneurons co-excited by group Ib and II afferents (Harrison & Jankowska, 1985b; Davies & Edgley, 1994; Stecina *et al.*, 2008a,b).

Some of these differences might be related to the different locations of interneurons sampled in different studies as the probability of synaptic contacts from fibres that terminate predominantly in a certain region of the spinal cord should be higher in that region. As mentioned above, a much higher proportion of interneurons co-excited by group I and II afferents was found in the L4-5 segments, whereas a much higher proportion of interneurons with input from group I but apparently not group II afferents was found in the L6-7 segments, which may reflect a certain rostrocaudal gradation within the whole population. However, we have not found indications for spatial segregation of these interneurons within Rexed's laminae in the coronal plane. Fig. 2 shows that the somata of interneurons with selective or predominant input from group II afferents in the L4 and L5 segments (Fig. 2A and B) and the somata of interneurons with selective or predominant input from group I afferents in the L6 segment (Fig. 2C) were located in corresponding regions of the grey matter.

Similarly, no differences were found in the laminar distribution of either excitatory or inhibitory interneurons with input from group I and/or II afferents. These interneurons were identified as excitatory or inhibitory using two main experimental approaches. The most recent approach utilized immunocytochemistry of intracellularly labelled interneurons [immunoreactivity of their terminals for vesicular glutamate transporters (VGLUT 2) in excitatory neurons, or gephyrin or glutamic acid decarboxylase in inhibitory interneurons and on reconstruction of their axonal projections (Bannatyne *et al.*, 2009)]. Only relatively small samples of intermediate zone interneurons, selected on the basis of antidromic activation from motor nuclei and monosynaptic input from group I and/or II afferents, could be analyzed in this way. In earlier studies electrophysiological techniques were used to identify these interneurons. Electrotonically spread population EPSPs and inhibitory postsynaptic potentials (IPSPs) evoked by single interneurons in motoneurons were recorded from motoneuron axons using sucrose gap and spike-triggered averaging (Brink *et al.*, 1981, 1983a; Cavallari *et al.*, 1987). No major differences in the patterns of convergence on excitatory and inhibitory interneurons were found in these studies; both showed input from either group Ib or group II afferents, or from both.

In Fig. 2A–C the location of glutamatergic and glycinergic interneurons is indicated by open symbols while filled symbols show the estimated locations of interneurons found to be excitatory or inhibitory by defining their actions on motoneurons. The location of interneurons projecting in parallel to Clarke's column and to motor nuclei (most likely inhibitory; Hongo *et al.*, 1983a,b; Jankowska & Puczynska, 2008) is shown in Fig. 2D and E for comparison. These were labeled by retrograde transport of horseradish peroxidase (HRP) from Clarke's column (open symbols) or were antidromically activated from Clarke's column (filled symbols). They are classified as inhibitory as only inhibitory interneurons with input from group I and II afferents were found to affect dorsal spinocerebellar tract neurons in Clarke's column (Hongo *et al.*, 1983a,b; Jankowska & Puczynska, 2008). In the adult cat, only two individual interneurons have been found to be GABAergic (or rather GABA and glycinergic;

Bannatyne *et al.*, 2009) among several samples of inhibitory interneurons with input from group I or II afferents, but GABAergic interneurons were reported to constitute a considerable proportion of inhibitory premotor interneurons with monosynaptic input from primary afferents located in laminae V/VI in neonatal mice; these interneurones might be homologues of intermediate zone interneurons in the cat (Wilson *et al.*, 2009; see also Lundfald *et al.*, 2007).

Distinct populations of interneurons with selective input from group II afferents located outside the intermediate zone

Monosynaptic input from group II afferents not accompanied by input from group I afferents has been found in only a small proportion of intermediate zone interneurons, but is a common feature of interneurons located within the dorsal horn (laminae IV-V of Rexed (Rexed, 1954) or within lamina VIII and at the border between laminae VII and VIII (Edgley & Jankowska, 1987b; Bannatyne *et al.*, 2006; Jankowska *et al.*, 2009).

The question therefore arises whether the selective input from group II afferents to dorsal horn and lamina VIII interneurons defines them as interneuronal populations distinct from intermediate zone interneurons co-excited by group I and II afferents, or might be compatible with 'intra-population' variations within the same interneuronal population.

