In the Name of God

the Compassionate and the Merciful

Electrophysiological studies of spinal reflex pathways from group II muscle afferents

by

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Dedicated to

my wife and children

Dedicated to my father who

passed away during my

PhD course

Contents **Page**

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Declaration

I certify that the authorship of this thesis is my own and that I carried out the experiments which were reported. None of the material presented has been submitted for any other degree or professional qualification. Some of the material has been published and reprints are included in the thesis as an appendix.

Summary

This thesis has investigated aspects of the circuitry located in the spinal cord by which signals originating from group II muscle afferents are processed. Studies have focused on two main problems:

Part 1. The organisation of neuronal systems mediating group II reflex actions in the lumbar enlargement of the rat spinal cord have been investigated and compared with that in the cat with the aim of determining whether general principles of the organisation of these systems are common to different mammalian species. Group II afferents of different hind-limb muscle nerves were found to evoke cord dorsum and field potentials in particular segments of the spinal cord. Group II afferents of the quadriceps and deep peroneal nerves evoked synaptic potentials mainly at the rostral end of the lumbar enlargement (L1-rostral L3 segments) while the group II afferents of the gastrocnemius-soleus and hamstring muscle nerves produced their synaptic actions mainly at the caudal end of the lumbar enlargement (caudal L5). Group II afferents of the tibialis posterior and flexor digitorum longus nerves produced their synaptic actions mainly in the central lumbar segments (caudal L3 - L4). These results show that in principal the topographical organisation of neurones in group II reflex pathways of the rat is similar to that of the cat.

Part 2. The actions of group II muscle afferents on neurones within the lower - lumbar segments of the cat spinal cord have been investigated with the aim of locating candidate last - order interneurones in group II reflex pathways.

Only group II afferents of the tibialis posterior nerve produced large cord dorsum potentials comparable to those evoked by other group II afferents in midlumbar and sacral segments. However, field potentials evoked by group II muscle afferents of the quadriceps, tibialis-posterior, gastrocnemius-soleus, flexor digitorum longus, posterior biceps-semitendinosus and popliteus were encountered throughout the L6 and L7 segments. Group II potentials were evoked in both the dorsal horn (laminae V-VI) and intermediate zone (laminae V-VII) at latencies compatible with monosynaptic actions of the fastest conducting group II muscle afferents.

The properties of interneurones receiving excitatory input from group II muscle afferents have been investigated using both extracellular and intracellular recordings. Almost half the sample of interneurones (38 of 76) were characterised by an ipsilateral ascending projection within the lateral funiculus to the L4 level. Both L4-projecting and non-projecting interneurones were located mainly in an area dorsal and lateral to the main region in which interneurones with input from group I muscle afferents are located.

Group II afferents of quadriceps and deep peroneal muscle nerves provided the most effective excitation (discharging 70-80% of neurones) while group II afferents of tibialis posterior, posterior biceps-semitendinosus and gastrocnemius-soleus were also effective sources of excitation (discharging 45- 55% of neurones). At least some of the group II EPSPs were monosynaptic.

Input to individual interneurones was multimodal and characterised by considerable convergence. Seventy-five percent of interneurones were discharged by group II afferents of two or more muscle nerves and 43 % by group II afferents of three or more nerves. In addition, group I muscle afferents evoked small EPSPs in over one quarter of the interneurones and virtually all were strongly excited by cutaneous afferents. Evidence of excitatory input from joint, interosseous and group III muscle afferents was also obtained but inhibition was rarely observed.

These interneurones are considered likely to function as last-order interneurones in group II reflex pathways.

Intra venous	i.v.
Intra muscular	i.m.
Interosseous	i.o.
Hertz	Hz
Kilo gram	Kg
Kilo Hertz	KHz
Kölliker-Fuse	KF
Lateral gastrocnemius	LG
Locus coeruleus	LC
Lumbar	L
Medial Gastrocnemius	MG
Mesencephalic locomotor region	MLR
Microampre	µA
Microvolt	µV
Microsecond	µs
Milligram	mg
Millimetre	mm
Millisecond	ms
Millivolt	mV
Personal computer	PC
Plantaris	Pl
Popliteus	Pop
Posterior biceps	PB

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Millimetre	mm
Millisecond	ms
Millivolt	mV
Personal computer	PC
Plantaris	Pl
Popliteus	Pop
Posterior biceps	PB

Post synaptic potentials	PSPs
Primary afferent depolarisation	PAD
Pudendal	Pud
Quadriceps	Q
Raphe nuclei	RN
Sartorius	Sart
Semitendinosus	ST
Sub coeruleus	SC
Spike triggered averaging	STA
Superficial peroneal	SP
Sural	Sur
Spinocervical tract	SCT
Stimulus artefact	SA
Tibialis anterior	TA
Threshold	T
Thoracic	Th.
Tibialis posterior	TP
Ventral spinocerebellar tract	VSCT
Wheat germ agglutinin conjugated with horseradish peroxidase	WGA-HRP

Chapter 1.0. General Introduction

1.0. Introduction

1.0.1 Classification of muscle afferent fibres

General history. Both anatomical and electrophysiological methods have been used to classify nerve fibres. The wide range of diameters of the various afferent fibres in muscle and cutaneous nerves was shown by Sherrington (1894) and Eccles & Sherrington (1930). Eccles and Sherrington (1930) performed a series of experiments on chronic de - efferented cats. They constructed afferent size histograms for muscle and cutaneous nerves and showed that they tended to have a tri - modal or bi - modal distribution respectively (i.e. grouped within certain diameter ranges). The first electrophysiological evidence for the existence of different groups of myelinated fibres was provided by Erlanger and Gasser (1924,1937). They recorded compound action potentials with up to four waves from peripheral nerves of frogs. They also recorded compound action potentials with two waves from saphenous nerves of dogs (Erlanger and Gasser, 1937). They pointed out that these waves represented compound action potentials, propagating in nerve fibres having different conduction velocities.

Current system of classification of muscle afferent fibres

The system of classification of muscle afferent fibres in use at the present time is based on that originally proposed by Lloyd (1943 a & b) and Lloyd & Chang (1948). Lloyd and Chang (1948) performed a series of experiments on chronic de - efferented cats. They measured the diameter of

the myelinated fibres in muscle nerves and plotted histograms of the number of fibres falling within different size ranges. The resulting fibre diameter spectrum had three peaks and it was according to these that myelinated muscle afferent fibres were classified. Group I contain large diameter fibres (20 - 12μm), group II contain medium diameter fibres (12 - 6μm) and group III contains small diameter fibres (6 - 1μm). Non - myelinated afferents (less than 1μm diameter) were classified as group IV (Lloyd, 1943b). Since fibre diameter is related to conduction velocity (see below), in electrophysiological studies, fibres belonging to these groups have often been identified according to conduction velocity (CV). The corresponding conduction velocity range for group I fibres are 72 - 120 m/s, for group II fibres are 24 - 72 m/s and for group III fibres are 4 - 24 m/s. Group IV fibres have conduction velocities of less than 2 m/s.

Relation between fibre diameter and conduction velocity

The classical observation that there is a virtually linear relation between conduction velocity and fibre diameter was made by Hursh (1939). He derived the following equation to describe this relation;$\theta = 6D$ where θ is conduction velocity and measured in m/sec and D is the outside fibre diameter in μm. Hursh's (1939) observations were made on a variety of different nerves in cat and kitten but his published conversion factor best fits the largest diameter fibres in adult nerve trunks. Subsequently, Boyd and Davey (1968) presented evidence that the relation differs slightly for fibres of different sizes ($\theta = 5.7D$ for fibres with conduction velocity of 50 - 90 m/s and

θ = 4.5D for fibres conducting at 20 - 40 m/s). A recent suggestion is that conduction velocity should be considered in relation to diameter of the axoplasm inside the myelin sheath rather than the outside diameter of the fibre (Boyd & Kalu, 1973). Kalu (1973) stimulated dorsal root and recorded from fibres of different conduction velocity. Afterwards, the conduction velocity data were compared with the fibre diameter measurements of both the inside and outside diameter of the fibre in the same nerve. The ratio of inside diameter to outside diameter was greater for the largest fibres than for the intermediate and small fibres. It was suggested that all cat myelinated nerve fibres roughly obey the relation $\theta \propto d$ (d is the diameter of axoplasm inside the myelin sheath), (Boyd & Kalu, 1973; Arbuthnott, Boyd & Kalu, 1977).

Relation between fibre diameter and function

Afferent fibres with different sizes and conduction velocities tend to innervate various groups of receptors. Group I contains fibres from spindle primary endings (Ia fibres) and those from Golgi tendon organs (Ib fibres). Group II fibres arise mostly from spindle secondary endings but also from non - spindle receptors. Group III consists of small myelinated fibres and group IV fibres are unmyelinated; these terminate exclusively as free nerve endings in muscle. The relation between fibre diameter and function is not absolute: for example Ia and Ib fibres have overlapping conduction velocities and some afferents from the secondary endings of the muscle spindles conduct within the group I range.

Muscle spindle secondary endings

Skeletal muscles are richly supplied with receptors in the muscle belly, muscle spindles, which are arranged in parallel with the extrafusal muscle fibres. Muscle spindles consist of a collection of modified muscle fibres with both sensory and motor innervation. The modified muscle fibres are called intrafusal fibres and are of two main types; nuclear bag and nuclear chain fibres. There are two types of sensory endings in muscle spindles: primary endings that lie upon both the nuclear bag and chain fibres and secondary endings that lie predominantly upon the nuclear chain fibres. The motor innervation of the muscle spindle is from two main types of gamma motoneurones; dynamic gamma motoneurones which innervate bag fibres (dynamic bag type) and static gamma motoneurones which innervate bag (static bag type) and chain fibres (Gladden, 1976 &1992; Hunt, 1990; Banks, 1994).

The muscle spindle is essentially a stretch receptor but there are differences in the responses of the primary and secondary endings to muscle stretch: secondary endings are mainly sensitive to muscle length, show little or no dynamic response and little or no adaptation to a maintained muscle stretch. The primary endings on the other hand are sensitive to both absolute muscle length and to the rate of change of muscle length and are very sensitive to small displacements. These differences in the response properties of the primary and secondary endings are a reflection of the location of the receptors on the intrafusal fibres; the primary endings lie on the equatorial regions of the intrafusal fibres while the secondary endings lie

on the polar regions. Because the equatorial region contains very few myofibrils it shows little resistance to stretch and the primary endings therefore respond readily to small displacements. In addition, during sustained stretch, the equatorial region tends to return toward its original length (internal creep in dynamic bag fibres) and therefore the primary endings adapt (dynamic response). Because the polar regions where the secondary endings are situated contain a higher concentration of myofibrils, they are more rigid (no internal creep) and the secondary endings therefore show mainly a static response (Boyd, 1976).

An important role of the gamma system is to allow the spindle to maintain its high sensitivity over a wide range of muscle lengths during different types of movements. Intrafusal fibres contain myofibrils and are therefore capable of contraction in response to activity in gamma motoneurones. Contraction mainly occurs at the polar regions that therefore produce stretch of the central region of the fibre. Accordingly, fusimotor activity helps maintain the sensitivity of the muscle spindle during contraction of skeletal muscle when the intrafusal fibres will tend to be slackened. Dynamic and static gamma motoneurones regulate the sensitivity of the spindle afferents either to dynamic or to static phases of stretch. Primary endings are influenced by both types of gamma motoneurones because the Ia fibre innervates both bag and chain fibres while secondary endings are influenced only by static gamma motoneurones because secondary endings innervate mainly chain fibres. There is evidence that the nervous system is able to adjust the balance between activation of static and dynamic gamma

motor neurones during the performance of different types of motor task (for review see Hulliger, 1984).

In electrophysiological studies it is possible to differentiate afferents supplying primary endings from those supplying secondary endings by their greater dynamic sensitivity. This can be achieved either by measurements of the dynamic index (firing rate at the end of stretch compared to that at final length held for 0.5 sec) or by recording the response to vibration applied to the tendon; primary endings (due to their dynamic properties) respond to vibrations of high frequency (200 - 500 Hz) and low amplitude (less than 10μm) while secondary endings respond only to vibrations of high amplitude and low frequency (Matthews, 1972). More recently it has been shown that the responses of primary and secondary endings are affected differently by succinyl choline (Gladden, 1976; Taylor, Durbaba & Rodgers 1992 & 1993). Succinyl choline produces a powerful dynamic action on the primary endings by causing a prolonged contraction of the nuclear bag fibres (Granit, Skoglund & Thesleff, 1953; Rack & Westbury, 1966).

Non - spindle group II afferents

Some group II afferent fibres terminate in connective tissue within and around the muscle in structures other than spindles, and the percentage of non - spindle group II afferents varies in different muscle nerves. Two main methods have been used to investigate the incidence of non - spindle group II muscle afferents. Firstly, electrophysiological recordings have been made from teased dorsal root filaments and the responses of the receptors

to natural stimuli (i.e. stretch, manipulation, muscle contraction) have been investigated (Hunt, 1954; Cleland, Hayward & Rymer 1990). Secondly, the number of muscle spindles in a given muscle have been compared with the number of the group II afferents in the nerve to the same muscle, any excess of group II afferents then being attributed to unidentified receptors, presumably free nerve endings or paciniform corpuscles (Barker, Ip & Adal 1962).

At the present time, information on the incidence of non - spindle group II afferents is available for only a limited number of hind - limb muscle nerves. Some reports in which the anatomical approaches were used to investigate the medial gastrocnemius (MG) and soleus (Sol) muscle nerves are conflicting. On the one hand, Boyd & Davey (1968) have reported that in Sol and MG muscle nerves, most group II afferents are from muscle spindles. On the other hand, Barker et al. (1962) have reported that about 35% of group II afferents in Sol are from non - spindle receptors. This discrepancy may arise because Barker et al. (1962) assumed one secondary ending per muscle spindle while Boyd & Davey (1968) assumed an average of 1.6 secondary endings per muscle spindle. However electrophysiological experiments tend to support the view that there are few non - spindle group II afferents in MG, lateral gastrocnemius (LG) and Sol muscle nerves (Hunt, 1954; Paintal, 1960). In addition, Cleland et al. (1990) reported only a few touch and stretch sensitive afferents of non - spindle origin in the MG muscle nerve, most of which had conduction velocities at the lower end the group II range. Similarly a relatively small number of non -

spindle group II afferents have been reported in semitendinosus (ST) and peroneus longus (PL) muscle nerves (Coppin, Jack & McIntyre, 1969; Jack & MacLennan, 1971 cited in Jack, 1978).

There is, however, a higher degree of contamination in some nerves such as that to tibialis anterior (TA) where about 30% of the group II afferents are from non - spindle receptors (MacLennan, 1972 cited in Jack, 1978). Another finding is that the flexor digitorum longus (FDL) muscle nerve is joined by a small branch containing afferents supplying pacinian corpuscle receptors in the interosseous membrane (Boyd & Davey, 1968). Similarly, small nerve branches from the knee joint join the quadriceps (Q) and sartorius (Sart) muscle nerves (Freeman & Wyke, 1967).

Practical problems associated with the identification of group II muscle afferents and spindle secondary afferents

As described above, group II muscle afferents are classified according to their conduction velocities and diameters. However, there are a number of problems associated with the use of this system. Firstly, the dividing line between group I and group II fibres (12 μm diameter, 72m/s CV) is based on fibre diameter histograms which have only been prepared for a limited number of nerves and although the same values are commonly extrapolated to other nerves, the division may not be the same. Secondly, fibre diameter or CV is not precisely related to function. Although, most group II fibres probably innervate the muscle spindle, some terminate as non - spindle endings. In addition, there is some overlap between the diameter

and conduction velocities of afferents from spindle primaries and those from the spindle secondaries when they are measured close to the spindle. This has led some investigators to divide electrophysiologically identified spindle afferents according to their conduction velocities into group I muscle spindle afferents and group II muscle spindle afferents. For example Jack (1978) has used an arbitrary dividing line of 0.7 times the fastest conducting fibres in the nerve while Matthews (1972) has suggested a value of 0.6 times.

Relation between excitability and fibre diameter

Electrical stimulation has been the main tool used for investigating the central connectivity and reflex action of afferent fibres in muscle and cutaneous nerve. Electrical stimulation produces a very synchronous volley of impulses in the afferent fibres that makes postsynaptic effects in spinal neurone more readily observed. It also enables the timing of these postsynaptic effects and correspondingly the number of synapses involved to be determined. In addition, by adjusting the strength of electrical stimuli, it is possible to activate different combination of muscle afferents fibres.

There is a direct relationship between fibre diameter and excitability to electrical stimulation. The largest diameter afferents are the most excitable and so require the lowest stimulus intensity while fibres of progressively smaller diameter are recruited at progressively higher stimulus strengths. This has proved useful experimentally because it means that by gradually increasing the strength of stimuli applied to a muscle nerve, it is possible to recruit group I afferents alone or in combination with group II,

group III and group IV afferents (Lloyd, 1943 a, b; Hunt, 1954; Bradley & Eccles 1953). It is customary to relate the strength of stimuli applied to a muscle nerve to that required to activate the most excitable (largest diameter) afferents in the nerve. This stimulus intensity that is referred to as nerve threshold (T) is normally determined by recording the afferent volley from the surface of the cord at the dorsal entry zone.

In most muscle nerves at stimulus strengths of 1.5 - 2.5T all of the group I afferents will be recruited and it is not possible to activate Ia and Ib afferents separately except in some exceptional cases (Bradley & Eccles 1953). The most excitable group II afferents are recruited at 1.5 - 2.0T and therefore there is an overlap between the threshold of the least excitable group I and the most excitable group II fibres. It is safe to assume that group II afferents are recruited maximally at 5T without significant recruitment of group III muscle afferents. In electrophysiological studies, effects appearing at about 2T and growing between 2 - 5T are usually attributed to group II muscle afferents (Eccles & Lundberg, 1959a; Coombs, Curtis & Landgren, 1956; Fu, Santini & Schomburg 1974; Fu & Schomburg, 1974; Ellaway, Murphy & Tripathi, 1982; Edgley & Jankowska 1987a; Lundberg, Malmgren & Schomburg, 1987 a, b). The most complete and widely cited data for a range of muscle nerve is given in the table 1.0. of Jack (1978). However, in this work spindle afferents were classified as group I and group II on the basis of an arbitrary dividing line and peripheral threshold determined in intact rather than cut muscle nerves.

Selective activation of group II afferents with conventional electrical stimulation methods is not possible because the strength of stimuli required to recruit group II afferents will also recruit group I afferents. However attempts have been made to devise procedures that can selectively activate group II afferents. DC anodal block has been used to suppress the conduction of impulses in group I fibres while leaving the thinner group II fibres relatively unaffected. (Cangiano & Lutzemberger, 1972; Jack & Roberts, 1974; Kato & Fukushima, 1976). Group II action can also be studied using the double volley technique in which a pair of stimuli are delivered to a muscle nerve. The first (2T) rendering group I afferents refractory and the second (5T) therefore, evoke a volley only in group II afferents (Bradley & Eccles 1953; Fu et al. 1974; Fu & Schomburg, 1974; Ellaway et al. 1982; Edgley & Jankowska 1987a).

1.0.2. Reflex actions of group II muscle afferents

The flexion reflex pattern of actions

Early studies on the reflex action of different afferent fibres started with recordings of the activity evoked in motor axons (peripheral nerve or ventral root recordings) by electrical stimulation of peripheral nerve (Lloyd 1943b). Using these techniques, group II afferents of both flexor and extensor muscle nerves were found to produce excitation of flexor α motoneurones. These early observations were extended using methods of intracellular recording and monosynaptic reflex testing (Eccles & Lundberg, 1959a; Holmqvist & Lundberg, 1961; Lundberg, Malmgren & Schomburg 1987a, b & c). These techniques showed that group II muscle afferents not only produce excitation of flexor α motoneurones but also inhibition of extensor motoneurones and that both flexor and extensor group II afferents were equally effective. These studies in which the effects of higher threshold (group III, IV) muscle afferents, cutaneous and joint afferents were also investigated led to the idea that group II muscle afferent pathways are part of a much larger system of reflex pathways which were termed the flexor reflex afferent (FRA) system (Eccles & Lundberg, 1959a; Holmqvist & Lundberg, 1961). This was because electrical stimulation of group II, III, IV muscle afferents, cutaneous and joint afferents were all found to produce excitation of flexor motoneurones and inhibition of extensor motoneurones (a pattern appropriate for limb flexion). These reflex actions appeared to be mediated at least in part by common reflex pathways involving common interneurones

and to be controlled by common descending pathways. The convergence from different groups of afferent fibres onto neurones of the flexor reflex afferent system has been confirmed using natural stimulation of cutaneous receptors combined with electrical stimulation of muscle afferents (Steffens & Schomburg, 1993). One of the problems with the term FRA is perhaps that it suggests a rigid action whereas in fact individual afferents contributing to the FRA system can also produce other reflex actions through different pathways. The expression of these different pathways may depend upon the particular preparation in which the investigation has been performed.

Alternative group II reflex pathways

Although the flexion reflex pattern of actions of group II muscle afferents dominates in anaesthetised or decerebrate spinal preparations, opposite reflex actions are also sometimes observed; i.e. an excitatory pathway to extensors and an inhibitory pathway to flexors (Eccles & Lundberg, 1959a; Lundberg et al. 1987 a, b & c). In the **high spinal unanaesthetised cat**, a particularly high incidence of the excitation in extensor motoneurones has been reported (Wilson and Kato 1965). The greater prevalence of extensor excitation in this preparation appears to be due to the absence of anaesthetic since Hongo and Pettersson (1988) have reported that there is no difference in the incidence of extensor excitation in decerebrate cats with a high spinal (cervical) section and those spinalised at the more conventional upper lumbar level.

Convergence from group II, III and IV muscle afferents, cutaneous and joint afferents has also been observed in the alternative FRA pathways to both flexor and extensor motoneurones (Lundberg et al. 1987 b & c). Lundberg et al. (1987 b & c) reported both excitation and inhibition from group II and III muscle afferents and from joint afferents in the same GS motoneurones suggesting that afferents from the same receptors activates both excitatory and inhibitory pathways to motoneurones. In decerebrate preparations in which the spinal cord is kept intact the usual flexor reflex pattern of actions (i.e. flexor excitation and extensor inhibition) are largely suppressed (Eccles & Lundberg 1959b; Holmqvist & Lundberg, 1961). These differences were attributed to the action in the decerebrate animal of tonic discharges from higher centres that act to inhibit transmission through spinal interneurones (Eccles & Lundberg 1959b). It is presumed that these different reflex pathways can be selected by the nervous system for different motor tasks.

Crossed extensor reflex

An early observation was that the flexion withdrawal reflex that can be evoked in the decerebrated animal is accompanied by extension of the opposite limb. This is the so called crossed extensor reflex (Creed et al. 1932). The crossed reflex actions of group II muscle afferents on motoneurones have been studied by Perl (1958); Holmquist (1961); Arya, Bajwa & Edgley (1991); Aggelopoulos & Edgley (1995).

In high spinal unanesthetised animals, conventional crossed extensor reflexes were recorded following stimulation of peripheral nerve strong enough to recruit group II afferents (Perl, 1958). In low pontine lesions, releases of inhibitory pathways from the FRA to contralateral flexor and extensor motoneurones have been reported, whereas in more caudal lesions a conventional crossed excitation reflex has been found (Holmqvist, 1961 cited in Holmquist & Lundberg, 1961). Arya et al. (1991) recorded intracellularly from motoneurones and observed the expected crossed extensor pattern of actions (excitation in extensor and inhibition in flexor motoneurones) in the low spinal anaesthetised cat whilst in animals with an intact spinal cord almost all of the motoneurones, both extensors and flexors were inhibited **(i.e. crossed inhibition reflex)**. These actions were evoked predominantly by group II afferents of the contralateral quadriceps nerve.

Do group II muscle afferents contribute to the stretch reflex?

The matter of the contribution of group II muscle afferents to the stretch reflex is controversial. Matthews (1969, 1972); McGrath & Matthews, (1970) found that in the decerebrate cat the reflex response to stretch was often stronger than that to vibration. He assumed the Ia fibres would be fully activated by vibration and attributed the extra tension developed during stretch to the action of group II fibres not activated during vibration. These experiments were criticised by Grillner (1970) on the grounds that additional tension during muscle stretch could be produced as a result of the mechanical properties of muscle. Jack and Roberts (1978) performed a

similar set of experiments to Matthews (1969) but using vibration combined with gamma efferent stimulation instead of muscle stretch. They reported that although efferent stimulation produced an increase in secondary spindle firing, no additional tension occurred. Matthews (1983 & 1984) has also performed experiments with a similar rationale in human subjects. When a contracting human muscle is stretched its electromyogram (E.M.G) shows an early latency response (M1) which is generally agreed to depend on the spinal reflex actions of Ia afferents. This first component is followed by a second longer latency response (M2 component) which Matthews reported is more effectively evoked by muscle stretch than vibration (flexor pollicis longus muscle). He argued that the M2 response may be due to group II muscle spindle afferents with the extra delay being due to a longer peripheral conduction time. However, this interpretation has subsequently been withdrawn by Matthews (1989), (see below).

There is also evidence from human experiments against a unique role for group II afferents in the production of the long latency component of the stretch reflex. First, if it were true that slowly conducting afferents are wholly responsible for the longer latency response, then those muscles nearest the spinal cord would be expected to have the shortest latency M2 responses but this is not the case (Marsden, Merton & Morton, 1973). The second piece of evidence comes from experiments involving ischemic block of the hind - limb that affects large afferents before small afferents. It was found that both M1 and M2 components of the stretch reflex (in triceps surae muscle nerve) were eliminated after ischaemia at the same time (Fellows,

Dömges, Töpper, Thilmann & Noth, 1993). Furthermore, cooling the arm (Matthews, 1989) slows the conduction velocity of small diameter afferents before effecting that of the larger diameter fibres and should therefore delay the onset of the long latency component of the stretch reflex more than that of the first component. In fact, cooling the arms delays both components of the reflex by the same amount (Matthews, 1989). An alternative suggestion for the longer latency of the M2 component might be that it involves a transcortical loop (Marsden, Merton & Morton, 1976). In patients in which the movements of both hands are controlled by only one (the left) cerebral hemisphere, stretching the flexor policis longus muscle of the right hand elicits a long latency stretch reflex in both the right and left (unstretched) hands. Such reflexes were not however, observed in more proximal muscles e.g. of the wrist, (Rothwell, Thompson & Day, 1991).

More recent evidence in favour of the involvement of group II muscle spindle afferents in the stretch reflex has been provided by Schieppati, Nardone & Corna (1995); Corna, Grasso, Nardone & Schieppati (1995). In the standing position, toe up and toe down rotation of a platform induces short latency and medium latency responses in pretibial flexor and extensor muscles. The short latency response is the counterpart of the monosynaptic stretch reflex; although the origin of the medium latency response is still debated. Schieppati et al. (1995) argue that it is most likely due to activity in group II afferents on the basis of conduction velocity and central delay measurements. Corna et al. (1995) investigated this further by looking at the effects of an $\alpha 2$ adrenergic receptor agonist (tizanidine) on the size of short

latency and medium latency responses. In the cat, the effects of ionophoretic application of noradrenergic agonists were selective in that group II field potentials were depressed while group I field potentials simultaneously recorded at the same location were not affected (Bras, Jankowska, Noga & Skoog, 1990). Corna et al. (1995) found that on average medium latency responses were decreased (pretibial flexors) one hour after tizanidine administration while the short latency responses were unaffected (pretibial extensor). They considered this to be evidence that the medium latency response is mediated by group II muscle afferents. It is therefore, possible that both group II spinal reflex pathways and a transcortical loop contribute to the longer latency component of the stretch reflex. The relative contribution of these two mechanisms may differ between the proximal and distal muscles and depend upon the motor tasks to be performed.

1.0.3. projections and central connections of group II muscle afferents

Monosynaptic connection of group II muscle spindle afferents with motoneurones

Kirkwood and Sears (1974,1975) used the technique of spike triggered averaging (STA) to demonstrate that secondary endings of muscle spindles have direct projections to homonymous motoneurones. They showed that motoneurones participating in the intercostal stretch reflex and motoneurones of triceps surae draw monosynaptic excitation from secondary as well as primary endings (Kirkwood and Sears 1974). The synaptic connection of spindle group II afferents from the MG muscle with hind - limb motoneurones has also been investigated by Stauffer, Watt, Taylor, Reinking & Stuart (1976); Munson, Fleshman & Sypert (1980); Sypert, Fleshman & Munson (1980). Stauffer et al. (1976) considered that EPSPs (in GS motoneurones) with latencies of 1.4 ms or less (0.3 - 1.4 ms, mean 0.93 ms) were evoked monosynaptically. Longer latency EPSPs and IPSPs (up to 4 ms) were attributed to di or trisynaptic connections. Sypert et al. (1980) and Munson et al. (1980) divided spindle group Ia and II afferents into two different functional connectivity groups based on the proportion of homonymous motoneurones receiving a monosynaptic projection and the size of EPSPs. Functional connectivity for the entire group Ia (fast and slow) was greater than the group II muscle spindle afferents. The faster conducting group II afferents (CV>52 m/s) had more projections to

motoneurones and evoked larger EPSPs than the slower conducting afferents (CV<52 m/s).

Projection and central connection of group II muscle spindle afferents with interneurones

Monosynaptic connections from secondary endings of muscle spindles to motoneurones of the hind - limb are relatively weak (Lundberg, Malmgren & Schomburg, 1977; Sypert et al. 1980); most of the actions of group II afferents on motoneurones occurring via interneurones. The projections and sites of termination of group II muscle afferents have been investigated using a number of approaches; these include recording of field potentials (focal synaptic potentials, FSPs) evoked by group II muscle afferents, intra - axonal staining of group II afferents with horseradish peroxidase (HRP) and intracellular recording from interneurones excited by group II muscle afferents. Studies using these approaches show that group II afferents of different muscle nerves have preferred projection areas within different segments of the spinal cord. In the following account the action of group II afferents in the midlumbar, sacral and lower - lumbar segments are therefore considered separately.

Field potentials

i) Field potentials in the lower - lumbar region (L6 - L7). Although group II field potentials were first recorded in the lower - lumbar segments, this is now perhaps the least well studied region. Coombs et al.(1956); Fu et al. (1974) recorded FSPs evoked by group II afferents of biceps and

semitendinosus (BST) and gastrocnemius (GS) muscle nerves inthe L6 and L7 spinal segments. Field potentials were recorded in two main locations; one in the dorsal horn and the other in the ventral horn. Antidromic activation of group II afferents by stimuli applied at the location where field potentials could be recorded, together with measurements of latency showed that potentials were evoked monosynaptically (Fu & Schomburg, 1974). The latencies of field potentials in the dorsal horn were shorter(0.6 - 1 ms from the group II incoming volleys) than those in the ventral region(1.2 - 2.5 ms) indicating that there was considerable slowing of the collaterals of group II afferents, presumably due to branching on the way to more ventral regions. Field potentials recorded in the ventral region were preceded by larger group I potentials. In general, the group II potentials in the lower - lumbar region are small (<150 μV) in comparison to the group II potentials that have subsequently been recorded in other spinal segments.

ii) Field potentials in the midlumbar region (L3 - L5). Edgley and Jankowska (1987 a) showed that the midlumbar segments of the spinal cord constitute a major relay for signals from group II muscle afferents. Electrical stimulation of group II muscle afferents of Q, sartorius (Sart), deep peroneal (DP, tibialis anterior and extensor digitorum longus together) and Flexor digitorum longus (so called FDL et al. which includes tibialis posterior and popliteus) evoked cord dorsum potentials (CDPs) over the L3, L4 and L5 spinal segments. Within these segments field potentialscould be recorded in the dorsal horn (laminae IV - V) and in the intermediate zone and ventral horn (laminae VII - VIII). Large potentials (0.5 - 1 mV) were evoked in the

dorsal horn while in the intermediate zone the group II potentials were smaller (>0.3 mV) and preceded by group I potentials. As in the lower - lumbar region, latencies of deeper potentials were longer than those recorded in the dorsal horn. Antidromic activation of group II afferents of TA and extensor digitorum longus (EDL) muscle nerves by stimuli applied at the location that field potentials could be recorded together with the measurement of latency and lack of temporal summation following high frequency stimuli indicated that the potentials were evoked monosynaptically. Field potentials could also be evoked in the dorsal horn by small amplitude stretches. These field potentials were recorded at locations where electrical stimulation of muscle nerve produced group II but not group I field potentials. This suggests that spindle group II afferents were responsible for the stretch evoked potentials and must contribute also to the potentials evoked by electrical stimuli. In contrast, short latency (large amplitude) field potentials were evoked by stretch in the region of Clarke' column, where electrical stimulation of the Q nerve evoked large field potentials, showing the involvement of group I muscle afferents. Further evidence that muscle spindle secondaries contribute to the field potentials evoked by electrical stimulation of group II muscle afferents has been presented by Harrison, Jami & Jankowska (1988). They showed that field potentials were also induced by spindle secondary endings when activated by electrical stimulation of fusimotor axons at all locations at which field potentials were obtained from group II afferents stimulated electrically.

iii) Field potentials in the sacral region (S1 - S2). The actions of group II muscle afferents in the sacral region have also been studied by recording CDPs and field potentials evoked by electrical stimulation of muscle nerves (Jankowska and Riddell 1993). Group II afferents of PBST and GS were the most effective of the nerves tested. The sites in which the largest CDPs and field potentials evoked by group II afferents have recorded varied in relation to the L7 - S2 spinal segments but were consistent in their location relative to the pudendal motor nucleus (Onuf's nucleus), being largest at its rostral end, since the rostral end of Onuf's nucleus correspond to the caudal end of PBST motor column (Romanes, 1951). Group II afferents of PBST and GS evoked large field potentials (0.5 - 1 mV) in the dorsal horn (laminae IV - V) throughout the L7 - S2 spinal segments. At the more rostral end of this region (caudal L7/rostral S1) other nerves (Q, Gracilis and Plantaris) also produced dorsal horn group II field potentials which were however, smaller than those produced by PBST and GS and in addition small group II field potentials (<0.3 mV) preceded by group I field potentials could be recorded in the intermediate zone (V - VI). The latencies of field potentials in the dorsal horn were shorter (0.5 - 0.7 ms, from the group II incoming volleys) than those in the intermediate zone (1.1 - 2 ms). As in the midlumbar group II field potentials, antidromic activation of group II afferents of ST and LGS muscle nerves by stimuli applied at the location that field potentials could be recorded together with the measurement of latency and lack of temporal summation following high frequency stimuli indicated

that the potentials were evoked monosynaptically. Field potentials could also be evoked in the same region by small muscle stretches likely to activate secondary endings suggesting that spindle group II afferents were responsible.

Intra - axonal staining of group II muscle afferents

A small sample of group II afferents have been studied using intra - axonal injection of HRP (Fyffe, 1979, Brown, 1981, Hongo, 1992; Mannen, Ishizuka, Hongo, Kudo, Sasaki & Yamashita, 1981) and so far, only group II afferents terminating in the lower - lumbar and rostral sacral segments have been investigated. The pattern of termination of group II afferents revealed by these studies corresponds well to the region in which monosynaptic field potentials can be evoked. The studies show that group II afferents vary in their pattern of termination; some terminate in the dorsal horn while others terminate in the dorsal horn, intermediate region and sparsely within the motor nuclei. It appears that the main region of termination displayed by an individual group II afferent collateral depends on its location in relation to the motor column of the homonymous muscle. At the rostral and the caudal ends of the motor columns, terminal boutons are more numerous in the dorsal horn whereas at the level of the motor column more boutons occurred in the intermediate region than the dorsal horn (data for medial gastrocnemius, MG and plantaris, Pl muscle nerves, Ishizuka, Hongo, Kudo, Sasaki, Yamashita & Mannen, 1984).

1.0.4 Interneurones in reflex pathways from group II muscle afferents

Several populations of group II interneurones have been identified in the regions of spinal cord corresponding to those in which group II field potentials have been recorded.

i) Group II interneurones in midlumbar segments

Interneurones with monosynaptic input from group II afferents have been found in both the dorsal and ventral grey matter of midlumbar segments and these are now the most extensively studied populations of group II interneurones.

Intermediate zone interneurones. This population of interneurones is located in laminae VI - VII (intermediate zone). They have relatively large cell bodies (mean = 42 μm) and dendrites that radiate in all directions up to 1000 μm from the cell body (Bras, Cavallari, Jankowska and Kubin 1989). Most of these interneurones are monosynaptically activated by group II afferents. Group II afferents of Q, tibialis anterior - extensor digitorum longus (TA - EDL) and sartorius (Sart) muscle nerves provide the most effective inputs to these interneurones, producing EPSPs in more than half. Group II afferents of FDL and gracilis muscle nerves also provide input to a proportion of these interneurones (40%) while in contrast, group II afferents of ankle extensors and knee flexors evoke EPSPs in only very few.

Individual interneurones are often excited by group II afferents of several nerves (Edgley & Jankowska, 1987b).

Although, the dominant input to these interneurones is from group II muscle afferents, most also receive convergent input from other types of sensory afferents. A proportion of interneurones (62%) is excited by group I muscle afferents (both Ia & Ib) of a wide range of muscle nerves. However, in most neurones the largest group II EPSPs are more than twice the size of the largest group I EPSPs and when recorded extracellularly group II afferents discharged 72% of interneurones while group I afferents only discharged 15%. In addition to muscle afferent inputs, 40% of the interneurones are excited by cutaneous afferents (most monosynaptically) and about half are excited by joint and interosseous afferents (Edgley & Jankowska, 1987b).

Many group II interneurones in the intermediate zone can be antidromically activated by stimuli applied in the motor nuclei of lower - lumbar segments. Furthermore, the intermediate zone of midlumbar segments is one of the areas in which last order premotor interneurones are transneuronally labelled from hind - limb motor nuclei by wheat germ agglutinin conjugated with horseradish peroxidase (WGA - HRP); (Harrison, Hultborn, Jankowska, Katz, Storai and Zytnicki, 1984; Jankowska & Skoog, 1986; Harrison, Jankowska & Zytnicki 1986). Cavallari, Edgley & Jankowska (1987) used the spike triggered averaging technique (STA) and sucrose gap recording from ventral roots to test the hypothesis that midlumbar interneurones in the intermediate zone project to hind - limb motoneurones.

They recorded in parallel, the activity of interneurones that were excited by ionophoretic application of an excitatory amino acid and compound post synaptic potentials electrotonically conducted along ventral root axons. They showed that activity in interneurones was associated with both positive and negative ventral root potentials indicating that some of the interneurones had excitatory actions upon motoneurones whereas others had inhibitory actions. Intra - axonal labelling has confirmed that these neurones produce axon collaterals that arborize within motor nuclei of the L5 - S1 spinal segments. The finding that these interneurones are monosynaptically activated by group II muscle afferents and that they in turn have a direct projection to motoneurones, indicates that the minimal linkage in excitatory and inhibitory reflex pathways from group II muscle afferents is disynaptic. The latencies of activation of midlumbar interneurones by group II afferents and the conduction time along their descending axons suggest that they could be responsible for the earliest EPSPs and IPSPs that are evoked in motoneurones by group II afferents of Q and DP nerves (Edgley & Jankowska, 1987b; Lundberg et al. 1987a).

All of the types of sensory afferents that evoke EPSPs in midlumbar intermediate interneurones also evoke IPSPs (Edgley and Jankowska, 1987 b). The latencies of these effects in general suggest disynaptic inhibition. The IPSPs evoked by group II muscle afferents might be in part the result of mutual inhibition between sub populations of group II interneurones in midlumbar segments. This follows from the finding that short latency IPSPs are evoked in some L4 interneurones by weak stimuli applied within the

motor nuclei (Cavallari et al. 1987). Intracellular labelling of midlumbar interneurones has shown that in addition to their projection to motoneurones in lower - lumbar segments, they also produce axon collaterals in the midlumbar segments in laminae VII - VIII and it is possible that some of these may contact other intermediate zone interneurones (Bras et al. 1989).

There is evidence that inhibitory post synaptic potentials (IPSPs) of both group I and group II origin in midlumbar group II interneurones might be mediated by interneurones located in more caudal segments. Firstly, interneurones with appropriate inputs and an ascending projection to the midlumbar segments are located in lower - lumbar segments (Hongo, Jankowska, Ohno, Sasaki, & Yamashita 1983 a; Brink, Harrison, Jankowska, McCrea & Skoog, 1983; Harrison & Riddell, 1989). Secondly, Harrison, Connolly & Jefford (1993) recorded IPSPs evoked by group I and group II muscle afferents in midlumbar neurones after lesioning the dorsal columns at the L5 - L6 border. The lesion would have interrupted afferent input to midlumbar interneurones so that IPSPs must have been mediated via interneurones in more caudal segments with axons ascending via the lateral funiculus.

Dorsal horn interneurones. This group of interneurones which is located in lamina IV, V and VI of the dorsal horn have smaller cell bodies (mean = 32 μm) and more limited dendritic trees (up to 500 μm) than the intermediate zone interneurones (Edgley & Jankowska, 1978 b; Bras et al. 1989). Like the intermediate zone interneurones, most dorsal horn group II

interneurones are monosynaptically activated by group II muscle afferents. Those nerves that provide the most effective excitation of intermediate interneurones also provide the main input to the dorsal horn interneurones (Edgley & Jankowska, 1987b). In contrast to the intermediate zone interneurones, dorsal horn interneurones do not receive inputs from group I afferents. They do however, receive convergent input from cutaneous and joint afferents. Because, they are located more dorsally, more interneurones receive inputs from cutaneous afferents (60%) and most are excited monosynaptically (Edgley & Jankowska, 1987b).

In contrast to the interneurones in the intermediate zone, the dorsal horn interneurones can not be antidromically activated from motor nuclei and activity in these interneurones is not correlated with ventral root potentials (Cavallari et al. 1987; Edgley & Jankowska 1987b). This is in agreement with the result of trans synaptic labelling that indicates that few if any last order premotor interneurones are located in the dorsal horn (Harrison et al. 1984; Jankowska & Skoog, 1986; Harrison, Jankowska, & Zytnicki, 1986). Intracellular staining of these interneurones show that they are funicular neurones and have either ascending or descending axons running in either lateral or ventral funiculi. Their initial axonal collaterals branch primarily in the dorsal horn or in lamina VII but not close to the motor nuclei. Since some of these collaterals appear to make contact with neurones in the intermediate zone, it is possible that they have connections with group II premotor interneurones but this has yet to be confirmed (Bras et al. 1989). Since some of the interneurones also have a contralateral projection (Bras et

al. 1989), they might also be responsible for mediating inputs from contralateral group II afferents to intermediate zone interneurones.

Dorsal horn group II interneurones are a functionally heterogeneous population of interneurones and appear to include both excitatory and inhibitory neurones. Immunocytochemical studies show that some of the neurones contain glycine while others do not contain either GABA or glycine and are therefore likely to be excitatory (Maxwell et al. 1997). Both electrophysiological evidence (Jankowska & Riddell, 1995, see also below) and ultrastructural evidence (Maxwell et al. 1997) indicates that some dorsal horn group II interneurones are involved in presynaptic inhibition of group II muscle afferents.

Lamina VIII interneurones in midlumbar segments with a crossed projection. Most last order premotor interneurones have connections with ipsilateral motoneurones but some project to contralateral motoneurones as shown by trans synaptic labelling with WGA - HRP. All of the contralaterally projecting last order premotor interneurones are located in lamina VIII (Harrison et al. 1986; Jankowska & Skoog, 1986) and one group is located within the midlumbar segments. Electrophysiological studies of these interneurones show that they can be antidromically activated from contralateral motor nuclei in the lower - lumbar segments and that some are monosynaptically excited by group II muscle afferents of the same nerves as excite other neurones in midlumbar segments (Jankowska & Noga, 1990).

There is evidence that lamina VIII interneurones in midlumbar segments mediate the crossed inhibition of group II origin in extensor and flexor motoneurones that is seen in cats with an intact spinal cord (Aggelopoulos & Edgley 1995). Aggelopoulos and Edgley (1995) found that interruption of the dorsal columns at L5 dramatically attenuated the crossed group II inhibition recorded in motoneurones. The fact that midlumbar interneurones mediate this crossed inhibition and the fact that the minimum latencies of the IPSPs are comparable to those of IPSPs evoked by ipsilateral group II afferents means that this must be a disynaptic pathway. It follows that lamina VIII interneurones must be responsible since they are the only interneurones that would be capable of mediating such disynaptic actions on motoneurones.