Classification of dorsal horn interneurons with group II input as functionally distinct is supported not only by their input but also by their characteristic axonal projections. Only exceptionally were they found to have direct connections with alpha-motoneurons, in contrast to connections made by the majority of intermediate zone interneurons with either group I and II or only group II input. This is schematically indicated in the top diagrams in Fig. 3B and has been indicated by three kinds of observations: first, that only intermediate zone interneurons were labeled by transneuronal transport of wheat germ agglutinin-conjugated HRP introduced to alpha motoneurons (see e.g. (Harrison *et al.*, 1986; Alstermark & Kummel, 1990) or by retrograde transport of markers injected into motor nuclei (Hoover & Durkovic, 1992); second, that unlike intermediate zone neurons, dorsal horn interneurons were not antidromically activated by stimuli applied in motor nuclei several segments away (Edgley & Jankowska, 1987b); and third, that the terminal projection areas of intracellularly labeled intermediate zone interneurons extended to motor nuclei (Czarkowska *et al.*, 1976; Bras *et al.*, 1989a; Jankowska *et al.*, 1993b; Bannatyne *et al.*, 2009) while projections of dorsal horn interneurons were generally outside motor nuclei (Bannatyne *et al.*, 2006). Synaptic transmission to dorsal horn interneurons was likewise found to be modulated differently than to intermediate zone interneurons, both by monoamines (Bras *et al.*, 1989b, 1990) and by GABAergic presynaptic inhibition (Jankowska *et al.*, 2002). The only inconsistent feature has been the demonstration that some inhibitory dorsal horn interneurons (represented by the top right neuron in Fig. 3A) have bilateral projections that extend to the motor nuclei in the same segment, where three of them were found to make synaptic contacts with choline acetyltransferase-labeled neuronal profiles (Bannatyne *et al.*, 2006). However, if these contacts were with gamma rather than alpha motoneurons this would not contradict the general conclusion that dorsal horn interneurons are not premotor interneurons.

Lamina VIII interneurons with selective input from group II afferents represent a distinct interneuronal population on other grounds. In contrast to dorsal horn interneurons all their features are consistent with them being premotor interneurons. They were labeled by both transneuronal and retrograde transport from motor nuclei (Harrison *et al.*, 1986; Alstermark & Kummel, 1990; Hoover & Durkovic, 1992), were antidromically activated by stimuli applied in distant motor nuclei (Jankowska *et al.*, 2005), and those labeled intracellularly showed terminal projection areas within motor nuclei (Jankowska *et al.*, 2009). However, unlike intermediate zone premotor interneurons they have practically exclusively crossed axonal projections, at least in adult cats (see Jankowska *et al.*, 2009). This is schematically indicated in the bottom diagrams of Fig. 3B.

These commissural interneurons operate in association with another distinct population of lamina VIII commissural interneurons characterized by monosynaptic input from reticulospinal and vestibulospinal neurons and sometimes also from group I afferents but not from group II afferents (Jankowska *et al.*, 2005). In these commissural interneurons excitatory input from group I afferents turned out to be segregated from that from group II afferents, although both subpopulations are inhibited by neurons activated by group I as well as group II afferents. Commissural interneurons activated by group II afferents were found to be scarce and hard to record from, but to have potent crossed actions on contralateral motoneurons (Arya *et al.*, 1991), either excitatory or inhibitory depending on which of the two alternative crossed reflex pathways are operating under various experimental and behavioural conditions (Aggelopoulos *et al.*, 1996b). Input from group I afferents to other lamina VIII commissural interneurons may function primarily to support their activation by descending fibers because it was only rarely found to result in their discharge by itself (Harrison & Zytnicki, 1984; Harrison *et al.*, 1986; Arya *et al.*, 1991).

Relationships between input from group Ib and group II afferents, transmitter phenotype and axonal projections

Because, as discussed above, no relationship has been found between predominant input from group I or group II afferents and transmitter phenotype or the locations of the intermediate zone interneurons, the possibility that the axonal projections of excitatory and inhibitory subpopulations of these interneurons differ was considered.

As indicated schematically in Fig. 3B such differences have been revealed because excitatory intermediate zone interneurons were found to project ipsilaterally, bilaterally or contralaterally while all inhibitory neurons sampled projected only ipsilaterally (Bannatyne *et al.*, 2009).

All intermediate zone interneurons were found to be funicular or to be propriospinal neurons with axons that descended and/or ascended for a few segments (but not beyond the lumbar segments). Of these, the ipsilaterally projecting axons of the excitatory interneurons entered only the lateral funiculus while the axons of inhibitory interneurons (which all projected ipsilaterally) entered either the lateral or ventral funiculi. The axons of bilaterally projecting intermediate zone interneurons branched, with one axonal branch crossing via the ventral commissure and ascending, descending (or both) within the contralateral ventral funiculus before entering the contralateral ventral horn. The second axonal branch remained within the ipsilateral grey matter and had terminal projection areas within a short distance of the soma. Dorsal horn interneurons with bilateral projections differed in this respect because both of their axonal branches entered the white matter and both ascended and/or descended; the crossed one in the contralateral ventral funiculus and the uncrossed one in the lateral funiculus (Bannatyne *et al.*, 2006).

All of the subpopulations of intermediate zone interneurons with different patterns of projections included interneurons co-excited by

group I and II afferents. Predominant input from group I or from group II afferents is thus not specifically related to either excitatory or inhibitory intermediate zone interneurons, or to subpopulations with different patterns of axonal projections, in keeping with the nonsegregated distribution of input from group I and II afferents within the whole pool of these interneurons.

Are reflex actions from group I and group II afferents compatible with their mediation by the same functional population of spinal interneurons?

Comparison of synaptic actions evoked in individual motoneurons, interneurons and spinocerebellar neurons

If one population of interneurons relays the reflex actions of both group I and II afferents to motoneurons, then both types of afferent should evoke disynaptic EPSPs and IPSPs in motoneurons. In addition, simultaneous stimulation of group I and II afferents in a single muscle nerve should evoke postsynaptic potentials with two components, the first attributable to faster conducting (group Ia and/or Ib) and the second attributable to slower conducting (group II) fibres. In addition, the first components should be evoked at lower stimulus intensities and the second components by stronger stimuli in view of the lower thresholds of group I compared to group II afferents. Dual-component postsynaptic potentials (PSPs) compatible with these requirements were described in the earliest studies of motoneuron responses, while using both relatively weak stimuli (Eccles *et al.*, 1957) and stimuli suprathreshold for group II afferents (Eccles & Lundberg, 1959; Lundberg *et al.*, 1987a). The later components of the PSPs evoked at stimulus intensities 1.4–1.8 threshold (within the higher range of stimuli needed to excite group Ib afferents) were originally interpreted as evoked trisynaptically, or due to double discharges of the interneurons (Eccles *et al.*, 1957). However, as the stimuli that evoked these later components could encroach upon the lowest threshold group II afferents (see e.g. Jack, 1978; Lundberg *et al.*, 1987a), effects of these stimuli would also be compatible with re-excitation of the same interneurons by slower conducting group II afferents. Stimuli suprathreshold for group II afferents evoked even more distinct double-component PSPs. It should also be pointed out that the latencies of both EPSPs and IPSPs of group II origin are compatible with disynaptic coupling when intraspinal conduction is taken into account (Edgley & Jankowska, 1987a; Edgley *et al.*, 1988) even though only the EPSPs had originally appeared to fulfill this condition (Lundberg *et al.*, 1987a). Interneurons co-excited by group I and II afferents may thus contribute to reflex actions of group I and II afferents to motoneurons.

When PSPs evoked by group I afferents are not followed by any distinct later components attributable to group II afferents this does not necessarily require that they were mediated by interneurons with selective input from group I afferents; it might be that the interneurons were not discharged by group II afferents. Several potential mechanisms might underlie this. (i) Actions from group II afferents on the same interneurons might be filtered away by the modulatory actions of monoamines or presynaptic inhibition, both of which are potent on group II afferent terminals. (ii) Interneurons discharged by group I afferents might be refractory at the time of arrival of nerve volleys in the fastest group II afferents and therefore discharge only once. (iii) Many intermediate zone interneurons are subject to disynaptic inhibition by group I afferents at the time of arrival of nerve volleys in the fastest group II afferents (e.g. Fig. 1D; see also Brink *et al.*, 1983b; Edgley & Jankowska, 1987b).