II) Group II interneurones in sacral segments

This group of interneurones is located in lamina III - V of the grey matter in the caudal L7 to S2 spinal segments (in a region overlying the pudendal motor nuclei). These interneurones have medium sized cell bodies (32.2 - 37.8 μm) and dendrites that radiate in all directions up to 520 μm (Jankowska, Riddell, Szabo - Läckberg and Hammmer. 1993b). They are powerfully excited by group II muscle afferents, the great majority monosynaptically. In contrast to midlumbar interneurones, the most effective input to sacral interneurones is from group II afferents of PBST which excite virtually all of the interneurones. In addition afferents of GS, Q and gracilis produce EPSPs in about half of the interneurones while afferents of plantaris

(PI), anterior biceps semi membranusus (ABSM), deep peroneal (DP) and sartorius (Sart) produce EPSPs in less than a third. Like the dorsal horn interneurones in midlumbar segments, none of the sacral dorsal horn interneurones are excited by group I muscle afferents. In addition to their group II inputs, sacral interneurones are also excited by cutaneous afferents but in contrast to the midlumbar segments where afferents of the superficial peroneal (SP) and saphenous nerves provide the most effective cutaneous inputs, afferents of cutaneous femoris (CF), pudendal (Pud) and sureal (Sur) provide the main cutaneous inputs to sacral interneurones (Jankowska and Riddell, 1994).

Intracellular staining of sacral interneurones shows that they are morphologically heterogeneous and that they are funicular neurones with axons running within the lateral or ventral funiculus for several millimetres. Axons running within the lateral funiculus descend or ascend and collaterals of these axons arborize primarily within the dorsal horn and the intermediate zone. All of those axons which run within the ventral funiculus are ascending and collaterals of these axons arborize in both the intermediate zone and ventral horn, in a few cases passing close to sacral motor nuclei (Jankowska et al. 1993b). Group II interneurones in sacral segments therefore appear to have mainly local actions like the interneurones in the dorsal horn of midlumbar segments. It seems unlikely that they have direct actions upon hind - limb motoneurones since, they are located dorsal of the area within which last order premotor interneurones are transneuronally labelled and most show no terminal branching near the motor nuclei. Furthermore, the

latencies of PSPs evoked in motoneurones by stimulation of PBST, CF and Pud nerves (which should be mediated mainly by this group of interneurones) are compatible with a tri - but not with a disynaptic coupling. However, some of the interneurones near the rostral border of Onuf's nucleus might have direct actions on motoneurones of the external urethral and anal sphincter muscles judging by the projection of their axon collaterals (Jankowska et al. 1993b). Like the midlumbar interneurones, sacral interneurones are likely to comprise a functionally heterogeneous group; some having excitatory actions and others inhibitory actions. There is evidence that some of the sacral dorsal horn interneurones mediate presynaptic inhibition of group II muscle afferents (Jankowska & Riddell, 1995, see below).

1.0.5. Segmental inhibitory control of group II interneurones

Post synaptic inhibition of peripheral origin

Post synaptic inhibition has been seen in all of the populations of group II interneurones that have been studied in midlumbar and sacral regions. A general feature of these IPSPs is that they are most frequently evoked by those sensory afferents that provide the main excitatory input to the interneurones (Edgley & Jankowska 1987b; Jankowska & Riddell, 1994, see above).

Presynaptic inhibition

Presynaptic inhibition is a means of modulating transmission between sensory fibres and neurones in the spinal cord. Presynaptic inhibition is believed to be produced by activity at axo - axonic synapses formed by the terminals of spinal GABAergic interneurones with the terminals of primary afferent fibres. Release of GABA at these synapses produces a long lasting depolarisation of the afferent terminal and also leads to a decrease in the amount of transmitter released at the terminal by a nerve impulse. Primary afferent depolarisation (PAD) can be detected by placing the proximal end of a cut dorsal root on an electrode and recording the depolarising potential that spreads electrotonically from the site of generation in the nerve terminals. This potential is known as the dorsal root potential (for review see Schmidt, 1973). It can also be detected directly by intra - axonal recording from the afferent fibres but this is a technically difficult procedure. An alternative method for detecting PAD is to monitor the

excitability of afferent terminals: stimuli applied within the cord set up an antidromic volley that can be recorded in the dorsal roots and because presynaptic inhibition involves depolarisation of nerve terminals, a given stimulus will produce a larger antidromic volley in the presence of presynaptic inhibition. A refinement of this technique is to monitor the current intensity required to antidromically activate the terminal of a single muscle afferent fibre recorded in peripheral nerve.

The circuitry involved in presynaptic inhibition of group I muscle afferents has been extensively analysed (reviewed by Rudomin, 1990). However, it is only recently that the neuronal systems mediating presynaptic control of group II muscle afferents have been investigated. PAD of the central terminals of group II muscle afferents is most readily evoked by group II muscle afferents. Cutaneous afferents are also effective in evoking PAD in the majority of group II afferents whereas group I muscle afferents (both Ia and Ib) are much less effective (Harrison & Jankowska, 1989; Riddell, Jankowska & Huber, 1995). This pattern appears to be the same for all of the group II afferents that have currently been studied; afferents of both flexor and extensor nerves and afferents terminating in midlumbar and in sacral segments.

The organisation of neuronal systems producing PAD of group II muscle afferents has been investigated by comparing the sources of PAD acting on group II afferents terminating in midlumbar and sacral regions (Riddell et al. 1995). The effectiveness with which afferents of a range of different nerves depolarised group II muscle afferents fibres of PBST and

GS terminating in the sacral region were similar. However, the effectiveness with which a range of nerves produced PAD of DP group II afferents terminating in the midlumbar segment was found to differ significantly from the effect of the same nerve on afferents terminating in the sacral segment. This suggests a topographical organisation of the neuronal system producing PAD in group II afferents such that fibres terminating in a given region are most effectively depolarised by other fibres terminating nearby while fibres terminating at a distance are much less effective. This implies that neurones responsible for PAD have fairly local actions (Riddell et al. 1995).

The similar sources of presynaptic control of group II afferents and of inputs to interneurones in group II reflex pathways in midlumbar and sacral regions suggest that interneurones in reflex pathways from group II muscle afferents and the neurones responsible for PAD are likely to be activated in parallel. In functional terms this would mean that the level of presynaptic inhibition of group II afferents could be automatically regulated to match the degree of activation of neurones in reflex pathways and this organisation would be particularly well suited to provide an automatic negative feedback control of these pathways (Jankowska & Riddell, 1995).

1.0.6. Descending inputs to group II interneurones

Descending control of group II interneurones

Supraspinal structures can greatly influence the activity of spinal reflex pathways. Eccles & Lundberg (1959b) reported that the usual flexion reflex actions seen in the spinal animal are largely suppressed in the decerebrate preparation. Furthermore, Holmqvist & Lundberg (1961) reported that reflex pathways from Ib, group II, group III and higher threshold cutaneous and joint afferents have different reflex actions after lesions at various levels of the brain stem. These authors attributed these differences to the presence, in the decerebrate animal, of tonic discharges from higher centres that act to inhibit transmission through spinal interneurones (see section on Reflex actions of group II muscle afferents).

The descending control of the group II reflex pathway has been investigated further using various techniques. It has long been believed that the descending control of group II reflex pathways may in part involve monoaminergic systems (Andén, Jukes, Lundberg, & Vyklicky, 1966). Recently, the effects of ionophoretic application of monoaminergic agonists on group II field potentials recorded in the midlumbar region have been investigated by Bras, Jankowska, Noga and Skoog (1990). Comparison of the effects of various types of noradrenergic and serotonergic agonists suggested that transmission from group II muscle afferents in dorsal and ventral parts of the grey matter is controlled by different monoamines. Serotonergic agonists were found to depress group II field potentials

recorded in the dorsal horn while having little effect on field potentials in the ventral horn. Conversely, noradrenergic agonists depressed field potentials in the intermediate/ventral regions while having little effect on group II field potentials in the dorsal horn. Furthermore, the effects of noradrenergic agonists were selective in that group II field potentials were depressed while group I field potentials simultaneously recorded at the same location were not affected. Application of serotonergic agonists within the sacral segments was also found to depress group II field potentials recorded in the dorsal horn of this region while noradrenergic agonists were largely without effect (Jankowska, Szabo - Läckberg and Dyrehag, 1994).

Electrical stimulation within widespread regions of the brain stem has been reported to produce depression of group II field potentials recorded in midlumbar segments (Noga, Bras and Jankowska, 1992; Noga, Jankowska and Skoog, 1995). Among the sites from which the most effective inhibition is produced are those that are the main source of descending monoaminergic neurones; including the locus coeruleus/sub coeruleus and Kölliker - Fuse nuclei which contain a high proportion of noradrenergic neurones and the raphe nuclei which are predominantly serotonergic. Stimuli applied in areas containing serotonergic and noradrenergic neurones might have been expected to produce the same selective effects on dorsal horn and intermediate zone group II potentials as that produced by serotonergic and noradrenergic drugs. However, in practice such stimuli have similar effects on group II field potentials recorded at both of these locations. Noga , Bras & Jankowska (1992) suggested that

this discrepancy might be explained by the extensive interconnections that exist between the various brain stem sites in which stimuli were applied.

Since, the region in which the field potentials depressed by brain stem stimuli were recorded is known to contain last order premotor interneurones, it was presumed that such stimuli would also produce a depression of transmission in group II reflex pathways. This was further investigated by looking at the effect of stimulation within brain stem sites on PSPs recorded intracellularly from motoneurones (Jankowska, Riddell, Skoog & Noga, 1993a). This study showed that both the inhibitory and excitatory pathways from group II muscle afferents are similarly affected. Furthermore, not only the reflex actions of group II afferents relaying in the midlumbar region (i.e. those of Q and DP group II afferents) but also those of group II afferents relaying in other spinal segments (e.g. those of PBST and GS group II afferents) appeared to be equally affected by the descending systems. This suggests a system capable of producing a general inhibition of group II reflex actions in the spinal cord.

Although monoamine containing regions are among the most effective of the brain stem sites from which depression can be produced, electrical stimulation is by no means a selective method of activating monoaminergic systems. Among the various problems are: 1. Not all of the regions in the stimulated areas are monoaminergic; some contain other neurotransmitters. 2. Some of the monoaminergic neurones colocalise other transmitters. 3. Stimuli applied in a given region can lead to activation of neurones in other regions; principally those receiving a projection from the

stimulated region or those projecting to the stimulated region. These problems raise the question of to what extent the depression of group II field potentials produced by the electrical stimuli applied in the brain stem involves monoamines. Evidence in favour of the involvement of monoamines includes: 1. Brain stem regions known to contain a high proportion of monoaminergic neurones are among those from which the most effective depression can be evoked. 2. Monoamines also produce a depression of transmission from group II afferents. 3. The application of $\alpha 2$ antagonists partially reduces the depressive effects of brain stem stimulation (Skoog & Noga, 1992). 4. The most effective and selective depressive effects of brain stem stimulation on group II actions occur at relatively long conditioning - test intervals. This is consistent with the involvement of monoaminergic fibres which are generally slowly conducting (e.g. <7m/s for spinally projecting monoaminergic neurones of the locus coeruleus/sub coeruleus; Rude & Gobel, 1980). 5. Direct contacts between monoaminergic fibres and group II interneurones and dorsal spinocerebellar tract neurones have been demonstrated in the cat and rat (Jankowska, Maxwell, Dolk & Krutki, 1995; Maxwell & Jankowska, 1996). There is also evidence that a presynaptic mechanism of inhibition contributes to the depression of group II reflex pathways evoked by stimuli applied within brain stem sites. Stimuli applied in locus coeruleus/sub coeruleus and raphe nuclei have been shown to increase the excitability of the central terminals of group II muscle afferents to electrical stimuli (Riddell, Jankowska & Eide, 1993). It is not known

whether monoamines are involved in this presynaptic inhibitory action. There is no evidence that monoamines can act directly to produce a depolarisation of group II muscle afferents but it is possible that they activate the GABAergic PAD interneurones which contact the terminals of group II afferents. Alternatively, it is possible that non - monoaminergic descending systems activated in parallel with monoaminergic systems are responsible for evoking PAD of group II afferents (see Riddell et al. 1993 for discussion).

Convergence of inputs from different descending motor pathways on group II activated midlumbar interneurones

Descending pathways are not only involved in controlling (inhibiting) the activity of interneurones but can also activate (i.e. excite) interneurones and thereby influence motoneurones. It has been suggested by Lundberg (Lundberg, 1979; Lundberg et al. 1987,c) that subsets of excitatory and inhibitory group II interneurones may be used by the brain to mediate motor commands.

The organisation and convergence of inputs from different descending motor pathways on individual midlumbar group II interneurone has been investigated by Davies & Edgley (1994) using intracellular recording. The great majority of interneurones were monosynaptically excited by electrical stimulation of at least one of the descending pathways tested. Monosynaptic excitation from reticulo, vestibulo and rubrospinal pathways was common but rubrospinal and reticulospinal fibres activated largely different population of interneurones. Corticospinal inputs to these

neurones were seen less frequently than that from the other descending pathways and were only seen in neurones also receiving monosynaptic input from rubrospinal pathways. These observations suggest that the control of distal musculature during volitional tasks and the control of axial musculature involved in postural tasks, may involve largely different populations of midlumbar group II interneurones. Further evidence of the involvement of midlumbar group II interneurones in postural control has been provided by Yates (Yates, Kaspeer, Brink and Wilson, 1988; Yates, Kasper & Wilson, 1989). These authors showed a strong involvement of midlumbar group II interneurones in the labyrinthine and neck reflexes. Their studies showed that a considerable proportion of the interneurones in L4 with group II input from Q, Sart and TA muscle nerves were activated by tilt or rotation of the neck. These interneurones were located in lamina VI and VII and some were antidromically activated from lower - lumbar motor nuclei. It is therefore, very likely that these interneurones correspond to the last order premotor interneurones described by Edgley and Jankowska (1987b).

Effects of electrical stimulation in the cuneiform nuclei (mesencephalic locomotor region)

Descending systems are involved in locomotion as well as in posture and voluntary movements. In the decerebrate cat, electrical stimuli applied in one circumscribed area of the mesencephalon, called the mesencephalic locomotor region (MLR) causes the animal to walk when placed on a treadmill. Furthermore, electrical stimulation of the MLR in the paralysed

decerebrate cat produces a pattern of activity in motoneurones that resembles that seen in stepping (i.e. alternating periods of activity in motoneurones of flexor and extensor muscles; Shik, Severin & Orlovsky, 1966; Grillner, 1973).

Edgley, Jankowska & Shefchyk (1988) investigated the effects of stimuli applied within the cuneiform nucleus which is the anatomical correlate of the MLR on field potentials and on post synaptic potentials evoked in L4 interneurones. Distinct short latency field potentials were recorded mainly within the ventral horn just ventral to where field potentials evoked from group II muscle afferents were maximal. Due to the short latency of the potentials evoked by stimulation in cuneiform nuclei, they were considered likely to be mediated by a disynaptic reticulo spinal pathway (Armstrong, 1986).

Shefchyk, McCrea, Kriellaars, Forttier & Jordan (1990) studied the behaviour of L4 group II interneurones with a projection to L7 motor nuclei during fictive locomotion. Two thirds of the interneurones studied were rhythmically active, firing during the flexion phase of the step cycle and falling silent during the extension phase. The remaining one third of the interneurones showed evidence of a phasic modulation of their peripheral input. In some neurones, electrical stimulation of group II afferents evoked activity in the neurones prior to fictive locomotion which was then suppressed during fictive locomotion while others were excited by group II afferents during the flexion phase but not in the extension phase of the step cycle (Shefchyk et al. 1990).

The powerful input to L4 interneurones from group II muscle afferents of muscles (i.e. iliopssoas, Q and Sart) which are stretched during hip extension indicate that these interneurones are likely to be activated when the limb is extended. This has led to the suggestion that one function of these interneurones might be to mediate the switch from the stance phase to the swing phase of the step cycle (Edgley & Jankowska, 1987b; Aggelopoulos, Bawa, Edgley, 1996). If this is the case, the descending inputs to these interneurones might allow adjustment of the timing of the switch from stance to swing and thus provide a route for supraspinal modification of the gait. However, Perreault , Angel, Guertin, & McCrea (1995) have reported that electrical stimulation of group II afferents of Sart during the extension phase of fictive locomotion prolongs extension. Furthermore, electrical stimulation of flexor nerves (TA, PBST or Sart) during the flexor phase of fictive locomotion leads to termination of activity in flexor and a resetting to extension.

1.0.7 Ascending pathways with input from group II muscle afferents

Information from muscle, joint and skin receptors provides an awareness of movements and positions of joints. With respect to information from group II spindle afferents, it has been recognised that a signal related to muscle length is especially useful for position sense (Matthews, 1982). However, there is little firm evidence that group II muscle afferents contribute to kinaesthesia and until recently even the extent to which information from these afferents is forwarded to higher centres in the brain remained to be clarified.

Group II muscle afferents from the hind - limb do not project to the dorsal column nuclei (Fern, Harrison & Riddell, 1988) and therefore any information from these afferents which reaches supraspinal centres must be conveyed by second order neurones. Ascending neurones excited by group II muscle afferents have now been identified in both midlumbar and sacral regions of spinal cord but not so far in the lower - lumbar segments.

I) Spinocerebellar tract neurones

There are in fact a number of different sets of spinocerebellar tracts neurones involved in transmitting proprioceptive information from the hind - limb to cerebellum (Oscarsson, 1973; Matsushita, Hosoya & Ikeda 1979). Two of the best known of these are Clarke's column neurones which

constitute one of the sets of dorsal spinocerebellar tract neurones and ventral spinocerebellar tract neurones.

Clarke's column. Clarke's column in the cat extends from Th1 (first thoracic segment) to about the rostral end of the L4 (forth lumbar) spinal segment. The main input from muscle nerves to Clarke's column neurones is contributed by group I afferents; some neurones are exclusively excited by Ia afferents, others receive an exclusive input from group Ib afferents while some neurones are excited by both. However, group II afferents produce only weak actions in a minority of neurones (Eccles, Oscarsson, Willis, 1961; Oscarsson, 1973).

DSCT neurones in L5 - L6 segments. A further group of DSCT neurones located within the L5 - L6 segments at level caudal to Clarke' column has been investigated by Aoyama, Hongo & Kudo (1988). The majority of neurones receive monosynaptic excitatory inputs from group I muscle afferents (mostly from both Ia and Ib afferents of more than two nerves) and cutaneous afferents. However, few neurones were reported to be excited by group II muscle afferents as well.

Dorsal horn spinocerebellar tract (DSCT) neurones in midlumbar region. There is a set of DSCT neurones located in the dorsal horn of the midlumbar region which unlike other sets of DSCT neurones so far investigated are strongly excited by group II muscle afferents (Edgley & Jankowska 1988; Edgley & Gallimore, 1988). In many ways the inputs to these neurones resemble those of the dorsal horn group II interneurones

seen in this region. Most neurones were excited by group II afferents of three to five nerves and also by cutaneous afferents. In addition to their projection to the cerebellum, some neurones of this population also give rise to an axon collateral that projects to an area of medulla called nucleus Z. These neurones therefore provide one route by which information from group II muscle afferent can reach higher centres (Asif & Edgley, 1992).

So far, the involvement of the spinocerebellar tract in relaying information from group II muscle afferents appears to be largely restricted to the midlumbar segments. Very few spinocerebellar tract neurones within the sacral segments appear to receive input from group II muscle afferents (Riddell, Jankowska, Hammer and Szabo - Läckberg, 1994) and so far, there are no reports of spinocerebellar tract neurones in the lower - lumbar region with input from group II muscle afferents.

Ventral spinocerebellar tract neurones (VSCT). Group I afferents (mainly Ib afferents) also provide the major monosynaptic excitatory input to VSCT neurones. Group II and group III muscle afferents and afferents from cutaneous and joint nerves produce strong inhibitory actions (Eccles, Hubbard & Oscarsson, 1961).

II) Spinocervical tract neurones (SCT)

The spinocervical lemniscal pathway is one of the major routes by which somatosensory information reaches the cerebral cortex. The spinocervical tract arises from cells in lamina III, IV and V of the dorsal horn with ascending axons in the ipsilateral dorsolateral funiculus which terminate

in the lateral cervical nucleus in the upper cervical segments. Relay neurones in the lateral cervical nucleus forward information to the thalamus which in turn project to the cortex. Input from cutaneous afferents (hair follicle receptors) is a major feature of the SCT neurones (Brown, 1981).

Harrison & Jankowska (1984) investigated the possibility that non - cutaneous afferents may provide input to SCT neurones within the lower - lumbar segments. They reported that SCT neurones in these segments receive virtually no input from group I muscle afferents but a few receive input from group II muscle afferents, joint and interosseous (i.o.) afferents. More recently, Riddell et al. (1994) investigated ascending tract neurones processing information from group II muscle afferents in sacral region. The great majority (93%) of group II activated ascending tract neurones in the sacral segments had axons ascending in ipsilateral funiculi and neurones of the SCT constituted 82% of them. Although, the most potent excitation of the SCT neurones was evoked by monosynaptic input from cutaneous afferents (afferents of CF and Sur nerves), most of the neurones were also effectively activated by group II muscle afferents. The most effective group II muscle afferents were those of PBST, GS and Q which evoked monosynaptic EPSPs in more than half of the neurones. Hammer, Szabo - Läckberg & Jankowska (1994) have since reported that SCT neurones in midlumbar segments of the spinal cord are also activated by group II muscle afferents. About two thirds of the SCT neurones investigated were monosynaptically excited by group II afferents of the Q and DP muscle nerves.

The finding that SCT neurones process information from group II muscle afferents suggest that this information is conveyed to cortical levels. The organisation of group II muscle and cutaneous afferent input to neurones of the SCT might thus constitute the first stage in the integration of information from muscle, skin and joint receptors which is the basis for a sense of position of a given joint or body part (Riddell et al. 1994).

Chapter 2.0. Topographical organisation of neurones in group II reflex pathways of the rat spinal cord

2.0.1. Introduction

2.0.1. Introduction

Recent studies of group II reflex pathways in the cat spinal cord have led to the identification of several populations of interneurones with a powerful excitatory input from group II muscle afferents. An interesting organisational feature that has emerged from these studies is that the processing of information from group II afferents of different combinations of hind - limb muscle nerves appears to take place in particular segments of the spinal cord. For example, group II afferents of the nerves to the quadriceps and pretibial flexor muscles produce their strongest actions on interneurones at the rostral end of the lumbosacral enlargement (in midlumbar segments; Edgley & Jankowska, 1987a, b) while group II afferents of gastrocnemius and posterior biceps - semitendinosus mainly contact interneurones at the caudal end of the lumbosacral enlargement (in sacral segments; Jankowska & Riddell, 1993, 1994).

In comparison to the rostral and caudal regions of the lumbar enlargement where group II reflex circuits have now been extensively investigated (See chapter 1.0, Section on interneurones; Jankowska, 1992 for review of midlumbar interneurones), much less is known about the role of interneurones within the central segments of the lumbar enlargement (L6 and L7 of the cat spinal cord). Field potentials are produced by group II afferents of gastrocnemius and posterior biceps - semitendinosus in this region (Fu, Santini & Schomburg, 1974) but are considerably smaller than those produced by the same afferents in the sacral segments (Jankowska &

Riddell, 1993). There are also several brief accounts of interneurones in the L6 and L7 segments with excitatory input from group II muscle afferents of various hind - limb nerves (Fukushima & Kato, 1975; Harrison & Jankowska, 1985; Lundberg, Malmgren & Schomburg, 1987b; Harrison & Riddell, 1989; Harrison, Connolly & Guzman - Villalba, 1994). The most recent of these studies suggest that group II afferents in nerves innervating posterior muscles of the lower leg with actions mainly at the ankle and digits (flexor digitorum longus and associated muscle nerve branches; FDL et al.) may be among those with the strongest actions in these segments (Harrison & Riddell, 1989; Harrison et al. 1994).

So far, the interneurones interposed in group II reflex pathways have only been studied in the **cat** (See chapter 1.0, section on interneurones in reflex pathways from group II muscle afferents). This chapter of thesis is an account of attempts made to investigate the topographical organisation of neurones in group II reflex circuits of the **rat** spinal cord. This study has the following aims: Firstly to determine whether the organisation of group II reflex pathways in the rat is similar to that in the cat and thus to what extent the organisation of spinal reflex circuits might follow a common plan in different mammalian species. Secondly, to conduct a systematic investigation of the central region of the lumbar enlargement where the actions of group II muscle afferents have not yet been fully explored. And thirdly to obtain basic information on the organisation of group II reflex pathways in the rat that would provide the necessary background for further studies in this species.

2.0.2. Method

2.0.2. Methods

Useful results were obtained from experiments performed on 22 male Wistar rats, 300 - 500g weight.

Anaesthesia

General anaesthesia was induced with a single dose of sodium pentobarbitone (Sagatal; Rhône Mérieux; 50 mg/kg i.p.). In some cases Halothane (1 - 2%) in a 50/50 mixture of O_2/N_2O was also given to ensure deep anaesthesia during the initial surgical procedures. The depth of anaesthesia was assessed during the dissection by monitoring withdrawal reflexes, the corneal reflex, arterial blood pressure and an electrocardiogram (ECG). Additional doses of anaesthetic were given (5 - 10 mg/kg i.v.) if a withdrawal reflex was seen on pinching or if blood pressure increased on pinching or was abnormally high. During the recording phase of the experiment, when the animal was paralysed, administration of anaesthetics was continued at a rate commensurate with that required before paralysis. The adequacy of this regime was checked by continuously monitoring the arterial blood pressure and by periodically allowing the animal to recover from paralysis.

Preparatory surgery

The animal was placed in the supine position on an operating table and secured with rubber bands attached to each of the limbs. The animal's core temperature was continuously measured by a rectal probe and

maintained by a homeothermic control unit at between 37°- 38°C. The ECG was monitored during dissection through needle electrodes which were inserted through skin of the left and right forelimb and the right hind - limb (when a surgical level of anaesthesia had been obtained heart rate was typically between 390 - 420 beats/minute). Before starting the surgery, Decadron (dexamethasone sodium phosphate; Merk Sharp & Dome, 2 -3 mg/kg i.m.) was injected to reduce the swelling of nervous tissue (see for example Stamford, 1992). Atropine sulphate, (Macarthy medical) at a dose of 0.05 mg/kg i.p was also injected to reduce bronchial constriction and salivary secretions. Before making skin incisions fur was removed from the relevant areas using an animal fur clipper. Fine surgery including cannulation, nerve dissection and laminectomy were performed with the aid of a dissecting microscope (Zeiss S5). A cautery (Eschmann equipment, TDB 50) or soldering iron was used to prevent bleeding from small vessels and ligatures placed around larger vessels before cutting.

Cannulation

A carotid artery, jugular vein and the trachea were cannulated. A mid - line incision was made through the skin overlying the trachea, the skin was retracted and the superficial muscles covering the trachea were separated. A carotid artery was exposed by blunt dissection and about 2 cm of the artery freed from surrounding connective tissue, being careful not to damage the vagus nerve. The artery was ligated distally and a cannula inserted proximally (i.e. towards the heart) to permit continual monitoring of arterial

blood pressure which was maintained above 90 mm Hg (usually at 100 -120 mm Hg). A continuous infusion of a 100 mM solution of bicarbonate containing 5% glucose (2 - 3 ml/kg/hr) was also administered through the arterial cannula throughout the experiment to help maintain the acid base balance and fluid level of the animal. This infusion also avoided any tendency for the arterial cannula to block. A jugular vein was cannulated to allow intravenous administration of anaesthetics and other drugs. The skin and muscles overlying the trachea were retracted and a small length of the trachea was separated from the surrounding tissues. The trachea was opened and a cannula inserted and tied in position. From time to time excessive secretions in the trachea were removed by suction. All cannulae were secured to the skin by threads in order to prevent them being accidentally dislodged and skin incisions were closed with Michel clips.

Nerve dissection

A variety of different muscle and cutaneous nerves were dissected according to the aims of the experiment. The terminology used here is that described by Greene (1959) for hind - limb nerves in the rat. To dissect branches of the femoral nerve the animal was left in the supine position and an incision was made on the antero - medial side of thigh from the groin proximally to about the knee distally. Before entering the thigh the femoral nerve divides into anterior and posterior divisions. The anterior branch consists of muscular branches to iliacus and pectineus and the posterior branch consists of a muscular branch to the various parts of quadriceps

femoris (Q) together with the saphenous nerve which is cutaneous. The Q motor branch was separated from the iliacus, pectineus and saphenous nerves and mounted in a tunnel electrode.

To dissect branches of the sciatic nerve, the animal was turned and placed in a prone position. A skin incision was made on the lefthind - limb from the ischial tuberosity to about 2 cm caudal of the popliteal fossa. The skin was retracted by retractor and/or by clipping weights to skin or muscles. To provide access to the hamstring nerve (Ham), the caudo - femoralis muscle which is a long oblique muscle in the posterior thigh was removed. Selective stimulation of muscle afferents of the hamstring nerve is complicated (in this species) by a cutaneous nerve branch which runs for most of its length together with muscular branches. To selectively activate muscle afferents, each of the muscle branches has to be mounted on a separate electrode. The following muscular branches were therefore dissected: posterior biceps femoris (PB), semitendinosus (principal head, ST) and semi - membranosus (SM). The cutaneous branch of the hamstring nerve was also mounted. Within the knee and lower leg the following nerves were dissected: lateral gastrocnemius - soleus (LGS), medial gastrocnemius (MG, sometimes mounted together, GS), plantaris (Pl), flexor digitorum and halucis longus (FDL, no attempt was made to identify a separate branch equivalent to that which supplies the interosseus membrane in the cat), tibialis posterior (TP), popliteus (Pop, or when mounted together with tibialis posterior, TP - Pop), sural (Sur), superficial peroneal (SP), pudendal (Pud), and posterior cutaneous of thigh (Post Cut).

The tibialis anterior and extensor digitorum longus nerves were mounted together as deep peroneal (DP) and a nerve branch equal to extensor digitorum brevis (EDB) in the cat was also identified, separated and mounted.

Laminectomy

A mid - line skin incision was made from thoracic vertebrae to the sacral region. Before the laminectomy, local anaesthetic (xylocaine, 2 mg/ml) was infiltrated into the tissues surrounding the vertebrae to prevent severe depression of blood pressure which could not be prevented by additional anaesthetic (Schouenborg & Sjölund, 1983). The skin was retracted and the longissimus dorsi muscles were separated from the lumbar multifidus muscles and the multifidus muscles were removed from the vertebral column. Afterwards, the vertebral column was raised and in this way the dorsal aspect of the inter - vertebral joints opened and the posterior arch was cautiously removed, using small bone rongeurs and bone cutting instruments. Bone wax and plasticine were used to prevent the seeping of blood from the cut edge of vertebrae and gel foam was also used to prevent bleeding. The laminectomy involved removal of bone from three or four vertebrae between Th13 and L4 depending on the segments of the spinal cord to be exposed.

Preparations for recordings

After completing the surgery; the animal was transferred to the recording frame. The animal's head was secured by an incisor bar. The vertebral column was fixed rigidly by two clamps either end of the laminectomy; one clamp gripped the spinal processes of lower - lumbar vertebrae and the other the body of a lower thoracic vertebra (e.g. Th12).

Paraffin pools were formed around the posterior side of the left hind - limb and the laminectomy by attaching threads passed through the edge of the skin incision to part of the frame. The pools were filled with liquid paraffin at about 37°C so as to cover all exposed tissues. This insulation prevented current spread to other tissue on electrical stimulation of peripheral nerves. In addition, heat loss and drying of tissue was avoided. The temperatures of the pools were maintained by heating lamps controlled thermostatically through thermistors which were lodged in the leg and back pools.

Before beginning recording it was necessary to reduce movements of the thorax by paralysing and artificially ventilating the animal. The trachea was cleared of excessive secretions by suction and the animal was paralysed with gallamine triethiodide (a non depolarising neuromuscular blocking agent, Flaxedil , May & Baker; i.v. 24 mg/kg and supplementary doses of 8 mg/kg every 30 minutes). The animal was artificially ventilated with oxygen/enriched air via a T - piece which was connected to a respiratory pump (small animal ventilator, Harvard). The level of carbon dioxide was monitored (portable fast response CO_2 analyser FM1, ADC)

and was kept about 4% throughout the experiment by adjusting the ventilation rate.

Stimulating and recording procedures

Nerve stimulation

A diagrammatic representation of the experimental arrangement showing the position of stimulating and recording electrodes is shown in Fig. 1. Peripheral nerves were mounted on bipolar silver ball electrodes with the cathode proximally. Electrical stimulation was applied with 0.1 ms rectangular current pulses, singly or in trains. Stimulus strengths were expressed relative to threshold (T) for the most excitable afferents in the nerve, determined from recordings of afferent volleys from the surface of the spinal cord.

Surface recordings

The dura was opened along the length of the exposed spinal cord and silver ball electrodes were placed in position with the reference electrode on back muscles. A silver wire (earth electrode) was also inserted in back muscles. Afferent volleys and cord dorsum potentials (CDPs, negativity upwards) were recorded with the electrodes placed on the surface of the dorsal columns close to the dorsal root entry zone (Fig. 1 A). To map the rostro - caudal distribution of CDPs, the recording electrodes were moved along the dorsal columns in 1 mm steps.

Field potential recordings

Openings (patches) were made in the pia mater by tearing the pia with two pairs of fine watch maker's forceps. The patches were made in areas free of blood vessels close to where the largest group II cord dorsum potentials could be recorded following stimulation of the relevant peripheral nerve. Field potentials were recorded using glass microelectrodes filled with 2M NaCl (2 - 3 μm tip diameter, 2 - 4 MΩ resistance). The microelectrode was connected to the headstage of a DC amplifier and mounted on a stepping motor by which the electrode could be advanced in steps from 2 - 12 μm. The stepping motor was mounted on a combined arc and micromanipulator which enabled the angle and position of the electrode to be precisely adjusted. To map the distribution of field potentials within the grey matter the microelectrode was introduced through the dorsal columns (Fig. 1 A). The medio - lateral angles of electrode tracks were varied systematically from 0° to 35° (laterally and medially) while maintaining the same entry position on the surface and field potentials were recorded at intervals of 200μm or less along the full length of each electrode track (up to 2.5 mm depending on angle). This procedure is illustrated in Fig. 2. In this way all but the most dorsal regions of grey matter were explored.

Group II field potentials in the L5 segment (i.e. caudal region) could not be reached using a dorsal column entry site; this is because the arrangement of the overlying dorsal roots at this level necessitates a more medial entry point than at more rostral levels (i.e. rostral region) and it is then difficult to angle the electrode sufficiently to reach lateral aspects of the

dorsal horn. Successful recordings of group II potentials at this level were obtained by entering the spinal cord through the lateral funiculus after gently reflecting overlying dorsal roots.

Recordings were further amplified using an AC amplifier, digitised and stored on digital audio tape (DTR - 1801 digital tape recorder) and personal computer (1401 plus, Cambridge Electronic Design, CED,sampling rate 24 - 40 KHz). On and off line analysis, such as averaging from 16 - 32 individual potentials, and measurements of the latency and amplitude of potentials were performed using an oscilloscope emulation program (signal averaging, sigavg).

Single afferent fibre recordings from dorsal roots

The fibre composition of the LGS muscle nerve was investigated electrophysiologically in four experiments. The LGS nerve was dissected and mounted on bipolar electrodes and stimulated in the usual manner. The main region of entry of LGS fibres was located by determining where the afferent volley was largest and dorsal root filaments (mainly from L4 and rostral L5 roots) were then cut close to their entry into the spinal cord. Filaments were teased apart and mounted on bipolar silver wire electrodes to achieve recordings in which the impulses of individual afferent fibres could be distinguished (Fig. 1. B). Where filaments contained fibres with ongoing activity, averaging was used to aid identification of electrically evoked activity. An attempt was made to record from as many LGS fibres as possible in a single experiment. Peripheral thresholds of fibres were related

to those for the most excitable fibres in the nerve as determined by recordings of the afferent volley directly from the main sciatic nerve trunk and conduction latencies for impulses evoked by supra threshold stimuli were measured off - line. Particular care was taken in these experiments to maintain the temperature of the paraffin pools above 36°C. At the end of the experiment the conduction distance for impulses in the recorded fibres was measured (see below).

Identification of spinal segment

The segmental locations of recording sites were determined from the position of marking electrodes left in the patches. The laminectomy was extended rostrally to about the twelfth thoracic (Th12). The last rib was found with the aid of a scalpel in order to locate the Th13 dorsal root ganglion which usually lies at the same level. In addition, the laminectomy was extended caudally to the L6 vertebra (the last lumbar vertebra in the rat which lies level with the pelvic bone) and the L5 ganglion which usually lies at this level was identified. Other ganglia were identified by working rostrally toward the Th13 ganglion. Spinal roots were cut and traced to their level of entry into the spinal cord. The lengths of spinal segments were measured with millimetre paper and a plan indicating the location of marking electrodes was drawn.

Conduction distance measurements

The leg incision alongside the main nerve trunk was extended toward the back incision as close as possible to the vertebral column. Thereafter, the conduction distance of the relevant peripheral nerve was measured from the cathode electrode to the relevant patch by laying a piece of cotton thread along the nerve.

In experiments in which single fibre recordings were made from dorsal root fibres the conduction distance was measured by complete dissection of the conduction path through the sciatic notch. The conduction distance was then measured several times and an average measurement was calculated.

Histology

At the end of experiments, marking electrodes were left **in situ** at recording sites and the animal perfused through the arterial cannula with 4% buffered formol saline. The animal was left overnight with the back pool filled with fixative to cover the spinal cord. The next day, the spinal cord was extracted being careful not to dislodge the marking electrodes and kept in a container filled with fixative. Blocks of spinal cord containing the marking electrodes were prepared by cutting away residual spinal roots and peeling away pia membranes. Blocks about three millimetres in length were mounted on a vibratome stage (series 1000, sectioning system) and serial transverse sections (50 - 100 μm) were cut and mounted on glass slides.

Staining and dehydration

Slides were left to dehydrate at room temperature. The following procedure was usually used for staining:

i) 70% alcohol (Methyl alcohol), 2 minutes.

ii) 0.5% Cresyl violet in acetate buffer (pH 3.8 - 4.0; kept in the oven at 56 C° for half an hour before use), for about 1 minute.

iii) 2 changes of acetate buffer, 1 minute each.

iv) 2 changes of 95% alcohol, 1 minute each.

v) 2 changes of absolute alcohol, 2 minutes and 3 minutes.

vi) Proportion of 50% to 50% of xylene/alcohol, dip in briefly

vii) 2 changes of xylene, 2 minutes.

Thereafter, glass slides were coverslipped with DPX.

Microscopic examination

Sections were examined with a light microscope (Zeiss, Axioplan) and camera lucida drawings were made of the positions of marking electrodes and boundaries of the grey matter. Since cresyl violet mainly stains Nissl particles, the neuronal cell bodies in the grey matter appear bluish in colour. Maps of the areas of grey matter within which field potentials were recorded were constructed from information on the angle of electrode tracks in relation to the marking electrode and the depth of recordings along each track. The locations of surface potentials evoked by afferents of GS and muscle branches of hamstring nerve were in addition related to the rostral end of the pudendal motor columns (see Jankowska &

Riddell, 1993). This was identified by examining a series of sections and determining the point at which the distinctive, compact columns of small neurones in the ventral horn which constitute the pudendal motonuclei (see Schrøder, 1980; Jordan, Breedlove & Arnold, 1982; McKenna & Nadelhaft, 1986) gave way to larger, more evenly distributed neurones (see Fig. 13).

2.0.3. Results

2.0.3. Results

Group II cord dorsum and field potentials were recorded throughout the lumbar enlargement. Each of the nerves for which the actions of group II afferents were investigated produced their greatest effects in particular segments of the lumbar enlargement. For convenience, in the following account, the actions of group II afferents in rostral, caudal and central regions of the lumbar enlargement are therefore considered separately.

Part 1.0 Rostral lumbar segments

Group II afferents in the Q and DP nerves (which were investigated in four and three animals respectively) produced synaptic actions predominantly at the rostral end of the lumbar enlargement in the L1, L2 and rostral L3 segments.

Cord dorsum potentials evoked by the quadriceps nerve

Effects of graded electrical stimuli. Stimulation of the Q muscle nerve produced distinct negative cord dorsum potentials (CDPs) which followed the incoming volleys in records from the surface of the spinal cord. Fig. 3 A & B shows examples of such CDPs which appeared at 2.0 - 2.5T, grew in amplitude as the stimulus intensity was raised and reached a maximal amplitude at 4 - 5T. While data on the stimulus strengths required to recruit group II afferents in rat is lacking, this range of stimulus strengths (2 - 5T) is precisely that over which the majority of group II muscle afferents are recruited on electrical stimulation of hind - limb muscle nerves in the cat

(Jack, 1978, Ellaway, Murphy and Tripathi, 1982; Lundberg et al. 1987a). Accordingly, the potentials described here are considered equivalent to the potentials of group II origin previously described in the cat (Edgley & Jankowska, 1987a; Jankowska & Riddell, 1993). Although the presumed group II potential did not grow in amplitude at higher stimulus strengths, a small, later component occurred on the falling phase when the stimulus strength was increased to 10T or more (Fig. 3. B, uppermost record). This later component is presumed to result from activation of group III muscle afferents.

Rostro - caudal distribution. The distribution of group II CDPs recorded following stimuli applied at 5T to the Q nerve was mapped in 1 mm steps along the surface of the spinal cord. A plot of the amplitude of potentials recorded in this manner is shown in Fig. 4 A. As can be seen, CDPs were recorded over a length of spinal cord spanning from Th13 to L3 segments with the largest potentials in this case occurring over L1 and L2. Fig. 4 B shows the distribution of CDPs recorded from four animals. The largest CDPs were recorded over the middle of the L2 segment in 3 animals and over the middle of the L1 segment in one animal. Potentials with amplitudes 75% or more of the maximal (black bar in Fig. 4 B) were recorded over most of the length of L2 in all four animals and in addition over most of the L1 segment in two animals. The mean maximal amplitude of group II CDPs was 104μV (see table 1).

Latencies. The latencies of group II CDPs measured from group I volleys ranged between 1.0 - 1.2 ms. In two experiments in which group II

afferent volleys could be identified (see below), the CDPs followed these volleys at latencies of 0.42 and 0.48 ms (see table 1).

Afferent volleys

Following stimuli producing a group I volley close to maximal (2T or more), a separation of the group I afferent volley into two separate components was usually observed (Fig. 6, upper most records). This most likely reflects separate Ia and Ib components of the volley as originally described for afferents of the semi - membranosus nerve in the cat (Bradley and Eccles, 1953). In two experiments a further component of the afferent volley appeared and grew in amplitude in parallel with the group II CDPs as the stimulus intensity was increased (arrows in Fig. 3 A and B).These components are considered to represent group II afferent volleys which under appropriate conditions can also be seen in the cat (Fu et al. 1974; Edgley & Jankowska, 1987a).

The largest afferent volleys were consistently recorded at the middle of the L3 segment, a few millimetres caudal of where the largest group II CDPs were evoked. A similar arrangement occurs in the cat where the largest CDPs in the midlumbar segments are recorded rostral of the main entry point of the fibres that produce them (Edgley & Jankowska, 1987a). The electrophysiological evidence (in the rat) on the level of entry of Q afferents concurs with anatomical evidence showing that HRP anterogradely transported from the Q muscle nerve produces labelling of cells within the L2 and L3 (mainly L3) dorsal root ganglia (Peyronnard et al. 1986).

Cord dorsum potentials evoked by the deep peroneal nerve

Examples of cord dorsum potentials which were evoked following stimulation of the DP nerve are shown in Fig. 5 A. Similar to those potentials evoked by the Q nerve, these potentials appeared where the group I volley was already close to maximal amplitude (1.8 - 2T) and grew progressively larger in amplitude with stronger stimuli up to 4 - 5T.

Fig. 5.C shows the distribution of CDPs evoked by afferents of DP in three animals. Note that these potentials were distributed more caudally than those evoked by Q afferents (Fig. 4 B). The mean maximal amplitude of group II CDPs was 48μV (table 1). These were recorded mainly over the caudal half of the L2 segment and also extended over L3 in two animals (Fig. 5 C).

Latencies of the CDPs measured from group I volleys ranged from 1.02 - 1.85 ms while latencies from group II volleys (which were seen in all three experiments; arrow in Fig. 5 A.) ranged from 0.55 - 0.7 ms (table 1).

The largest group I afferent volleys were always seen caudal (caudal L3 to mid L4) to where the largest group II CDPs were evoked from the DP nerve. This concurs with anatomical evidence showing that HRP anterogradely transported from the TA - EDL muscle nerves produces labelling of cells within the L4 dorsal root ganglion (Peyronnard et al. 1986).