PSPs with the characteristics of disynaptic potentials of group II origin not preceded by earlier and lower threshold components that would be attributable to group I afferents might be explained (i) if group I input alone were insufficient to discharge the interneurons, (ii) if the PSPs were evoked via more rostrally located interneurons in which input from group I afferents is both weaker and less frequent and (iii) if transmission from group I afferents to these neurons were hampered by presynaptic inhibition, so that stimuli below threshold for group II afferents were insufficient to discharge them. Under these conditions input from both group I and II afferents might result in longer latency responses linked to activation of group II afferents. Polysynaptic actions of group II afferents could of course be relayed by a variety of other interneurons, e.g. Ia inhibitory interneurons or interneurons in pathways from the flexor reflex afferents (FRA; Eccles & Lundberg, 1959; Lundberg *et al.*, 1987b,c; Schomburg, 1990).

The conclusion that the synaptic actions of Ib afferents on motoneurons are relayed by interneurons that are co-excited by group II afferents would also require that synaptic actions evoked by these afferents mutually facilitate each other. To test whether such facilitation exists, the effects of stimulation of group I and II afferents of different nerves had to be used to avoid the complications of refractory periods for pairs of stimuli to the same nerve and the choice of effective combinations is fairly limited (Edgley & Jankowska, 1987b). Some of the combinations tested turned out nevertheless to be effective (Jankowska *et al.*, 1996).

Synaptic actions of group I afferents on other spinal neurons have also been found to be evoked in parallel with synaptic actions of group II afferents and with indications that they are evoked by the same intermediate zone interneurons. For instance, the evidence for mutual inhibitory interactions between intermediate zone interneurons with input from group Ib afferents (Jankowska *et al.*, 1981a; Brink *et al.*, 1983b) or group II afferents (Edgley & Jankowska, 1987b; Bajwa *et al.*, 1992) involves interneurons co-excited by group Ia and Ib afferents (Fetz *et al.*, 1979; Czarkowska *et al.*, 1981; Jankowska *et al.*, 1981a; Jankowska & McCrea, 1983; Harrison & Jankowska, 1985a; Powers & Binder, 1985) as well as group II afferents.

IPSPs from both group I and II afferents have also been found in ventral spinocerebellar tract neurons (Eccles *et al.*, 1961b; Burke *et al.*, 1971; Lundberg & Weight, 1971) and dorsal horn dorsal spinocerebellar tract neurons (Edgley & Jankowska, 1988). In Clarke's column dorsal spinocerebellar tract neurons IPSPs were originally only found to be evoked from group Ib afferents (Eccles *et al.*, 1961c). As these IPSPs were shown to be mediated by the same intermediate zone interneurons that mediate inhibition of Ib origin in motoneurons (Hongo *et al.*, 1983a,b), it was predicted that inhibition from group II afferents would be evoked in dorsal spinocerebellar tract neurons by these interneurons, and this was indeed recently demonstrated (Jankowska & Puczynska, 2008). We are thus not aware of any postsynaptic actions evoked from group Ib afferents that are not replicated by group II afferents and that could be mediated by interneurons with selective input from group Ib afferents.

Comparison of patterns of reflex actions from group Ib and group II afferents and their supraspinal and propriospinal control

Similar patterns of reflex action of group I and II afferents would support our contention that these are mediated by a single functional population of interneurons, while different patterns would speak against it.

Originally Lloyd (1943a,b) and Laporte & Lloyd (1952) related the monosynaptic actions of muscle afferents to the actions of group I afferents (defined as the largest afferents, with diameters 20-12 μm) and polysynaptic actions to smaller afferents which were collectively referred to as group II and III. Lloyd's group II afferents that evoked di- or polysynaptic actions might thus have included both group Ib tendon organ afferents and group II muscle spindle secondaries, as defined in later work. These were shown to induce longer latency facilitation (mainly of flexor monosynaptic reflexes) and inhibition (mainly of extensor monosynaptic reflexes) than the shortest latency excitatory and inhibitory actions of the lowest threshold afferents (subsequently identified as muscle spindle primary afferents).