Cord dorsum potentials evoked by extensor digitorum brevis nerve

In the cat, the main nerve to extensor digitorum brevis (EDB) contains a high proportion of non - muscular afferents (Lundberg, Malmgren

& Schomburg, 1975). This nerve is therefore usually dissected away from the closely associated branches to EDL when the actions of muscle afferents from pre - tibial flexor muscles are to be studied (e.g. Edgley & Jankowska, 1987a, b). This is especially important when CDPs are to be used to assess the actions of group II muscle afferents because non - muscular afferents tend to produce distinct CDPs. Whether in the rat afferents of the EDB nerve also innervate non - muscular tissue has been difficult to determine using gross teasing methods of dissection (see Green, 1959). As a precaution, in the present study the nerve branch to EDB was therefore dissected away from EDL and stimulated separately.

As can be seen from Fig. 5. A (lower most record) & B, in comparison to CDPs evoked by pure muscle nerves, the CDPs evoked by EDB were much larger (122 - 139μV, mean 131μV) and had a rounded shape similar to that evoked by stimulation of cutaneous afferents in the cat (Bernhard, 1953; see also below). These potentials were evoked at lower stimulus strengths than those attributed to group II muscle afferents and were of virtually maximal size at 2T. The plots in Fig. 5 B and C show that potentials evoked by EDB were distributed over the caudal L3 and rostral L4 segments, several mm caudal to the largest group II potentials evoked by the DP nerve. These observations indicate that in the rat, considerable numbers of non - muscular afferents run within the nerve branch innervating the EDB muscle.

Field Potentials (focal synaptic potentials)

The central projections of Q group II afferents within the spinal cord were investigated by recording focal synaptic potentials (field potentials) which are generally considered to result from the flow of current at synapses where postsynaptic potentials are generated in neurones (Eccles, Fatt, Landgren & Winsebury, 1954).

Following stimulation of the Q nerve at 5 T, distinct field potentials were regularly recorded at segmental locations corresponding to those over which the largest cord dorsum potentials were induced. Fig. 6 shows examples of field potentials recorded within the dorsal horn and intermediate region/ ventral horn.

Effects of graded electrical stimuli. As can be seen in Fig. 6, the appearance and increase in size of field potentials was clearly related to stimulus strength. Field potentials recorded in the dorsal horn (middle records) followed the deflection caused by the afferent volley and consisted of a single component which did not appear until the stimulus intensity was 1.8 - 2.0 T which was often maximal or close to maximal for group I afferents as judged by the size of afferent volleys. Further increases in stimulus strength over the range 3 - 5T, which presumably activate more group II afferents, produced much larger field potentials. By analogy with the actions of graded stimuli in the cat (see above), these dorsal horn field potentials are attributed to the actions of group II muscle afferents.

The field potentials evoked in the intermediate region and ventral horn (Fig. 6 lower records) had two components; an early component which was evoked at low stimulus strengths (e.g. 1.5T) and a later component

which appeared only when stimulus strengths were increased to 3 - 5T. The early component of these potentials are attributed to the actions of group I afferents and the later components to the actions of group II afferents.

Distribution of field potentials in the grey matter. Fig. 7 shows the results of a systematic mapping in the transverse plane of the area of grey matter in which field potentials evoked by afferents in the Q nerve could be recorded. Fig. 7 B shows the distribution of group II and group I field potentials recorded in the grey matter of the L2 segment and Fig. 7 A shows recordings made at different depths along an electrode track passing through the centre of the area in which group II field potentials could be recorded. A similar mapping of field potentials in the L1 and L2 segments was performed in three experiments.

Group II field potentials were recorded in the dorsal horn, intermediate zone and ventral horn, mainly in more lateral aspects of the grey matter. The largest field potentials (up to 600μV; see table 2) occurred within a region of the dorsal horn corresponding to laminae IV & V of Molander, Xu & Grant (1984). Examples of field potentials recorded in the dorsal horn are shown in Fig. 6, (middle records) and Fig. 7.

Deeper in the grey matter, within the intermediate zone and parts of the ventral horn corresponding to laminae VI - VII of Molander et al. (1984), group II field potentials were generally smaller (<500μV; see table 2) and in some regions, were preceded by group I field potentials. The later varied in amplitude but were not usually larger than the group II potentials except

within a small ventromedial area. Examples of field potentials recorded in the intermediate zone and ventral horn are shown in Fig. 6, (lowerrecords) and Fig. 7.

Latencies. In the dorsal horn the minimal latencies of group II field potentials were 1.07 - 1.20 ms with respect to group I incoming volleys (table 2) and less than 0.5 ms with respect to group II volleys (2 experiments). However, as illustrated by the records in Fig. 7 A, the latencies of group II field potentials increased progressively with depth by up to 0.9 ms in the intermediate zone and ventral horn.

Motonucleus fields

Towards the caudal end of the region in which cord dorsum and group II field potentials were encountered (i.e. L2 - L3 border), large group I field potentials were recorded in the intermediate nucleus and large motoneurone fields within the motonucleus. The motonucleus was identified by its location (depth 2.4 mm), by its very short latency (1.15 ms from stimulus artefact) which must be due to the antidromic conduction of impulses along motor fibres and from the Renshaw - like field potentials which were recorded nearby. More rostrally (i.e. in L1), no evidence of the Q motonucleus was seen. These observations are in agreement with the anatomical evidence of Nicolopoulos - Stournaras & Iles (1983) that the rostral end of the Q motonucleus lies in rostral L2. Field potentials evoked by group II afferents of the Q nerve are therefore located close to the rostral end of the Q motor column.

Interneurones and ascending tract neurones

In some experiments, extracellular recordings were made of the discharges of single neurones evoked by group II afferents of the Q nerve. Fig. 8 shows examples of recordings made from neurones in the dorsal horn (A) and ventral horn (B). As can be seen, stimuli of 3.5T (middle records) produced virtually no discharge of the neurones while following stronger stimuli (5T; upper records), presumably sufficient to activate most group II afferents, discharges of the neurones were superimposed on group II (but not group I) field potentials.

Part 2.0. Caudal lumbar segments

Group II afferents in the hamstring and GS nerves (which were investigated in four and eight animals respectively) produced synaptic actions predominantly at the caudal end of the lumbar enlargement in the L5 and L6 segments.

Cord dorsum potentials evoked by the hamstring nerve

Investigation of the action of afferents supplying the hamstring muscles of the rat is complicated by the presence of a cutaneous nerve which runs with the muscle branches for much of their length and is not easily separated from them. For this reason branches of the hamstring nerve were individually mounted for stimulation according to the arrangement shown in Fig. 9 A.

Cord dorsum potentials evoked by muscle branches. Fig. 9. B shows examples of CDPs evoked by stimulation of the hamstring nerve. Combined stimulation of the muscle branches of the hamstring nerve (PB+ST+SM) evoked potentials which appeared at about 2T and reached a maximal amplitude at between 4 and 5T. Stimulation of the individual branches alone showed that group II afferents of both PB and ST contributed to the CDPs whilst stimulation of SM was virtually without effect.

As can be seen in Fig. 9 D, CDPs could be recorded over a length of spinal cord extending from L4 - S1 segments but were largest mainly within L4 and L5 (Fig. 9 E). The mean maximal amplitude of group II CDPs was 34μV (see table 1) and were therefore of considerably smaller amplitude

than the CDPs evoked in the rostral lumbar segments by group II afferents of Q and DP.

The latencies of CDPs were 1.1 - 1.57 ms with respect to group I volleys and 0.84 ms from the group II afferent volleys discernible in one animal (table 1).

Cord dorsum potentials evoked by the cutaneous branch. Fig. 9 C shows an example of the CDPs evoked following stimulation of the cutaneous branch of the hamstring nerve. These potentials had a time course typical of those of cutaneous origin and were much larger (maximally 114 - 248μV; mean 190μV) than the CDPs produced by stimuli applied to muscle nerve branches. The potentials always appeared at about threshold and were of a maximal amplitude at 2T. Fig. 9 D shows an example of the distribution of the cutaneous CDPs which, in each of the four animals in which they were recorded, occurred mainly within the L5 segment and were maximal at a very similar level to the largest group II CDPs evoked by muscle branches.

Cord dorsum potentials evoked by the pudendal nerve

Large CDPs were also recorded following stimulation of the pudendal nerve (Fig. 9 C, lower record). The largest of these potentials (456 - 675μV, mean = 551μV; n = 4) were recorded in the S1 segment (open arrow heads in Fig 9 E).

Cord dorsum potentials evoked by other cutaneous nerves

That the CDPs produced by stimulation of the cutaneous branch of the hamstring nerve and the pudendal nerve are typical of those evoked by cutaneous afferents is evident from the results of stimulating two cutaneous nerves, the superficial peroneal (SP) and posterior cutaneous nerve of the thigh (which is equivalent to the caudal femoralis nerve of the cat). Examples of CDPs evoked by afferents in these nerves are shown in Fig. 10 together with information on their distribution.

Cord dorsum potentials evoked by the gastrocnemius - soleus nerve

Stimulation of the LGS nerve (4 animals) or GS nerve (4 animals) evoked clear CDPs over the caudal lumbar segments. As for other group II potentials, these appeared at 2 - 2.5T and reached a maximal amplitude at 4 - 5T (Fig. 11 A).

Fig. 11 B shows the rostro - caudal distribution of the CDPs which were clearest mainly over the L5 segment and sometimes extended into caudal L4 and rostral L6. CDPs of maximal amplitude (mean = 39.5μV) occurred within L5 (five animals) or close to the L5 - L6 border (three animals). The rostro - caudal distribution of these CDPs is therefore similar to that of the CDPs evoked by group II afferents of the hamstring muscle nerve. The latencies of the CDPs were 1.3 - 1.7 ms with respect to group I volleys and 0.82 ms with respect to the group II volleys seen in one animal (table 1).

Cord dorsum potentials evoked by the plantaris nerve

Fig. 12 A shows examples of CDPs evoked by stimulation of group II muscle afferents of the plantaris (Pl) nerve, the effect of which was investigated in a single experiment. These potentials were similar to those evoked by stimulation of GS nerve and like these potentials occurred mainly over the L5 segment (Fig. 12 B & C).

Afferent volleys

The largest hamstring and GS afferent volleys were usually recorded within the L4 and L5 segments either at the same level or slightly rostral to the locations at which the largest group II CDPs were evoked. This is a similar arrangement to that which exist in the cat (Jankowska & Riddell, 1993).The electrophysiological evidence (in the rat) that GS afferents enter mainly at L5 are in agreement with anatomical evidence showing that the majority of GS dorsal root ganglion cells lie in the L5 ganglion (Molander and Grant, 1987).

Location of the CDPs evoked by group II afferents of the Hamstring and GS muscle nerves in relation to the pudendal motor columns

As can be seen from Fig. 9 E & Fig. 11 C (filled arrow heads), the locations at which group II afferents of the hamstring and GS muscle nerves induced the largest cord dorsum potentials showed some variation in relation to the caudal lumbar segments. In the cat, a similar variation in the location of group II CDPs with respect to the sacral segments has been attributed to varying degrees of pre - and post - fixation of the lumbar plexus in different

animals (see Discussion of Jankowska & Riddell, 1993). In the cat study, the position of CDPs of maximal amplitude were, however, shown to be consistent in relation to an internal landmark; the rostral end of the pudendal motor columns (Jankowska & Riddell, 1993). Although the motoneurone columns in the rat and cat are located in different spinal segments, the position of different motoneurone columns with respect to one another appears to be very similar in both species (c.f. Romanes, 1951;Nicolopoulos - Stournaras & Iles, 1983). The pudendal motor columns therefore provide an internal landmark by which the location of CDPs can be compared in the two species.

The location of CDPs of maximal amplitude in relation to the position of the pudendal motor columns were investigated in ten animals; four in which CDPs were evoked by afferents of the hamstring muscle nerve and 6 in which the potentials were evoked by afferents of LGS/GS. Fig. 13 shows a camera lucida drawing of a transverse section of the spinal cord at the level of the pudendal motor nucleus (rostral S1) in which the compact collections of cells forming the dorsomedial nucleus (DMN) and the dorsolateral nucleus (DLN), together comprising the pudendal motonuclei, could be clearly identified (Schrøder, 1980; Jordan, Breedlove & Arnold, 1982; McKenna & Nadelhaft, 1986). The rostral end of these nuclei was defined as the most rostral of five consecutive sections, any three of which contained larger, evenly dispersed neurones in place of the small neurones of the pudendal motor columns. Sections corresponding to the location where CDPs of maximal amplitude were recorded in the same experiment were then also

determined with reference to marking electrodes left at this or a nearby location. The distance between the two was then calculated from the number of 200μm thick sections separating the relevant sections. The largest CDPs evoked by stimulation of both the hamstring and LGS/GS muscle nerves were consistently found close to the rostral end of the pudendal motor columns, within 1 mm in all 10 animals and within 500μm in 7 animals.

Field potentials evoked by muscle branches of the hamstring nerve

Group II field potentials

Following combined stimulation of muscle branches of the hamstring nerve at 5T, field potentials were recorded within regions of the spinal cord corresponding to those over which the largest cord dorsum potentials were recorded. Fig. 14 A & B shows examples of group II field potentials recorded within the dorsal horn of the rostral L5 segment.

Effects of graded stimuli. As in the example shown in Fig 14 A the field potentials appeared at around 2T and were maximal at 5T. As for CDPs, individual stimulation of branches to PB and ST evoked field potentials while stimulation of the SM branch was virtually without effect (Fig. 14 B). Further examples of dorsal horn field potentials evoked by combined stimulation of the muscle branches of the hamstring nerve are shown in Fig. 15 A & B.

Distribution of field potentials in the grey matter. Fig. 14 F shows the distribution of group II field potentials recorded in the grey matter

of the rostral L5 segment. Group II field potentials were recorded in the dorsal horn mainly in more lateral parts of the grey matter. The largest field potentials (up to 310μV; see table 2) occurred within a region of deep dorsal horn corresponding to laminae III, IV and V of Molander et al. (1984).

Latencies. In the dorsal horn the minimal latency of group II field potentials was 1.15 ms with respect to group I incoming volleys (table 2) and 0.72 ms with respect to group II volleys.

Group I field potentials

In the ventral region of the grey matter, within the intermediate zone, small group I field potentials (up to 110μV) were recorded (Fig. 14 F). These potentials were evoked at low stimulus strengths and reached a maximal amplitude at 2T.

As shown in Fig. 14 E, the latency of group I field potentials (0.62 ms with respect to group I volleys) was always shorter than the latency of group II field potentials seen in the dorsal horn.

Field potentials evoked by the cutaneous branch

Stimulation of the cutaneous branch of the hamstring nerve evoked large field potentials (up to 1 mV) at the same location in the dorsal horn as that where field potentials were evoked by muscle branches of the hamstring nerve (Fig. 14 D).

Field potentials evoked by the gastrocnemius - soleus nerve.

Group II field potentials

Fig. 17 and Fig. 18 show examples of field potentials evoked by stimulation of the LGS or GS nerves in the dorsal horn and intermediate region at the L5 - L6 border. As can be seen, in the dorsal horn large field potentials appearing at 1.8 - 2.0T and reaching a maximal amplitude at 5T were recorded. The largest of these (211μv; see table 2) were recorded in the dorsal horn mainly in more lateral aspects of laminae IV - V of Molander et al. (1984), a similar area to that within which potentials evoked by muscle branches of the hamstring nerve were recorded (see Fig. 17 F and Fig. 18 E)

More ventrally in the grey matter within the intermediate zone (corresponding to lamina VI) a few examples of group II fields preceded by group I field potentials were recorded (Fig. 17. B & C). These group II potentials were always much smaller (largest, 91μV) than the potentials recorded in the dorsal horn.

In the dorsal horn, the minimal latency of group II field potentials was 1.1 ms with respect to group I volleys (table 2) and 0.45 ms with respect to group II volleys (seen in one experiment). In the intermediate zone, the minimal latency of group II field potentials was slightly longer, being 1.2 ms with respect to group I volleys and 0.55 ms with respect to group II volleys.

Group I field potentials

In some parts of the intermediate region, group I field potentials which were not followed by group II potentials were recorded, Fig. 17 D & Fig. 18 B. These potentials, the largest of which were up to 260μV in

amplitude occurred mainly in more medial parts of laminae VII of Molander et al (1984), see Fig. 17 F and Fig. 18 E. Group I field potentials always had a shorter latency (minimally 0.5 ms with respect to group I volleys) than the group II field potentials recorded in the dorsal horn.

Field potentials evoked by the plantaris nerve

Fig. 19 A & B shows examples of field potentials recorded within the dorsal horn and intermediate zone following stimulation of the plantaris (Pl) nerve. These potentials were recorded in a similar region to those evoked by stimulation of the hamstring and GS nerves but were restricted to a localised area (Fig. 19 C).

Motonucleus fields in the caudal lumbar segments

Towards the most rostral end of the region in which group II cord dorsum and field potentials were produced following stimulation of the hamstring and GS nerves (i.e. caudal L4), large motoneurone fields were recorded within the motonucleus (Fig. 16. D, Fig. 17 D and Fig. 18 D). The motonuclei were identified from their location in the ventral horn and from the latencies of the field potentials which were shorter than synaptically evoked group I potentials and could only therefore be due to antidromic conduction of impulses along motor fibres (Fig. 16 E, Fig. 17 F and Fig. 18 E). Furthermore, as shown in Fig. 16, B & C and Fig. 18 C Renshaw - like field potentials showing the characteristic ripples produced by repetitive discharges of Renshaw cells were recorded in the vicinity of motonuclei. These occurred in an area mainly medial to the motonucleus as one would

expect from information provided by labelling of Renshaw cells in the cat and rat (Jankowska & Lindström, 1971; Harrison et al. 1984; Jankowska, 1985).

These observations on the motonucleus are in agreement with the anatomical evidence of Nicolopoulos - Stournaras & Iles (1983) that the biceps semitendinosus and GS motonuclei lie mainly within the L4 - L5 segments, with the semitendinosus motonucleus extending more caudally than GS (as far as L6).

Since, the caudal end of the hamstring motor columns is equivalent to the rostral end of the pudendal motor columns, these electrophysiological observations correspond to the finding that the largest group II CDPs occur close to the histologically defined rostral end of the pudendal motor columns.

Part 3.0 Central lumbar segments

Group II muscle afferents in the tibialis posterior (TP), popliteus (Pop) and flexor digitorum and halucis longus (FDL) nerves produced cord dorsum and field potentials predominately within the central lumbar segments (i.e. L3 and L4 segments) lying in - between the rostral and caudal lumbar regions so far described.

Cord dorsum potentials evoked by the TP nerve

Stimulation of the TP muscle nerve alone (2 animals) or together with Pop (3 animals) produced large CDPs which followed the incoming volleys in records from the surface of the spinal cord (Fig. 20 A & B). As for other group II potentials, these CDPs appeared at about 2T, grew in amplitude as the stimulus intensity was increased and were of maximal amplitude by 5T. Since in contrast, stimulation of the Pop nerve alone was relatively ineffective (see below), potentials evoked by stimulation of TP and Pop together must be mainly due to the actions of group II afferents of TP.

In contrast to other CDPs of group II origin, those evoked from TP showed two peaks in their rostro - caudal distribution (see Fig. 20 C and arrow heads in Fig. 20 D). Large potentials (75% of max., black bars in Fig. 20 D) were usually recorded over a length of the spinal cord extending from caudal L2 to rostral L5 segments. The mean maximal amplitude of CDPs evoked by group II afferents of TP was 36μV (see table 1) and were therefore comparable in amplitude to group II CDPs recorded in the rostral and caudal lumbar segments.

The latencies of group II CDPs measured from the group I volleys ranged from 1.1 - 1.5 ms. The latencies of group II CDPs from group II volleys (identified in all experiments) ranged from 0.45 - 0.77 ms (see table 1).

Cord dorsum potentials evoked by the popliteus and the flexor digitorum and halucis longus nerves

Stimulation of the Pop or FDL nerves were much less effective in evoking field potentials within the central lumbar segments than stimulation of TP. Group II afferents of the popliteus nerve (two experiments) produced very small (maximal amplitude <15μV) cord dorsum potentials mainly within the L3 segment (see Fig. 21).

The surface potentials that followed stimulation of FDL differed from any so far described in that they consisted mainly of a positive deflection (see Fig. 22 A). Increasing the stimulus intensity within the range producing group II CDPs on stimulation of other nerves produced only very small negative displacements (mean 17μV, n = 7) of these otherwise positive waves (compare top two records of Fig. 22 A). Evidence of weak group II actions of this type were seen mainly close to the L4/L5 border (Fig. 21 B & C).

Afferent volleys

The largest afferent volleys recorded in the central lumbar segments following stimulation of the TP, Pop and FDL nerves were usually recorded at L4 and rostral L5 segments caudal to the locations in which the largest CDPs were evoked by these nerves.

Field potentials evoked by the TP nerve

Group II field potentials

Following stimulation of the TP nerve at 5T, clear field potentials were regularly recorded at segmental locations corresponding to those over which the largest CDPs were induced.

Fig. 23 E shows an outline of the spinal cord at caudal L3 on which is indicated the areas in which field potentials were recorded. The largest field potentials of group II origin (up to 192μV; table 2) were recorded in an area extending from laminae IV - V of the dorsal horn into the intermediate region (shaded area in Fig. 23 E, records in Fig. 23 A). In more ventral areas of the grey matter, corresponding to laminae VI - VII, smaller group II fields potentials (Fig. 23 C), were recorded which were preceded at some locations by shorter latency group I field potentials (Fig. 23 B).

In the dorsal horn the minimal latency of group II field potentials was 1.22 ms with respect to group I volleys (table 2), and 0.50 ms with respect to group II volleys. The minimal latency of group II fields in the intermediate and ventral region was only slightly longer.

Group I field potentials

In the medial part of intermediate nuclei (laminae VI - VII), large group I field potentials (up to 270μV) which were not accompanied by group II potentials were recorded (Fig. 23 D). The minimal latency of these potentials was 0.77 ms with respect to group I volleys.

Field potentials evoked by the Pop nerve

No clear examples of field potentials evoked by group II afferents of the Pop nerve were seen at any of the locations in which recordings were made within the central lumbar segments. The only potentials observed were small group I field potentials (largest 81 μV; latency from group I volleys, 0.5 ms) in the intermediate region of mid L4.

Field potentials evoked by the FDL nerve

Group II field potentials

Following stimulation of the FDL nerve, small field potentials were sometimes recorded within the deep dorsal horn (see Fig. 24) and intermediate zone of central lumbar segments. These potentials were less frequently encountered and always considerably smaller (largest 80 μV) than group II potentials evoked by afferents of TP.

In the dorsal horn, the minimal latency of group II field potentials was 1.2 ms with respect to group I volleys and 0.5 ms with respect to group II volleys (table 2). In the intermediate zone, the minimal latency of group II potentials was 1.35 ms with respect to group I volleys and 0.65 ms with respect to group II volleys.

Group I field potentials

In rostral L4, close to where the motonucleus was encountered (see Fig. 25), large group I field potentials (up to 209μV) were recorded in the medial part of lamina VI and VII. The minimal latency of these group I field potentials was 0.5 ms with respect to group I volleys.

Field potentials evoked by the gastrocnemius nerve

Group II field potentials

Although the main actions of group II afferents of the GS nerve were in the caudal lumbar segments, small group II field potentials were also recorded in the L4 segment. Fig. 26 shows examples of group II field potentials (A - C) recorded in the deep dorsal horn of rostral L4. As illustrated in Fig. 26, the potentials recorded here were much smaller (largest 154μV) and spatially much more restricted (shaded area in Fig. 26 E) than GS field potentials evoked in the caudal lumbar segments.

Group I field potentials

More ventrally in the grey matter within the intermediate nucleus, large group I field potentials (up to 400μV) were seen, Fig. 26 E. These potentials were recorded at the same location as those produced by group I afferents of FDL. This region presumably therefore corresponds to the group I intermediate nucleus in the lower - lumbar segments (L6 - L7) of the cat spinal cord which has been the subject of extensive investigation (Eccles et al. 1954).

Synaptic linkage of field potentials

Latencies. The shortest latencies of dorsal horn field potentials were 1.07 - 1.40 ms with respect to group I afferent volleys, and 0.54 - 0.75 ms with respect to group II afferent volleys where these could be identified (see table 2). Since some of this delay must be due to conduction along the central branches of group II fibres in the dorsal columns and along the relatively slowly conducting collateral branches travelling ventrally into the grey matter (Fu and Schomburg, 1974), it may be concluded that the time remaining is sufficient for only one synaptic delay (about 0.3 ms). At least the earliest components of group II field potentials in the dorsal horn seem therefore to be evoked largely monosynaptically.

Frequency following. Further evidence of monosynaptic actions was sought by verifying that at least the main component of group II field potentials (peaks of potentials) followed a train of stimuli without temporal summation. This was investigated by recording responses to a train of stimuli of 200 - 300 Hz and observing the amplitude of the resulting field potentials. Such tests were performed on between three and six occasions for each nerve and each site (rostral, caudal and central lumbar segments) at which group II afferents were found to evoke group II field potentials. No evidence of temporal summation was observed. An example of one of these tests is illustrated in Fig. 17 E.

Axonal and terminal potentials

At some recording locations, field potentials evoked by group II muscle afferents were preceded by additional short duration potentials. These potentials occurred in two forms; either as biphasic, positive - negative excursions (see Figs. 14 A - C, 15 A & B, 18 A) or, more commonly, as triphasic waves with a predominantly negative excursion (see Figs. 15 C & D, 26 B). Triphasic potentials were 0.3 ms or less in duration (see table 3) while the biphasic potentials were only slightly longer at about 0.4 ms (table 3). These potentials closely correspond in both form and duration to the biphasic terminal potentials and triphasic axonal potentials which can be recorded in conjunction with focal synaptic potentials or EPSPs evoked by single group Ia and group II muscle afferents in the cat (Munson & Sypert, 1979 a, b; Munson, Fleshman & Sypert, 1980).

The single fibre potentials which have been described in the cat are characterised by a very small amplitude; less than 15μV for axonal potentials and less than 50μV for terminal potentials. In contrast most of the potentials recorded in the rat were well in excess of 50μV (see table 3) suggesting that they are likely to represent the activity in a number of afferent fibres simultaneously activated by the electrical nerve stimulation. Furthermore, in some cases, axonal potentials varied in amplitude with stimulus strength paralleling the changes in size of field potentials (e.g. Fig. 14 & Fig. 15)

Given that fibre and field potentials must be recorded in very close proximity to the structures by which they are generated (Munson & Sypert,

1979a) it seems reasonable to assume that the axons and terminals responsible for the fibre potentials also evoke the field potentials which they precede. However, since the present examples represent compound fibre and field potentials it cannot be excluded that some component of the fibre potentials is due to activity in axons of passage that terminate elsewhere. Nevertheless, in support of a causal relationship between fibre potentials and field potentials the delay between them was generally less than 0.5 ms (see table 3), a similar range to that between single fibre potentials of Ia afferents and potentials in their target neurones (Munson & Sypert, 1979b).

Single afferent fibre recordings

The results so far presented establish that cord dorsum and field potentials equivalent to those attributed to the actions of group II muscle afferents in the cat are also evoked in the spinal cord of the rat (see also Discussion). In the rat, as in the cat, actions attributable to group II muscle afferents appeared at stimulus strengths of about 1.8 - 2.5T (depending on the nerve) and were maximal at 4 - 5T. These results imply that rat muscle nerves contain a population of afferents equivalent to the medium diameter myelinated fibres which comprise the group II range of the cat. This was investigated in four animals by recording from single afferents of the LGS nerve. In order to obtain the largest possible samples of afferents, recordings were made from fibres in teased dorsal roots and the conduction velocity and peripheral thresholds of each afferent fibre determined by electrical stimulation of the nerve (up to 5T).

Recordings were obtained from between 60 and 89 single fibres in each experiment. Examples of recordings are shown in Fig. 27. The data for each experiment is shown in the pairs of plots in Fig. 28 (A - D). Plots showing data for the peripheral thresholds and corresponding conduction velocities of each of the fibres in the samples are plotted on the right of Fig. 28. Although some scatter is evident in these plots each clearly shows that slower conduction velocities are associated with higher peripheral thresholds as described for cat muscle afferents (see Jack, 1978; Eccles et al. 1959; Ellaway et al. 1982). From these plots it can be determined that stimuli of the order of 1.8 - 2.0T, about threshold for most group II cord dorsum and field potentials, would recruit fibres with conduction velocities of around 50 m/s.

The histograms on the left side in Fig. 28 show the distribution of conduction velocities for the samples of muscle afferents. These show some tendency towards a bimodal distribution which may reflect groupings equivalent to the group I and group II populations in the cat. Although no obvious boundaries occur in the histograms a division at around 50 m/s seems reasonable for three of the four nerves studied with a somewhat lower dividing line (around 45 m/s) being more appropriate for the fourth.

Furthermore confirmation that 45 - 50 m/s is an appropriate conduction velocity value for the fastest conducting group II afferents in rat muscle nerves can be obtained from observation of the latencies of group II components of afferent volleys. A group II component could be identified in only one of the afferent volley evoked by LGS. The conduction time for this group II component (2.3 ms) together with the conduction distance for the

nerve (91.5 mm) suggests a contribution from fibres conducting at (40 m/s). Observations of group II afferent volleys evoked on stimulation of other nerves (Q, DP, Hamstring, TP and Pop) suggest that fibres conducting in the range 40 - 50 m/s also comprise the fastest conducting component of group II afferents in these nerves.

These results therefore suggests two populations of muscle afferents one comprised of fibres conducting at between 90 and about 50 m/s which is activated by weak electrical stimuli from nerve threshold to about 1.8 - 2.0T, and another composed of fibres with conduction velocities between about 50 and 15 m/s which are activated by stimuli between 2 and 5T. The later group are responsible for the cord dorsum and field potentials which followed electrical stimulation of muscle nerves. Sometimes additional potentials could be recorded following stimuli at 10T, these presumably reflect the action of group III muscle afferents

Figures and tables

Fig. 1. Diagrammatic representation of the experimental arrangement. A, cord dorsum and field potential recordings. Bipolar ball electrodes were used to apply electrical stimuli to peripheral nerves. Cord dorsum potentials were recorded through a monopolar ball electrode placed on the spinal cord and field potentials were recorded using glass microelectrodes. B, single fibre recording. Bipolar ball electrodes were used to apply electrical stimuli to the LGS nerve. Single fibre recordings were made from the distal ends of teased dorsal roots laid on bipolar electrodes and afferent volleys were recorded from the sciatic nerve.

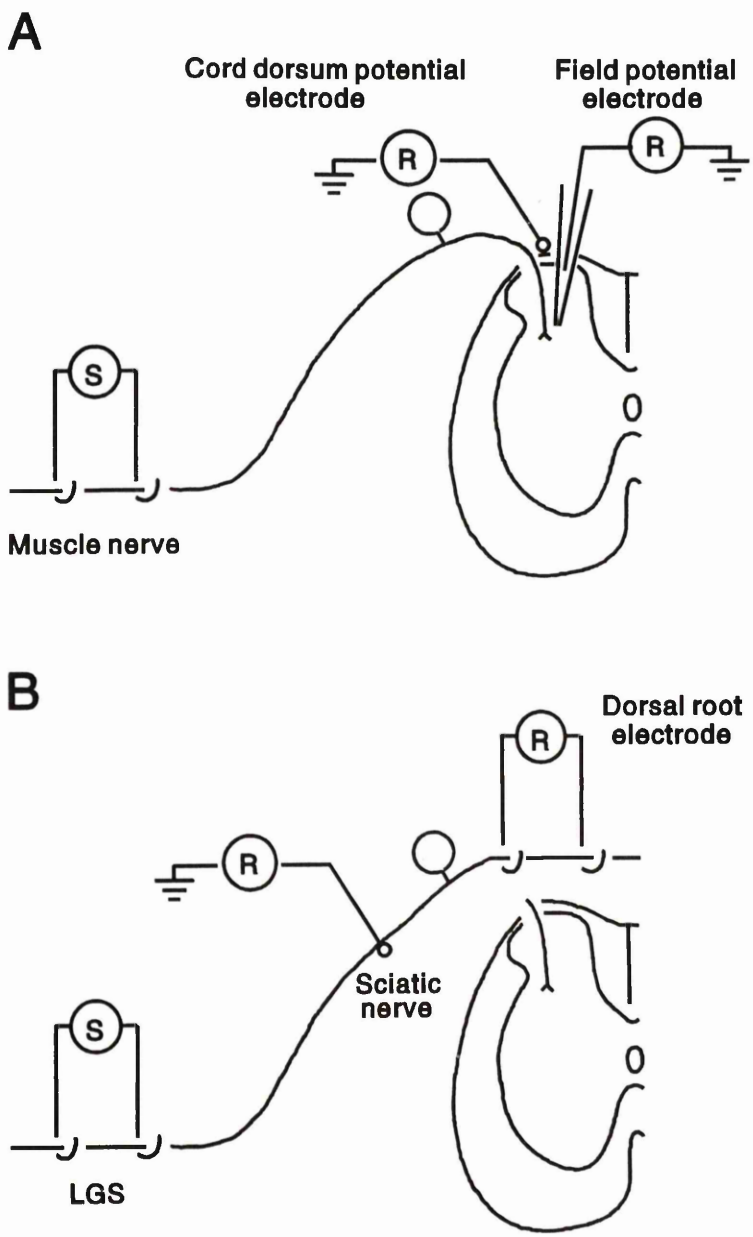

A
Cord dorsum potential electrode
Field potential electrode
R
R
S
Muscle nerve
B
Dorsal root electrode
R
R
Sciatic nerve
S
LGS

Fig. 2. Procedure used to record systematically throughout grey matter The microelectrode was introduced at various angles (e.g. 15° medially to 30° laterally) from the same cord dorsum entry position. Recordings of field potentials were made at 200μm intervals or less along each electrode track. The outline is taken from an actual experiment and the grid superimposed according to the position of a marking electrode left at a known angle.

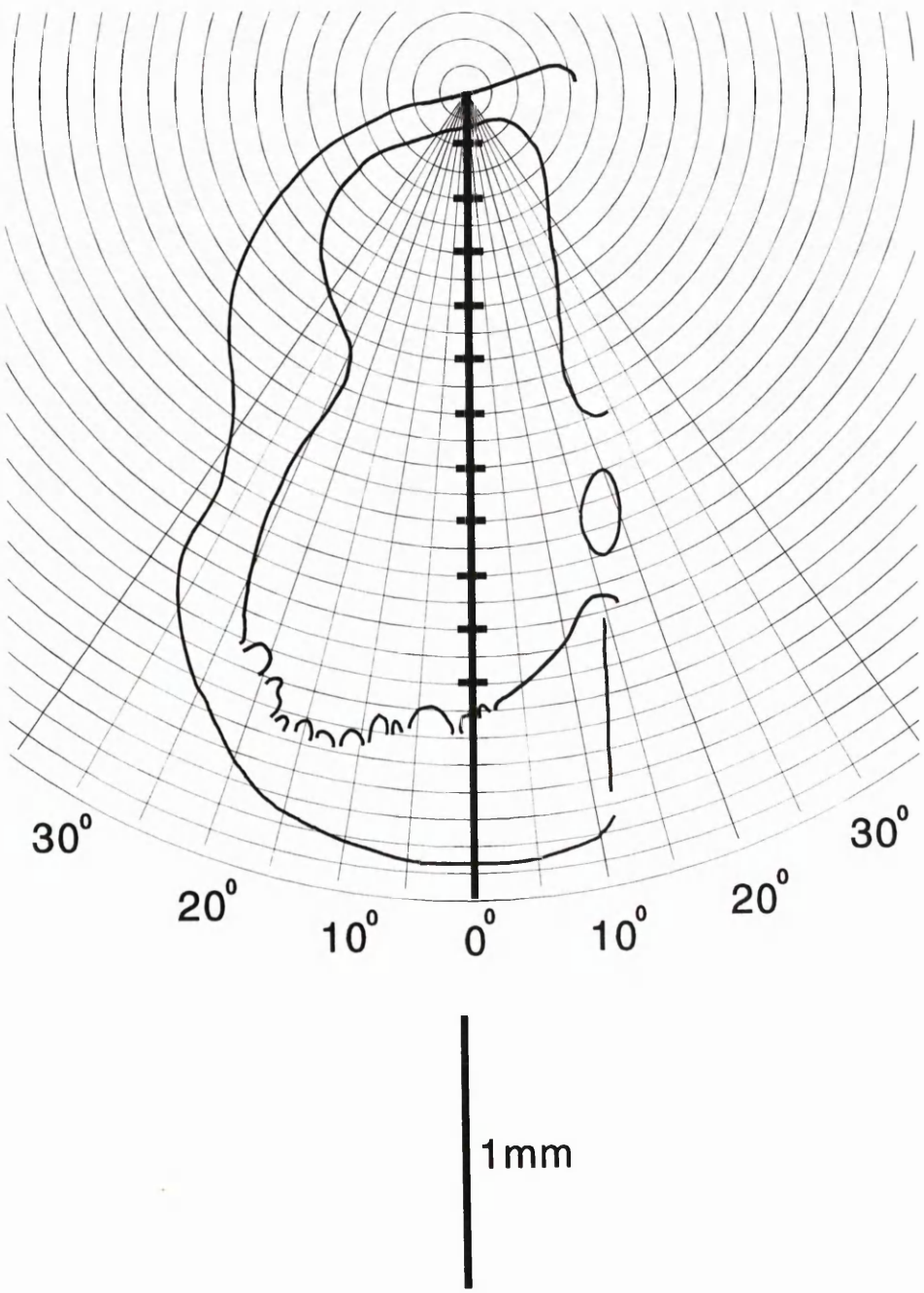

30°
20°
10°
0°
10°
20°
30°
1mm

Fig. 3. Cord dorsum potentials (CDPs) evoked by group II afferents of the quadriceps (Q) nerve. A and B, examples of recordings from the surface of the L2 spinal segment following stimuli of different strengths (as indicated). In this and later figures, surface recordings are shown with negativity upward. Group II afferent volleys are indicated by filled arrows. All recordings are averages of 32 sweeps. C, example of recording, with the parameters measured during all experiments indicated: (1) stimulus artefact, (2) onset of group I afferent volley, (3) group I afferent volley, (4) group II afferent volley, (5) group II CDPs, (6) peak amplitude for CDPs, (7) latency of group I afferent volley, (8) latency of group II CDPs with respect to group I afferent volley and (9) latency of group II CDPs with respect to group II afferent volley.

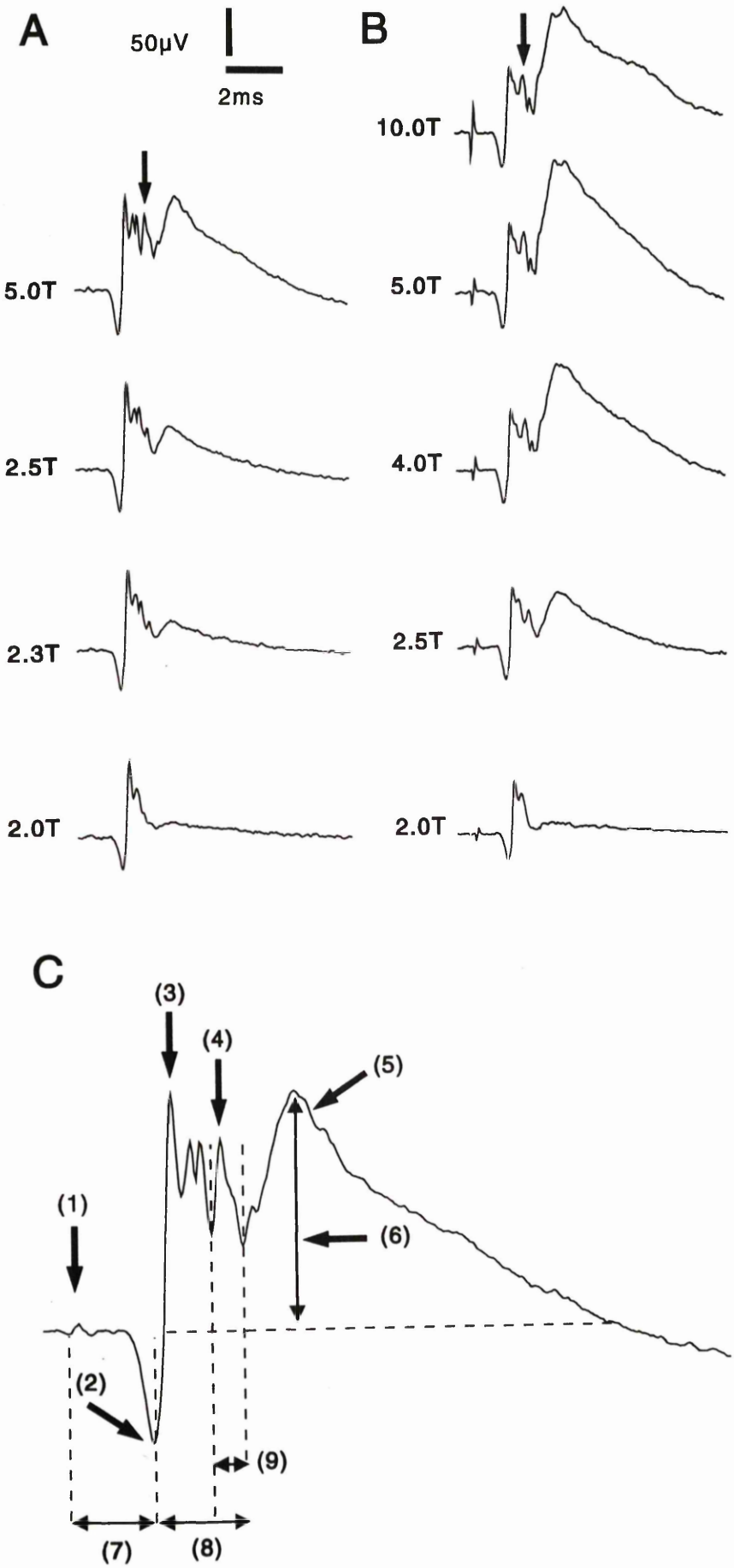

A
50μV
2ms
5.0T
2.5T
2.3T
2.0T
B
10.0T
5.0T
4.0T
2.5T
2.0T
C
(1)
(2)
(3)
(4)
(5)
(6)
(7)
(8)
(9)

Fig. 4. Rostro - caudal distribution of CDPs evoked by group II afferents of quadriceps nerve. A, plot of amplitudes of CDPs evoked by stimuli applied at 5T and recorded at 1 mm intervals along the spinal cord (upper most in B). B, location of the largest CDPs evoked by stimuli of 5T in four animals. Arrow heads indicate the positions where the largest potentials of group II origin were recorded and the black bars indicate the length over which potentials were at least 75% of peak amplitude.

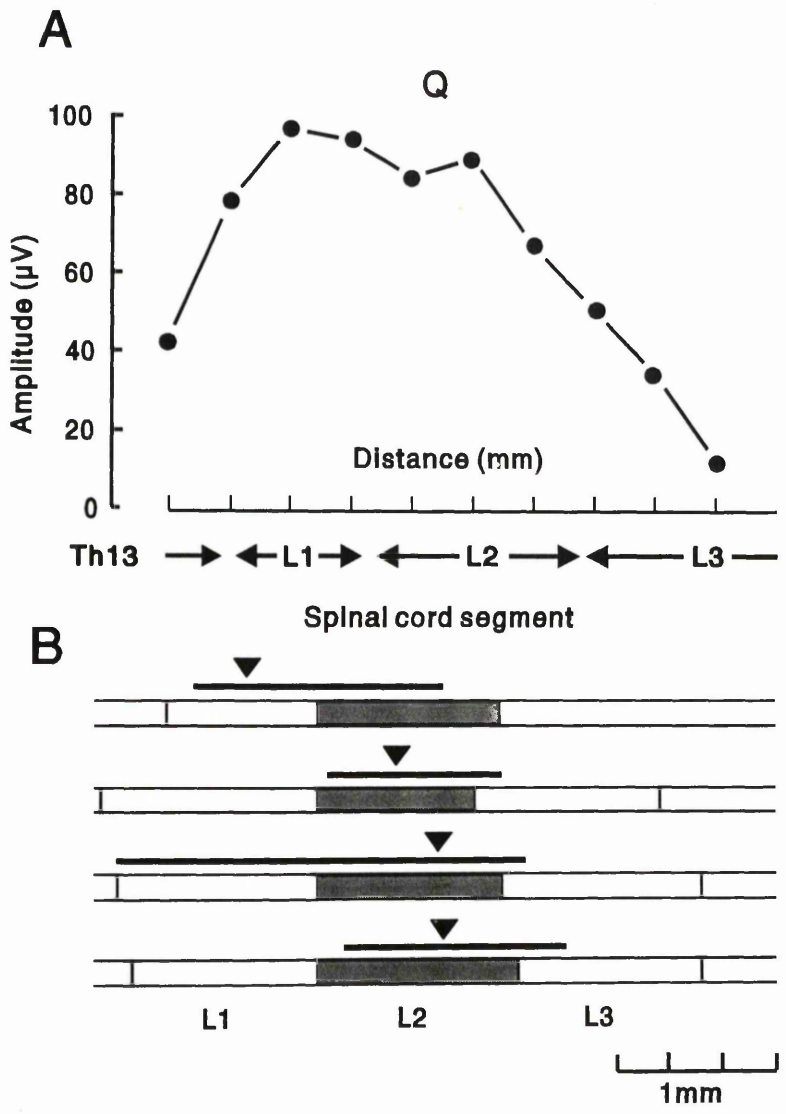

A
Q
100
80
60
40
20
0
Amplitude (μV)
Distance (mm)
Th13
L1
L2
L3
Spinal cord segment
B
L1
L2
L3
1mm

Fig. 5. Cord dorsum potentials evoked by group II afferents of the deep peroneal (DP, tibialis anterior - extensor digitorum longus) and extensor digitorum brevis (EDB) nerves. A, examples of recordings from the surface of the caudal L2 segment following stimuli of different strengths (as indicated) applied to DP or EDB (bottom most record). A group II afferent volley is indicated by the arrow. All recordings are averages of 32 sweeps. B and C, rostro - caudal distribution of CDPs. B, plot of amplitudes of CDPs evoked by stimuli 5T (DP), recorded at 1 mm intervals along the spinal cord in one experiment. Note that the right hand ordinate applies to EDB (2T). C, location of the CDPs evoked by stimuli 5T (DP) in three animals. Filled arrow heads indicate the positions where potentials evoked by DP were largest (black bars indicate the length over which potentials were at least 75% of the peak amplitude) and open arrow heads indicate where potentials evoked by EDB (2T) were largest.

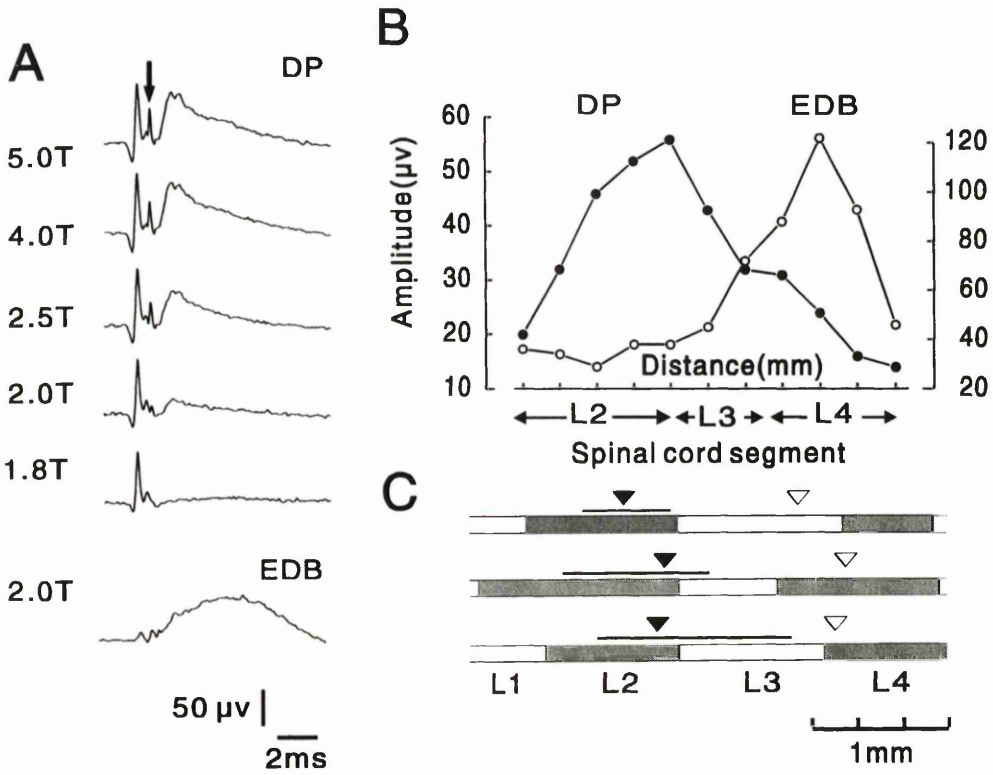

A
DP
5.0T
4.0T
2.5T
2.0T
1.8T
EDB
2.0T
50 μv
2ms
B
DP
EDB
Amplitude(μv)
60
50
40
30
20
10
120
100
80
60
40
20
Distance(mm)
L2
L3
L4
Spinal cord segment
C
L1
L2
L3
L4
1mm

Fig. 6. Field potentials evoked by afferents of the quadriceps (Q) nerve. Afferent volley (upper records) and field potentials recorded in the dorsal horn (middle records) and ventral horn (lower records) of the L2 segment. The traces are composed of superimposed records evoked by stimuli applied to the Q nerve at different strengths. In the lower records potentials evoked by group I and group II afferents are denoted I and II respectively. Each record is an average of 32 sweeps. The voltage and time calibration in the middle and lower records (A) is 200μV and 1 ms respectively. B, example of recording, with the parameters measured during all experiments indicated: (1) calibration pulse, (2) stimulus artefact, (3) reflection of group I afferent volley, (4) group I field potentials, (5) group II field potentials, (6) peak amplitudes for field potentials, (7) latency of group I field potentials with respect to group I volley and (8) latency of group II field potentials with respect to group I volley.

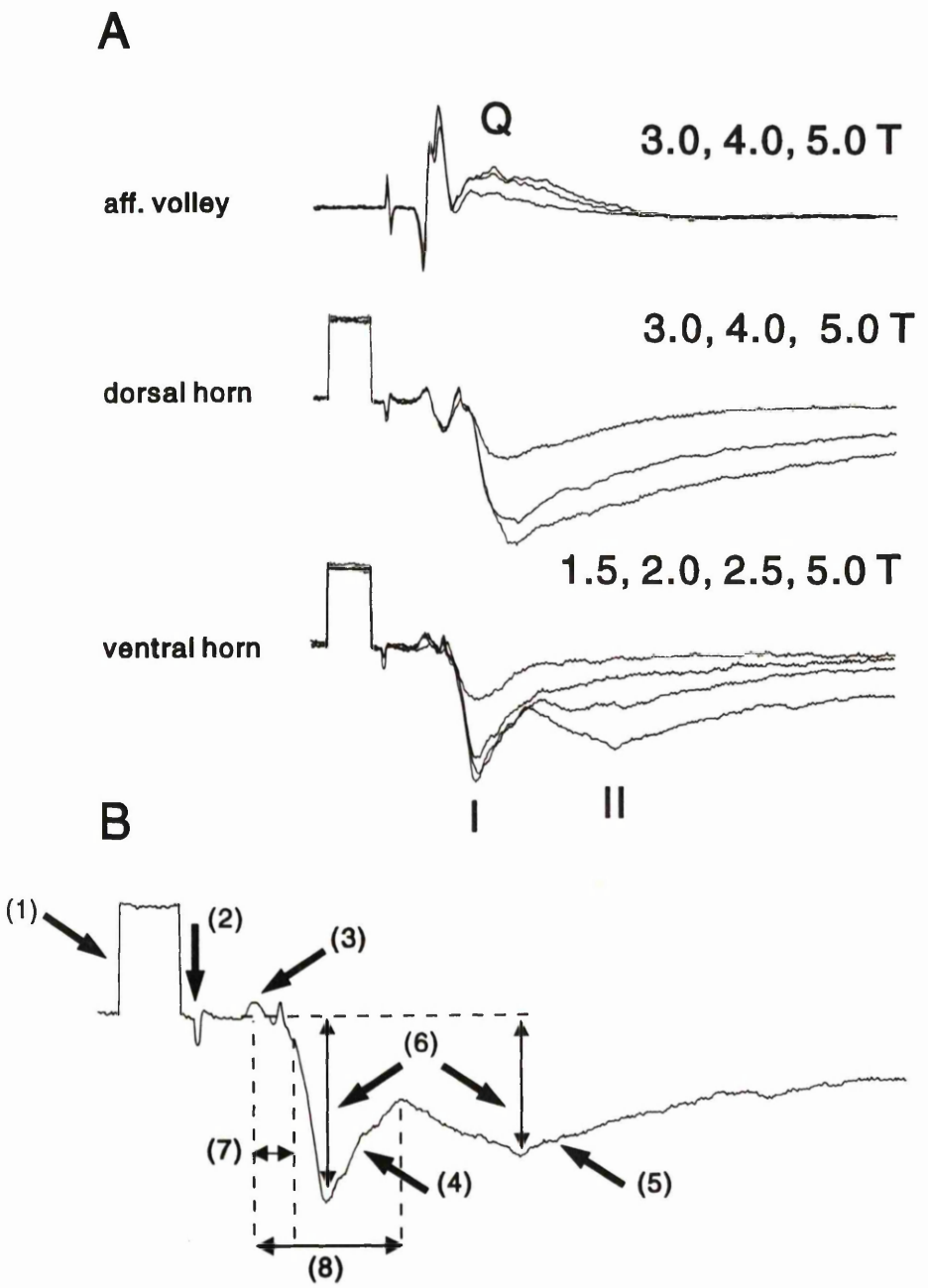

A
Q
3.0, 4.0, 5.0 T
aff. volley
3.0, 4.0, 5.0 T
dorsal horn
1.5, 2.0, 2.5, 5.0 T
ventral horn
I
II
B
(1)
(2)
(3)
(4)
(5)
(6)
(7)
(8)

Fig. 7. Field potentials evoked by afferents of the Q nerve in the L2 segment. A, records of field potentials (averages of 32 sweeps) evoked by stimuli 5T, recorded at different depths within the grey matter. Note the gradual increase in onset latency of the group II potentials and the appearance of a group I potential (I) ventrally. Calibration pulse; 200μV and 1 ms all records. B, outline of the spinal cord showing the area in which group II (>200μV) and group I field potentials (100μV) were recorded during systematic tracking in the grey matter. The electrode track and each recording position is also indicated in B.

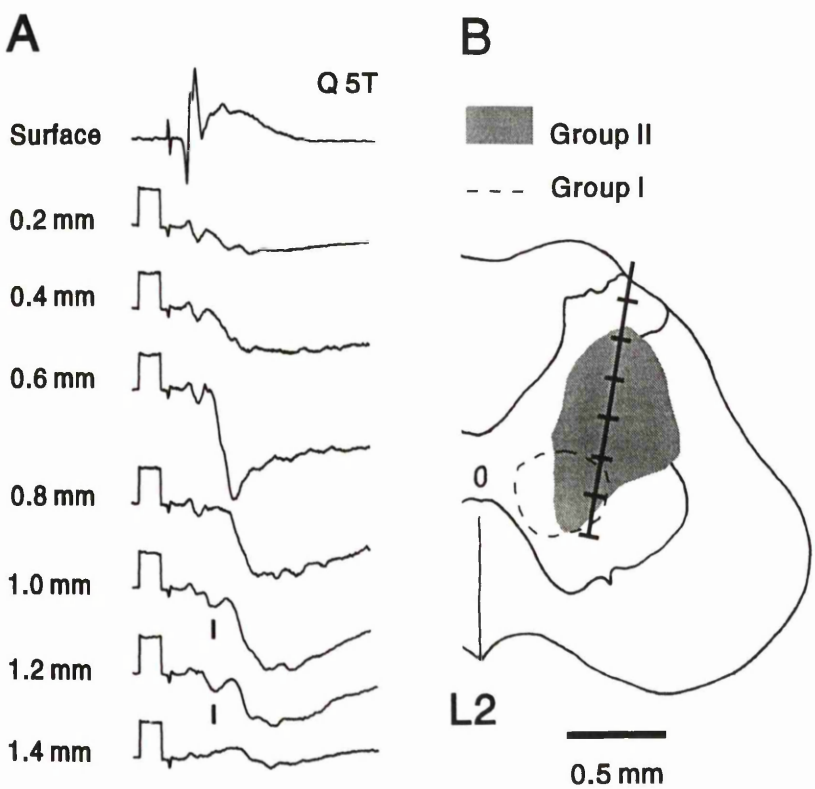

A
Q 5T
Surface
0.2 mm
0.4 mm
0.6 mm
0.8 mm
1.0 mm
1.2 mm
1.4 mm
B
Group II
Group I
0
L2
0.5 mm

Fig. 8. Extracellular discharges evoked by group II afferents of quadriceps nerve. A and B, top and middle traces are superimposed single sweep recordings (n = 4) of the extracellular discharges of neurones. Note that extracellular spikes are superimposed on dorsal and ventral field potentials and that the discharges recorded in the ventral horn coincide with the group II but not group I field potentials. The voltage and time calibration is 200μV and 1 ms respectively.

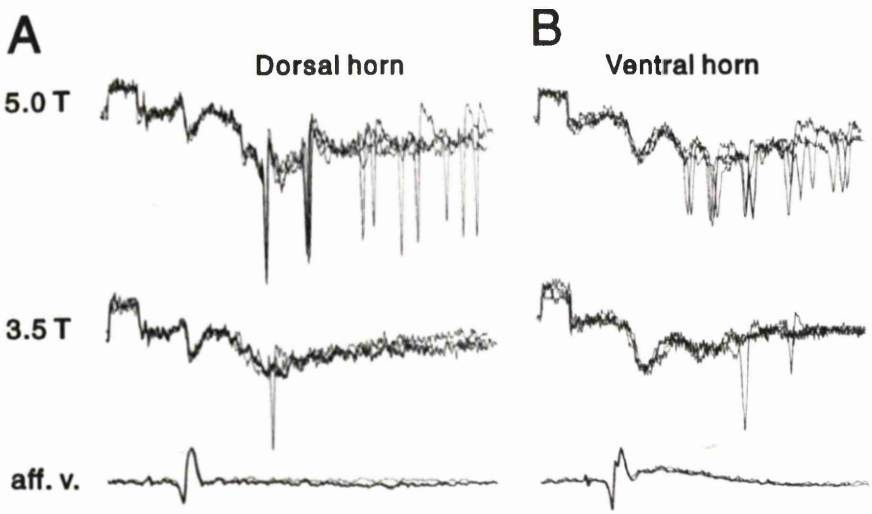

A
Dorsal horn
5.0 T
3.5 T
aff. v.
B
Ventral horn

Fig. 9. Cord dorsum potentials evoked by afferents in branches of the hamstring nerve. A, arrangement of stimulating electrodes on branches of the hamstring nerve. B, recordings of CDPs evoked by group II afferents in different muscle branches of the hamstring nerve. The top two records show CDPs evoked by combined stimulation of branches to posterior biceps, semitendinosus (principal head) and semi - membranosus and the lower three records show potentials evoked by stimulation of these branches individually. C, CDPs recorded following stimuli applied to the cutaneous branch of the hamstring nerve and the pudendal nerve. All recordings are averages of 32 sweeps. D, plot of amplitudes of CDPs evoked by combined stimulation of muscle branches at 5T and stimulation of the cutaneous branch at 2T, in the same experiment. Recordings were made at 1 mm intervals along the spinal cord. Note that the right hand ordinate applies to the cutaneous branch. E, location of the CDPs evoked by stimuli at 5T (muscle branches) in four animals. Filled arrow heads indicate the positions where potentials evoked by muscle branches were largest (black bars indicate the length over which potentials were at least 75% of the peak amplitude) and open arrow heads indicate where potentials evoked by the pudendal nerve (2T) were largest.

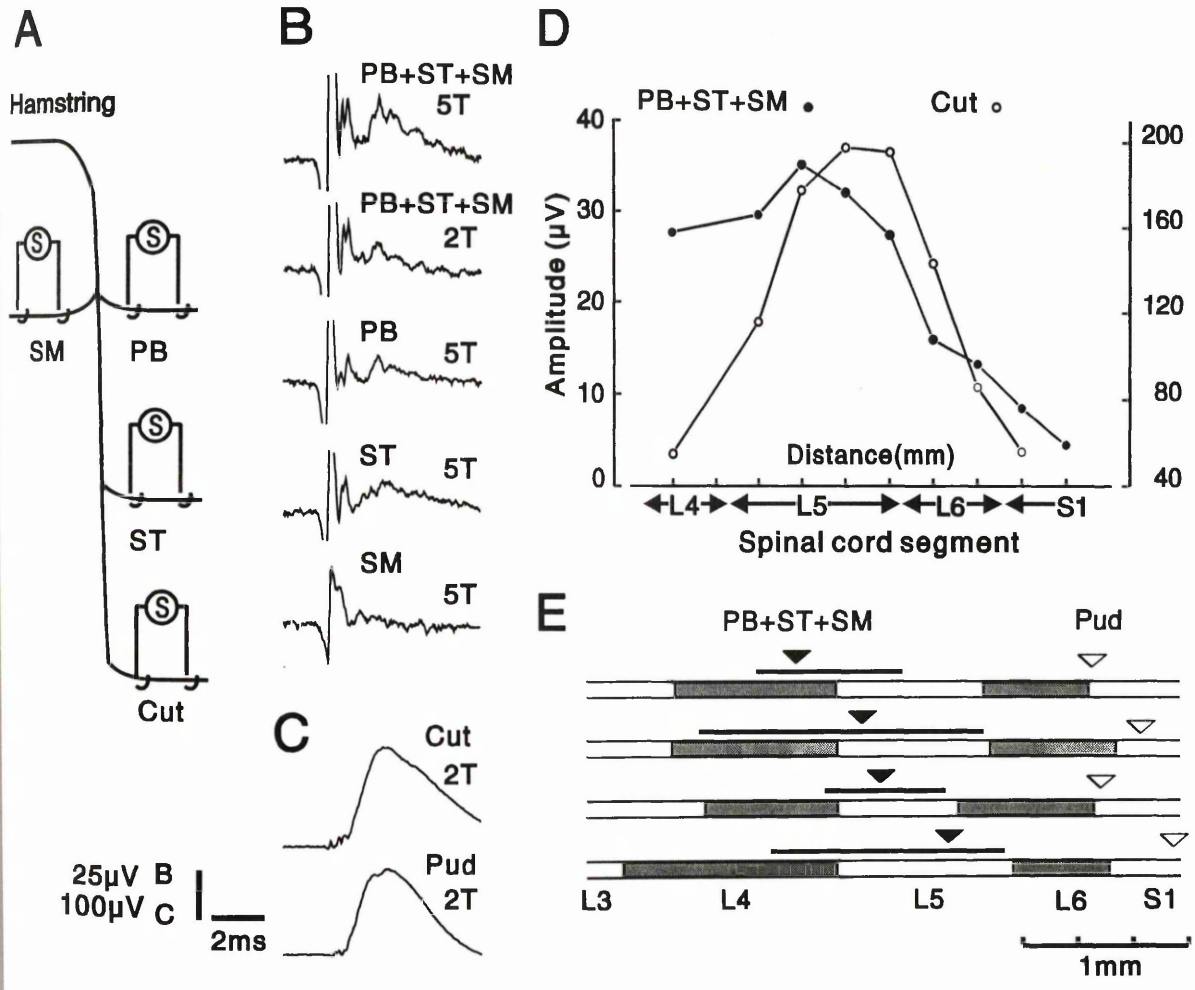

A
Hamstring
SM
PB
ST
Cut
B
PB+ST+SM
5T
PB+ST+SM
2T
PB
5T
ST
5T
SM
5T
C
Cut
2T
Pud
2T
25μV B
100μV C
2ms
D
PB+ST+SM
Cut
Amplitude (μV)
40
30
20
10
0
200
160
120
80
40
Distance(mm)
L4
L5
L6
S1
Spinal cord segment
E
PB+ST+SM
Pud
L3
L4
L5
L6
S1
1mm

Fig. 10. Cord dorsum potentials (CDPs) evoked by superficial peroneal (SP) nerve and posterior cutaneous nerve of thigh (Post. Cut). A, recording from the surface of the L4 segment following stimuli applied to SP at 2T. B and C, rostro - caudal distribution of CDPs. B, plot of amplitudes of CDPs evoked by stimuli 2T applied to the SP nerve, recorded at 1 mm intervals along the spinal cord in one experiment. C, locations of CDPs evoked by stimuli 2T applied to the SP nerve. Arrow heads indicate the positions where the largest potentials were recorded. D, recording from the surface of the L5 segment following stimuli applied to Post. Cut nerve at 2T. E, plot of amplitudes of CDPs evoked by stimuli 2T applied to the Post. Cut nerve, recorded at 1 mm intervals along the spinal cord in one experiment. All recordings are averages of 32 sweeps

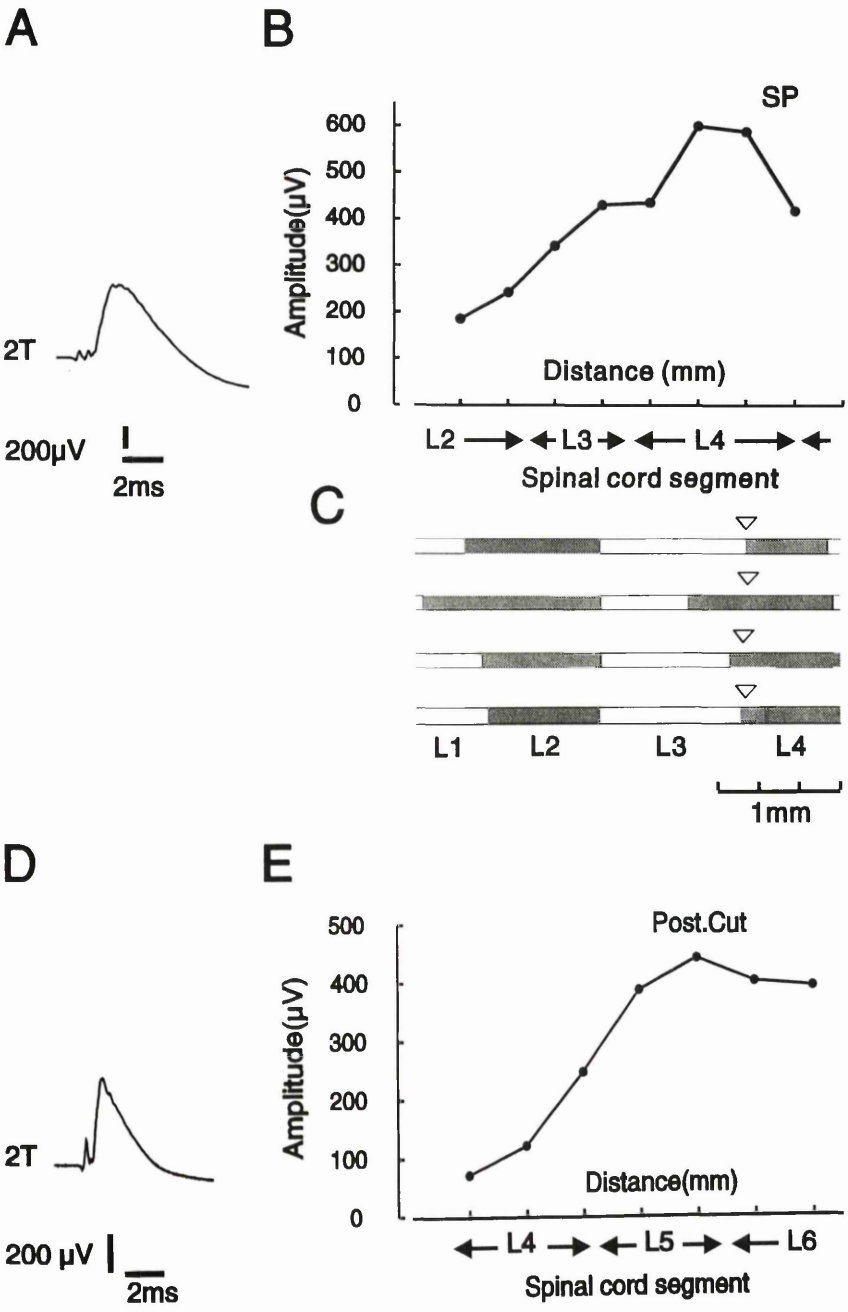

A
B
2T
200μV
2ms
SP
600
500
400
300
200
100
0
Amplitude(μV)
Distance (mm)
L2
L3
L4
Spinal cord segment
C
L1
L2
L3
L4
1mm
D
E
2T
200 μV
2ms
500
400
300
200
100
0
Amplitude(μV)
Post.Cut
Distance(mm)
L4
L5
L6
Spinal cord segment

Fig. 11. Cord dorsum potentials (CDPs) evoked by group II afferents of the gastrocnemius - soleus (GS) nerve. A, recordings from the surface of the L5 segment following stimuli of different strengths applied to the lateral gastrocnemius - soleus (LGS) nerve branch. All recordings are averages of 32 sweeps. B and C, rostro - caudal distribution of CDPs. B, plot of amplitudes of CDPs evoked by stimuli 5T applied to the LGS branch, recorded at 1 mm intervals along the spinal cord in one experiment. C, locations of CDPs evoked in eight animals by stimuli 5T applied to the GS or LGS nerves. Arrow heads indicate the positions where the largest potentials of group II origin were recorded and black bars indicate the length over which potentials were at least 75% of peak amplitude.

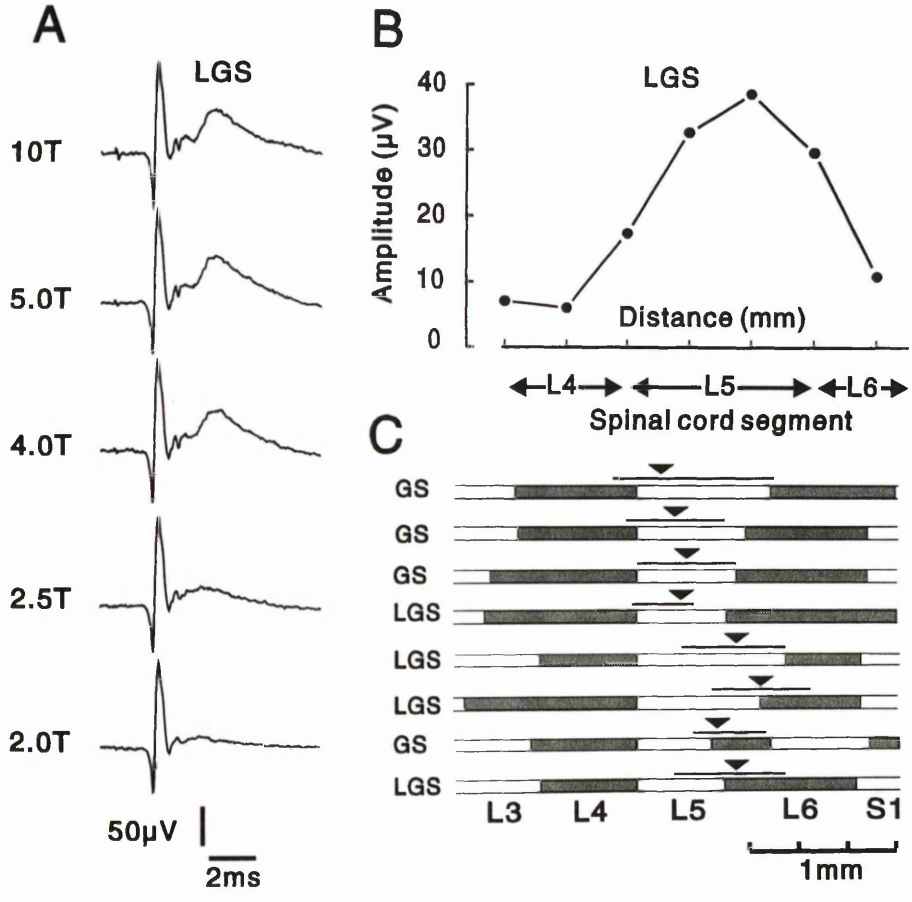

A
LGS
10T
5.0T
4.0T
2.5T
2.0T
50μV
2ms
B
LGS
Amplitude (μV)
40
30
20
10
0
Distance (mm)
L4
L5
L6
Spinal cord segment
C
GS
GS
GS
LGS
LGS
LGS
GS
LGS
L3
L4
L5
L6
S1
1mm

Fig. 12. Cord dorsum potentials (CDPs) evoked by group II afferents of the plantaris (Pl) nerve. A, recordings from the surface of the L5 segment following stimuli of different strengths applied to the Pl nerve. All recordings are averages of 32 sweeps. B and C, rostro - caudal distribution of CDPs. B, plot of amplitudes of CDPs evoked by stimuli 5T, recorded at 1 mm intervals along the spinal cord in one experiment. C, locations of CDPs evoked by stimuli. Arrow head indicates the positions where the largest potential of group II origin was recorded and a black bar indicates the length over which potentials were at least 75% of peak amplitude.

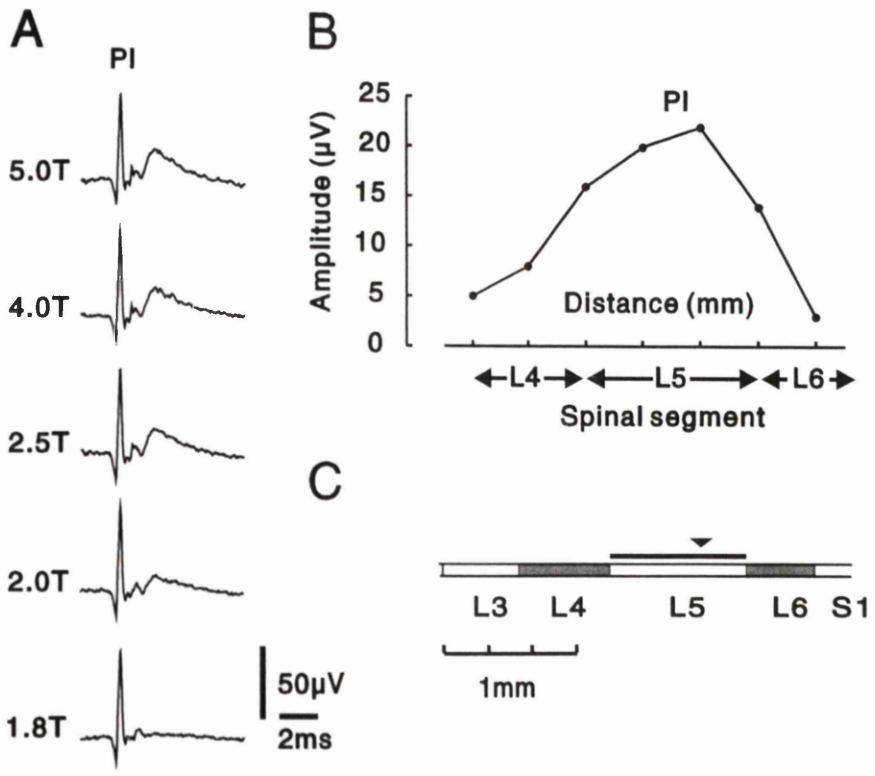
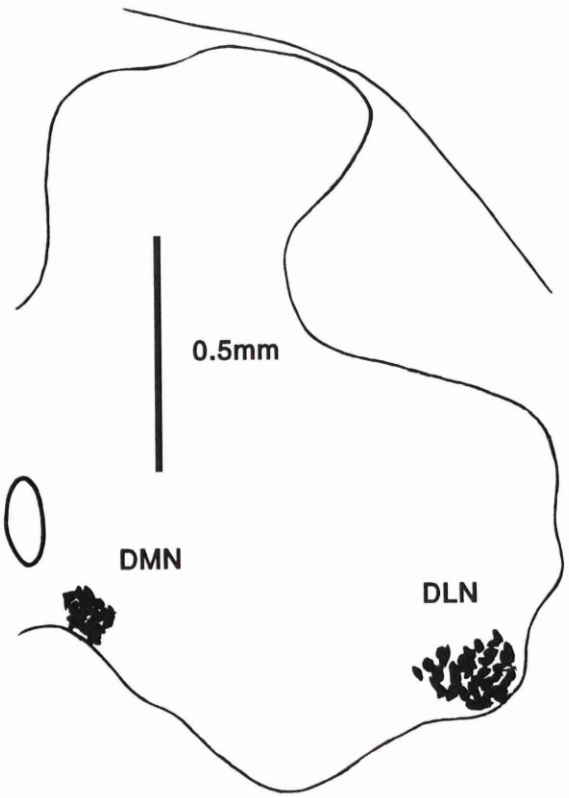

A
PI
5.0T
4.0T
2.5T
2.0T
1.8T
50μV
2ms
B
PI
Amplitude (μV)
25
20
15
10
5
0
Distance (mm)
L4
L5
L6
Spinal segment
C
L3
L4
L5
L6
S1
1mm

Fig. 13. Location of motonuclei associated with pudendal nerve. The diagram shows a camera lucida drawing of a transverse section of the spinal cord at the level of the pudendal motor nucleus stained with cresyl violet (Nissl stain). Nerve cells at the locations of the dorsomedial nucleus (DMN) and dorsolateral nucleus (DLN) are shown. Cells from three 100µm thick sections are superimposed.



Rostral S1

Fig. 14. Field potentials evoked by afferents in branches of the hamstring nerve in the rostral half of the L5 segment. A, effects of combined stimulation of muscle branches of the hamstring nerve at different stimulus strengths. B, effects of stimulation of individual branches of the hamstring nerve at 5T. Note that field potentials were evoked by group II afferents of the PB and ST nerve branches. C, terminal potential from the top record of A (arrow), shown at a faster time base and higher amplification. D, field potential evoked by afferents of the cutaneous branch of the hamstring nerve at the same location. E, field potentials evoked by group I afferents in muscle branches at a more ventral location. Calibration pulse; 200μV, 1 ms. All recordings are averages of 32 sweeps. Arrows indicate the onset of terminal potentials. F, outline of the spinal cord showing the area in which group II and group I field potentials (>100μV) were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A - E are indicated.

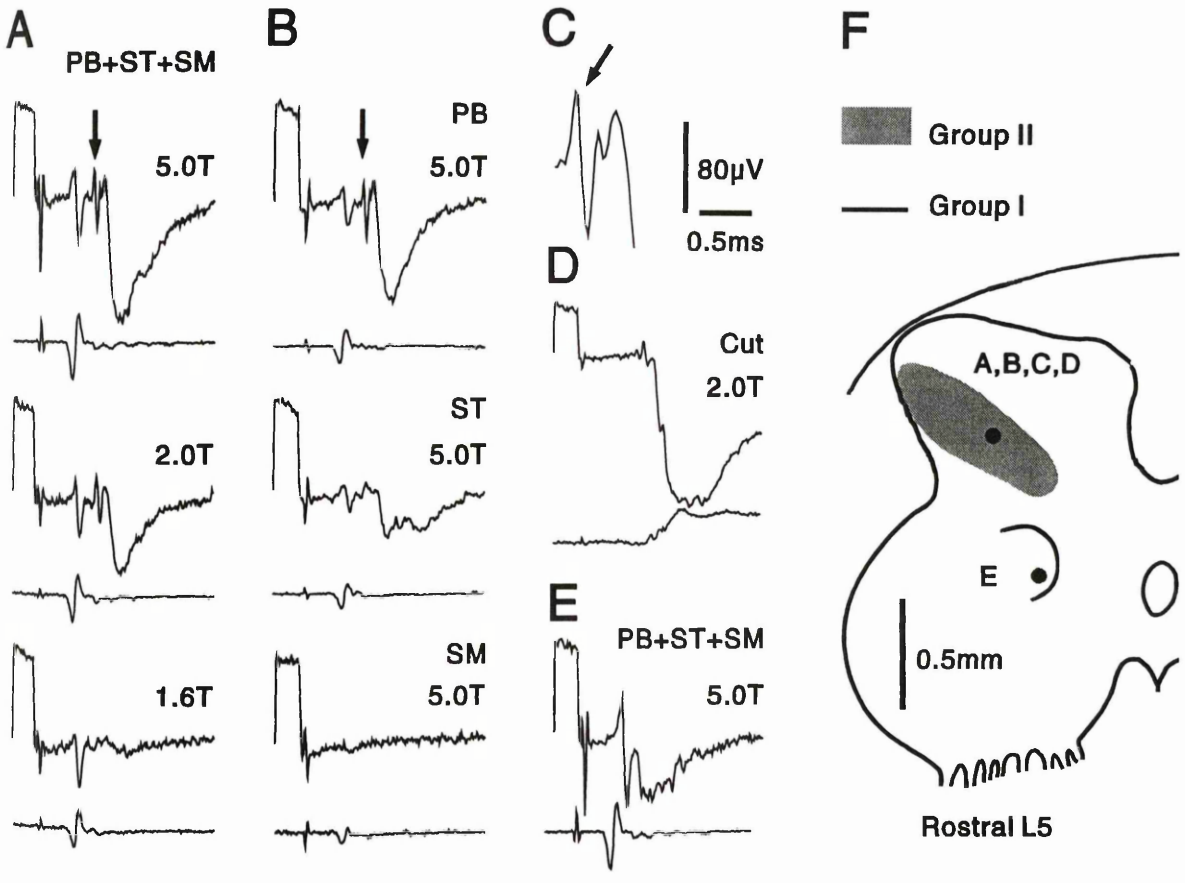

A
PB+ST+SM
5.0T
2.0T
1.6T
B
PB
5.0T
ST
5.0T
SM
5.0T
C
80μV
0.5ms
D
Cut
2.0T
E
PB+ST+SM
5.0T
F
Group II
Group I
A,B,C,D
E
0.5mm
Rostral L5

Fig. 15. Field potentials evoked by combined stimulation of branches of the hamstring nerve. A, field potentials evoked by stimuli of different strengths. B, terminal potential indicated by the arrow in A, shown at a faster time base and higher amplification. C, field potentials evoked by stimuli of different strengths at a different location from the potentials shown in A. D, axonal potential indicated by the arrow in C, shown at a faster time base. All recordings are averages of 32 sweeps. Arrows indicate the onset of terminal potentials (biphasic, A & B) or axonal potentials (triphasic, C & D).

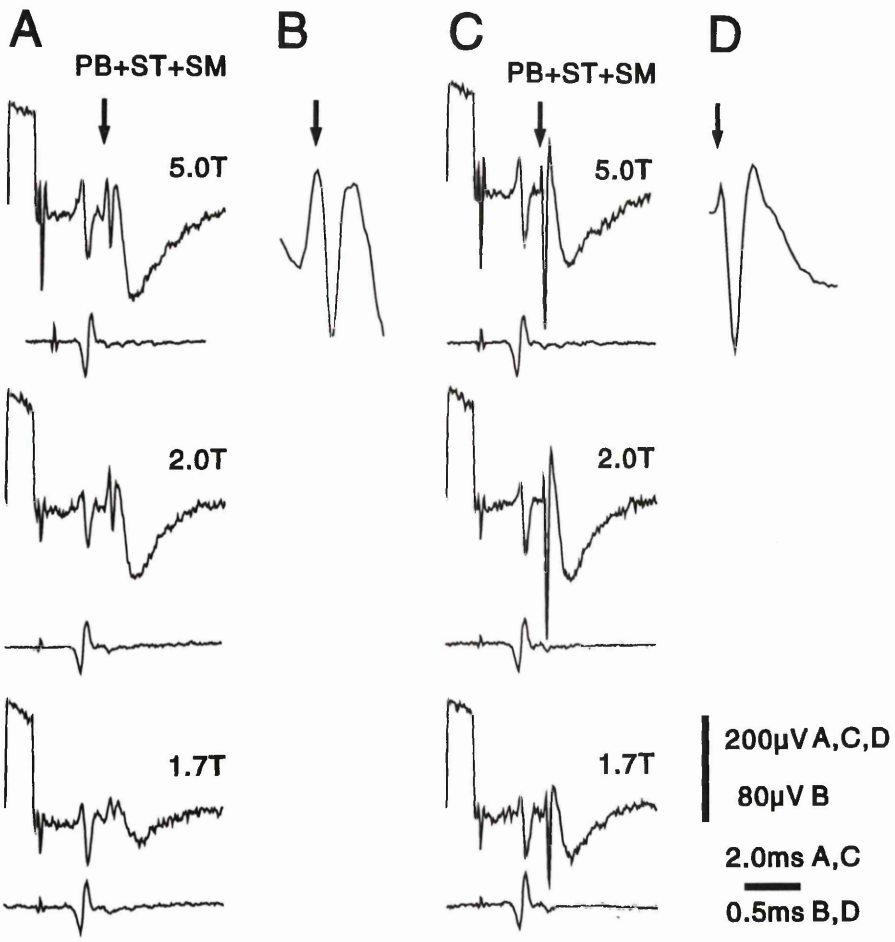

A
PB+ST+SM
5.0T
2.0T
1.7T
B
C
PB+ST+SM
5.0T
2.0T
1.7T
D
200μV A,C,D
80μV B
2.0ms A,C
0.5ms B,D

Fig. 16. Field potentials evoked by muscle branches of the hamstring nerve at the level of the motonucleus. A, field potential evoked by group I afferents in the intermediate region. B, and C, field potentials evoked by Renshaw neurones (note characteristic ripples produced by repetitive discharge pattern typical of Renshaw cells). The field shown in C was recorded at a location where the motonucleus field could also be recorded. D, motonucleus field. Note that this recording is shown at a lower gain than those above it. All recordings are averages of 32 sweeps. E, outline of the spinal cord at caudal L4 showing the recording locations of the potentials shown in A - D. Also shown are the regions in which field potentials (>100μV) were recorded in the intermediate region (shaded area), the region in which Renshaw fields were recorded (dash line) and the region where motonucleus fields were recorded (solid line).

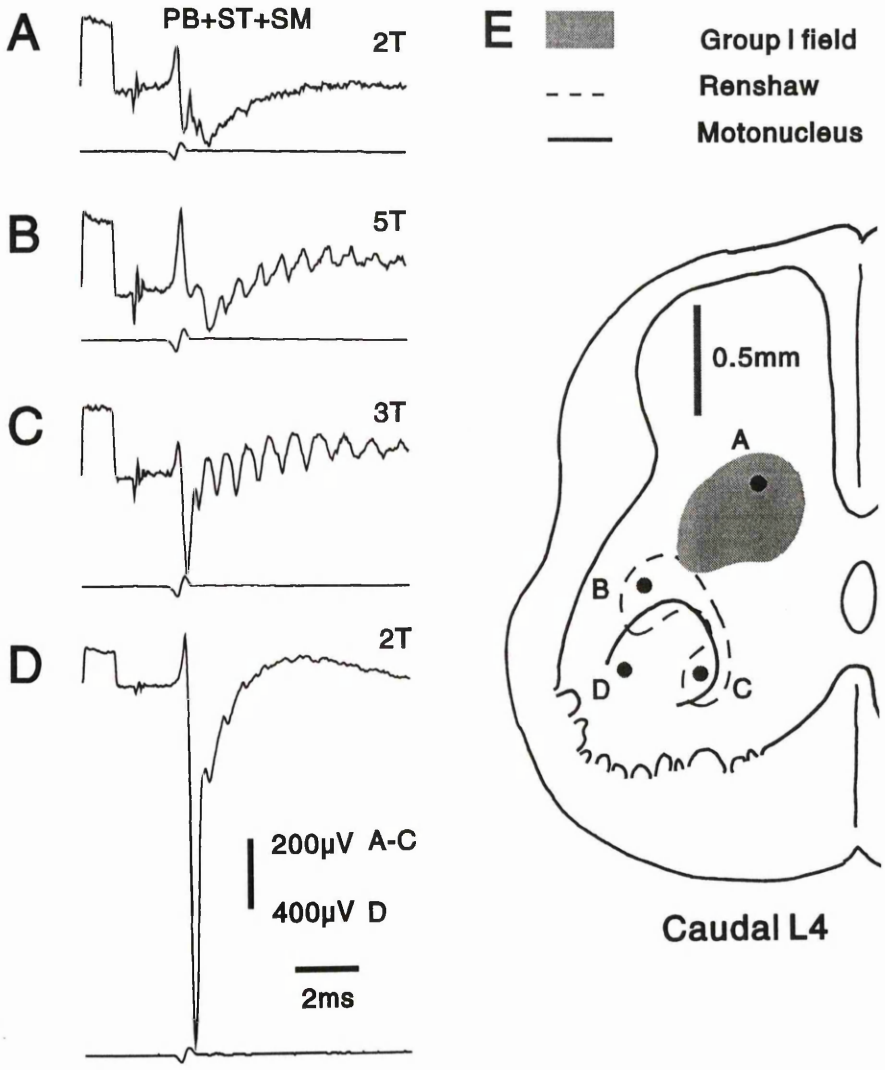

A
PB+ST+SM
2T
B
5T
C
3T
D
2T
200μV A-C
400μV D
2ms
E
Group I field
Renshaw
Motonucleus
0.5mm
A
B
C
D
Caudal L4

Fig. 17. Field potentials evoked by afferents of the LGS nerve at the border between the L5 and L6 segments. A, field potentials evoked by stimuli of different strengths. B, C & D, field potentials evoked by stimuli of 5T recorded at progressively deeper locations. Note that the field potentials shown in A are produced by group II afferents alone, those in B and C by group I (I) and group II (II) afferents and those in D by group I afferents alone. E, potentials evoked by a train of stimuli, same recording location as A; note the lack of change in amplitude of the group II field potential with successive stimuli. Calibration pulse; 200μV, 1 ms (A - D) and 2 ms (E). All recordings are averages of 32 sweeps. F, outline of the spinal cord showing the area in which group II and group I field potentials (>100μV) were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A - D are indicated.