By using graded electrical stimulation of muscle nerves to activate group Ia, Ib and II afferents, and by combining this with intracellular recording from motoneurons, more detailed patterns of actions of these afferents were revealed (Eccles *et al.*, 1957). With respect to actions of Ib (tendon organ) afferents it was concluded that afferents originating from extensor muscles are more potent than afferents from flexors, and that inhibition is more frequently evoked in extensors and excitation in flexors. Nevertheless, excitation is sometimes seen in extensors and inhibition in flexors (Laporte & Lloyd, 1952; Eccles *et al.*, 1957; Hongo *et al.*, 1969; Jankowska *et al.*, 1981c; Harrison *et al.*, 1983; McCrea, 1986).

Dominant inhibition of extensor motoneurons and excitation of flexor motoneurons has also been found in response to stimulation of group II afferents (muscle spindle secondaries), but with excitatory actions on some extensor and inhibitory actions on some flexor motoneurons (Eccles & Lundberg, 1959; Wilson & Kato, 1965; Kanda & Rymer, 1977; Lundberg *et al.*, 1987a; Hongo & Pettersson, 1988) evoked in parallel with similar alternative actions of Ib afferents. Reflex actions of group Ib and II afferents are thus compatible with actions mediated by the same interneurons. However, group II actions evoked at longer latencies are often induced in parallel with actions of afferents that evoked flexor or extensor reflexes, so they originally were linked primarily with interneurons mediating actions from high-threshold muscle, skin and joint afferents denoted flexor reflex afferents (Eccles & Lundberg, 1959; Lundberg *et al.*, 1987c).

Supraspinal control of the reflex actions of group Ib and II afferents likewise shows similarities: both are greatly depressed by decerebration and re-established after caudal medullary lesions or spinalization (for review see Lundberg, 1982). The possibility that reticulospinal neurons act primarily on interneurons in the shared FRA pathways and only via them on interneurons mediating disynaptic actions of either group Ib or group II afferents has nevertheless been considered (Engberg *et al.*, 1968; Lundberg, 1982).

Modulation of the reflex actions of group Ib and II afferents by propriospinal neuronal systems has been investigated less systematically. Effects mediated by long propriospinal tract neurons were analysed primarily on Ib and general FRA actions, both found to be facilitated (Jankowska *et al.*, 1973, 1983), and interneurons with group II input are also activated by long propriospinal tract neurons mediating neck reflexes (Brink *et al.*, 1985; Suzuki *et al.*, 1986; Yates *et al.*, 1989). Effects mediated by short propriospinal tract neurons were analysed primarily in terms of group II actions (Cavallari & Pettersson, 1989), but enhancement of actions from not only group II but also from group I afferents was found following lesions of the axons of propriospinal neurons in the L2 and L3 segments. Thus these propriospinal neurons induce tonic inhibition of interneurons in pathways from both group Ib and group II afferents.

Comparison of patterns of reflex actions from group Ib and group II afferents during locomotion

Potent modulation of reflex actions from both group Ib and II afferents has also been found to occur during locomotion. It was first demonstrated as suppression of inhibition evoked in motoneurons by stimulation of group Ib afferents and the release of excitation from these afferents (Pearson & Collins, 1993; Gossard *et al.*, 1994; McCrea *et al.*, 1995; Angel *et al.*, 1996; Quevedo *et al.*, 2000; Angel *et al.*, 2005). Initially, these effects appeared to be specific to the synaptic actions from group Ib afferents, but suppression of inhibition from group II afferents and release of excitatory actions of these afferents (Perreault *et al.*, 1995) and associated changes in excitability of interneurons with group II input ((Shefchyk *et al.*, 1990); K. Stecina & D.A. McCrea, personal communication) have subsequently been demonstrated. In addition, the latencies of the earliest EPSP appearing during locomotion and the origin of these EPSPs from both Ia and Ib afferents (Guertin *et al.*, 1995; McCrea *et al.*, 1995; Degtyarenko *et al.*, 1998; Quevedo *et al.*, 2000) are appropriate for mediation by intermediate zone interneurons.

However, the effects of stimulation of group I and II afferents on locomotion were found to differ. One difference was that some of these effects (prolongation of the extensor phase and subsequent resetting of the locomotor rhythm by stimuli delivered during this phase and also advancement of the extensor phase by stimuli delivered during the flexor phase) were evoked following stimulation of group I afferents in extensor nerves but not by stimulation of group II afferents of the same nerves when stimulus intensity was increased (Guertin *et al.*, 1995). Similar effects were only evoked by flexor group II afferents and some group I afferents (Guertin *et al.*, 1995; Perreault *et al.*, 1995), while other flexor group I afferents had the opposite effect, prolonging the flexion phase when stimulated during flexion (Guertin *et al.*, 1995; Perreault *et al.*, 1995; Stecina *et al.*, 2005).

Provided that disynaptic actions of group I and II afferents and various forms of resetting of the locomotor rhythm by stimulation of group Ib and II afferents are mediated by the same population of premotor interneurons, different patterns of actions of these afferents during swing and stance phases of the locomotor cycle reveal an important feature of the recruitment of these interneurons. They indicate that individual intermediate zone interneurons that target motoneurons innervating different muscles may be selected for action in a task-dependent manner and not only during locomotion (see e.g. Degtyarenko *et al.*, 1998; Quevedo *et al.*, 2000; Rybak *et al.*, 2006; McCrea & Rybak, 2008) but also during any movements (see e.g. Lundberg, 1975, 1982; Jankowska, 1992; McCrea, 1992). Whether this reflects a subdivision of the population into distinct subpopulations, task-dependent modulation or task-dependent recruitment of multipurpose interneurons of the same population, as in the turtle (Berkovitz, 2005), remains an open question.

Differential modulation of reflex actions of group Ib and II afferents by monoamines is compatible with mediation of these actions by the same as well as by distinct interneurons

Spinal reflexes are powerfully modulated by descending monoaminergic neuronal systems providing behavioural flexibility. Systemically applied monoamines or their precursors produce profound and highly differentiated changes in spinal reflexes (for review see Lundberg, 1982; Schomburg, 1990; Schomburg & Steffens, 1998). One of the main features of these modulatory actions is that monoamines have very strong effects on the reflex actions from the FRA (sometimes, but

not always, parallel to the actions of group II afferents) but weak (sometimes none, sometimes in the opposite direction) effects on reflex actions of group I afferents. As illustrated in Fig. 4, stimuli applied in locus coeruleus–subcoeruleus may almost abolish IPSPs evoked by stimulation of group II afferents but have a negligible effect on IPSPs evoked by stimulation of group Ib afferents. These differences raise the question of whether differently modulated synaptic actions of group I and II afferents on motoneurons are indeed relayed by the same interneurons. It is therefore relevant that both the depressed synaptic actions of group II afferents and the unaffected actions of group Ib afferents were disynaptically evoked. They could thus be mediated by the same population of intermediate zone interneurons activated first by faster conducting group I afferents and then by slower conducting group II afferents (see above). However, this would require that noradrenaline (NA; replicating the effects of neurons in the locus coeruleus) counteracted activation of intermediate zone interneurons by group II afferents without interfering with their activation by group I afferents, i.e. it would have to act selectively. As shown in Fig. 4C this was indeed found to be the case when NA (or a precursor or agonist) was applied ionophoretically on individual interneurons (Bras *et al.*, 1989b, 1990). Differential effects evoked by locus coeruleus stimulation could also be related to more potent GABAergic presynaptic inhibition of transmission from group II than from group I afferents (Riddell *et al.*, 1993). Different effects of NA and 5-hydroxytryptamine (serotonin) on transmission from group II afferents to dorsal horn and intermediate zone interneurons (Bras *et al.*, 1989b, 1990; Jankowska *et al.*, 2000) are on the other hand consistent with operation of the dorsal horn interneurons as specific relay neurons of synaptic actions of group II afferents (Bras *et al.*, 1989b). It is also consistent with the evidence (see above) for distinct roles played by these interneurons.