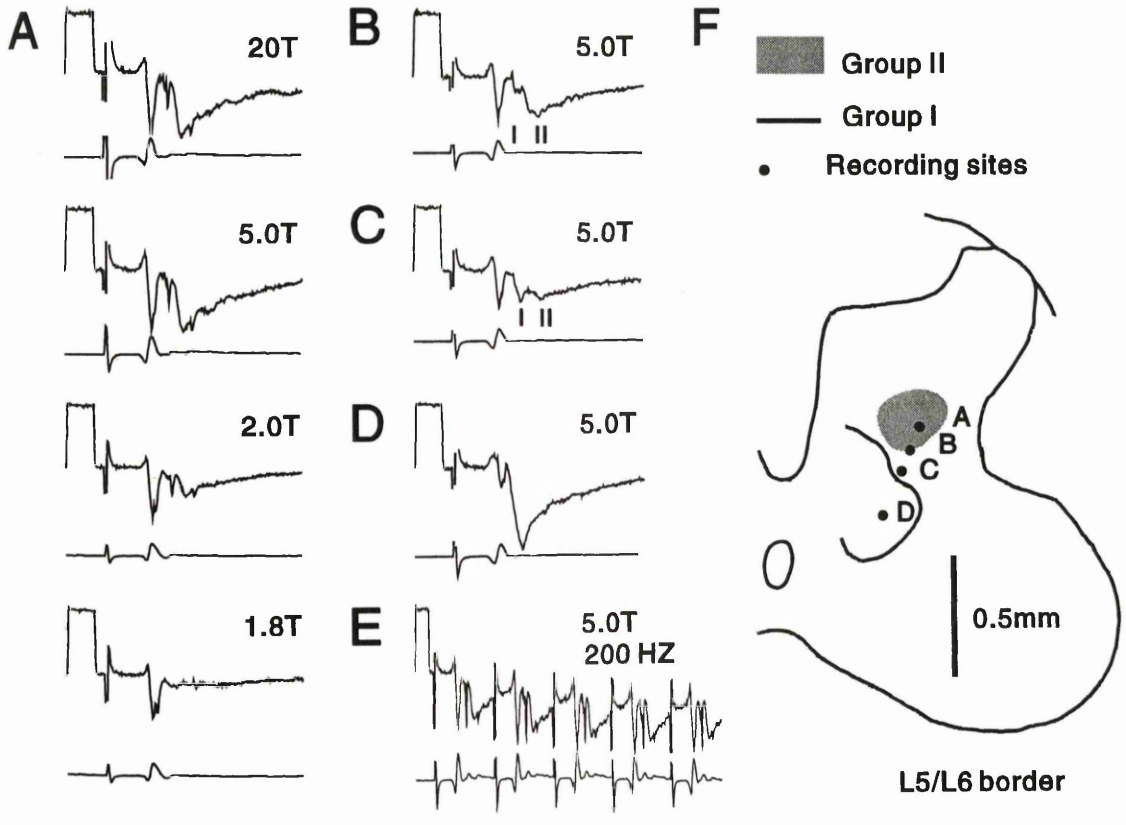

A
20T
5.0T
2.0T
1.8T
B
5.0T
I II
C
5.0T
I II
D
5.0T
E
5.0T
200 HZ
F
Group II
Group I
Recording sites
A
B
C
D
0.5mm
L5/L6 border

Fig. 18. Field potentials evoked by afferents of the GS nerve at the border between the L5 and L6 segments. A, field potentials evoked by stimuli of different strengths. B, C & D, field potentials evoked by stimuli of 5T recorded at deeper locations. Note that the field potentials shown in A are produced by group II afferents alone (terminal potentials shown by arrow). In B, field potentials are produced by group I afferents alone. C, field potentials evoked by Renshaw neurones (note characteristic ripples produced by repetitive discharge pattern typical of Renshaw cells). The field shown in C was recorded at a location where the motonucleus field could also be recorded. D, motonucleus field. Note that this recording is shown at a lower gain than those above it. All recordings are averages of 32 sweeps. E, outline of the spinal cord showing the area in which group II and group I field potentials (>100μV) were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A - D are indicated.

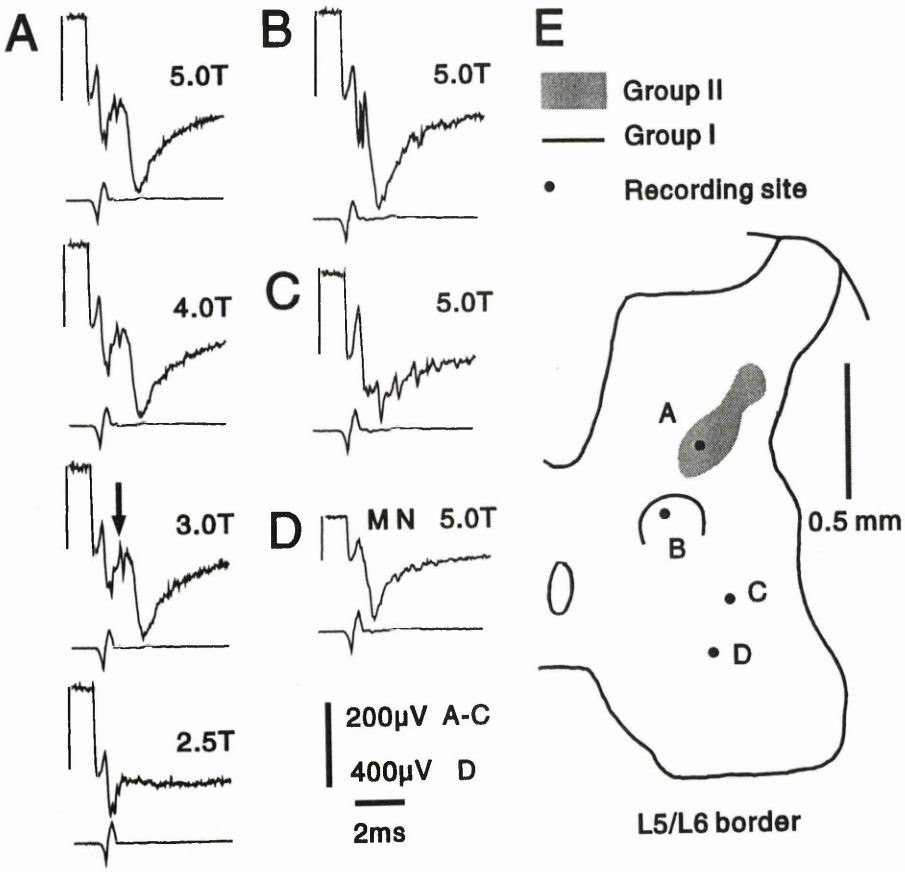

A
5.0T
4.0T
3.0T
2.5T
B
5.0T
C
5.0T
D
M N 5.0T
200μV A-C
400μV D
2ms
E
Group II
Group I
Recording site
A
B
C
D
0.5 mm
L5/L6 border

Fig. 19. Field potentials evoked by afferents of the plantaris (Pl) nerve at rostral L5. A, field potentials evoked by stimuli of 5T but not at 2T. B, field potentials evoked by stimuli 5T recorded at deeper locations. Note that the field potentials shown in A are produced by group II afferents alone and in B by group I afferents alone. Calibration pulse; 200μV, 1 ms. All recordings are averages of 32 sweeps. C, outline of the spinal cord showing the area in which group II and group I field potentials (>100μV) were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A & B are indicated.

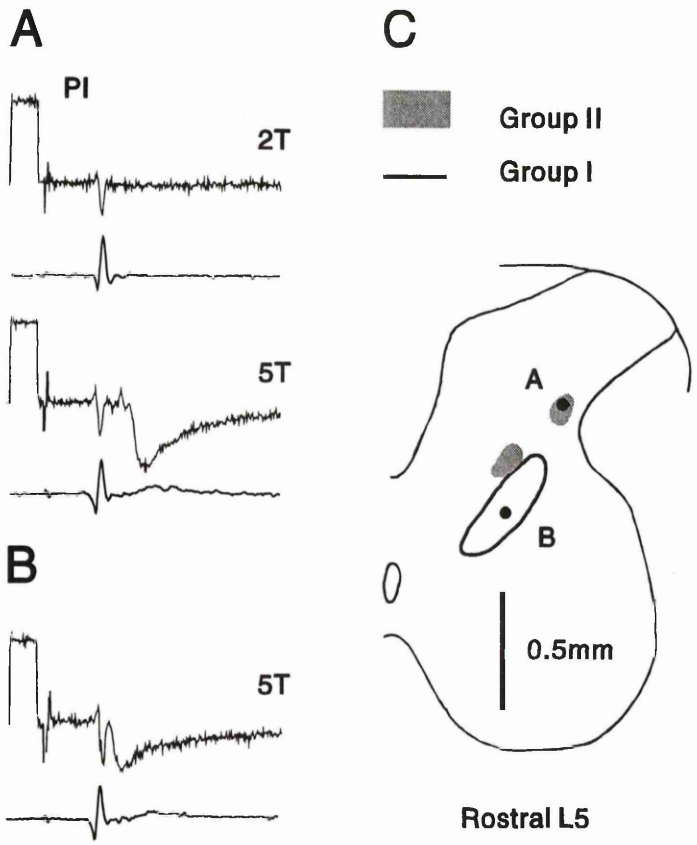

A
PI
2T
5T
B
5T
C
Group II
Group I
A
B
0.5mm
Rostral L5

Fig. 20. Cord dorsum potentials (CDPs) evoked by afferents of tibialis posterior (TP) or tibialis posterior and popliteal nerves (TP - Pop) stimulated together. A and B, recordings from the surface of the spinal cord following stimuli of different strengths applied to TP (A, L3 - L4 border) and TP - Pop (B, mid L4) nerves. All recordings are averages of 32 sweeps. Arrow in B indicates group II afferent volley. C, plot of amplitudes of CDPs evoked by stimuli 5T applied to the TP nerve, recorded at 1 mm intervals along the spinal cord in one experiment. D, locations of CDPs evoked in five animals by stimuli 5T applied to the TP or TP - Pop nerves. Arrow heads indicate the positions where the largest potentials of group II origin were recorded; two separate peaks were consistently observed. Black bars indicate the length over which potentials were at least 75% of the larger of the two peaks.

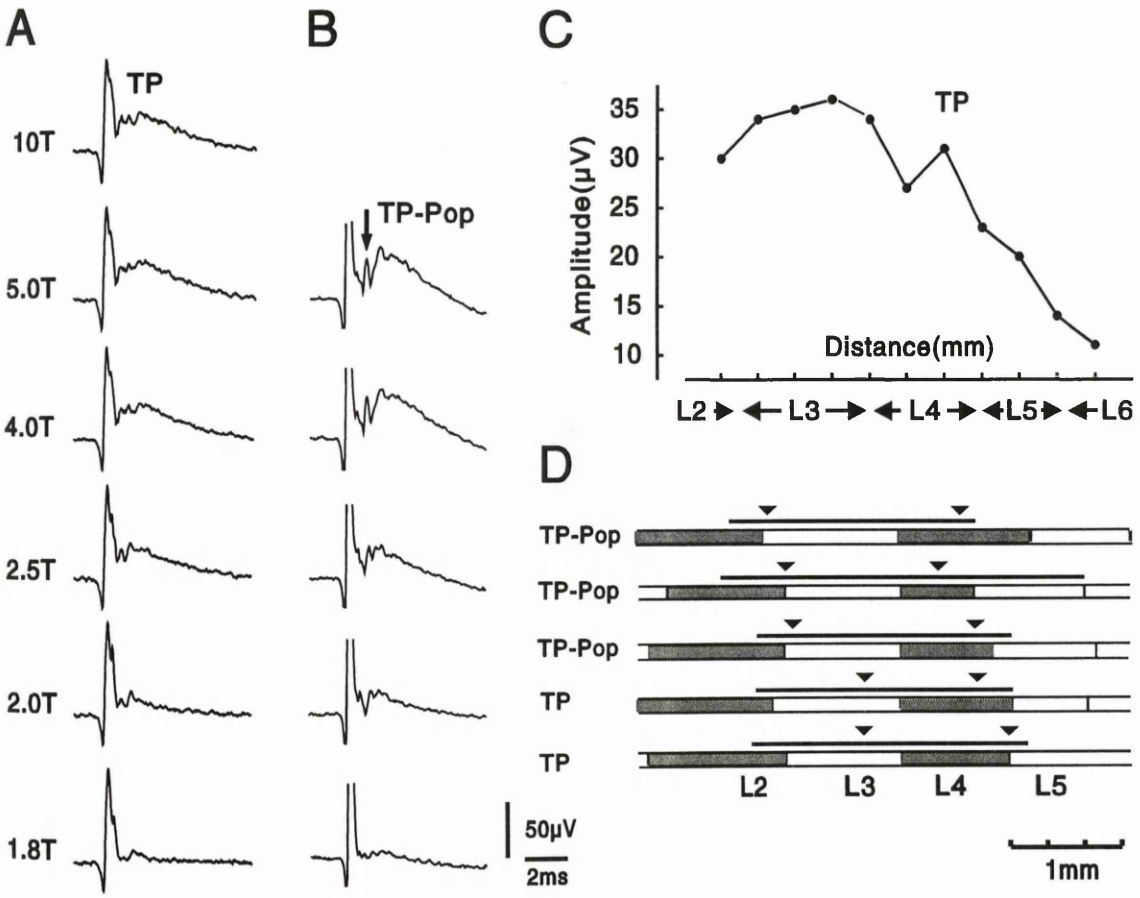

A
B
C
D
TP
10T
5.0T
4.0T
2.5T
2.0T
1.8T
TP-Pop
50μV
2ms
Amplitude(μV)
35
30
25
20
15
10
Distance(mm)
L2
L3
L4
L5
L6
TP-Pop
TP-Pop
TP-Pop
TP
TP
L2
L3
L4
L5
1mm

Fig. 21. Cord dorsum potentials (CDPs) evoked by afferents of popliteus (Pop) muscle nerve. A, recordings from the surface of the spinal cord at caudal L3 following stimuli of different strengths. Note that stimulation of this nerve produced smaller CDPs than the TP nerve. All recordings are averages of 32 sweeps. B, plot of amplitudes of CDPs evoked by stimuli 5T, recorded at 1 mm intervals along the spinal cord in one experiment. C, locations of CDPs evoked in two animals by stimuli 5T. Arrow heads indicate the positions where the largest potentials of group II origin were recorded. Black bars indicate the length over which potentials were at least 75% of the larger of the peak amplitude.

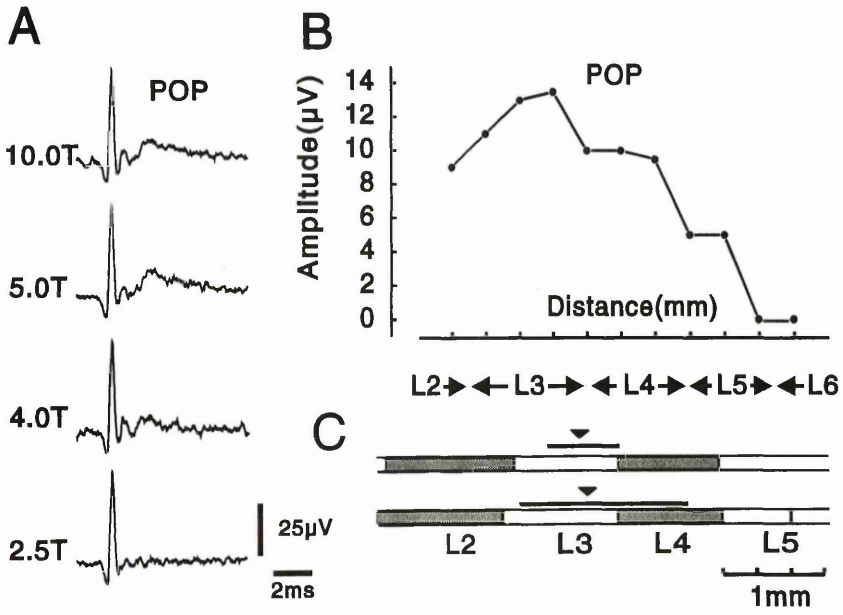

A
POP
10.0T
5.0T
4.0T
2.5T
25μV
2ms
B
POP
Amplitude(μV)
14
12
10
8
6
4
2
0
Distance(mm)
L2
L3
L4
L5
L6
C
L2
L3
L4
L5
1mm

Fig. 22. Cord dorsum potentials (CDPs) evoked by afferents of flexor digitorum longus (FDL) muscle nerve. A, recordings from the surface of the spinal cord at rostral L5 segment following stimuli of different strengths. Note that stimulation of this nerve produced a negligible CDPs. All recordings are averages of 32 sweeps. B, plot of amplitudes of CDPs evoked by stimuli 5T, recorded at 1 mm intervals along the spinal cord in one experiment. C, locations of CDPs evoked in seven animals by stimuli 5T. Arrow heads indicate the positions where the largest potentials of group II origin were recorded.

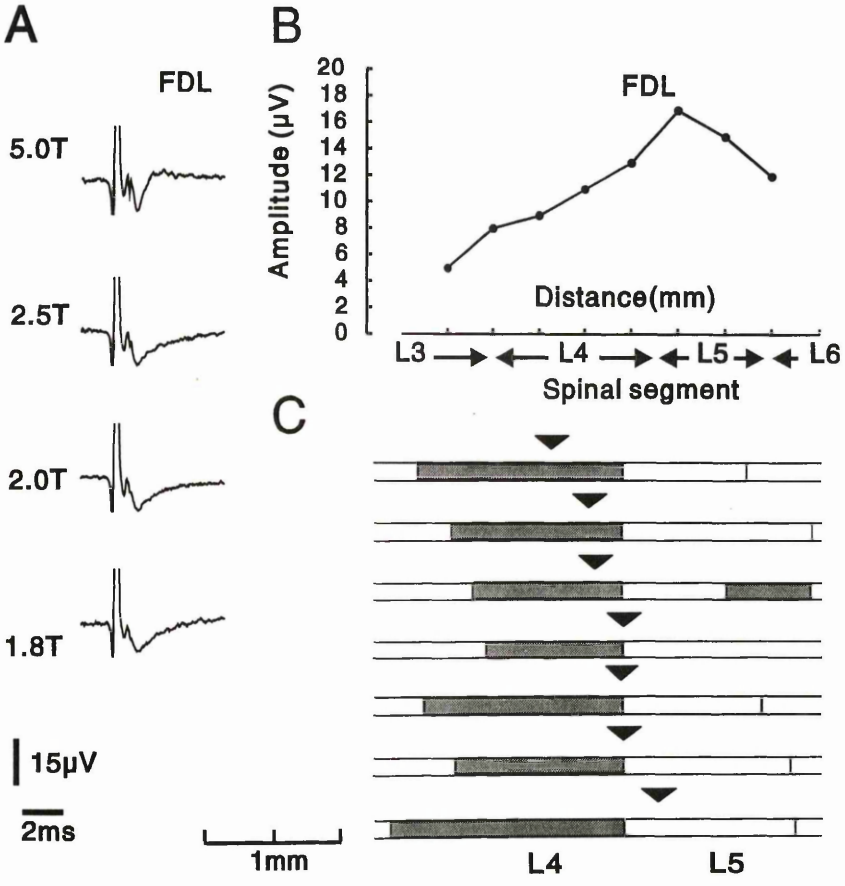

A
FDL
5.0T
2.5T
2.0T
1.8T
15μV
2ms
B
Amplitude (μV)
20
18
16
14
12
10
8
6
4
2
0
FDL
Distance(mm)
L3
L4
L5
L6
Spinal segment
C
1mm
L4
L5

Fig. 23. Field potentials evoked by afferents of the tibialis - posterior nerve in the caudal half of the L3 segment. A, B, C & D, field potentials evoked by stimulation of the TP nerve at different strengths and recorded at different locations (shown in E). The field potentials shown in A and C were produced by group II afferents alone, those in B by group I (I) and group II (II) afferents and those in D by group I afferents alone. Calibration pulse; 200μV, 1 ms. All recordings are averages of 32 sweeps. E, outline of the spinal cord showing the area in which group II and group I field potentials (>100μV) were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A - D are indicated.

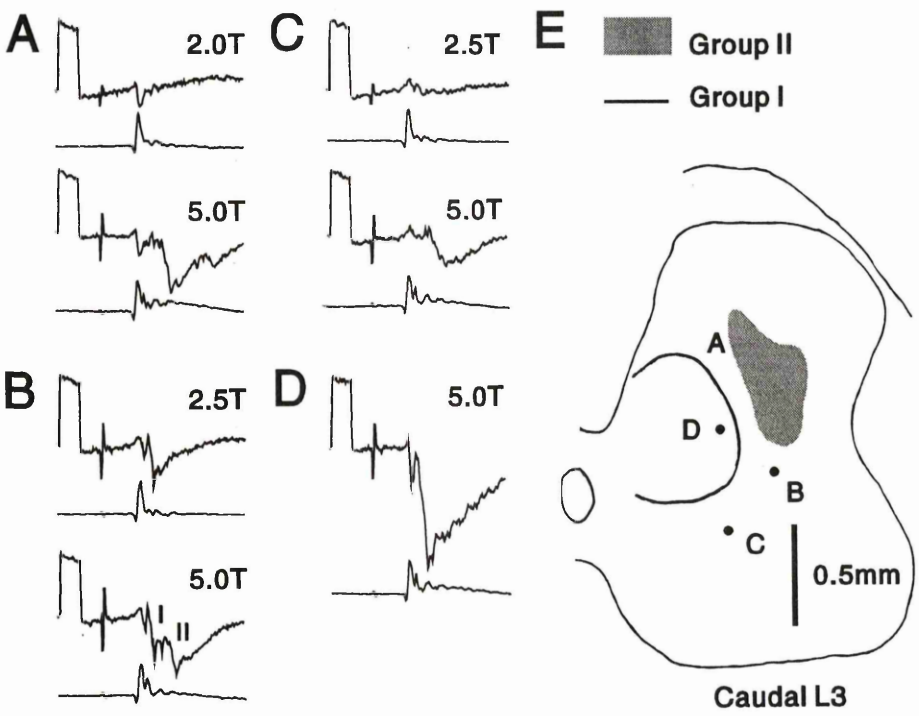

A
2.0T
5.0T
C
2.5T
5.0T
B
2.5T
5.0T
I
II
D
5.0T
E
Group II
Group I
A
D
B
C
0.5mm
Caudal L3

Fig. 24. Field potentials evoked by afferents of the FDL nerve in rostral L5. A, field potentials produced by group II afferents alone. B, field potentials produced by group I afferents alone. Calibration pulse; 200μV, 1 ms. All recordings are averages of 32 sweeps. C, outline of the spinal cord showing the area in which group II (79μV) and group I (>100μV) field potentials were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A and B are indicated.

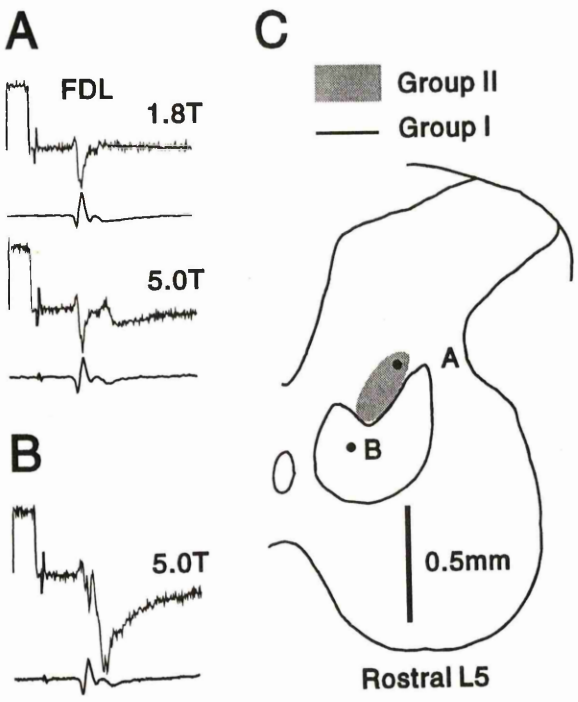

A
FDL
1.8T
5.0T
B
5.0T
C
Group II
Group I
A
B
0.5mm
Rostral L5

Fig. 25. Field potentials evoked by afferents of the FDL nerve in rostral L4. A, B and C, field potentials evoked by stimulation of the FDL nerve at different strengths and recorded at different locations (shown in C). The field potentials shown in A were produced by group I afferents. B and C, motonucleus field. Calibration pulse; 200μV, 1 ms. All recordings are averages of 32 sweeps. C, outline of the spinal cord showing the area in which group I field potentials and motonucleus fields were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A, B and C are indicated.

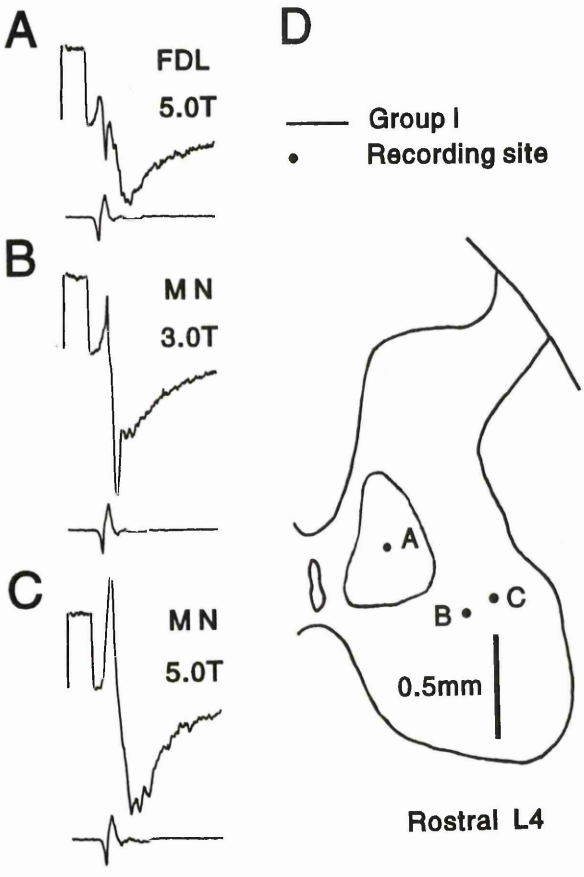

A
FDL
5.0T
B
M N
3.0T
C
M N
5.0T
D
Group I
Recording site
A
B
C
0.5mm
Rostral L4

Fig. 26. Field potentials evoked by afferents of the gastrocnemius - soleus nerve in the rostral part of the L4 segment. A, B & C, group II field potentials recorded at three closely spaced locations (shown in E) in the dorsal horn. Note the clear axon potential in B (arrow). D, field potential evoked more ventrally by group I afferents of the same nerve. All recordings are averages of 32 sweeps. E, outline of the spinal cord showing the area in which group II and group I field potentials (>100μV) were recorded during systematic tracking in the grey matter. The recording sites for the potentials shown in A - D are indicated.

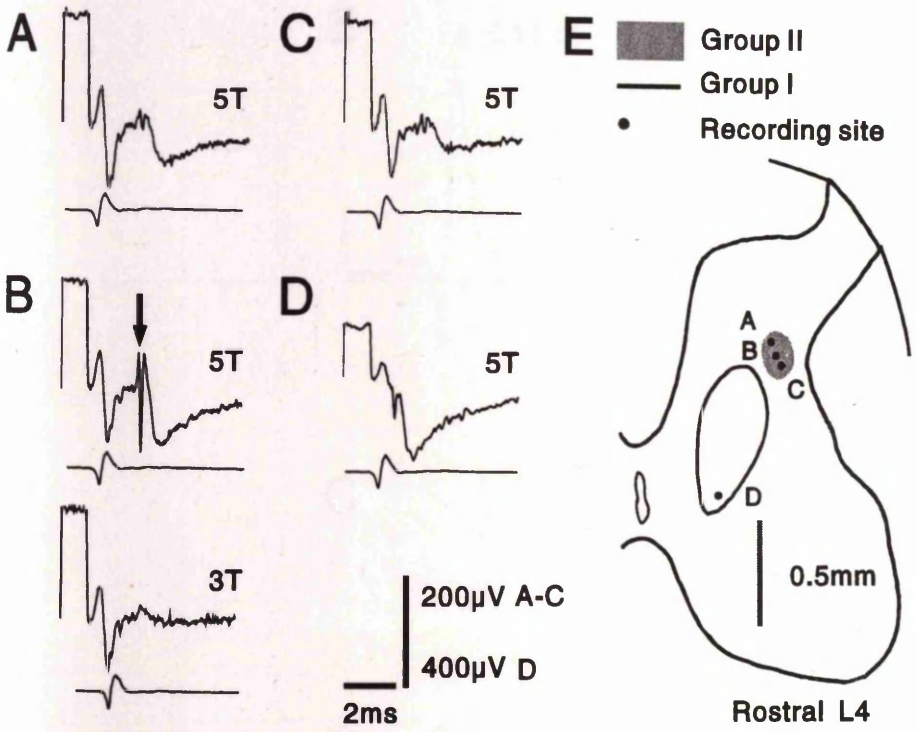

A
5T
C
5T
B
5T
3T
D
5T
200μV A-C
400μV D
2ms
E
Group II
Group I
Recording site
A
B
C
D
0.5mm
Rostral L4

Fig. 27. Single fibre recordings. A, B and C, single fibre recordings made from teased dorsal root following stimuli of 5T applied to the LGS nerve. Thresholds and conduction velocities for individual fibre as denoted. A and B are single sweep recordings while C is an average of 16 sweeps; amplitude and time calibration as indicated.

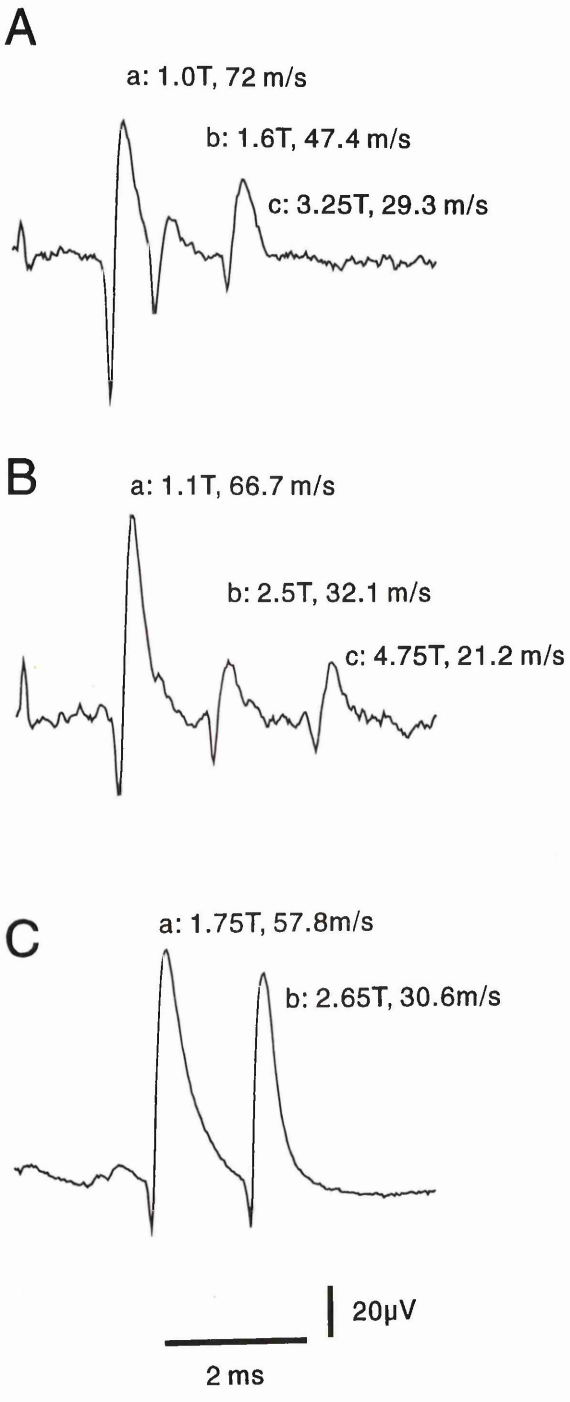

A
a: 1.0T, 72 m/s
b: 1.6T, 47.4 m/s
c: 3.25T, 29.3 m/s
B
a: 1.1T, 66.7 m/s
b: 2.5T, 32.1 m/s
c: 4.75T, 21.2 m/s
C
a: 1.75T, 57.8m/s
b: 2.65T, 30.6m/s
20μV
2 ms

Fig. 28. Afferent fibre composition of the LGS nerve. Rows A - D show data obtained in four experiments in which the conduction velocities and thresholds of as many as possible of the afferent fibres in the LGS nerve activated by stimuli of up to 5T were determined. The histograms on the left show the distribution of the samples of fibres according to conduction velocity (5 m/s bins). Note that in each nerve there was a tendency for a bimodal distribution with a dividing line of about 55 m/s fitting three of the four nerves. The plots on the right show data for the threshold and conduction velocity of each fibre (a few fibres for which accurate measurements of threshold were not made are omitted). Curves best fitting the data points were drawn using regression analysis. The numbers of afferent fibres sampled from each nerve are indicated.

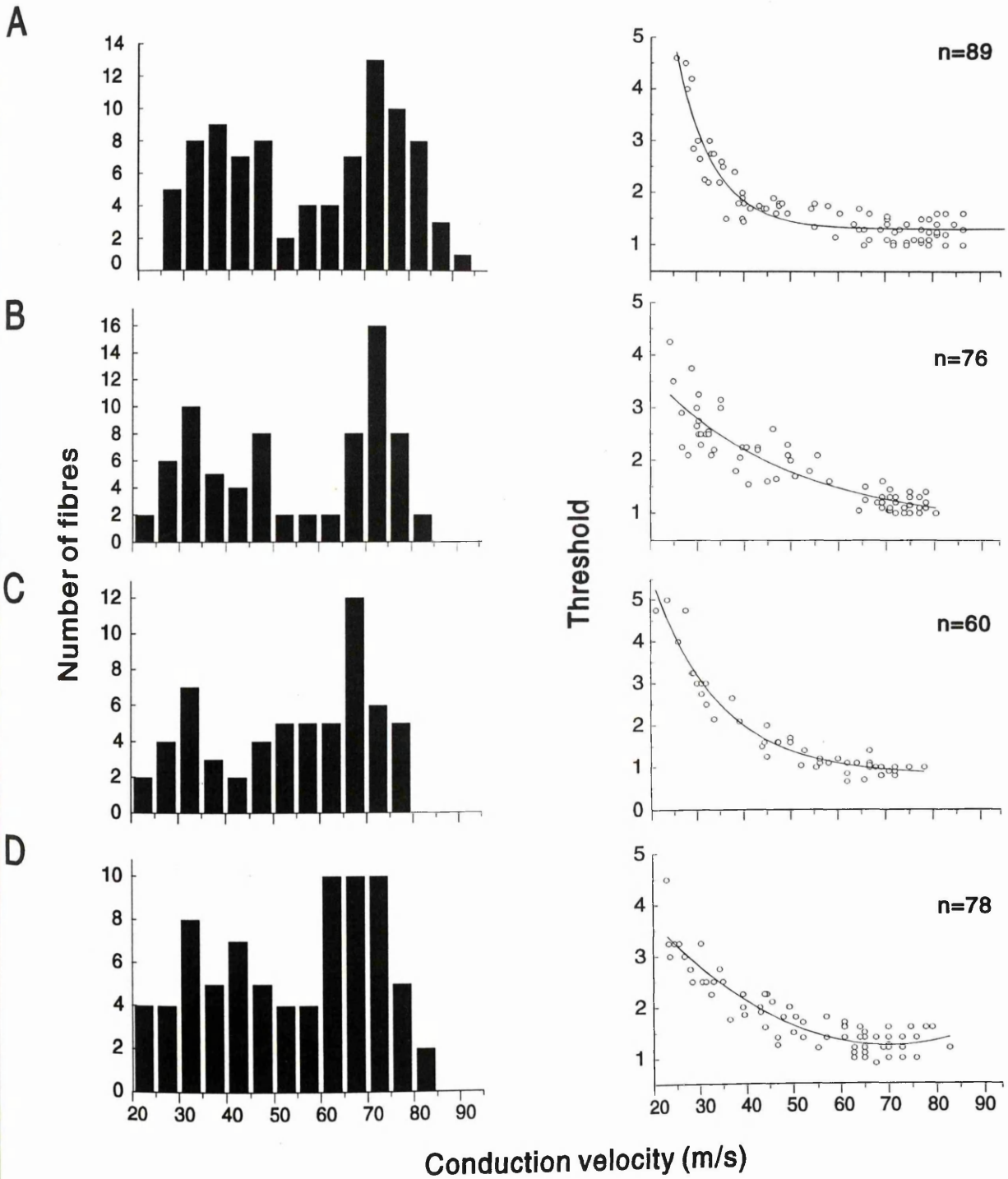

A
B
C
D
Number of fibres
Threshold
n=89
n=76
n=60
n=78
Conduction velocity (m/s)

Fig. 29. Diagram summarising the organisation of group II neuronal systems within the lumbar enlargement of the rat spinal cord. Group II field potentials were recorded in three regions in the rostral, caudal and central lumbar segments. The main muscle nerves containing group II afferents terminating in each of these regions are indicated together with an indication of whether contacts were made with neurones in the dorsal horn, intermediate area or both. The dashed lines represent the weaker projection of group II afferents with actions in the rostral lumbar or caudal lumbar segments, to the central lumbar region. This later information is extrapolated from the cat (see chapter 3.0) except for the action of GS which was examined in the central lumbar segments of the rat spinal cord as well as more caudally. Also shown is the relationship between the termination of group II muscle afferents and the level of the Q, BST, GS and pudendal motor columns.

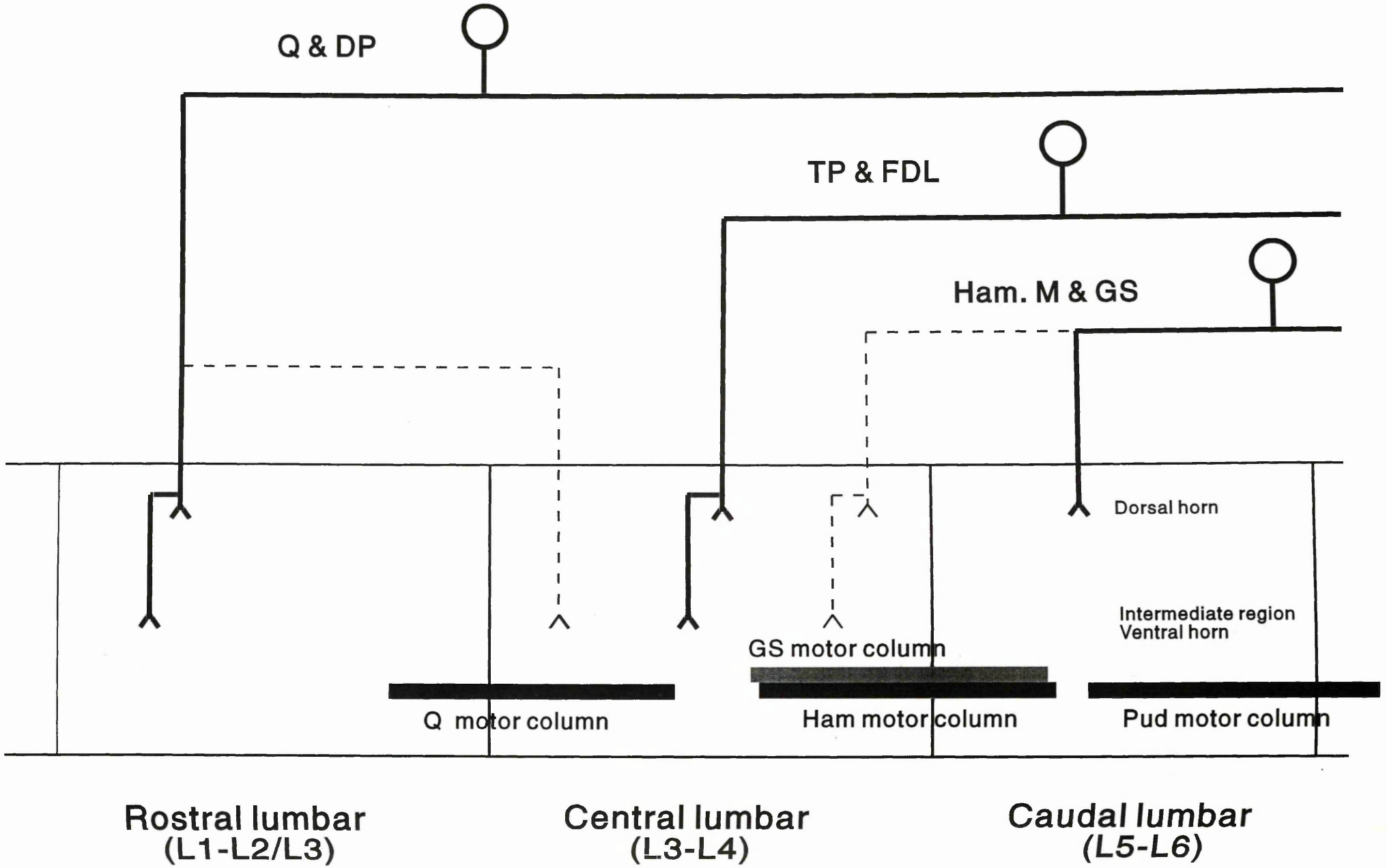

Q & DP
TP & FDL
Ham. M & GS
Dorsal horn
Intermediate region
Ventral horn
GS motor column
Q motor column
Ham motor column
Pud motor column
Rostral lumbar
(L1-L2/L3)
Central lumbar
(L3-L4)
Caudal lumbar
(L5-L6)

Table 1. Latencies of group II CDPs measured with respect to the stimulus artefact, group I and group II afferent volleys. Latency values represent the shortest (i.e. minimal) latencies seen in each animal. Mean maximal amplitudes of cord dorsum potentials are shown in the far right column. The mean values shown are calculated from values for the largest potentials seen in each experiment. FDL and Pop evoked minimal group II CDPs and so are not included in the table (see text).

Table 1. Latencies and amplitudes of group II cord dorsum potentials.

Nerve	Lat. from stimulus (ms)			Lat. from group I volleys (ms)			Lat. from group II volleys (ms)			Mean maximal amplitude (μV)
	mean	range	n	mean	range	n	mean	range	n	
Q	2.18	2.06 - 2.27	4	1.10	1.0 - 1.20	4	-	0.42, 0.48	2	104
DP	3.38	3.04 - 3.80	3	1.31.	1.02 - 1.85	3	0.65	0.55 - 0.70	3	48
Hamstring	2.62	2.45 - 2.90	4	1.28	1.10 - 1.45	4	-	0. 84	1	34
GS/LGS	3.05	2.70 - 3.25	8	1.47	1.30 - 1.70	8	-	0.82	1	40
TP/TP-Pop	3	2.70 - 3.10	5	1.23	1.10 - 1.50	5	0.53	0.45 - 0.77	5	36

Table 2 A & B show data for the latencies and amplitudes of field potentials evoked by group II afferents of different nerves in the dorsal horn (A) and intermediate region and ventral horn (B) of lumbar segments. The latency and amplitudes measurements were made during systematic transverse mapping of the grey matter within appropriate segments of the spinal cord. Latency values represent minimal latencies observed i.e. the shortest single observation from one of many recordings made at different locations in a single animal. Amplitude measurements represent the largest single value seen in each animal

Table 2 A . Latencies and amplitudes of group II field potentials in the dorsal horn.

Nerve	Lat. from stimulus (ms)			Lat. from group I volleys (ms)			Lat. from group II volleys (ms)			Amplitude (μV)		
	mean	range	n	mean	range	n	mean	range	n	mean	range	n
Q	2.18	2.07 - 2.30	3	1.13	1.07 - 1.20	3	0.60	0.54 - 0.67	3	488	297 - 606	3
Ham	-	2.37,2.75	2	-	1.15,1.32	2	-	0.89,0.72	2	-	222, 310	2
GS/LGS	2.73	2.50 - 2.82	4	1.2	1.10 - 1.40	4	0.55	0.45 - 0.75	4	170	81 - 211	4
TP/TP-Pop	2.92	2.77 - 3.12	3	1.20	1.10 - 1.27	3	0.51	0.40 - 0.57	3	145	80 - 192	3

Table 2 B. Latencies and amplitudes of group II field potentials in the intermediate region / ventral horn.