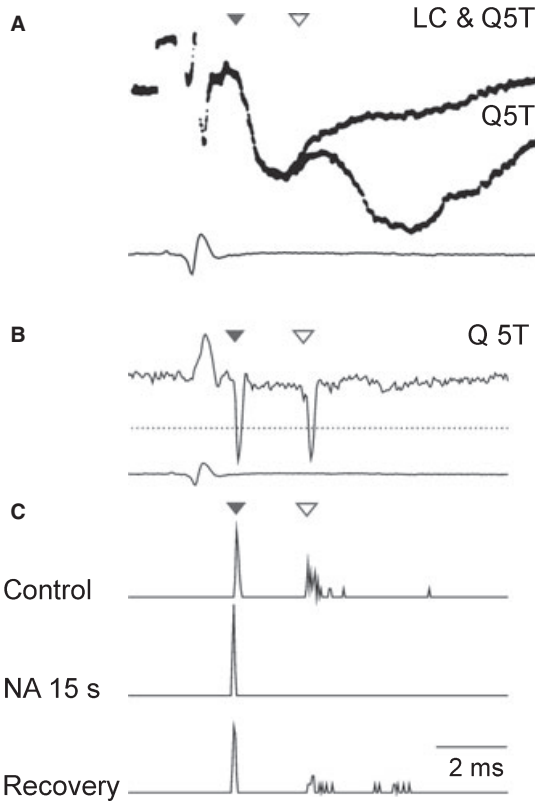

FIG. 4. Examples of differential modulation of synaptic actions of group I and II afferents on motoneurons and intermediate zone interneurons. Responses in (A) a motoneuron and (B and C) an intermediate zone interneuron, both of which were evoked by both group I and group II afferents (filled and open arrowheads indicate the early effects of group I and later effects of group II afferents respectively). (A) Intracellular records from a gastrocnemius–soleus motoneuron, illustrating the effects of stimuli applied in the region of origin of the descending noradrenergic neurons, in locus coeruleus/subcoeruleus (LC) on IPSPs evoked by stimulation of both group I and II afferents in the quadriceps (Q); lower trace, afferent volley from the cord dorsum. Note that control records displayed both an early (group I) and a late (group II) component: the late component was very substantially suppressed when Q stimulation was preceded by LC conditioning stimulation (at a conditioning–testing interval of 160 ms). Modified from Jankowska *et al.* (1993a). (B) Records from an intermediate zone interneuron responding with an early (group I) and a later (group II) spike to the same intensity of Q stimulation, and record of afferent volleys from the cord dorsum. (C) Peristimulus time histograms of spike potentials (exceeding the discrimination level indicated by the dotted line) for the interneuron illustrated in B. They show responses evoked by 20 consecutive stimuli before, during and after ionophoretic ejection of NA from a second micropipette positioned close to the same interneuron. Note that the late responses disappeared during application of NA while the early responses persisted. Modified from fig. 5 in Jankowska *et al.* (2000).

Concluding comments on integrative functions of intermediate zone interneurons relaying synaptic actions of group I and II afferents

Proprioceptors are highly specialised to transduce and encode specific features of muscle mechanics. Considering that the information contained in the discharge of afferents from each of the types of receptors that converges onto intermediate zone interneurons is so precise and specific (see Matthews, 1972), it has been a subject of continuing concern that this specific information will be lost when combined by neurons that mix information from several different

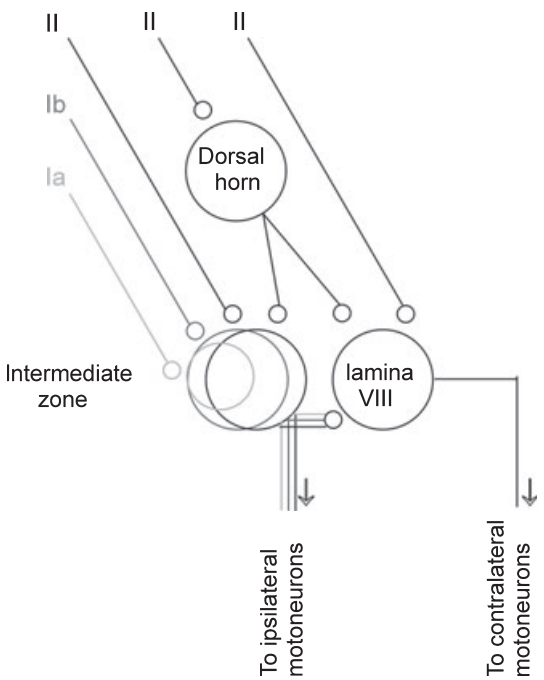

FIG. 5. Simplifying diagram of relationships between intermediate zone interneurons co-excited by group I and II afferents and dorsal horn and lamina VIII interneurons with input from group II afferents. The diagram only takes into account the distribution of the excitatory input to these neurons. However, interactions have been found between excitatory and inhibitory interneurons with input from group Ib and II afferents.