Nerve	Lat. from stimulus (ms)			Lat. from group I volleys (ms)			Lat. from group II volleys (ms)			Amplitude (μV)		
	mean	range	n	mean	range	n	mean	range	n	mean	range	n
Q	3.2	2.95 - 3.40	4	2.27	2.0 - 2.45	4	1.74	1.47 - 1.92	4	263	50 - 464	4
GS/LGS	-	2.82	1	-	1.2	1	-	0.55	1	-	91	1
TP/TP-Pop	3.12	2.77 - 3.50	4	1.40	1.22 - 1.52	4	0.71	0.52 - 0.95	4	141	130 - 192	4

Table 3 A & B. The columns from left to right show nerve evoking fibre potentials (axonal and terminal potentials), latencies of fibre potentials measured with respect to group I volley, interval between fibre potential and field potential, duration and amplitude of fibre potential.

Table 3 A. latency, duration and amplitude of axonal potentials.

Nerve	Lat. from group I volley (ms)	AP - field pot. interval (ms)	Duration (ms)	Amplitude (μV)
GS	1.2	0.45	0.3	174
GS	0.8	0.25	0.3	88
GS	1.62	0.3	0.25	128
GS	0.9	0.32	0.3	130
FDL	1	0.42	0.25	45
FDL	0.9	0.3	0.3	30
FDL	0.9	0.5	0.25	38
TP-Pop	0.65	0.35	0.2	150
TP	0.75	0.52	0.15	130
Ham.	0.72	0.32	0.27	74
-	-	mean, 0.37	mean, 0.26	mean, 99
-	-	n=10	-	-

Table 3 B. latency, duration and amplitude of terminal potentials.

Nerve	Lat. from group I volley (ms)	TP - field pot. interval (ms)	Duration (ms)	Amplitude (μV)
GS	0.65	0.37	0.4	61
TP	0.75	0.37	0.45	96
Ham	0.82	0.45	0.4	63
-	-	mean, 0.39	mean, 0.41	mean, 73
-	-	n=3	-	-

2.0.4. Discussion

Comparison of topographical organisation in cat and rat

Rostro - caudal distribution. A notable feature of the organisation of group II reflex circuits in the cat spinal cord is that group II afferents in nerves from different hind - limb muscles exert their strongest synaptic actions within particular segments of the lumbosacral enlargement. The results of the present study show that group II afferents of different muscle nerves of the rat hind - limb also have their particular sites of action in the spinal cord and furthermore, that their projections to different segments show an organisation remarkably similar to that described for the cat.

In the cat, group II afferents of Q and pretibial flexor muscles very powerfully excite neurones within the L4 and L5 segments (Edgley & Jankowska, 1987 a, b), but have weaker actions on neurones within the sacral segments (Jankowska & Riddell, 1993, 1994). Conversely, group II afferents of GS and PBST very strongly excite neurones in sacral segments (Jankowska & Riddell, 1993, 1994) but have weaker actions in the L6 and L7 segments (Fu et al. 1974) and are virtually without effect within L4 and L5 (Edgley & Jankowska, 1987 a, b). In effect therefore, the actions of group II afferents of Q and DP are concentrated at the rostral end of the lumbosacral enlargement (midlumbar segments), while the actions of GS and PBST are concentrated at the caudal end of the lumbosacral enlargement (sacral segments). In the rat, although different spinal segments are involved, the same general organisation is seen. Group II afferents of Q and DP produce

their strong actions in L1, L2 and the rostral half of L3 which represent the rostral end of the lumbar enlargement. In contrast, the strongest synaptic actions of group II afferents of GS and hamstring muscles were concentrated at the caudal end of the lumbar enlargement in the L5 and L6 segments.

Further confirmation that the organisation of group II reflex circuits shows the same rostro - caudal plan in the two species is provided by the relationship between the location of group II relay regions and specific motoneurone columns. The later provide internal landmarks of neuronal organisation within the spinal cord which are common to both species. In the cat spinal cord, group II afferents of the sacral relay region produce their greatest actions close to the rostral end of the pudendal motor column (Jankowska & Riddell, 1993). In the present study it was established that the same close relationship exists between the locations where the largest surface potentials produced by group II afferents of GS and hamstring muscles are recorded and the histologically determined rostral ends of the pudendal motor columns (see Jordan, Breedlove & Arnold, 1982; McKenna & Nadelhaft, 1986).

Distribution of field potentials in the grey matter. In addition to their characteristic projection to particular target segments, group II afferents showed a characteristic pattern of terminaticn within the spinal grey matter. In the rostral lumbar segments of the rat, group II afferents of the Q nerve were found to produce field potentials in two main areas; large field potentials were recorded within deep laminae of the dorsal horn while

smaller group II potentials, sometime preceded by group I potentials were recorded in areas of the intermediate zone and ventral horn. This pattern of actions fits well with the actions of group II afferents in the midlumbar segments of the cat spinal cord which present results indicate correspond with the upper lumbar region of the rat (see above). In the cat midlumbar cord, group II muscle afferents also contact neurones in both the dorsal horn and more ventrally within the intermediate zone and ventral horn (Edgley & Jankowska, 1987a). In the dorsal horn several groups of neurones processing information from group II muscle afferents have been identified, including neurones belonging to ascending tracts (Edgley & Jankowska, 1988; Edgley & Gallimore, 1988; Jankowska, Hammer & Szabo - Läckberg, 1995) and interneurones with mainly local actions (Edgley & Jankowska, 1987b; Bras et al. 1989). In parts of the intermediate zone and ventral horn, areas of termination of group II afferents overlap with those of group I muscle afferents. As a result many of the neurones located here receive convergent excitatory input from both group I and group II muscle afferents (Edgley & Jankowska, 19987a, b). Among them are a population of group II - activated last - order, pre - motor interneurones. These interneurones, some of which are excitatory and others inhibitory, have descending axons which project to the lower - lumbar segments where they contact motoneurones (Bras, et al. 1989; Cavallari et al. 1987). Although the properties of single group II - activated neurones in the rat spinal cord were not investigated, the close similarity of the sites at which group II afferents evoke synaptic

potentials in the upper lumbar segments of the rat and midlumbar segments of the cat suggests the existence of similar groups of neurones processing information from group II muscle afferents in both species.

In the L5 and L6 segments of the rat spinal cord the termination of group II afferents of GS and hamstring muscles shows a quite different pattern to that of Q afferents in the upper lumbar segments. The group II field potentials recorded here are confined mainly to the dorsal horn with small potentials occurring only in very restricted regions close to the border with L4. This corresponds to the pattern of actions of group II afferents seen in the sacral group II relay region of the cat spinal cord which is organised rather differently to that in the midlumbar segments (Jankowska & Riddell, 1993). As in the rat, synaptic potentials produced by PBST and GS group II afferents are confined to the dorsal horn although small fields occur in more ventral regions of the grey matter as the lower - lumbar segments are approached (Jankowska & Riddell, 1993). Target neurones of group II afferents in cat sacral dorsal horn include ascending neurones of the spinocervical tract (Riddell, Jankowska, Hammer & Szabo - Läckberg, 1994) and interneurones with mainly local actions confined to nearby segments (Jankowska & Riddell, 1994; Jankowska, Riddell, Szabo - Läckberg & Hammer, 1993b). There is evidence that some of the dorsal horn neurones may be responsible for mediating presynaptic inhibition of group II muscle afferents (Riddell, Jankowska & Huber, 1995; Jankowska & Riddell, 1995).

Amplitudes of potentials. One consistent difference between group II potentials observed in the rat and those previously reported in the cat was in their amplitudes. Both CDPs and field potentials recorded in the rat spinal cord were of considerably smaller size than their counterparts in the cat. For example, in the cat, field potentials recorded in the dorsal horn of midlumbar segments following stimulation of the Q nerve can be more than a 1 mV in amplitude (Edgley & Jankowska, 1987a). In comparison, the largest potentials evoked by the same afferents in the dorsal horn of the L1 and L2 segments in the rat were around 600 μV. Similarly, the largest group II field potentials evoked by PBST and GS afferents in the dorsal horn of sacral segments in the cat are between 400 - 600μA (Jankowska & Riddell, 1993) whereas in the equivalent region of the rat spinal cord, the largest group II field potentials recorded following stimulation of GS and hamstring nerves were around 200 - 300 μV. These differences are presumably the result of smaller numbers of group II afferents, spinal neurones and therefore synapses in the rat compared to cat spinal cord, although the use of different anaesthetics in the two species (sodium pentobarbital in the present studies and chloralose in the cat studies) may also be a contributory factor.

Actions of group II afferents in central lumbar segments

While the group II reflex circuits of midlumbar and sacral segments of the cat lumbosacral enlargement have now been extensively investigated, much less is known about the role of interneurones within the lower - lumbar segments (L6 and L7). Until recently neurones of these segments were thought to be contacted mainly by group II afferents of triceps surae (Fu & Schomburg, 1974; Fu, Santini & Schomburg, 1974; Fukushima & Kato, 1975). More recent evidence, mainly of a preliminary nature, suggests wider actions from group II afferents of a number of hind - limb muscle nerves with branches innervating posterior muscles of the lower leg (flexor digitorum longus and associated muscle nerve branches; FDL et al.) having particularly strong actions (Lundberg et al. 1987b; Harrison & Riddell, 1989; Harrison, Connolly & Guzman - Villalba, 1994; Riddell & Hadian, 1996). The present results of investigations in the central lumbar region in the rat (caudal L3, L4) which has been take to be equivalent to the lower - lumbar segments of the cat support this preliminary evidence.

Only group II afferents of TP evoked cord dorsum potentials over the central lumbar region comparable in amplitude to those produced on the surface of other regions of the spinal cord. Correspondingly the largest group II potentials recorded within the grey matter of the central lumbar segments were evoked by afferents of TP. These field potentials were of a similar amplitude to those evoked in the caudal lumbar segments by afferents of GS and hamstring nerves but unlike the actions of these

afferents, group II afferents of TP contacted neurones not only in the dorsal horn but also in areas of the intermediate zone and ventral horn. At some locations within the central lumbar region field potentials were also evoked by group II afferents of FDL and GS but these were always much smaller (less than half the amplitude) of the potentials evoked by TP. Field potentials evoked by GS were always much smaller and much more localised than potentials evoked by the same afferents in L5 or L6. This is consistent with the actions of group II afferents of GS in the cat which have much weaker effects in the lower - lumbar segments (Fu et al. 1974) than in the caudal group II relay region of the sacral segments (Jankowska & Riddell, 1993).

Group II afferents in rat muscle nerve

Afferent fibres in cat muscle nerve are conventionally divided into a number of groups (group I, group II etc.) according to fibre diameter. This terminology, originally proposed by Lloyd (Lloyd, 1943; Lloyd & Chang, 1948) has endured in part because the groups defined by fibre diameter correspond roughly to functionally different sets of fibres and also because the relationship between fibre diameter and excitability to electrical stimuli has provided a useful experimental tool for the study of the central actions of muscle afferents in the cat (see Matthews, 1972). It is generally assumed that similar groupings of sensory fibres exist in the muscle nerves of other species, but histological studies of muscle nerves of the rat have so far failed to demonstrate this convincingly.

Zelená and Hnik (1963) looked at the fibre diameter distribution of the soleus nerve and although they claimed to show a bimodal distribution of fibres with a division at 8 - 10 μm, this is not clear from the published plots. Similarly, Kaizawa and Takahashi (1970) who studied the distribution of afferents in the LGS and MG nerves and Mellström and Skoglund (1965) who investigated the fibre diameter distribution of the LG, MG and DP nerves also failed to detect obvious peaks in their plots.

Several sets of electrophysiological evidence from the present study indicate that in the rat, the myelinated afferents in muscle nerves form groupings equivalent to the group I and group II fibres identified in the cat. Firstly, throughout the spinal cord, two clear sets of synaptic actions were consistently observed following electrical stimulation of muscle nerves. The earliest of these were evoked at low stimulus intensities (<2T) and occurred within the intermediate nuclei or motonuclei; features consistent with the actions of group I muscle afferents observed in the cat (see Baldissera, Hultborn & Illert, 1981; Jankowska, 1992 for reviews). These early synaptic potentials were followed at slightly longer latencies, sometimes at the same recording location, by potentials that required stronger electrical stimuli; the range of stimuli (from 1.8T/2.0T to 4/5T) over which these potentials were evoked being directly comparable to the range of stimulus intensities recruiting group II afferents in the cat (Jack, 1978; Ellaway, Murphy and Tripathi, 1982; Lundberg, et al. 1987a).

Investigation of the fibre composition of the LGS nerve using electrophysiological methods revealed that the population of muscle

afferents activated by stimuli of up to 5T show a tendency towards a bimodal distribution of conduction velocities. This tendency is most likely underestimated because the fastest conducting fibres have very similar latencies and it was often therefore difficult to distinguish the activity of single fibres, while at the opposite end of the range the slower conducting fibres produce smaller action potentials which are difficult to isolate. It seems reasonable to suppose that the bimodal distribution of fibres that occurs in the conduction velocity histograms represent populations of fibres equivalent to the group I and group II fibre peaks seen in fibre diameter data from the cat. Precise boundaries between the two groupings in these histograms are not readily identified, but a division at around 50 m/s appears reasonable for three of the four plots (Fig. 28 left). This would be consistent with the observation that group II cord dorsum and field potentials evoked by stimulation of the LGS nerve begin to appear at about 1.8 - 2.0T since this is the intensity range where fibres conducting at around 50 m/s are recruited (Fig. 28, right). Together, these observations suggest that 50 m/s represents the upper end of the conduction velocity range for group II muscle afferents in rat muscle nerves. This value is also in close agreement with the findings of Andrew, Leslie & Thompson (1973) who proposed 55 m/s as a rough dividing line between samples of functionally identified muscle spindle primary and secondary afferents of various muscles in the rat hind - limb.

Furthermore, recognition of the difference between individual muscle nerves in the cat has led some to set a dividing line between muscle spindle

group I and group II fibres at 0.6 times the conduction velocity of the fastest conducting afferents of a given nerve (see Matthews, 1972). When this procedure is adopted with the present results the dividing lines fall close to 50 m/s.

Activation by stimuli 2T or more and conduction velocities of 40 - 50 m/s may therefore be suggested as reasonable criteria for identifying group II muscle afferents in the LGS nerve of rat. The applicability of these criteria to muscle nerves other than LGS remains to be tested. Nevertheless, two sets of observations suggest that a dividing line of about 50 m/s may equally apply to other nerves. Firstly, the thresholds of group II potentials evoked by the Q, hamstring, DP, TP and FDL nerves were similar (i.e. around 1.8 - 2.0T). Secondly, the conduction velocities of fibres contributing to group II components of afferent volleys for different nerves were about 40 - 50 m/s.

Receptor origin of group II muscle afferents

In cat muscle nerves, while the majority of fibres in the group II range originate from the secondary ending of the muscle spindle, a minority innervate non - spindle receptors (see Boyd & Davey, 1968; Matthews, 1972). Since electrical stimuli sufficient to activate group II afferents will excite fibres of both spindle and non - spindle origin indiscriminately, there is always some ambiguity as to the identity of the muscle afferents responsible for the central actions that result. In the cat, activation of muscle spindle secondaries by stretch or by fusimotor stimulation has been shown to evoke field potentials at the same location as electrical stimulation of group II muscle afferents (Edgley & Jankowska, 1987a; Harrison, Jami & Jankowska,

1988; Jankowska & Riddell, 1993). These observations provide strong evidence that muscle spindle secondary afferents contribute to the group II cord dorsum and field potentials evoked by electrical stimulation but leave open the possibility that non - spindle group II afferents might also be involved. There is no information on the nature or number of non - spindle afferents in rat muscle nerves, but given the almost identical organisation of group II reflex pathways in the rat and cat, it seems unlikely that there would be any major differences in the receptor origin of group II potentials.

Conclusion

Fig. 29 summarises the results of the investigation of group II field potentials within the lumbar enlargement of the rat spinal cord. The schematic diagram shows the three regions of the lumbar enlargement in which group II field potentials were recorded; the rostral, caudal and central lumbar segments. The main muscle nerves containing group II afferents terminating in each of these regions are indicated together with an indication of whether contacts were made with neurones in the dorsal horn, intermediate area or both. The dashed lines represent the weaker projection of group II afferents with actions in the rostral lumbar or caudal lumbar segments, to the central lumbar region. This information is extrapolated from the cat, since apart from FDL, TP and Pop only the actions of GS were systematically studied in this region (See Cat chapter). Also shown is the relationship between the termination of group II muscle afferents and the

level of the Q, BST and pudendal motor columns (Nicolopoulos - Stournaras & Iles, 1983; Jordan et al. 1982; McKenna & Nadelhaft, 1986).

The present results establish that both in terms of their segmental distribution and their distribution within the grey matter, the topographical organisation of neuronal systems mediating the reflex actions of group II muscle afferents in the rat is similar in principal to that of the cat. These observations raise the possibility that this aspect of spinal cord organisation may be a fundamental feature of the mammalian spinal cord. The observations also provide a useful basis for further investigations of spinal reflex circuitry in this species. It is anticipated that this information will be of particular value for experiments involving identification of neurotransmitters and/or receptors in spinal circuits since antisera and *in situ* hybridization probes are generally developed for use in the rat and because of species differences in the epitopes which the antisera or probes are designed to recognise do not always work successfully in other species.

The comparative information on the organisation of group II reflex circuits in the rat and cat provided by this study may also prove useful to those investigating spinal locomotor pathways using the neonatal rat spinal cord preparation (Kjaerulff, Barajon, Kiehn, 1994; Cazalets ; Borde, Clarac, 1995, 1996).

Chapter 3.0. Actions of group II muscle afferents in the lower-lumbar segments of the cat spinal cord

3.0.1. Introduction

3.0.1. Introduction

Apart from a weak monosynaptic projection to homonymous motoneurones (Kirkwood & Sears, 1974; Stauffer et al. 1976; Munson et al. 1980) the reflex actions of muscle afferents in the group II range are mediated by spinal interneurones. Several populations of interneurones with input from group II muscle afferents have now been investigated, but so far only one population of last - order pre - motor interneurones (i.e. interneurones connected directly to motoneurones) have been identified. These interneurones are located in midlumbar segments and have axons that project caudally to lower - lumbar levels where they produce excitation or inhibition of motoneurones (Edgley & Jankowska, 1987b; Cavallari et al. 1987; reviewed in Chapter 1.0.). In addition to their segmental inputs, midlumbar interneurones are also influenced by various descending motor pathways (Yates et al. 1989; Davies & Edgley, 1994) and by the central pattern generator circuits for stepping (Shefchyk et al. 1990). Interneurones in the midlumbar segments receive input predominantly from group II afferents of hip flexor, knee extensor and pretibial flexor muscles, a pattern of convergence which has led to the suggestion that they may be involved in triggering the transition from stance to swing during the step cycle (Edgley & Jankowska, 1987b; Aggelopoulos et al. 1996). The midlumbar interneurones are not, however, excited by group II afferents of knee flexors or ankle extensors so that the interneuronal circuits driven by signals from these

muscles (Lundberg et al. 1987a) must be located in other segments of the lumbosacral enlargement.

Interneurones with powerful input from semitendinosus and gastrocnemius group II afferents have recently been discovered in the sacral segments. These interneurones form a functionally heterogeneous population of interneurones, some of which may mediate presynaptic inhibition of group II muscle afferents (Jankowska & Riddell, 1994, 1995; Jankowska et al. 1994, Maxwell et al 1997). However, few if any of the group II interneurones in sacral segments appear to make direct contact with motoneurones so that in this part of the thesis investigations have been concentrated on the lower - lumbar (L6 - L7) segments.

Although reflex circuits in the lower - lumbar segments have been extensively studied, most investigations have focused on interneurones in pathways from group I muscle afferents. These studies have included unidentified neurones located in the intermediate nucleus (e.g. Eccles, Eccles & Lundberg, 1960; Hongo, Jankowska & Lundberg, 1966) and also functionally identified neurones interposed in pathways of reciprocal inhibition from group Ia muscle spindle afferents (Hultborn, Jankowska & Lindström, 1971; Jankowska & Roberts, 1972b) or non - reciprocal inhibition from group Ib tendon organ afferents (e.g. Hongo, Jankowska, Ohno, Sasaki, Yamashita & Yoshida, 1983a; Harrison & Jankowska 1985). Much less is known about interneurones with input from group II muscle afferents in the lower - lumbar segments although intracellular studies have revealed group II excitation in a small minority of interneurones with input mainly from

group I muscle afferents (about 10% of Ib interneurones; Harrison & Jankowska, 1985). There are also brief reports of interneurones in these segments which are powerfully excited by group II afferents but only weakly if at all by group I afferents (Lundberg et al. 1987b; Harrison et al. 1994). In addition, recordings made from axons in the lateral funiculus at L6 indicate that the lower - lumbar segments contain a population of group II - activated interneurones with an ascending projection to the midlumbar segments (Harrison & Riddell, 1989).

The aim of the present study was to investigate the action of group II muscle afferents in the lower - lumbar segments and in particular to attempt to locate candidate last - order pre - motor interneurones in group II reflex pathways.

3.0.2. Method

3.0.2. Methods

Useful results were obtained from experiments performed on13 female cats, 2.5 - 3.0 kg weight.

Anaesthesia

General anaesthesia was induced by passing a mixture of 4% Halothane in an O_2/N_2O mixture through a box in which the animal was housed. Once arterial and venous cannulae were in place, anaesthesia was switched to chloralose (70 mg/kg) and supplementary doses (10 mg/kg i.v.) were given throughout the remainder of the experiment when required. The depth of anaesthesia was assessed during the dissection by monitoring withdrawal and corneal reflexes, arterial blood pressure and an ECG. Additional doses of anaesthetic were given if a withdrawal reflex was seen on pinching or if blood pressure increased on pinching or was abnormally high. During the recording phase of the experiment, when the animal was paralysed, administration of anaesthetics was continued at a rate commensurate with that required before paralysis. The adequacy of this regime was checked by continuously monitoring the arterial blood pressure, diameter of the pupils and by periodically allowing the animal to recover from paralysis.

Preparatory surgery

The animal was placed in the supine position on an operating table and secured with cord attached to each of the limbs. The animal's core

temperature was continuously measured by a rectal probe and maintained by a homeothermic control unit at between 37° - 38°C. The ECG was monitored during dissection through needle electrodes which were inserted through skin of the left and right forelimb and the right hind limb (when a surgical level of anaesthesia had been obtained heart rate was typically between 120 - 150 beats/minute). Decadron (dexamethasone sodium phosphate; Merk Sharp & Dome) at a dose of 2 -3 mg/kg i.m. was injected to reduce the swelling of nervous tissue (see for example Stamford, 1992). Atropine sulphate, (Macarthy medical) at a dose of 0.05 mg/kg i.p was also injected to reduce bronchial constriction and salivary secretions. Before making skin incisions fur was removed from the relevant areas using an animal fur clipper. A cautery (Eschmann equipment, TDB 50) or soldering iron was used to prevent bleeding from small vessels and ligatures placed around larger vessels before cutting.

Cannulation

The carotid artery and jugular or radial veins were cannulated: A mid - line incision was made through the skin overlying the trachea. The skin was retracted and the superficial muscles covering the trachea were separated. The right carotid artery was exposed by blunt dissection and a length of artery freed from surrounding connective tissue and the vagus nerve being careful not to damage the nerve. The artery was ligated distally and a cannula inserted proximally (i.e. towards the heart) to permit continual monitoring of arterial blood pressure which was maintained above 90 mm Hg

(usually at 100 - 120 mm Hg). A continuous infusion of a 100 mM solution of bicarbonate containing 5% glucose (2 - 3 ml/kg/hr) was also administered through the arterial cannula throughout the experiment to help maintain the acid base balance and fluid level of the animal. This infusion also avoided any tendency for the arterial cannula to block. One or more veins (jugular and/or radial veins) were cannulated to allow intravenous administration of anaesthetics and other drugs. The muscles and connective tissue overlying the trachea were retracted and a small length of the trachea was separated from the surrounding tissues. The trachea was opened and a cannula inserted and tied in position. From time to time excessive secretions in the trachea were removed by suction. All cannulae were secured to the skin by threads in order to prevent them being accidentally dislodged. Skin incisions were closed with Michel clips.

Nerve dissection

A variety of different muscle and cutaneous nerves were dissected according to the aims of the experiment. To dissect branches of the femoral nerve the animal remained in the supine position and an incision was made on the antero - medial side of the thigh from the groin proximally to about the knee distally. The quadriceps femoris (Q) motor branch was separated from the saphenous which is a cutaneous nerve and sartorius muscle nerve and mounted in a tunnel electrode.

To dissect branches of the sciatic nerve, the animal was turned and placed in a prone position. A skin incision was made on the left hind- limb

from the ischial tuberosity to about 3 cm caudal of the popliteal fossa. The skin was retracted by retractor and or by clipping weights to skin or muscles. Hamstring muscle nerve branches were dissected and usually included posterior biceps and semitendinosus mounted together (PBST) and anterior biceps and semi - membranosus mounted together (ABSM). Other nerves dissected included gastrocnemius - soleus (GS), flexor digitorum and halucus longus (FDL, from which a branch to interosseous membrane was dissected and mounted as i.o.), tibialis posterior (TP), popliteus(Pop), tibialis anterior and extensor digitorum longus nerves which were mounted together as deep peroneal (DP), the caudal branch of the sural(Sur) nerve, and the superficial peroneal nerve (SP).

Laminectomy

A mid - line skin incision was made from thoracic vertebrae to the sacral region. The skin was retracted and the longissimus dorsi muscles were separated from the lumbar multifidus muscles. The multifidus muscles were removed from the vertebral column. Afterwards, the vertebral column was raised and in this way the dorsal aspect of the inter - vertebral joints opened and the posterior arch was cautiously removed, using bone rongeurs. Bone wax and plasticine were used to prevent the seeping of blood from the cut edge of vertebrae and gel foam was also used to prevent bleeding. The laminectomy usually involved removal of bone from the sixth, fifth, fourth, and part of the third lumbar vertebrae. One vertebral clamp was placed on the second vertebral body and a second clamp gripped the

processes of fourth and fifth thoracic vertebrae. A short laminectomy was also performed at the lower thoracic level (Th13).

Preparation for recordings

Many of the procedures and much of the equipment used while investigating the rat spinal cord were also used for experiments on the cat. After completing the surgery the animal was transferred to the recording frame. The animal's head was supported by a small pillow. Vertebral clamps gripping the vertebra were secured in position to a frame and paraffin pools were formed from skin flaps. Before beginning recording it was necessary to reduce movements of the thorax by paralysing the animal, providing artificial ventilation and performing a bilateral pneumothorax. The trachea was cleared of excessive secretions by suction and the animal was paralysed with gallamine triethiodide. The animal was artificially ventilated with oxygen/enriched air via a T - piece which was connected to a respiratory pump. The level of carbon dioxide was monitored (normocap 200 oxy Datex) and was kept about 4% throughout the experiment by adjusting the ventilation rate.

Stimulation and recording procedures

Nerve stimulation

Peripheral nerves were mounted on bipolar silver ball electrodesand electrical stimuli (0.1 ms rectangular pulse) were applied singly, in pairs or as trains.

Surface recordings

The dura was opened over the full length of the exposed spinal cord and silver ball electrodes were placed in position. Afferent volleys andgroup II CDPs were recorded from the surface of the cord. In those experiments that field potentials were systematically mapped, cord dorsum potentials were recorded along the rostro - caudal length of spinal cord in 2 mm steps.

Field potential recordings

Patches were made in the pia mater where the largest group II CDPs could be recorded from the surface of cord following stimulation of the relevant nerve. Field potentials were recorded using glass microelectrodes filled with 2M NaCl (2 - 3 μm tip diameter, 2 - 4 MΩ resistance) introduced through the dorsal columns close to the root entry zone. The distribution of field potentials within the grey matter was mapped systematically by recording at different angles (in 5^{o} steps up to 35^{o} from vertical) and depths (up to 4 mm) while maintaining the same entry position on the surface of the spinal cord (Fig. 1 A & Fig. 2). To detect group II potentials in caudal parts of the L7 segment it was often necessary to adopt a large angle (30^{o} - 35^{o} tip pointing laterally). This is because group II potentials were often located laterally in the grey matter at this level (see results) and the arrangement of dorsal roots at this level necessities a more medial entry point than at more rostral levels.

Intra - and extracellular recordings from group II neurones in L6 - L7 segments.

A diagrammatic representation of the experimental arrangement showing the position of stimulating and recording electrodes used for recording from interneurones is shown in Fig. 30. Small tears in the pial membrane overlying the lower - lumbar (L6 and L7) segments were made at a number of locations to facilitate the introduction of glass microelectrodes into the spinal cord. Because the border between the L7 and S1 segments can sometimes be difficult to identify without dissection, recordings of cord dorsum potentials evoked by group II afferents of PBST were used as a guide (see Jankowska & Riddell, 1993); the most caudal pial patches were made some 3 - 5 mm rostral of the location where the largest of these potentials could be recorded. Glass microelectrodes filled with 2M potassium citrate (1.5 - 2.5 μm tip diameter, 2 - 6 MΩ resistance) or in three experiments, 2% biocytin in 0.05M tris - HCl buffer containing 0.5M potassium chloride, were introduced through the dorsal columns as close as possible to the root entry zone.

Neurones were searched for while tracking through the dorsal horn and the intermediate zone within regions in which monosynaptic field potentials evoked by group II afferents could be recorded (see Cat results, part 1.0). A search was made for neurones discharged by electrical stimulation of muscle nerves at up to 5T. The neurones were usually first recorded from extracellularly in order to establish the peripheral nerves by

which they could be discharged and to test for an ascending projection. In order to identify interneurones with an ascending projection to the L4 level, stimuli (0.1 ms, usually up to 300μV) were applied within the lateral funiculus at this level via a tungsten electrode. The electrode was inserted into the lateral funiculus, close to the border with the dorsal columns at a depth of about 1.5 - 2.0 mm with its tip directed medially at an angle of 5° (Harrison & Jankowska, 1985).

The collision test (Paintal, 1959) was used to identify the origin of action potentials recorded in lower - lumbar interneurones following stimulation at L4. This test enables action potentials conducted antidromically along an ascending axon projecting to L4 (Fig. 30 A) to be distinguished from those evoked synaptically as a result of stimulating fibres of descending neurones, propriospinal neurones or primary afferent neurones (Fig. 30 B). The first step is to identify fixed short latency responses to stimuli applied at L4. Orthodromic impulses were then produced in the interneurones by stimulating a peripheral nerve and the timing of the peripheral nerve stimuli relative to the L4 stimuli adjusted such that orthodromic impulses precede of the L4 response by a few ms. The orthodromic impulse was then gradually moved closer to the L4 response (i.e. the interval between stimuli was decreased) to see whether the L4 response disappeared. Disappearance of the L4 response could then be due either to inhibition of synaptically evoked potentials or collision between orthodromic and antidromic impulses. To distinguish between these possibilities the interval (between the orthodromic and L4 evoked impulses)

at which the L4 evoked impulses disappeared was measured. Collision between an orthodromically and antidromically conducted action potentials will occur only within a certain time interval termed the critical interval. The critical interval is equal to the orthodromic conduction time plus the antidromic conduction time plus an absolute refractory period (see Brown & Fyffe, 1984). This period was calculated by taking the latency of the L4 evoked impulse, subtracting 0.2 ms for stimulus utilisation time (Jankowska & Roberts, 1972a), doubling the result and adding 0.5 ms for one absolute refractory period. Only those L 4 evoked responses which disappeared at the critical time were considered as antidromic. Records illustrating the collision test procedure are shown in Figs. 43 ,44C, 45A; Fig. 46 D.

Neurones were also tested for an ascending projection to Th13 segment. Stimuli (0.2 ms current pulse of up to 1 mA) were applied transdurally to the ipsilateral and contralateral lateral funiculi at Th13 irrespective of whether or not they were antidromically activated from L4. Neurones responding with antidromic impulses were excluded from the sample.

An attempt was then made to penetrate the neurones, either in the same electrode track or in subsequent track 20 - 100μm away. On successful penetration the absence of antidromic activation by thoracic stimuli and if, appropriate, antidromic responses to stimulation at L4 were confirmed and PSPs evoked by peripheral nerves were recorded. Recordings were further amplified using an AC amplifier,digitised and stored on digital audio tape and PC. On and off line analysis, such assingle sweep

recording, averaging of 16 - 32 individual potentials (signal averaging, sigavg) and measurements of the latency and amplitude of potentials were performed.

Identification of spinal segments

Recording sites were marked by leaving an electrode in the recording patch and their segmental locations determined by dissection of spinal roots. The laminectomy was extended laterally to expose the lumbosacral dorsal root ganglia. In addition, the laminectomy was extended caudally to the L7 vertebra (the last lumbar vertebra in the cat which lies level with the pelvic bone). The L7 ganglion (the largest of the lumbar dorsal root ganglia) was easily identified and other ganglia were identified by working rostrally. The spinal roots were then cut and traced to their level of entry into the spinal cord. Spinal segments were measured with millimetre paper and a plan of the spinal segments indicating the location of marking electrodes was drawn.

Conduction distance measurements

The conduction distances of the relevant peripheral nerves were measured between the cathodes of stimulating electrodes to the relevant recording patches as in the rat. In addition the conduction distances between recording sites and the L4 and Th13 electrodes were measured.

Histological reconstruction of recording sites.

A very similar procedure to that adopted in the rat was used to reconstruct the sites at which field potentials were recorded. Blocks about

six millimetres in length were mounted on a vibratome stage and serial transverse sections (100 - 200μm) were cut and mounted on glass slides. Slides were left to dehydrate at room temperature and stained with cresol violet as described in the section 2.0 (Rat). Sections were examined with a light microscope and reconstructions of the areas of grey matter within which field potentials were recorded were performed from information on the angle of electrode tracks in relation to the marking electrode.

Intracellular labelling of group II interneurones with biocytin

In three experiments group II activated neurones were intracellularly filled with biocytin (2% - 4% dissolved in 0.05M Tris - HCl buffer, pH 7.4 - 7.6) containing 0.5M KCl. Biocytin is a biotin - lysine complex of low molecular weight containing about 65% biotin. Biocytin is highly soluble in water and has a high affinity to the egg white protein, avidin. The latter molecule has been conjugated to several histochemical markers and can therefore be used as an intracellular marker (Horikawa and Armstrong, 1988). At the end of these experiments, the animal was perfussed through the descending aorta via a perfusion apparatus. This consisted of three separate glass containers into which were placed: 1. A warm rinse solution (37°C). 2. Warm fixative solution (formaldehyde or paraformaldehyde 1%, 37°C). 3. Cold fixative solution (about 0°C). The glass containers were connected to an air compressor so that these solutions could be introduced into the vascular system of the animal under pressure. To perfuse the animal it was first deeply anaesthetised and then the chest was opened in order to get access to the descending aorta and vena cava. A cannula was

introduced into the descending aorta and warm rinse solution was introduced under pressure. The vena cava was then cut to allow blood to drain and in this way the circulatory system of the animal was flushed free of blood. Thereafter, the warm and cold fixative solutions were introduced in turn. The spinal cord was then removed and blocks of tissue were prepared and kept in fixative solution for another 4 hours. The blocks were rinsed several times with phosphate buffered saline (pH 7.4); serial transverse sections (50 μm) were cut and sections were washed six times for 10 minutes each in phosphate buffered saline.

Avidin - HRP incubation and permeabilisation. The sections were incubated in Extr Avidin - peroxidase (Sigma; E - 2886, 1:1000 dilution in phosphate buffered saline) to which was added Triton - X100 (0.3%; Sigma) in order to increase the permeability of the tissue. The sections were left for 24 hours at room temperature with agitation (see Maxwell, 1992).

HRP reaction and intensification procedures. Sections were washed several times in phosphate buffered saline before performing HRP reaction using 3,3' - diaminobenzidine tetrahydrochloride (DAB) as chromagen . To increase the sensitivity of the DAB reaction nickel chloride (Sigma N - 5756; 0.07%) was added to the DAB solution. Sections were left to be incubated in the DAB solution for 10 minutes after which 0.005% H_2O_2 solution was added and the sections incubated for another 5 minutes.

Mounting and Coverslip. Before mounting, sections were washed twice for 10 minutes with 0.05M Tris buffer and twice for 10 minutes in 0.1M

phosphate buffer. Sections were left at room temperature for drying and then defatted with alcohol and Xylene before being coverslipped.

Microscopic examination. The sections were examined with a light microscope. Drawings of the location of four group II interneurones and an ascending tract neurone in the grey matter were made using a camera lucida.

3.0.3. Results

3.0.3. Results

Part 1.0.

Cord dorsum potentials

Electrical activation of group II afferents in nerves from hind - limb muscles produces distinct negative potentials (cord dorsum potentials) at the surface of midlumbar and sacral segments of the spinal cord (Edgley & Jankowska, 1987a; Jankowska & Riddell, 1993). These cord dorsum potentials reflect activity at synapses between group II afferents and neurones within the underlying grey matter, where field potentials with a similar time course to the surface potentials can be recorded.

Observations on potentials recorded from the surface of the lower - lumbar segments following stimulation of muscle nerves were made in seven animals. Previous reports that group II afferents of those nerves that most effectively evoke cord dorsum potentials at the sacral (PBST, GS; Jankowska & Riddell, 1993) and midlumbar (Q; Edgley & Jankowska, 1987a) levels, produced only small or negligible cord dorsum potentials at lower - lumbar levels were confirmed. Attention was therefore focused on the actions of group II afferents in certain muscle branches of the tibial nerve (FDL, TP & Pop) which recent evidence suggests may have a strong projection to the lower - lumbar segments (Harrison & Riddell, 1989; Harrison et al. 1994).

Cord dorsum potentials evoked by the tibialis posterior nerve.

Effects of graded stimuli. Stimulation of the TP muscle nerve at 5T produced distinct group II CDPs which followed the incoming volleys in records from the surface of spinal cord. Fig. 31 A shows examples of such CDPs which appeared at stimulus intensities close to twice nerve threshold, grew in amplitude as the stimulus intensity was raised and reached a maximal amplitude at about 4 - 5T. This range of stimulus strengths (2 - 5T) corresponds to that over which the majority of group II afferents are recruited (Jack, 1978, Ellaway et al. 1982; Lundberg et al. 1987a).

Further increases of stimulus strengths (up to 10T) did not increase the size of group II potentials but induced a later potential the early component of which can be seen in the lower most record of Fig 31 A. In two experiments, a second component of the afferent volley appeared in parallel with the group II CDPs when the stimulus strength was increased to maximal for group II afferents (indicated by arrow in Fig. 31 A). The largest CDPs (17 - 57μV, mean 39μV, n = 7) were generally similar or slightly smaller in amplitude than those evoked by PBST (27 - 66μV, mean 49μV, n = 7) at the sacral segments in the same experiments (see example in Fig. 31 D).

Rostro - caudal distribution. The distribution of group II CDPs recorded following stimuli applied at 5T to the TP nerve was mapped in 2 mm steps along the surface of the spinal cord. Fig. 32 A shows a plot of the amplitude of the potentials recorded in this manner. As can be seen, group II

CDPs were recorded over a length of spinal cord extending from L5 to S1 with the largest potentials in this case occurring over the L6 - L7 border.

Fig. 32 B shows the distribution of group II CDPs recorded in seven animals. The largest CDPs were recorded between caudal L6 and caudal L7 but were mostly seen within the L7 segment. The location of group II potentials evoked by PBST show a similar variation with respect to segmental topography but are fixed in relation to landmarks of the internal organisation of the spinal cord (the rostral ends of the pudendal motor columns; see Jankowska & Riddell, 1993). In the present investigation, cord dorsum potentials evoked by group II afferents of both TP and PBST were mapped in the same experiments and their locations could therefore be compared. As can be seen from Fig. 32B, the largest potentials produced by group II afferents of TP were consistently located between 6 and 8 mm rostral of those evoked by PBST (open arrow heads in Fig. 32B).

Although, the largest group II cord dorsum potentials evoked by afferents of TP were slightly smaller than those of PBST, they were distributed over a longer length of the spinal cord; potentials 75% of maximum were recorded over a 10 - 20 mm length of spinal cord, sometimes extending rostrally as far as the L5 segment (solid lines in Fig. 32 B).

Latencies. The latencies of group II CDPs measured from the group I volleys ranged from 1.25 - 1.5 ms (mean, 1.35 ms). These values are comparable to, or shorter than those of similar potentials recorded in the midlumbar and sacral segments (2.0 - 2.2 ms for potentials evoked by the Q

nerve and 1.5 - 2.2 ms for potentials evoked by the PBST nerve). This may be because the largest cord dorsum potentials occurred close to the main site of entry of TP muscle afferents (as judged by the amplitude of the afferent volley) and the intraspinal conduction path will therefore be relatively short.

In two experiments in which group II afferent volleys could be identified, the CDPs followed these volleys at latencies of 0.5 and 0.6 ms (see arrows in Fig. 31 A).

Cord dorsum potentials evoked by the popliteus nerve.

Cord dorsum potentials evoked by group II afferents of the Pop nerve were much smaller than those evoked by the TP nerve. Fig. 31 B shows examples of CDPs which appeared at 2T and grew in amplitude when the stimulus intensity was raised to 2 - 5T. Further increases of stimulus strengths (10T, tested in one experiment) evoked a later potential the early component of which can be seen in the lower most record of Fig. 31 B.

The largest CDPs (13 - 33 μV; mean 22μV, n = 7) were recorded at a similar location to those evoked by TP. The potentials occurred at latencies of 1.3 - 1.9 ms with respect to group I volleys and 0.65 ms with respect to a group II volley seen in one experiment (indicated by arrow in Fig. 31 B).

Cord dorsum potentials evoked by the flexor digitorum longus nerve.

Small group II CDPs were also recorded following stimulation of the FDL nerve (see Fig. 31 C). The largest of these (21 - 30μV, mean 27μV, n = 7) were recorded at a similar location to those evoked by the TP and Pop nerves. These potentials were evoked at latencies of 1.2 - 1.72 ms (mean 1.35 ms) with respect to group I volleys.

Field potentials.

The central projections of group II muscle afferents within the grey matter of lower - lumbar segments were investigated by recording focal synaptic potentials (field potentials) evoked by stimulation of muscle nerves at 5T in 10 animals. The grey matter of the L6 and L7 segments was searched systematically in both the transverse and rostro - caudal planes. In the transverse plane, all but the most dorsal regions of the grey matter were explored by recording along up to ten electrode tracks sharing the same entry point but covering a range of angles separated by 5°(up to 35° from vertical). Recordings were made at 200 μm intervals along each track, to depths of up to 4 mm, so that each transverse mapping involved 100 - 130 recordings. Such transverse mapping was repeated at various locations throughout the lower - lumbar segments. Fig. 33 A shows the rostro - caudal distribution of 30 sites where transverse explorations of the grey matter were made in the L6 - S1 segments. In view of variation in the segmental distribution of cord dorsum potentials, these sites are also shown in relation to the location of the largest cord dorsum potentials evoked by group II

afferents of PBST (Fig. 33 B). The plots show that all levels of the L6 and L7 segments were well sampled though with some bias towards the L7 segment.

Following stimulation of most of the muscle nerves tested, distinct field potentials attributable to the actions of group II afferents could be recorded within the grey matter. Field potentials were induced not only by afferents of TP, but also by several nerves producing only minimal potentials at the cord dorsum; examples are shown in Figs. 35 & 36 (TP), 37 & 38 (FDL), 39 (Pop), 40 & 41 (Q). As can be seen from Fig. 35 A, 36 A and 37 A, when the intensity of stimuli applied to muscle nerves was progressively increased, potentials appeared at between 1.8 - 2.0T and grew in amplitude with stronger stimuli reaching a maximum at 4 -5T. The appearance and amplitude of the field potentials is therefore clearly related to the range of stimulus strengths expected to recruit increasing proportions of group II muscle afferents (Jack, 1978; Ellaway et al. 1982; Lundberg et al. 1987a). Further evidence that these potentials originate from group II muscle afferents is provided by their latencies (Table 4), which are consistent with the actions of afferents conducting at velocities in the group II range (see below) and were always longer than those of potentials evoked at lower stimulus intensities (i.e. > 2T) by group I muscle afferents (see Table 4 and Figs. 37 A, 38 A, 40 and Fig. 41 A).

Figure 34 A shows the proportion of transverse mapping sites at which field potentials were evoked by each of the muscle nerves tested (data for ABSM which failed to evoke field potentials at any location is

omitted). Group II afferents of Q, GS, PBST and TP were most frequently effective, evoking field potentials at 60 - 80% of sites where their actions were systematically investigated, while field potentials evoked by group II afferents of FDL and Pop were recorded at 30 - 40% of sites. As shown in Fig. 34 B, the mean maximal amplitudes of field potentials evoked by each nerve were in the range 130 - 200 μV. This information on the frequency and mean amplitude of field potentials, provides an indication of the overall effectiveness of group II afferents of each of the muscle nerves tested as a source of input to neurones in the L6 and L7 segments. There was, however, some variation in both the rostro - caudal and transverse distributions of field potentials evoked by different nerves and in the amplitudes of field potentials evoked by the same nerve in different regions of the grey matter.