muscles as well as from different types of receptors. It is not easy to imagine how for example the precise information on dynamic changes in muscle length provided by primary and secondary endings is used when integrated by individual interneurons with information on active muscle tension from tendon organs as well as from several other receptors, and from several different muscles. Nevertheless, consistent findings from many different studies have shown that this is how many spinal interneurons integrate sensory information. As monosynaptic contacts on motoneurons are exclusively from group Ia and group II afferents, the precise information provided by these must be essential for monosynaptic reflexes. Likewise, monosynaptic contacts of group II but not group Ia or Ib afferents on dorsal horn interneurons and on lamina VIII interneurons indicate that the precise information from muscle spindle secondary afferents is particularly important for reflex actions mediated by them. In contrast, for the system of intermediate zone neurons we have described here, it is apparently essential for information from tendon organs to be integrated with that from muscle spindle primaries and/or secondaries to be used in a meaningful way (see discussion of this issue in Jankowska & McCrea, 1983). It appears to be only rarely used independently, e.g. by lamina VIII commissural interneurons with principal input from descending tract fibers (see above). However, because of the distributed input to intermediate zone interneurons, about one-half of them are involved in integrating information from group I and II afferents but the remaining neurons process it in a more selective way. In addition there are possibilities for information from tendon organs to be processed selectively, or at least more selectively, when other kinds of input are filtered away by presynaptic mechanisms. As illustrated in Fig. 4, input from group II afferents can be particularly effectively filtered out by monoaminergic modulation, thereby transforming intermediate zone interneurons co-excited by group I and II afferents into interneurons selectively excited by group I afferents. In this state they would still integrate information from group Ia as well as Ib afferents, both from several muscles, but ignore input from group II afferents. This form of transformation is unlikely to be fine-grained, with individual neurons switching but others not, but is likely to be a feature of specific behavioural states. A very schematic summary of the interrelations between the different functional populations of interneurons with excitatory input from group II afferents is illustrated in Fig. 5.

On the basis of the arguments we have presented above a further change in the terminology used to refer to spinal interneurons is proposed. When we demonstrated that what was traditionally denoted as 'Ib inhibition' is in fact mediated by interneurons co-excited by group Ia as well as Ib afferents, we proposed the terms 'Ia-like-Ib inhibition', 'Ia non-reciprocal inhibition' or 'group I non-reciprocal inhibition' (Jankowska *et al.*, 1981c; Jankowska, 1992). For the sake of convenience, and tradition, we continued to refer to the interneurons that mediate this action as 'Ib interneurons', while recognizing that other forms of input accompany input from group I afferents in these interneurons. Subsequent studies in the midlumbar segments revealed prominent effects evoked from group II afferents, so neurons at this location were referred to as 'group II interneurons', while recognizing that other forms of input accompany input from group II afferents in these interneurons. However, given our arguments above, it would be misleading to categorize intermediate zone interneurons with input from group I and group II afferents as either 'group Ib' or 'group II' interneurons. We therefore propose the names 'group Ia/Ib/II interneurons' or 'group I/II interneurons' to be used depending on the context.

Returning to the problems outlined in the Introduction, if the conclusion of this review that there are no reasons to subdivide the intermediate zone interneurons with input from group I and II afferents into distinct neuronal populations is accepted, there are obvious consequences for the analysis of their embryonic origin. In view of the different transmitter phenotypes and patterns of connectivity of some interneurons in this grouping, they might originate from different classes of embryonic neurons. However, all these would have one feature in common, becoming target cells of both group I and II afferents, in contrast to dorsal horn and lamina VIII commissural interneurons which accept synaptic contacts of only group II afferents.

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Abbreviations

EPSP, excitatory PSP; FRA, flexor reflex afferents; GABA, gamma-aminobutyric acid; HRP, horseradish peroxidase; IPSP, inhibitory PSP; NA, noradrenaline; PSP, postsynaptic potential.

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