Rostro - caudal distribution

Field potentials evoked by group II afferents of Q and TP were encountered with similar frequency throughout the L6 and L7 segments and also more caudally in the first sacral segment, where potentials are evoked in the dorsal horn by group II afferents of PBST (see Figs. 35 A, 36 A; see also Jankowska & Riddell, 1993). Potentials evoked by TP were of a similar amplitude at all levels but those evoked by Q tended to be larger in L6 (mean 231μV, n = 8) than in L7 (L7 mean 144μV, n = 5), a tendency that was clearer still when recording sites were related to the position of the largest PBST cord dorsum potential (9 - 6 mm, mean 262μV, n=7; 0 - 8 mm, mean 122μV, n = 6). Examples of field potentials evoked by group II

afferents of Q and recorded at different rostro - caudal levels in the same experiment are shown in Fig 40.

Group II potentials evoked by afferents of the remaining nerves were concentrated within caudal regions of the lower - lumbar segments. Field potentials evoked by afferents of GS, although occasionally encountered in L6, were seen with greater frequency in L7; a distribution that is in agreement with an earlier report (Fu et al. 1974). Field potentials evoked by group II afferents of PBST were evoked almost exclusively in L7 or within 8 mm rostral of the largest PBST cord dorsum potential. At locations close (within 2 mm) to where the largest cord dorsum potentials were evoked by group II afferents of PBST (i.e. within the most caudal part of L7 or within S1) large (> 500 μV) field potentials were evoked in the dorsal horn by afferents of both GS and PBST (see Fig. 36 A, bottom record and location in B); such potentials are expected at this location which corresponds to the rostral end of the sacral group II relay region (Jankowska & Riddell, 1993).

Field potentials evoked by group II afferents of FDL (Figs. 37 & 38) and Pop (Fig. 39) were less frequently encountered than those evoked by other muscle nerves tested. Although occasionally encountered within L6 they were seen with greatest frequency within L7 (4 - 8 mm rostral of the largest PBST potential).

Distribution in the transverse plane

During systematic mapping in the transverse plane, field potentials were encountered within two main areas: in the dorsal horn (dorsal group II potentials) and in the intermediate region/ventral horn (intermediate group II potentials).

Dorsal group II potentials. Field potentials recorded in the dorsal horn were largest just dorsal to the group I intermediate nucleus, in an area at the base of the dorsal horn corresponding to lamina V and VI (see Figs. 35 B, 37 B, 38 C & 39 B). Distinct group II potentials (>150μV) were generally encountered within a relatively small area, rarely more than 0.8 to 1.0 mm in medio - lateral or dorso - ventral extent. The field potentials usually occupied an area within the middle two thirds of the dorsal horn (Fig. 35 B) which sometimes extended almost to the lateral border of the grey matter (see example in Fig. 37 B).

Intermediate group II potentials. Within the intermediate zone and ventral horn, field potentials were encountered in an area immediately lateral to the group I intermediate nucleus which often extended from the base of the dorsal horn into the ventral horn; that is from the lateral part of lamina V through to the lateral part of lamina VII (Fig. 40 A, B & C, Fig. 41 B). As in the dorsal horn, the area within which distinct group II potentials were recorded was rarely more than 0.8 - 1.0 mm across and was often less than this in the medio - lateral plane.

Most of the group II field potentials recorded in the intermediate region and also some of those recorded in the dorsal horn, were preceded

by shorter latency potentials which grew to a maximal amplitude in parallel with the earliest component of the afferent volley and can therefore be attributed to the actions of group I muscle afferents. Examples of dorsal horn field potentials with both group I and group II components are shown in Figs. 37 A, 38 A 40 and 41 A.

Not all nerves produced dorsal group II field potentials and intermediate group II potentials with equal frequency and effectiveness. Pop produced only dorsal group II potentials while Q and FDL produced group II potentials almost exclusively in the intermediate region. GS produced intermediate group II potentials with twice the frequency of dorsal potentials (dorsal 7/25 mappings; intermediate 15/25 mappings) while group II afferents of PBST (dorsal 8/21 mappings; intermediate 10/21 mappings) and TP (dorsal 9/20 mappings; intermediate 7/20 mappings) produced both types of potentials with about equal frequency. Where they were effective at both sites, most nerves produced potentials of similar magnitude in both the dorsal horn and the intermediate region. However, TP was an exception to this, evoking field potentials in the dorsal horn (mean maximal amplitude 220 μV, n = 9) which were nearly twice the amplitude of those recorded more ventrally (mean maximal amplitude 120 μV, n = 7). Where field potentials were evoked by both group I and group II afferents at the same location, group I components were usually either similar, or smaller in amplitude than the potentials evoked by group II afferents.

It was quite common for field potentials evoked by group II afferents of more than one nerve to be recorded at the same transverse mapping site.

The effects of stimulation of 5 or 6 different nerves were investigated at 28 of the transverse mapping sites and field potentials evoked by two or more nerves (up to 6) were recorded at 20 (71%) sites while field potentials evoked by three or more nerves were recorded at 12 (43%). There was a greater tendency for potentials evoked by two or more nerves to be seen at sites in the L7 segment (83%) than in L6 (50%). Field potentials evoked by two or more nerves at the same transverse mapping level site often occurred within the same or overlapping regions of the grey matter. Of the 20 transverse mapping sites where field potentials were evoked by 2 or more nerves, group II potentials were recorded in the same area (dorsal horn or intermediate) at 17. This observation implies that spinal neurones located within such regions are likely to receive convergent input from group II afferents of more than one muscle nerve.

Latency and synaptic linkage

The minimal latencies of onset of field potentials evoked by muscle afferents of different nerves in the dorsal horn and in the ventral/intermediate region are shown in Table 4. Table 4, A shows the central latencies of the field potentials, which were measured with respect to the onset of the group I volley since group II components of afferent volleys were only rarely detectable. Table 4 B, shows the latencies of the same potentials with respect to application of the stimulus to the peripheral nerve.

The central latencies of the group II field potentials are accounted for by delays in the conduction path due to a) the later arrival of impulses in group II afferents at the dorsal roots compared to group I afferents

contributing to the afferent volley, b) conduction within the dorsal columns (where the afferents enter the cord at a distance from the recording location), c) intraspinal conduction in the grey matter and d) synaptic transmission. Consideration of these delays for each of the nerves investigated and comparison of the expected delays with the central latencies actually observed for dorsal horn group II potentials (1.02 - 2.80 ms) leads to the conclusion that the onset of the potentials are most likely evoked monosynaptically; even if it is assumed that the potentials are evoked by the fastest conducting group II muscle afferents, the time remaining after events a - c above would be sufficient for only one synaptic delay (0.3 ms, for further details of calculations see below part 2.0. interneurones).

In addition, the latencies of the potentials evoked by group II afferents of PBST and GS in the dorsal horn are very similar to those of potentials evoked monosynaptically by these afferents in the sacral segments (PBST, mean 1.55 ms; GS mean 1.87 ms; Jankowska & Riddell, 1993). This is to be expected if the potentials at both locations are evoked monosynaptically since the conduction path to the two regions is very similar. Similarly, the latencies of intermediate group II potentials evoked by Q afferents (mean 2.64 ms) are only marginally longer than those evoked monosynaptically in the midlumbar segments (mean 2.46, Edgley & Jankowska, 1987a). This again is consistent with the potentials in the lower - lumbar segments being evoked monosynaptically because Q afferents enter the cord at rostral L6 and caudal L5 so that the conduction path to the L4

and L5 segments will be very similar to that to the L6 and L7 segments. The latencies of field potentials evoked by group II afferents of FDL, TP and Pop are broadly similar to those evoked by group II afferents of PBST and GS despite the longer conduction distances of the former. This reflects the fact that the dorsal root entry of these afferents is mainly at the L6/L7 level (as judged by the largest afferent volleys) and conduction within the dorsal columns is therefore minimal.

The latencies of group I field potentials recorded at the same locations as intermediate group II potentials are typical of those that can be recorded in the group I intermediate nucleus in the lower - lumbar segments (Eccles, Fatt, Landgren & Winsbury, 1954). The latencies for different nerves are more similar than for group II potentials since they are all measured with respect to the group I incoming volley. The shortest latencies are for potentials evoked by afferents of TP and FDL which enter the cord very close to the recording sites. The longest delays are for potentials evoked by afferents of Q which enter some distance rostral (L5 - L6 border) of their target neurones, especially those in the L7 segment.

When the same nerve evoked group II field potentials in both the dorsal horn and more ventral regions of the grey matter, the central delays of the intermediate potentials were generally of longer latency (by about 1.0 ms, see Table 4.0) than the potentials evoked in the dorsal horn. This is in keeping with previous observations on field potentials evoked in these segments by group II afferents of GS (Fu, et al. 1974) and with the actions of group II afferents of other nerves in midlumbar and sacral segments (Edgley

& Jankowska, 1987a; Jankowska & Riddell, 1993). Although the longer delay could theoretically be explained by the addition of an interneurone in the pathway (i.e. a disynaptic linkage), it is more likely due to the additional time required for conduction along the ventrally directed collaterals of group II afferents. Fu and Schomburg (1974) have shown that there is a difference of up to 0.8 ms in the conduction time for group II afferents fibres when they are activated by intraspinal stimuli applied in the dorsal horn compared to the ventral horn indicating that branching of their intraspinal terminals is accompanied by an appreciable slowing in conduction velocity. The longer latencies of the ventral group II potentials are therefore also compatible with monosynaptic actions.

Further evidence that the field potentials were evoked monosynaptically was sought by verifying that at least the main components (up to the peak) of potentials followed a train of stimuli without temporal summation. This was investigated by recording responses to a train of stimuli of 200 - 300 Hz and observing the amplitude of the resulting field potentials. None of the field potentials showed any evidence of the temporal summation that would be expected if one or more interneurones were interposed in the pathway. An example of one of these tests on an intermediate group II potential evoked by FDL is shown in Fig. 38 B.

Further support for the conclusion that the field potentials are evoked at least in part monosynaptically is provided by both electrophysiological and morphological evidence (see Discussion) demonstrating that group II muscle afferents terminate within the regions of

grey matter where both the dorsal and intermediate group II potentials are recorded (Fu & Schomburg, 1974; Fyffe, 1979, see also Brown, 1981; Mannen, Ishizuka, Hongo, Kudo, Sasaki & Yamashita, 1981; Ishizuka, Hongo, Kudo, Sasaki, Yamashita & Mannen, 1984; Hongo, 1992).

Part 2.0.

Interneurones

The report is based on observations made on a sample of seventy - six interneurones recorded from within the grey matter of the L6 and L7 segments, all of which were excited by group II afferents of hind - limb muscle nerves. Fifty - five of the interneurones were recorded from extracellularly, twenty - eight were recorded from intracellularly and a small number (7) were recorded from both intra - and extracellularly. Neurones projecting rostral of the lumbar segments (see below), as determined by antidromic activation by stimuli applied at the Th13 level, are not included in the sample of interneurones.

Interneurones projecting to L4

Interneurones excited by group II muscle afferents were tested for an ipsilateral ascending projection to the rostral L4 level by applying stimuli within the lateral funiculi. Nearly half of the sample of interneurones (37/76) were found to be antidromically activated by such stimuli (thresholds 10 - 300 μA, mean 170 μA). However, since in most experiments stimuli were applied at L4 while searching for neurones, there may be a bias towards these interneurones in the sample.

Antidromic impulses evoked by stimulation at L4 were identified by their latency or by using the collision test (Paintal, 1959). The minimal latency of an orthodromic response to such stimuli would involve a) delay for the electrical stimulus to generate an action potential (utilisation time, 0.2

ms; Jankowska & Roberts, 1972a), b) time for axonal conduction between rostral L4 and the recording site (0.2 - 0.3 ms given a conduction distance of 20 - 30 mm and assuming a conduction velocity of 100 m/s) and c) a synaptic delay (0.3 ms). Impulses evoked at latencies of less than about 0.7 ms would therefore be too short to have been evoked orthodromically and can be considered antidromic (see Jankowska & Roberts, 1972a; Fern, Harrison & Riddell, 1988). Twelve of the 37 neurones were judged to be antidromically activated by these criteria.

A further 25 interneurones responded to stimulation at L4 at latencies of 0.7 ms or more and for these neurones the nature of the response to the L4 stimulus was confirmed by establishing that collision between orthodromic and antidromic impulses occurred within the critical interval. Examples of recordings made from interneurones during tests for an ascending projection are shown in Figs. 43C, 44C, 45A and 46 D. In Fig. 43 C, the top pair of records show an extracellularly recorded antidromic discharge (arrow) evoked at a fixed latency by stimulation at L4, which is preceded by orthodromic discharges evoked by stimulation of the Q nerve. The middle pair of records show that the antidromic discharge no longer occurs when the orthodromic discharge is evoked within the critical interval. In Fig. 44C, the top pair of records show an extracellularly recorded antidromic spike (arrow) evoked at a fixed short latency by stimulation at L4 and the middle pair of records illustrate the blocked antidromic spike subsequently recorded on intracellular penetration (see also Fig. 46 D). Fig. 45A shows an example of the collision test performed on an intracellularly

recorded interneurone in which the spike generating mechanism initially remained intact. Also shown in each of these figures is the lack of response to stimulation at the Th13 level.

The conduction velocities of the ascending axons were measured between L4 and the recording site in the lower - lumbar segments. These were calculated after subtracting 0.2 ms for the latent period of activation (see Jankowska & Roberts , 1972a) and ranged from 23 - 74 m/s (mean 45 m/s). These conduction velocities are similar to those of axons recorded from in the lateral funiculus which are likely to have belonged to the population of interneurones described here (mean 51 m/s; Harrison & Riddell, 1989). They are also similar to those of L4 projecting interneurones with monosynaptic input from group I muscle afferents which are located in the intermediate nucleus of the L6 and L7 segments (Ib inhibitory interneurones, mean 48 m/s; Brink, Harrison, Jankowska, McCrea & Skoog, 1983; Fern, Harrison & Riddell, 1988).

In addition to the interneurones excited by group II muscle afferents, recordings were made from two other types of neurone projecting to L4 which have not been included in the main sample. Firstly, in order to check the correct placement of the L4 electrode, recordings were sometimes made at the start of an experiment from Ib inhibitory interneurones (n=8). Secondly, recordings were made from three neurones projecting to L4 which, though discharged by cutaneous afferents, were not excited by stimulation of any muscle nerves.

Location of the interneurones

Rostro - caudal location. The rostro - caudal distribution of the sites where recordings were made from interneurones excited by group II muscle afferents are shown in Fig. 42. The histograms show the numbers of neurones recorded from in the rostral, middle and caudal thirds of the L6 and L7 segments. The histograms in Fig. 42 A & B show the rostro - caudal distribution of the intracellularly (A) and extracellularly (B) recorded samples of interneurones with an ascending projection to L4, while the histograms in Fig. 42 D & E show the same information for the non - projecting sample of interneurones. The histograms show that the neurones investigated were located throughout the L6 and L7 segments though with some bias towards L7 and caudal L6. Tracking within the more rostral parts of the L6 segment was avoided in order to reduce the likely - hood of recording from group II interneurones of the type that have been described in the midlumbar segments (Edgley & Jankowska, 1987b). For similar reasons, tracking was not performed within 2 mm rostral of where the largest group II cord dorsum potentials were evoked by group II afferents of PBST in order to avoid recording from the population of group II interneurones which are common in the dorsal horn of the sacral segments (Jankowska & Riddell, 1993, 1994).

Location within the grey matter

An indication of the distribution of the recorded interneurones within the grey matter is provided in Fig. 42 C & F. These show representative outlines

of the grey matter in the transverse plane on which the locations of intracellularly recorded interneurones with (C) and without (F) an ascending projection to the L4 level are indicated. The positions of the interneurones shown on the left - hand outlines were reconstructed from information on the angle and depth of the recording track in relation to marking electrodes left in the spinal cord (see Methods). The right - hand outlines show partial reconstructions of neurones that were intracellularly labelled with biocytin.

The interneurones were distributed mainly in the ventro - lateral part of the dorsal horn and the lateral part of the intermediate zone; the majority were located in the lateral half or one third of laminae V and VI of Rexed (1954), with a few lying more dorsally in laminae IV and a few more ventrally in lamina VII. The interneurones were therefore distributed in an area which lies dorsal and lateral to the main region in which interneurones with input from group I muscle afferents are located (indicated by dashed line in Fig. 42 C & F; Czarkowska, Jankowska & Sybirska, 1981; Jankowska, Johannison & Lipski, 1981) and which is similar to the region in which field potentials evoked by group II muscle afferents can be recorded (see part 1). There appears to be some tendency in the plots of Fig. 42 C & F for the non - projecting interneurones to lie more dorsally than the L4 projecting neurones. However, the mean recording depth for the larger samples of extracellularly recorded interneurones with and without an ascending projection were not statistically different (mean depths 2.05 and 1.98 mm, respectively).

Excitatory input from muscle afferents

Afferent fibre origin

The sources of muscle afferent input to the interneurones were investigated by applying stimuli of graded intensity to muscle nerves. Stimuli 2T are close to maximal for group I afferents of most muscle nerves while the majority of group II afferents can be recruited by increasing the stimulus intensity from 2T to 5T (Jack, 1978; Ellaway et al. 1982; Lundberg et al. 1987a). Group II muscle afferents can therefore be considered to contribute to the extracellularly recorded discharges of neurones when they are evoked by stimulation of muscle nerves at 5T, but not by stimulation of the same nerve at 2T, while group I afferents are likely to be primarily responsible for discharges evoked by stimuli less than 2T.

All 55 of the interneurones investigated using extracellular recording were discharged by group II afferents of one or more muscle nerves, but none were discharged by stimulation at 2T. In the majority of cases where they were precisely determined (84%, n= 86), stimuli threshold for evoking discharges were greater than 2.5T. Examples of extracellularly recorded discharges in two L4 - projecting interneurones are shown in Figs. 43 and 44. In the neurone illustrated in Fig. 43, discharges could be evoked on stimulation of several muscle nerves at 5T which were not evident when the same nerves were stimulated at 2T, even with pairs of shocks. In the neurones illustrated in Fig. 44, discharges (upper traces of each panel) evoked by stimuli 5T (but not at 2T, not illustrated) were closely correlated with EPSPs seen in intracellular recordings subsequently obtained (middle

traces). Records in Fig. 48, A & B shows examples of EPSPs recorded from a non - projecting interneurones which seen at 5T but not at 2T.

In order to test the possibility that group I afferents might have weak actions, insufficient to evoke a discharge following single stimuli at 2T, pairs of stimuli were delivered in quick succession (about 1.0 - 1.5 ms apart) in an attempt to produce temporal summation. The majority of the 33 neurones (similar numbers of L4 - projecting and non - projecting neurones) tested in this way failed to respond even to double stimuli (see lower pairs of records in Fig. 43), but a small number (7) were discharged by pairs of stimuli. However, the latencies of these discharges were usually longer (>2.5ms with respect to the second shock) than would be expected for monosynaptic group I input and the thresholds for the responses were of the order of 1.8T which is already sufficient to activate the most excitable group II muscle afferents. It is therefore possible that the few examples of additional discharges seen using paired stimuli may be due to activation of the most excitable group II afferents rather than temporal summation of sub - threshold actions from group I afferents.

The evidence obtained from extracellular recordings suggest that any input from group I muscle afferents to the interneurones is likely to be much weaker than that from group II afferents. This is supported by observations made during intracellular recordings from 28 interneurones. Excitatory postsynaptic potentials (EPSPs) evoked by group II muscle afferents appeared only when the stimulus intensity was raised to 1.8 - 2.5T (depending on the nerve) which was usually close to maximum for group I

afferents as judged by the afferent volley. Increasing the stimulus intensity produced EPSPs of increasing amplitude until a maximum amplitude was reached at intensities of about 5T. Examples of group II EPSPs evoked in response to stimuli of increasing intensity are shown in Fig. 45 B (left and middle columns) and Fig. 46 B.

For the majority of intracellularly recorded interneurones excited by group II muscle afferents, stimulation of a range of muscle nerves at intensities of up to 2T (i.e. intensities activating most if not all group I afferents) failed to evoke any PSPs. Fig. 44 and Fig. 47 show examples of recordings from L4 - projecting (Fig. 44) and non - projecting (Fig. 47) interneurones of this type. However, in about a quarter (8/28, 29%) of the intracellularly recorded interneurones (4 L4 - projecting and 4 non - projecting) there was evidence of convergence from both group I and group II afferents of muscle nerves. In some cases (6 nerves) both group I and group II EPSPs were evoked by the same nerve, as illustrated by the records in the middle column of Figs. 45 B & 49 A. In other examples (7 nerves) the group I EPSPs were evoked from nerves without any group II actions, as illustrated by the records in Fig. 45 B (right hand column) and Fig 46A. As can be seen from the illustrated examples, the group I EPSPs were evoked at shorter latencies and at lower stimulus strengths than those attributed to group II afferents. Consistent with the lack of extracellularly recorded discharges of neurones following stimulation of muscle nerves at 2T, group I EPSPs were always considerably smaller (less than half the amplitude) than the largest group II EPSPs evoked in the same neurone.

Threshold stimuli evoking group I actions, particularly those of PBST, were often of the order of 1.4T or more, which is higher than that required to excite the most sensitive group I fibres. The possibility that group Ib afferents might be primarily responsible for the group I excitation was therefore investigated for 4 EPSPs evoked by group I afferents of PBST, by taking advantage of the separation usually visible in the group I afferent volley between fibres innervating muscle spindle primary endings (Ia) and fibres innervating Golgi tendon organs (Ib, cf. Bradley & Eccles, 1953). Graded stimulation of PBST was found to produce PSPs that grew in parallel with the second (Ib) component of the afferent volley as illustrated by the recordings in Fig. 46A. These observations suggest that group Ib afferents contribute to the group I excitation of this population of interneurones. However, the possibility remains that group Ia afferents also contribute to the group I EPSPs, particularly those evoked by nerves other than PBST.

The actions of muscle afferents of high electrical threshold were only occasionally investigated by raising the stimulus intensity from 5T to 20T, which is sufficient to activate a proportion of group III muscle afferents (Lundberg et al. 1987a; Ellaway et al. 1983), produced considerable additional excitation in all five of the intracellularly recorded interneurones investigated in this way. Such group III EPSPs could be evoked from nerves also evoking group II EPSPs (as in the examples shown in Figures 46 A, B and 47 A, B) or from nerves where stimuli of 5T were ineffective.

Nerve origin

The proportions of extracellularly and intracellularly recorded interneurones excited by input originating from different muscle and cutaneous nerves are shown in the histograms of Fig. 50. Since the pattern of peripheral input to L4 - projecting and non - projecting interneurones was not obviously different, data for the two types of neurone have been pooled. Figure 50 A shows that group II afferents of Q and DP evoked discharges in the largest proportions of interneurones (nearly 80% and 70% respectively). Group II afferents of TP, PBST and GS (45 - 55%) and Pop and FDL (about 30%) were also an effective source of excitation of the interneurones while group II afferents of PI and ABSM were the least effective of those tested.

The frequency with which group II afferents of different muscle nerves evoked EPSPs in the intracellularly recorded interneurones is shown in Fig. 50 B. As can be seen, the order of effectiveness of group II afferents of different nerves as a source of EPSPs is very similar to their effectiveness in evoking discharges. Furthermore, the proportions of neurones in which group II EPSPs were evoked by a given nerve are, with the exception of Pop, quite similar to the proportion of neurones in which the same nerve produced discharges. Taken together, this evidence suggests that transmission from group II afferents of most muscle nerves to the interneurones is highly secure, usually being sufficient to evoke a discharge of the neurone.

The shaded portions of the histograms indicate the proportion of neurones in which EPSPs were evoked by group I afferents of muscle nerves.

As described above, these were seen much less frequently than group II EPSPs, even the most effective nerve (PBST) having group I actions on only 14% of neurones. Furthermore, the effectiveness of different nerves as a source of group I input did not match their effectiveness as a source of group II input. For example, stimulation of PI and ABSM, which produced the least effective group II actions, evoked group I EPSPs with the same frequency as stimulation of Q and DP which produced the most effective group II actions. In contrast, group I afferents of FDL, TP, Pop never produced group I EPSPs even though they were an effective source of group II input.

There was considerable convergence onto single interneurones from group II afferents of different muscle nerves. Group II afferents of two or more muscle nerves discharged 75% of the extracellularly recorded sample and evoked EPSPs in 90% of the intracellularly recorded interneurones while group II afferents of three or more muscle nerves discharged 43% of the extracellularly recorded sample and evoked EPSPs in 75% of the intracellularly recorded interneurones (see Fig. 52 A). There appeared to be a slightly lesser degree of convergence in the L4 - projecting interneurones than in the non - projecting interneurones; group II afferents of 3 or more muscle nerves evoked discharges in 30%, and EPSPs in 85% of L4 - projecting interneurone but in non - projecting neurones the equivalent proportions were 56% and 100%.

There was also a difference in the degree of convergence seen in neurones recorded in the L7 segment compared to those recorded in L6. Group II afferents of 3 or more muscle nerves discharged 51% of neurones in

L7 but only 25% of those in L6 and evoked EPSPs in 88% of neurones in L7 but only 58% of those in L6 (see Fig. 52 A). Figure 52 B shows that this is related to differences in the effectiveness of certain muscle nerves in the two segments. Group II afferents of Q, DP and Pop were similarly effective in both the L6 and L7 segments while group II afferents of GS, PBST and FDL produced EPSPs in a higher proportion of interneurones in the L7 segment than L6. These observations are consistent with the relative frequency with which group II field potentials evoked by these nerves tend to be recorded in the L6 and L7 segments (see part 1 field potentials).

The degree of convergent input to the interneurones is actually greater still if the actions of other types of afferent fibres on the interneurones is taken into account. In addition to input from group II muscle afferents, 29% were excited by group I muscle afferents and 82 % by cutaneous afferents of at least one nerve (see below). Although less systematically investigated, excitation from afferents of the posterior knee joint and interosseal nerves was also observed.

Synaptic linkage

The central delays (latencies) of EPSPs evoked by group II muscle afferents were measured in relation to the arrival of the group I afferent volley since volleys in group II muscle afferents could rarely be detected. The range of delays likely to be consistent with monosynaptic actions of the fastest group II afferents was estimated by considering: a) delay between the arrival of impulses in the fastest conducting group I and group II muscle afferents at the

dorsal roots, b) delay due to conduction within the dorsal columns, for those afferents entering the cord at a distance from the recording location, c) delay for intraspinal conduction (i.e. from the dorsal roots along branching collateral axons to axon terminals) and d) a delay for synaptic transmission. This is concluded based on the following analysis which performed for the latencies of potentials.

The delay between the arrival of impulses in the fastest conducting group I and group II afferent fibres (conduction velocities taken as 110 m/s and 70 m/s respectively) was calculated using measurements of conduction distance for each nerve. The level of entry of afferents of different nerves was determined by observing where the largest afferent volleys were evoked and conduction in the dorsal columns is taken to be half that in the periphery (Munson et al. 1980; Fern et al. 1988). Intraspinal delays for group II muscle afferents terminating in the lower - lumbar segments have been reported to range from 0.4 ms for terminals in the dorsal horn to 1.15 ms for terminals in the ventral horn (Fu & Schomburg, 1974). Synaptic transmission is considered to account for a further delay of 0.3 ms.

For GS (conduction distance 165 - 176 mm), impulses in the fastest conducting group II afferents should arrive at the cord about 0.9 ms after those in group I afferents; a similar delay has been observed experimentally (0.9 - 1.1 ms, table 1 of Fu et al. 1974).

For PBST which has a shorter conduction distance (105 - 121 mm) the delay will be a little shorter (about 0.6 ms). Afferents of both the GS and PBST nerves enter the cord mainly within S1 and caudal L7 so that impulses

reaching rostral L7 or caudal L6 must travel in the dorsal columns for up to 5 mm or more; this will involve an additional delay of about 0.2 ms. Central delays considered compatible with monosynaptic actions of the fastest conducting group II afferents are therefore 1.6 - 2.5 ms for GS and 1.3 - 2.2 ms for PBST.

Conduction distances measurements were not made for Q but will be similar to those for PBST (see also Edgley & Jankowska, 1987a, far right column in Table 1). However, Q afferents enter the cord mainly close to the L5 - L6 border so that impulses reaching caudal parts of L6 or rostral L7 must travel in the dorsal columns for up to 10 mm involving a delay of some 0.3 ms. A similar rationale applies to impulses in group II afferents of DP which will, however, be subject to greater delay with respect to the group I volley (1.0 ms) because of the longer conduction distance for this nerve (195 - 198 mm). Central delays considered compatible with monosynaptic actions of the fastest conducting group II afferents are therefore 1.3 - 2.3 ms for Q and 1.7 - 2.7 ms for DP.

The FDL, TP and Pop nerves have very similar conduction distances (178 - 197 mm) so that the arrival of impulses in group II afferents of these nerves at the cord will be delayed with respect to the group I volley by a similar amount (0.9 - 1.0 ms). The largest afferent volleys evoked by stimulation of these nerves were generally recorded in rostral L7 and no allowance is therefore made for conduction in the dorsal columns. Central delays considered compatible with monosynaptic actions of the fastest conducting group II afferents are therefore 1.6 - 2.4 ms.

It should be emphasised that these calculated ranges of central delays are only rough estimates since there are several sources of variation in the data from which they are derived. Firstly, there are variations in the peripheral conduction distances in different animals. Secondly, the conduction velocities of the fastest conducting group I and group II fibres vary among different nerves (e.g. Jack, 1978). Thirdly, the dorsal column conduction distances vary because the recording locations of neurones varied from rostral L6 to caudal L7. Finally, the intraspinal conduction delays vary according to the region (dorsal or ventral) of afferent termination.

The distribution of the latencies of discharges and EPSPs evoked by group II afferents of muscle nerves are shown in Fig. 51. The solid filled bars in the intracellular histograms indicate those EPSPs that are considered likely to have been evoked monosynaptically by the very fastest conducting group II afferents based on the arguments presented above. The histograms show that for most of the nerves investigated, a proportion of the EPSPs evoked by group II afferents occurred at latencies which were compatible with a monosynaptic connection. The one exception was the action of group II afferents of popliteus (shaded bars) which evoked EPSPs only at latencies of 3.0 ms or more. Group II afferents of one or muscle nerves produced EPSPs considered to be monosynaptic in 17 of the 28 (61%) intracellularly recorded interneurones. It is possible that the actual proportion of monosynaptic connections from group II muscle afferents may be greater than that indicated in the histograms since most group II afferents conduct more slowly than the 70 m/s on which the estimates are based. The black bars under the

histograms indicate the range of latencies of EPSPs that could be consistent with the monosynaptic actions of the full range of group II muscle afferents (i.e. with conduction velocities of 70 to 35 m/s, see Matthews, 1972). However, it should be emphasised that at the longer end of these ranges, monosynaptic actions of slowly conducting group II afferents can not be distinguished from possible di - or tri - synaptic actions of faster conducting group II afferents.

The latencies of all 13 of the group I EPSPs seen were within the range (0.6 - 1.0 ms with respect to group I volleys) expected of the monosynaptic actions of group I muscle afferents (see for e.g. Harrison & Jankowska, 1985).

Excitatory input from cutaneous, joint and interosseal afferents

Cutaneous afferents

In addition to muscle nerves, the actions of stimuli applied to one or more cutaneous nerves were investigated in virtually all of the sample of interneurones. Stimulation of cutaneous nerves, often at strengths close to nerve threshold, discharged 82% of the extracellularly recorded sample and produced EPSPs in all but one of the intracellularly recorded neurones tested (n=26); there was no difference between the L4 - projecting and non - projecting neurones examined. Examples of extracellular recordings of discharges evoked by cutaneous afferents are shown in Fig. 43 and Fig. 44 while examples of intracellularly recorded EPSPs are shown in Figs. 44, 46 C ,47,48 E and 49 E. The histograms of Fig. 50 show that cutaneous afferents of SP and sural were almost equally effective in evoking discharges (Fig. 50 A)

or EPSPs (Fig. 50 B) in the neurones and in fact, where the actions of both Sur and SP were tested on the same neurones, both were usually effective.

The histograms of Fig. 50 also show that cutaneous afferents of both the SP and sural nerves evoked discharges (Fig. 50 A) or EPSPs (Fig. 50 B) in the sample of interneurones as frequently as group II afferents of the most effective muscle nerves. Furthermore, as illustrated by the examples of intracellular records (Figs. 44, 46 C, 47, 48 E and 49 E), EPSPs evoked by cutaneous afferents of both Sural and SP were often as large, if not larger, than those produced by the most effective group II muscle afferents. Cutaneous afferents therefore provide a powerful source of input to most of the group II interneurones.

Cutaneous afferents of the SP nerve enter mainly at L7 so that, apart from a delay for synaptic transmission, the delay between the arrival of afferent impulses at the spinal cord and the synaptic potentials they evoke in interneurones will be due only to intraspinal conduction. This was assumed to be similar to the delay experienced by impulses in group II muscle afferents terminating in the dorsal horn and intermediate zone (0.4 - 0.8 ms; Fu & Schomburg, 1974). Afferents of the Sur nerve enter the cord mainly at S1; impulses in these afferents must therefore travel up to 10 mm in the dorsal columns to reach mid L6 and will therefore be delayed by an additional 0.3 ms or so. Central delays considered compatible with the monosynaptic actions of cutaneous afferents are therefore 0.7 - 1.1 ms for SP and 1.0 - 1.4 ms for Sur. As can be seen from the histograms in Fig. 51, about half of the EPSPs evoked by Sur and SP have latencies that lie within these ranges so that at

least a proportion of the cutaneous input to the interneurones is likely to be evoked monosynaptically. As with the actions of muscle afferents, this may be an underestimate if some of the longer latency EPSPs are evoked by slower conducting cutaneous afferents.

Joint and interosseous afferents

The effects of stimulation of the posterior nerve to the knee joint and the interosseous nerve (i.o.) were investigated less systematically than the actions of muscle and cutaneous afferents. Nevertheless examples of excitatory actions of these afferents were seen during extracellular and intracellular recording. Figure 47 D shows examples of intracellularly recorded potentials evoked by afferents of the joint nerve and Fig. 44 shows an example of both extracellular discharges and EPSPs evoked by afferents of the interosseous nerve. The latencies of EPSPs evoked by joint and i.o. afferents ranged from 1.3 to 3.2 ms (n=9) and 2.3 to 5.9 ms (n=6), respectively. The linkage of joint and i.o. afferents is more difficult to estimate than that of other afferents (see for example Harrison & Jankowska, 1985) but at least some of the EPSPs are likely to represent monosynaptic actions of these afferents.

Th13 projecting neurones.

Although the main purpose of this study was to investigate group II interneurones in the L6 - L7 segments, a few neurones were also encountered in this region. Recordings were made from 7 neurones antidromically activated by stimuli applied at the Th13 level. Six of these were excited by stimuli applied to lateral funiculus and one by stimuli applied contralaterally.

Four of the neurones were recorded extracellularly, three intracellularly and one both intra - and extracellularly.

Tests of projection

Figs. 54 C - E & 55 E extracellular records obtained while testing the rostral projection of the neurones. Fig. 54 C shows an antidromic impulse (indicated by arrow) produced by stimulation within the lateral funiculus at L4. Fig. 54 D shows examples of potentials which follow faithfully a train of stimuli (up to 650 Hz) applied at Th13. In Fig. 54 E, the top set of records shows the orthodromic impulses produce by stimulation of peripheral nerve and antidromic impulses produce by stimulation at Th13. The bottom set of records show the result of collision of the antidromic impulse with the orthodromic impulse within the critical interval. The records in Fig. 55 E shows an example of antidromic response (indicated by arrow) to stimulation at Th13 recorded from another neurone.

Conduction velocity.

Axonal conduction velocities measured between Th13 and the recording site (after having subtracted 0.2 ms for the latent period of activation) ranged from 34 - 78 m/s.

Location.

The neurones were encountered at rostro - caudal locations from caudal L6 to the middle of the L7 segment and at depths of 1.94 - 2.37 mm from the surface of the spinal cord. The neurones were located in the

ventrolateral part of the dorsal horn in lamina IV - V. A partial reconstruction of one neurone which was intracellularly labelled with biocytin is shown against an outline of the grey matter in Fig. 53.

Pattern of input.

The peripheral input to the Th13 neurones was similar to group II interneurones in this region. Taking extracellular and intracellular recordings together the neurones were excited by group II afferents of different nerves as follows; Q (6/7), PBST (6/7), TP (2/3), GS (4/7), FDL (2/6).

During extracellular recordings the neurones were strongly excited by stimuli applied to muscle nerves at 5T which activate group II muscle afferents but none were excited at 2T even when pairs of shocks were applied to produce temporal summation (Fig. 54 A and B). Figs. 54 and 55 show examples of extracellular and intracellular recordings made from these neurones in the L7 segment. Similarly, during intracellular recording EPSPs were evoked by stimuli applied at 5T to muscle nerves but little or no excitation occurred at 2T.

Fig. 55 shows evidence for convergence from group II and group III muscle afferents. In A, stimulation of the Q and DP muscle nerves evokes EPSPs which grow in amplitude between 3T and 5T and are therefore attributable to group II afferents. In B & C, stimulation of ABSM, PBST, GS and PI at 20T produce additional PSPs not seen at 5T which are attributable to group III afferents.

In addition to input from muscle afferents, stimulation of Sur (7/7), SP (2/2) and i.o. (1/1) nerves produced excitation of these neurones. Records in Figs. 54 B & 55 D show examples of extracellular and intracellular recordings which usually appeared at low threshold (2T).

Figures and tables

Fig. 30. Diagrammatic representation of the experimental arrangement used for investigating lower - lumbar interneurones. Bipolar ball electrodes were used to apply electrical stimuli to peripheral nerves (S1). Intra - and extracellular recordings were made from neurones in the lower - lumbar segment using glass microelectrodes (R). A tungsten electrode was inserted into the lateral funiculus at L4 to stimulate ascending axons of lower - lumbar interneurones (S2). To differentiate between interneurones and ascending tract neurones stimuli were also applied to the lateral funiculi at Th13 through ball electrodes placed on the surface of ipsi - and contralateral lateral funiculi (not shown). A and B, show two possible explanations for impulses recorded in lower - lumbar interneurones following stimuli applied to L4. A, shows a neurone with an ascending axon projecting to L4 by which action potentials could be antidromically conducted to the cell body. B, shows an axon (which might originate from descending neurones, propriospinal neurones or primary afferent neurones) synapsing with the lower - lumbar interneurones by which action potentials could be induced synaptically. The collision test was performed to distinguish between these two possibilities.

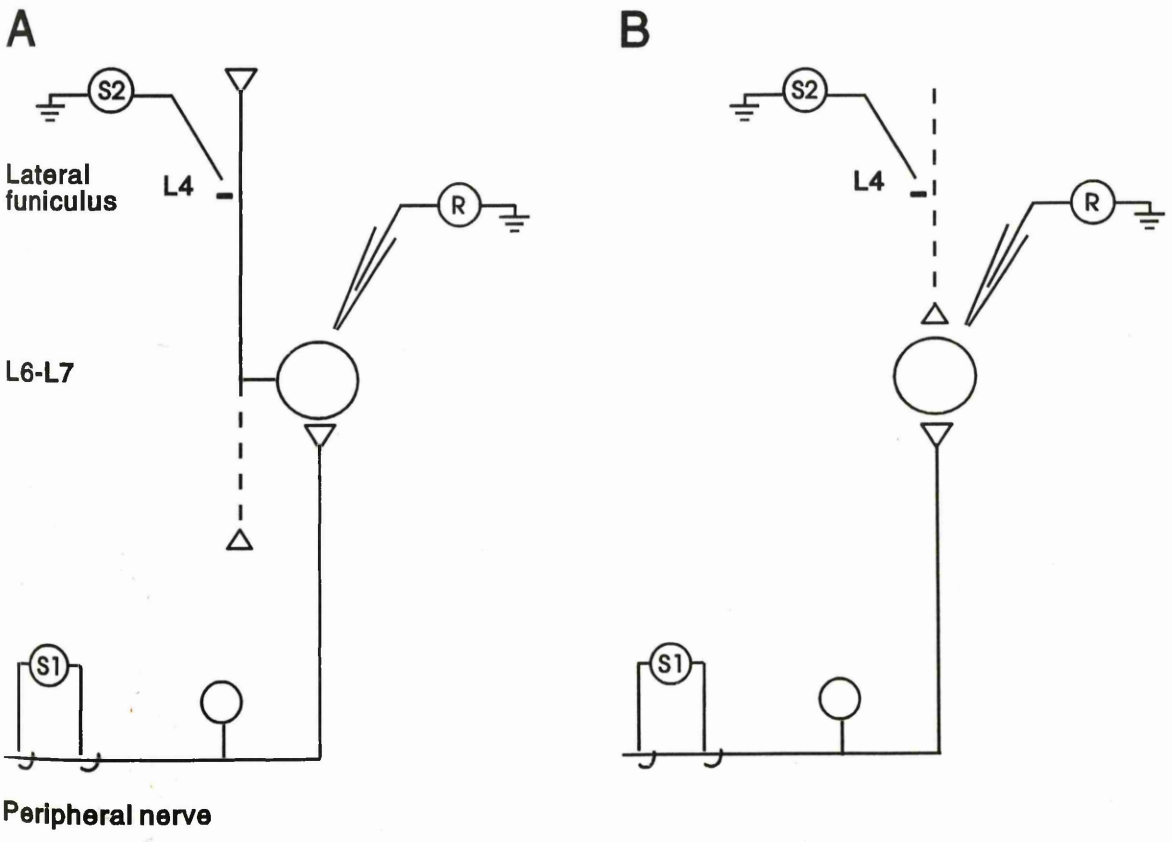

A
S2
Lateral
funiculus
L4
R
L6-L7
S1
Peripheral nerve
B
S2
L4
R
S1

Fig. 31. Cord dorsum potentials (CDPs) evoked by group II muscle afferents. A, B & C; CDPs recorded from the surface of the lower - lumbar segments following stimuli of different strengths applied to the tibialis posterior (TP, A), popliteus (Pop, B) and flexor digitorum longus (FDL, C) nerves. For comparison, a cord dorsum potential evoked by group II afferents of posterior biceps - semitendinosus which was recorded more caudally (rostral S1) is shown in D. All recordings are averages of 32 sweeps, negativity upwards. Time and voltage calibrations apply to all records. Arrows indicate group II afferent volleys.

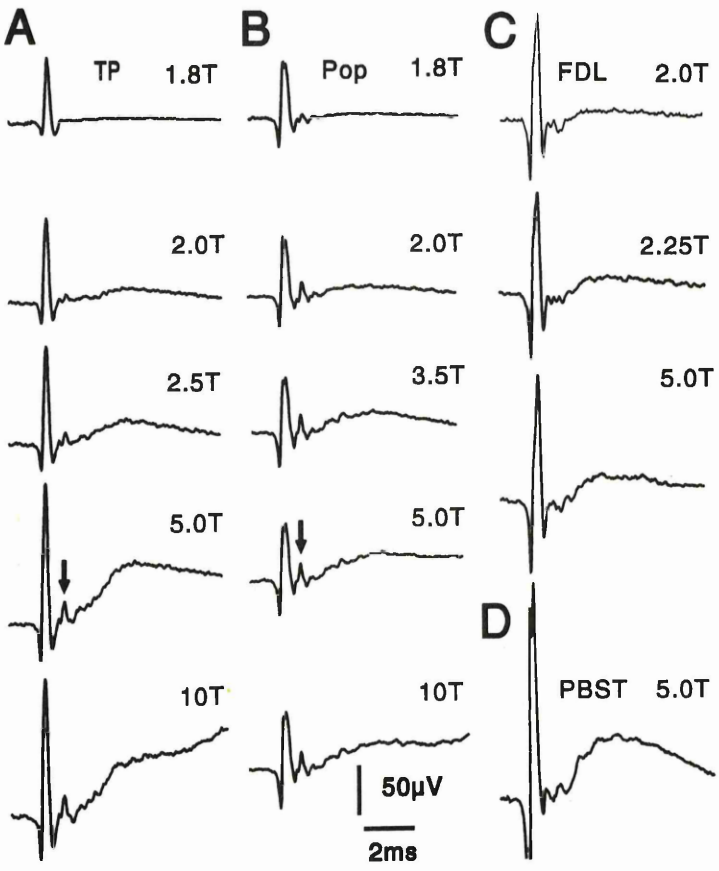

A
TP
1.8T
2.0T
2.5T
5.0T
10T
B
Pop
1.8T
2.0T
3.5T
5.0T
10T
50μV
2ms
C
FDL
2.0T
2.25T
5.0T
D
PBST
5.0T

Fig. 32. Rostro - caudal distribution of CDPs evoked by group II afferents of tibialis anterior nerve. A, plot of amplitude of cord dorsum potential evoked by stimuli 5T, recorded at 2 mm intervals along the surface of the lower - lumbar segments in one experiment. B, location of CDPs evoked by stimuli 5T in seven animals. Black arrow heads indicate the positions where the largest potentials of group II origin were recorded following stimuli applied to TP nerve and black bars indicate the length over which potentials were at least 75% of peak amplitude. White arrow heads indicate the positions where the largest potentials of group II origin were recorded following stimuli applied to PBST nerve.

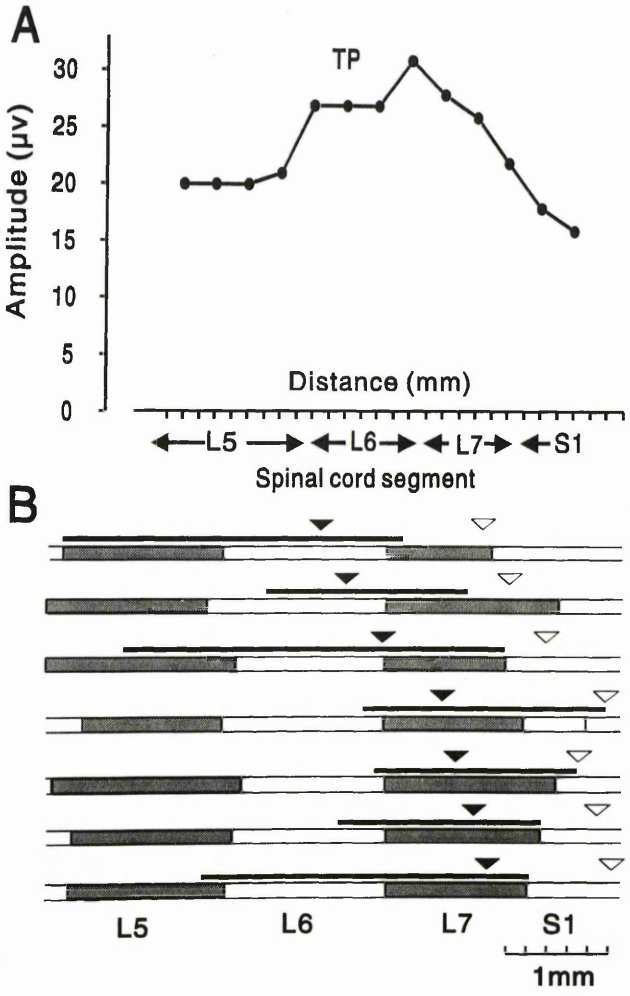
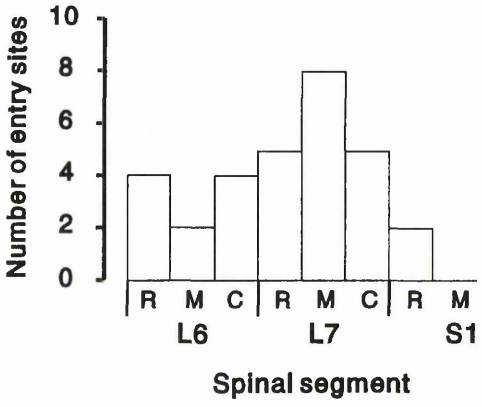

A
TP
30
25
20
15
10
5
0
Amplitude (μv)
Distance (mm)
L5
L6
L7
S1
Spinal cord segment
B
L5
L6
L7
S1
1mm

Fig. 33. Recording sites in the lower - lumbar segments. A & B, rostro - caudal distribution of 30 microelectrode entry sites in the lower - lumbar segments where the grey matter was systematically explored in the transverse plane. A, positions of entry sites in relation to segmental boundaries; rostral (R), middle (M) and caudal (C) third of the L6 - S1 segments. B, positions of entry sites in relation to the location of the largest cord dorsum potentials evoked by group II afferents of PBST (0 mm).

A


Number of entry sites
10
8
6
4
2
0
R M C R M C R M
L6 L7 S1
Spinal segment

B

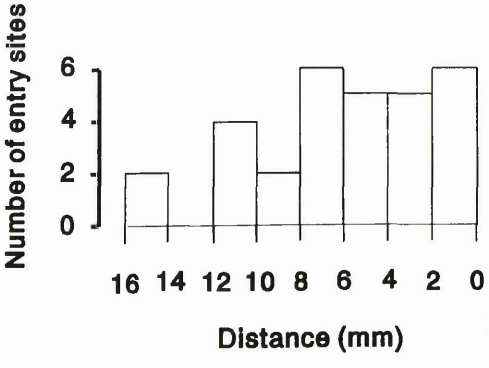

Number of entry sites
6
4
2
0
16 14 12 10 8 6 4 2 0
Distance (mm)

Fig. 34. Frequency of occurrence and amplitude of group II field potentials in lower - lumbar segments. A, relative frequency of occurrence of group II field potentials of different nerves (identified above each bar). Bars represent the percentage of transverse mapping sites at which field potentials were detected; the number of sites investigated are shown underneath each bar. B, mean maximal amplitudes of field potentials evoked by group II afferents of each nerve. These values are calculated from the largest field potentials recorded at each transverse mapping site; numbers of potentials from which means are calculated are shown underneath each bar. Data for ABSM is omitted because no field potentials were detected at any location.

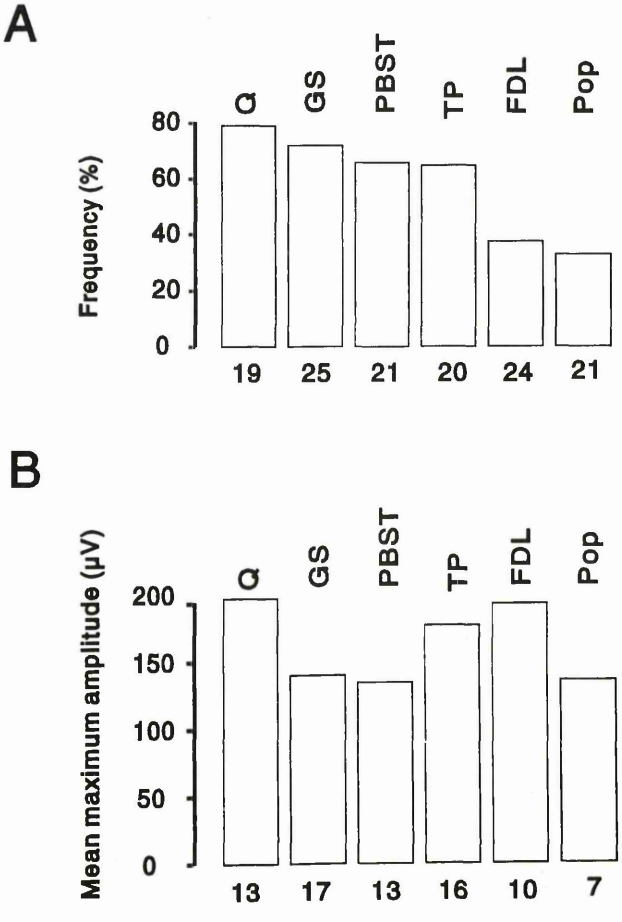

A
Frequency (%)
80
60
40
20
0
Q
GS
PBST
TP
FDL
Pop
19
25
21
20
24
21
B
Mean maximum amplitude (μV)
200
150
100
50
0
Q
GS
PBST
TP
FDL
Pop
13
17
13
16
10
7

Fig. 35. Field potentials evoked by group II muscle afferents of tibialis posterior (TP) nerve in caudal L7. A, examples of field potentials evoked by stimuli of different strengths, recorded at the same location. The top record of each pair is a field potential recording (negativity downward) and the bottom record is afferent volley (negativity upward). All records are averages of 32 sweeps. Calibration pulse; 1 ms, 200μV. B, outline of grey matter on which is indicated the areas in which field potentials evoked by group II (>200μV; shaded region) and group I (>150μV; dashed line) afferents of TP could be recorded. The recordings in A were made within the shaded region.

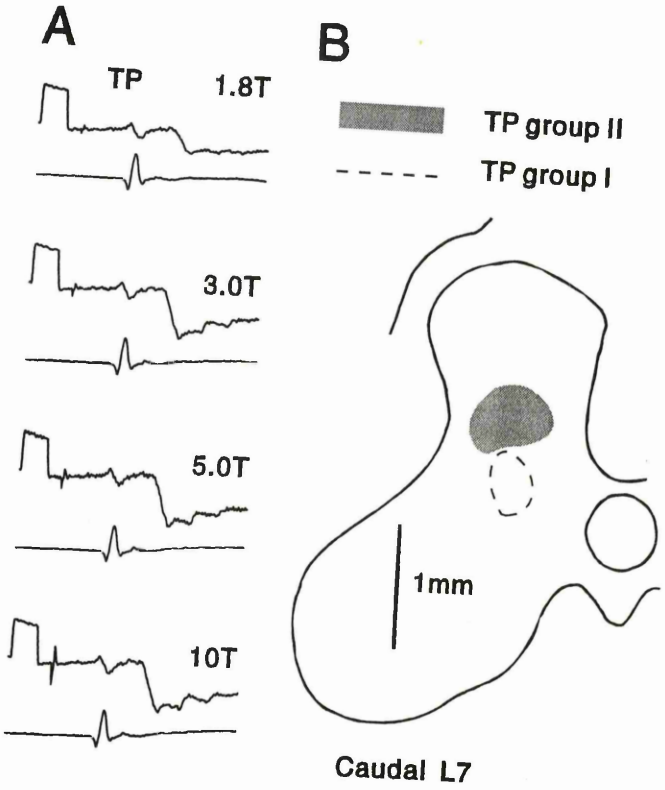

A
TP
1.8T
3.0T
5.0T
10T
B
TP group II
TP group I
1mm
Caudal L7

Fig. 36. Field potentials evoked by group II muscle afferents of tibialis posterior (TP) in rostral S1. A, examples of field potentials evoked by stimuli at different strengths; for comparison, the lower most pair of traces show a field potential evoked by group II afferents of PBST at the same location. All records are averages of 32 sweeps. Calibration pulse; 1 ms, 200μV. B, outline of grey matter on which is indicated the areas in which field potentials evoked by TP (>200μV; shaded region) and PBST (>400μV; solid line, >100μV; dashed lines) could be recorded. The recordings in A were made within the dorsal horn areas.

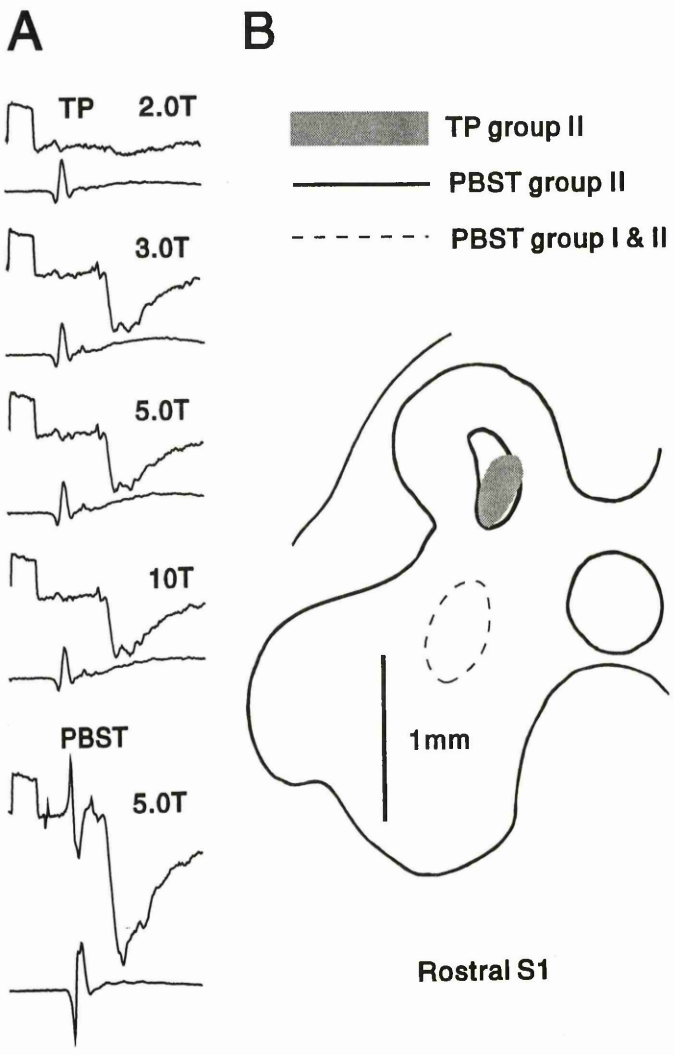

A
B
TP 2.0T
3.0T
5.0T
10T
PBST
5.0T
TP group II
PBST group II
PBST group I & II
1mm
Rostral S1

Fig. 37. Field potentials evoked by afferents of FDL nerve in the L7 segment. A, examples of field potentials; note that stimulation at 2T evoked only a small group I field while stimuli 5T evoked both group I and group II fields (denoted I and II, respectively). B, outline of grey matter on which is indicated the areas in which field potentials evoked by group II afferents (>100μV; shaded region) and group I afferents (>200μV; dashed line) of FDL, including those shown in A, could be recorded. All records are averages of 32 sweeps. Calibration pulse, 1 ms, 200μV.

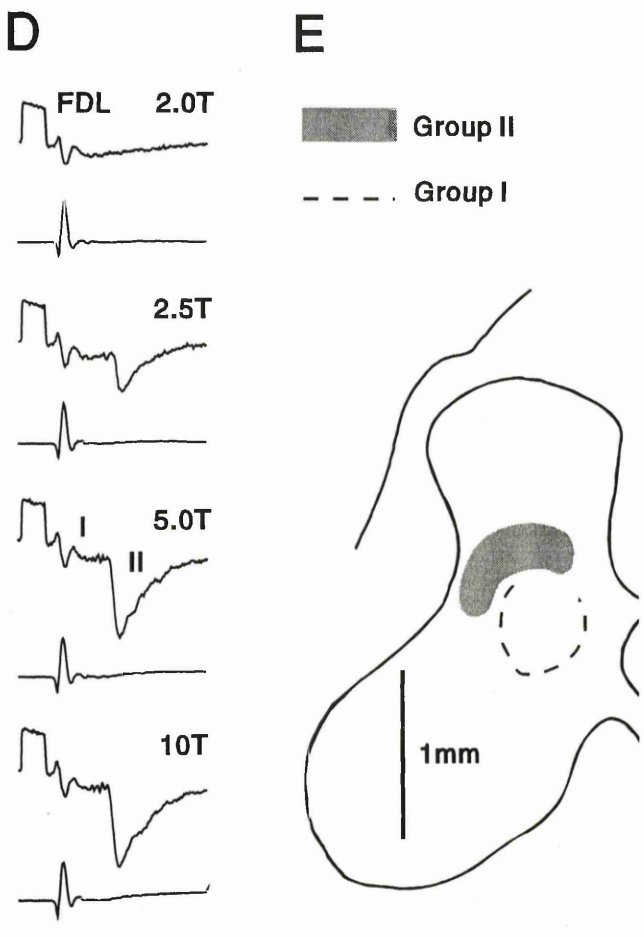

D
FDL
2.0T
2.5T
I
II
5.0T
10T
E
Group II
Group I
1mm
Caudal L7

Fig. 38. Field potentials evoked by afferents of the flexor digitorum longus (FDL) in the L7 segment. A, examples of field potentials evoked by afferents of FDL; note that stimulation at 2.25T evoked only a small group I field while stimuli 5T evoked both group I and group II fields (denoted I and II respectively). Calibration pulse; 1 ms, 200μV. B, The pair of records show the response to a train of stimuli at 333 Hz. C, outline of grey matter on which is indicated the area in which field potentials (>150μV) evoked by group II afferents of FDL, including those shown in A, could be recorded.

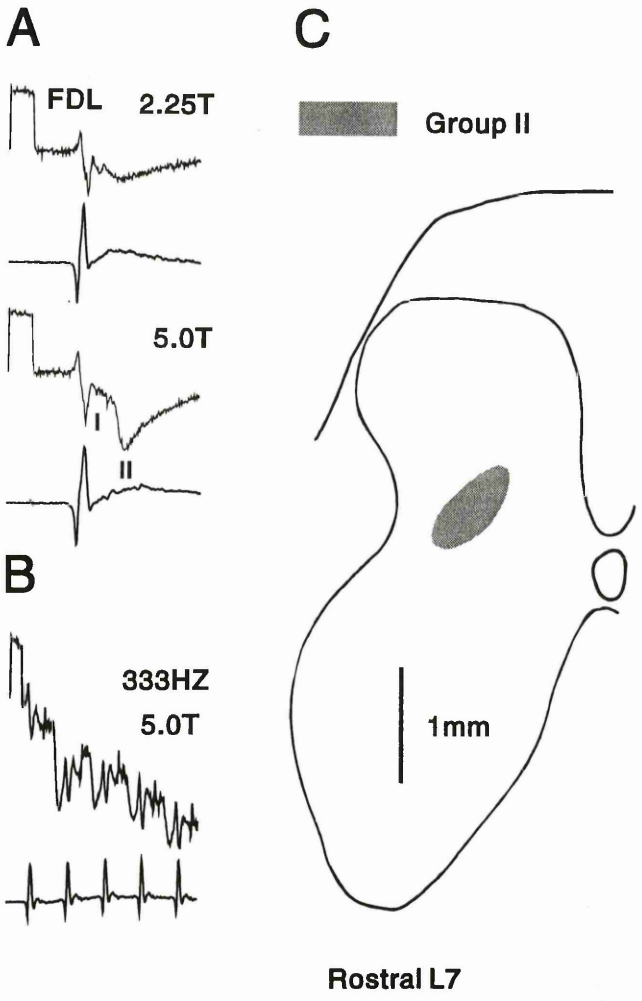

A
FDL
2.25T
5.0T
I
II
B
333HZ
5.0T
C
Group II
1mm
Rostral L7

Fig. 39. Field potentials evoked by group II muscle afferents of popliteus (Pop) nerve in caudal L7. A, examples of field potentials evoked by stimuli at 2T and 5T, recorded at the same location. The top record of each pair is a field potential recording (negativity downward) and the bottom record is afferent volley (negativity upward). All records are averages of 32 sweeps. Calibration pulse; 1 ms, 200μV. B, outline of grey matter on which is indicated the areas in which field potentials (>100μV) could be recorded. The recordings in A were made within the shaded region.

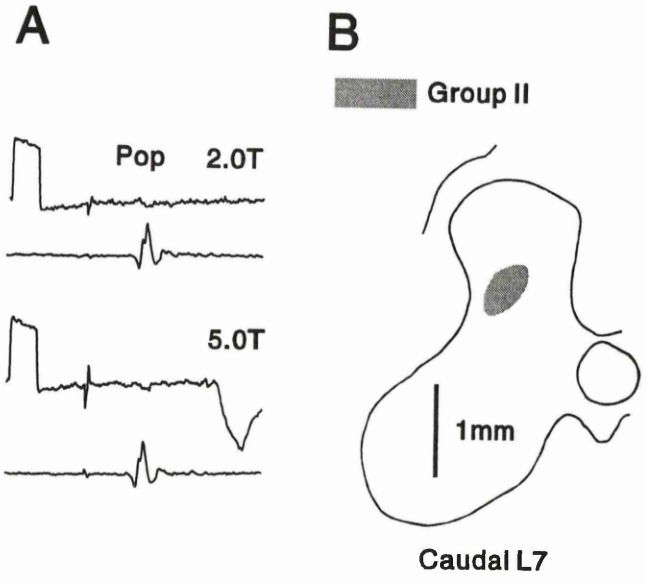

A
B
Group II
Pop
2.0T
5.0T
1mm
Caudal L7

Fig. 40. Field potentials evoked by afferents of the quadriceps nerve at different rostro - caudal levels of the lower - lumbar spinal cord. Panels, A, B & C, illustrate field potentials recorded at three different rostro - caudal levels in the same experiment. In each panel, the top pair of records show potentials evoked by stimuli maximal for group I afferents but sub - maximal for group II afferents, while the lower pair of records show potentials evoked by stimuli close to maximal for group II afferents. Note that the stronger stimuli produced both group I and II field potentials (denoted I and II, respectively). Note also the decrease in amplitude and increase in latency of the group II fields that occurs at progressively more caudal levels. Records are averages of 32 sweeps. Calibration pulse; 1 ms, 200μV. Underneath the records in each panel is an outline of the spinal cord on which is shown the area within which field potentials of group II origin, including those illustrated, could be recorded (>200μV; A & B, >100μV; C).

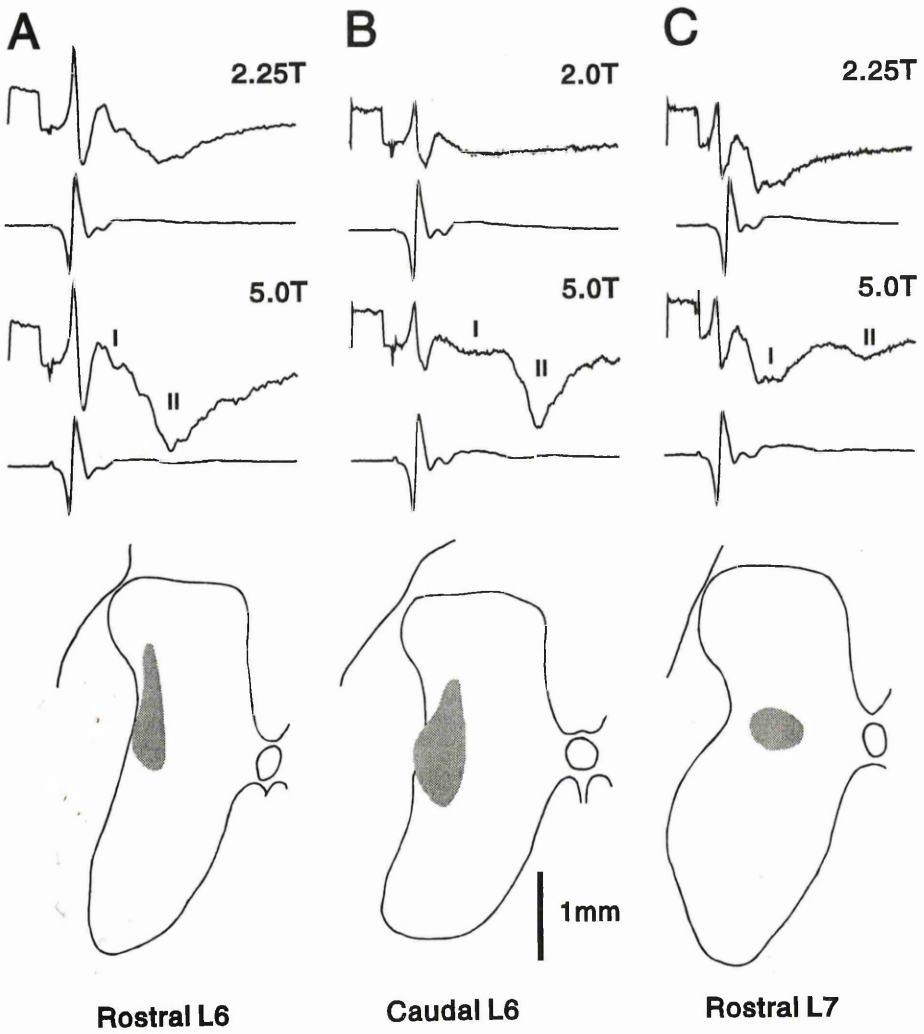

A
2.25T
5.0T
I
II
B
2.0T
5.0T
I
II
C
2.25T
5.0T
I
II
1mm
Rostral L6
Caudal L6
Rostral L7

Fig. 41. Field potentials evoked by afferents of the quadriceps nerve (Q). A, potentials recorded at the surface and at different depths within the grey matter following stimulation at 5T. Field potentials evoked by group I and group II afferents are denoted I and II, respectively. Records are averages of 32 sweeps. Calibration pulse; 1 ms, 200μV. B, outline of grey matter at caudal L6 on which are indicated the recording sites of the potentials shown in A.

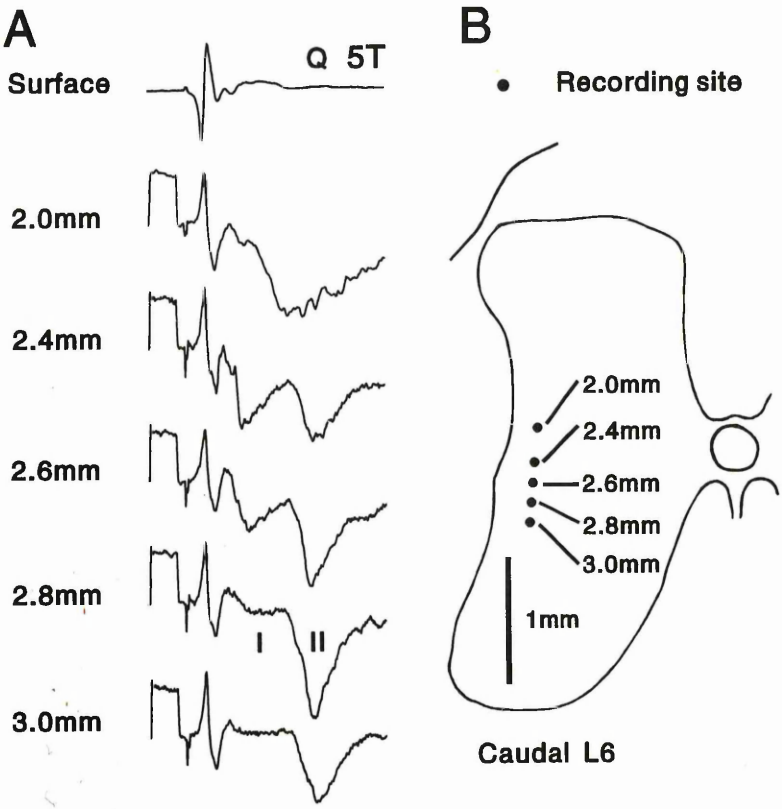

A
Surface
Q 5T
2.0mm
2.4mm
2.6mm
2.8mm
I
II
3.0mm
B
Recording site
2.0mm
2.4mm
2.6mm
2.8mm
3.0mm
1mm
Caudal L6

Fig. 42. Location of the interneurones investigated. A & B, rostro - caudal distribution of the sample of interneurones with a projection to L4. The histograms show the numbers of interneurones recorded in the rostral (R), middle (M) and caudal (C) third of the L6 - S1 segments. A, data for intracellularly recorded neurones. B, data for extracellularly recorded neurones (one neurone located in S1 not shown). C, locations of L4 projecting interneurones within the spinal grey matter. The outline to the left shows the locations of intracellularly recorded neurones, reconstructed using information on the recording angle and depth in relation to a marking electrode left in the recording track. Plots from different experiments were superimposed by alignment of the dorsal and lateral borders of the grey matter. The typical location of interneurones receiving excitatory input from group I muscle afferents is indicated by the circle drawn with a dashed line (data from Czarkowska, Jankowska & Sybirska, 1981; Jankowska, Johannison & Lipski, 1981). On the outline to the right are shown partial reconstructions of two neurones intracellularly labelled with biocytin. D, E & F, segmental distribution and location within the grey matter of the sample of non - projecting interneurones. Same format as A - C.

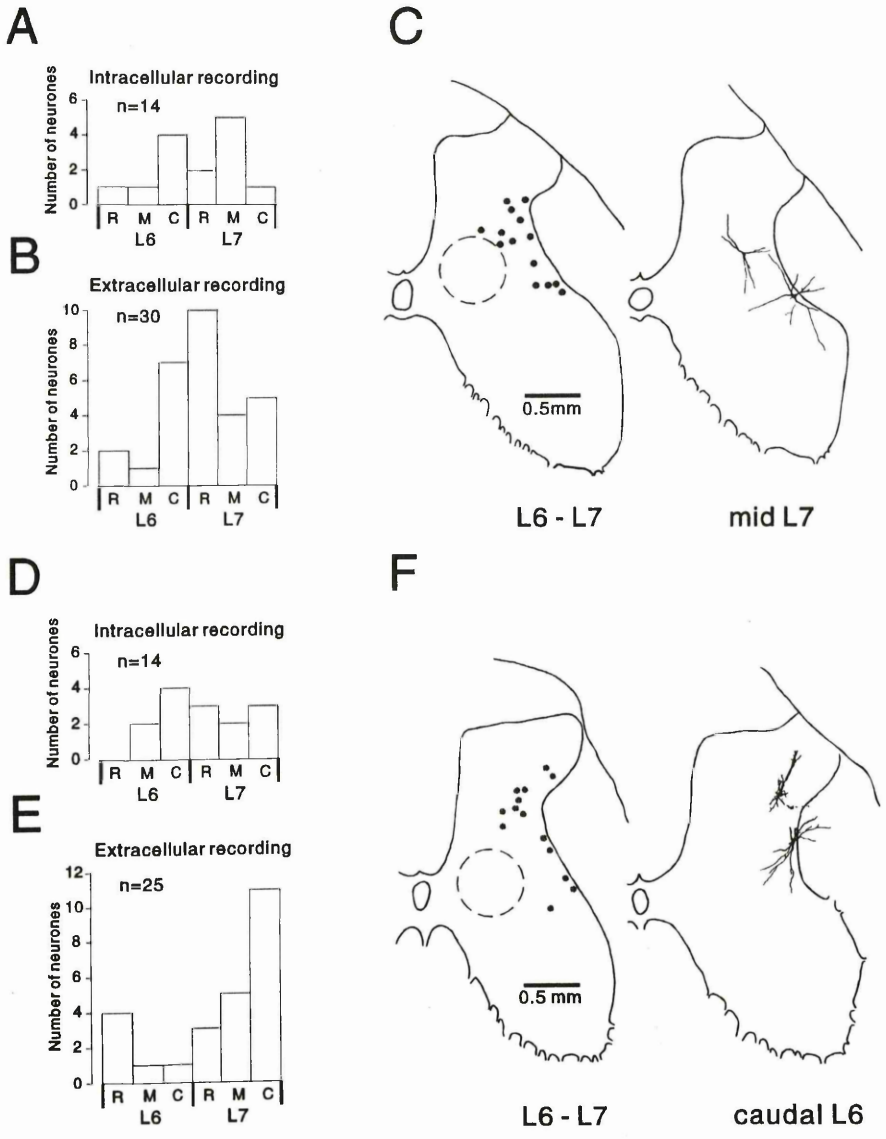

A
Intracellular recording
n=14
Number of neurones
R M C R M C
L6 L7
B
Extracellular recording
n=30
C
0.5mm
L6 - L7
mid L7
D
Intracellular recording
n=14
E
Extracellular recording
n=25
F
0.5 mm
L6 - L7
caudal L6

Fig. 43. Extracellular recordings from an L4 - projecting interneurone discharged by group II muscle afferents. In this and the following Figures, upper records in each pair are microelectrode recordings from the interneurone. Lower records are from the surface of the spinal cord and show the arrival of afferent volleys. Negativity is downwards in recordings of interneurones and upwards for records from the surface of the spinal cord. Each record is composed of four superimposed sweeps. The stimulus strength indicated against each set of traces is in multiples of threshold (T) for the most excitable fibres of a given nerve. Voltage calibrations apply to recordings of interneurones. A & B, peripheral input to the interneurone. Upper records show the effects of single stimuli applied to muscle nerves at 5T and cutaneous nerves at 2T, lower records show the lack of response to 2 shocks applied to the same muscle nerves at 2T. C, tests of projection. The top set of records show an antidromic impulse (indicated by arrow) produced by stimulation within the lateral funiculus at L4. The middle set of records show the result of collision of the antidromic impulse with orthodromic impulses occurring within the critical interval. The lower pair of records show the absence of an antidromic response to stimulation at Th13. Recording location; caudal L7.

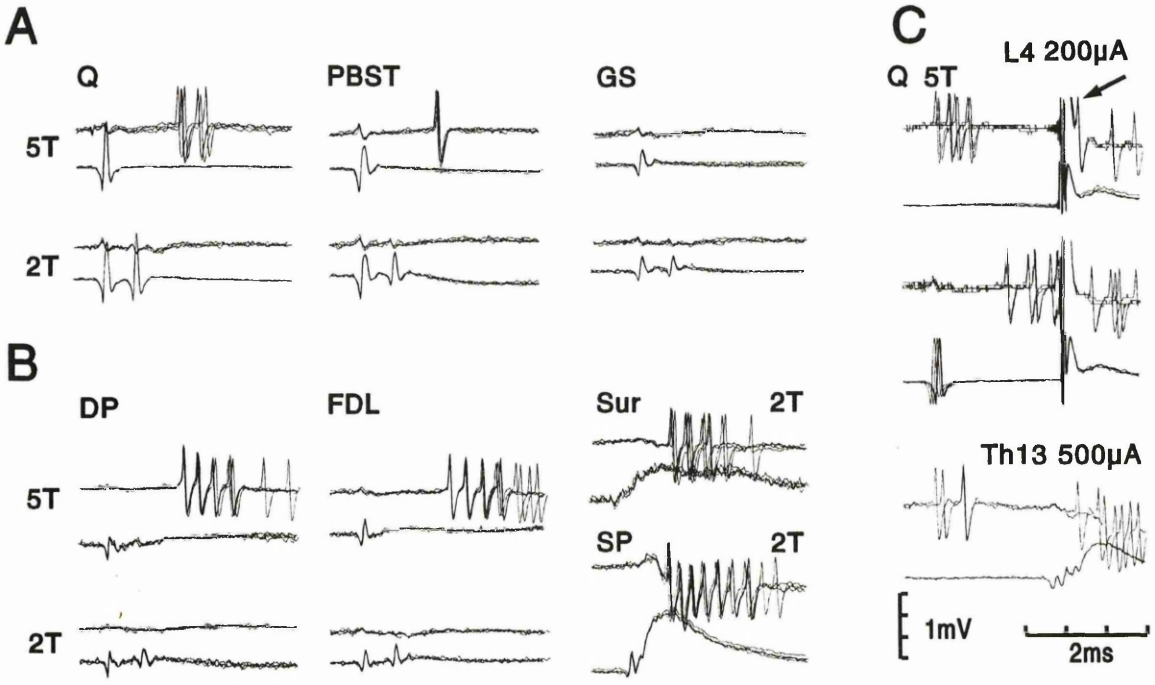

A
Q
PBST
GS
5T
2T
B
DP
FDL
Sur
2T
5T
SP
2T
2T
C
L4 200μA
Q 5T
Th13 500μA
1mV
2ms

Fig. 44. Extracellular and intracellular recordings from an L4 - projecting interneurone with excitatory input from group II muscle afferents. A & B, peripheral input from various muscle nerves, sural and interosseous nerves. Each panel of four records shows the responses to stimulation of the peripheral nerve indicated. The upper record of each panel is an extracellular recording of the discharge produced by a stimulus of 5T. The second record shows the PSPs evoked by the same stimulation during intracellular recording. The third record shows the effect of reducing the stimulus strength to 2T and the lower record shows the afferent volley. In this and the following Figures, negativity is downwards in extracellular and intracellular records from interneurones and upwards for records from the surface of the spinal cord. Note lack of PSPs following stimulation of muscle nerves at 2T indicating that group II afferents are responsible for the PSPs and discharges evoked at 5T. C, tests of projection; upper records of each pair are extracellular or intracellular recordings from the interneurone and lower records are from the surface of the spinal cord. The top and middle pairs of records show the antidromic response (indicated by arrows) of the interneurone to stimulation within the lateral funiculus at L4. The top set of records show an extracellularly recorded antidromic spike and the middle set of records show a blocked intracellularly recorded antidromic spike. The lower pair of records show the absence of an antidromic response (extracellular recording) to stimulation at Th13. Each trace is composed of four superimposed sweeps. Calibration scale shows sensitivity for extracellular (e.c.) and intracellular (i.c.) recordings. Recording location; caudal L7.

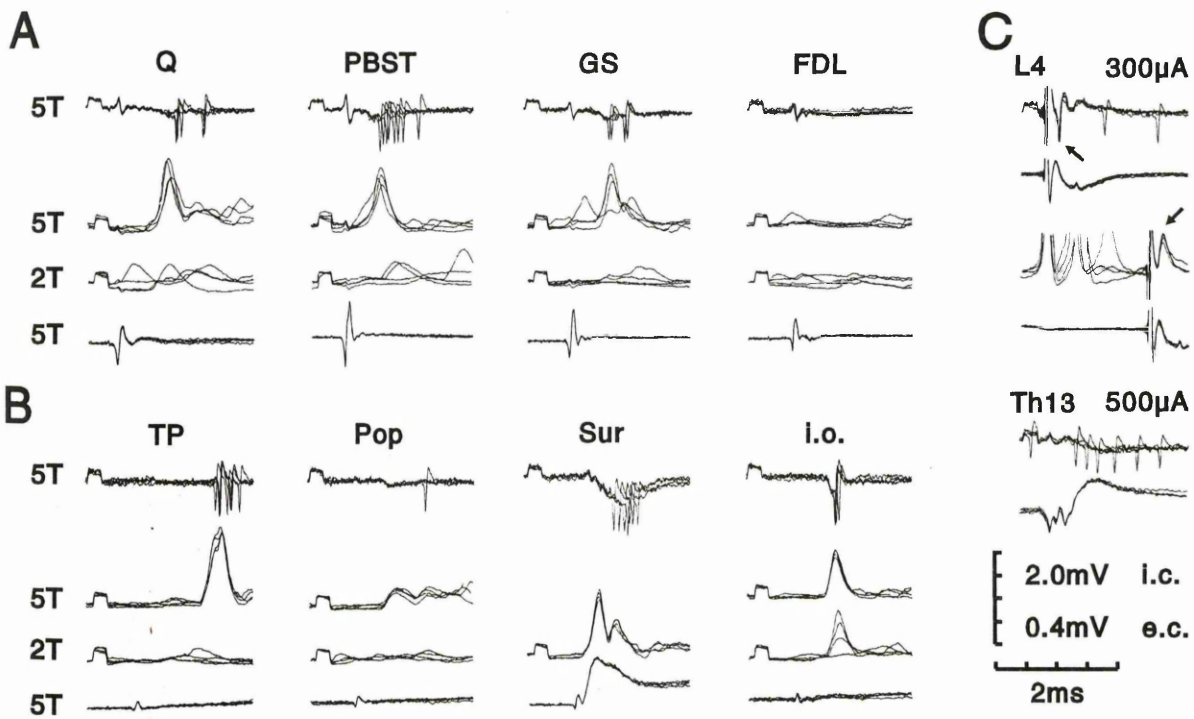

A
Q
PBST
GS
FDL
5T
5T
2T
5T
B
TP
Pop
Sur
i.o.
5T
5T
2T
5T
C
L4
300μA
Th13
500μA
2.0mV
i.c.
0.4mV
e.c.
2ms

Fig. 45. Intracellular recordings from an L4 - projecting interneurone with excitatory input from group I and group II muscle afferents. In both A and B, upper records of each pair are intracellular records from the interneurone and lower records are from the surface of the spinal cord (four superimposed sweeps). A, tests of projection. Records to left; orthodromic discharges evoked by group II afferents of the deep peroneal nerve, followed by an antidromic discharge in response to stimulation within the lateral funiculus at L4. Middle records; moving the timing of the L4 stimulus closer to that of the nerve stimulation leads to collision between orthodromic and antidromic impulses within the ascending axon and no antidromic response therefore appears. Records to right; lack of antidromic response to stimulation at Th13. B, EPSPs evoked by stimuli applied to the DP, GS and PI nerves at different stimulus strengths. Note that stimulation of DP evoked EPSPs attributable to group II afferents, stimulation of GS evoked EPSPs attributable to both group I and group II afferents and stimulation of PI evoked EPSPs attributable to group I afferents only. Recording location; rostral L7.

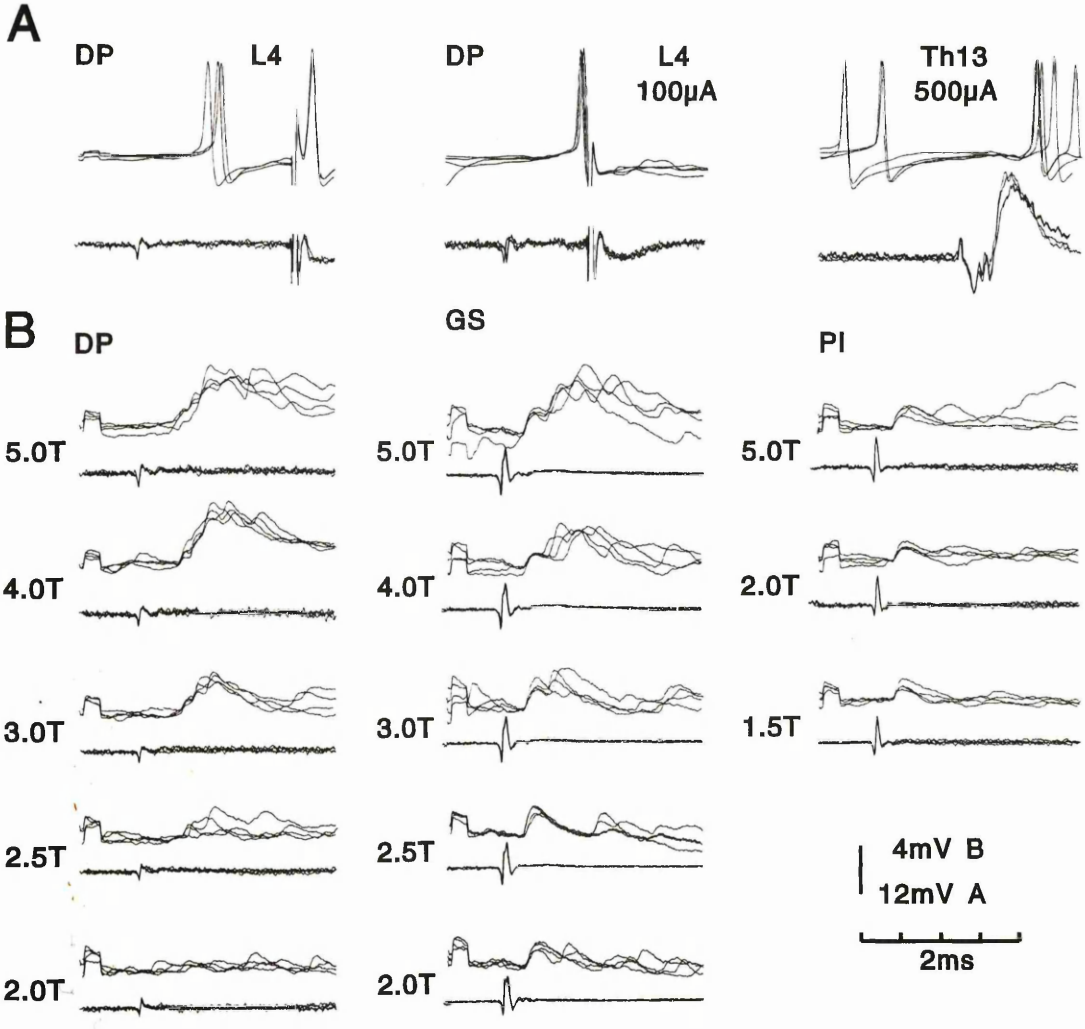

A
DP
L4
DP
L4
100μA
Th13
500μA
B
DP
GS
PI
5.0T
4.0T
3.0T
2.5T
2.0T
5.0T
4.0T
3.0T
2.5T
2.0T
5.0T
2.0T
1.5T
4mV B
12mV A
2ms

Fig. 46. Intracellular recordings from an L4 - projecting interneurone with excitatory input from group I and group II muscle afferents. Intracellular recordings from an interneurone projecting to L4. Upper traces of each pair are intracellular records from the interneurone and lower records are from the surface of the spinal cord (four superimposed sweeps). A & B, EPSPs evoked by stimuli applied to the PBST and DP nerves at different stimulus strengths. Note stimulation of PBST evoked a small early EPSPs which grew in parallel with the second (Ib) component of the group I volley (indicated by arrows in expanded records of volley). Stimulation of DP evoked EPSPs which grew in amplitude between 3T and 5T and are therefore attributable to group II afferents. Stimulation of both PBST and DP at 20T produced additional PSPs attributable to group III afferents. C, EPSPs produced by stimulation of cutaneous afferents. D, tests of projection. Top pair of records show blocked antidromic spike (indicated by arrow) produced by stimulation within the lateral funiculus at L4. Lower records show lack of antidromic response to stimulation at Th13. Time calibration; 4ms upper most records of A and B, 2 ms all other records. Recording location; mid L7.

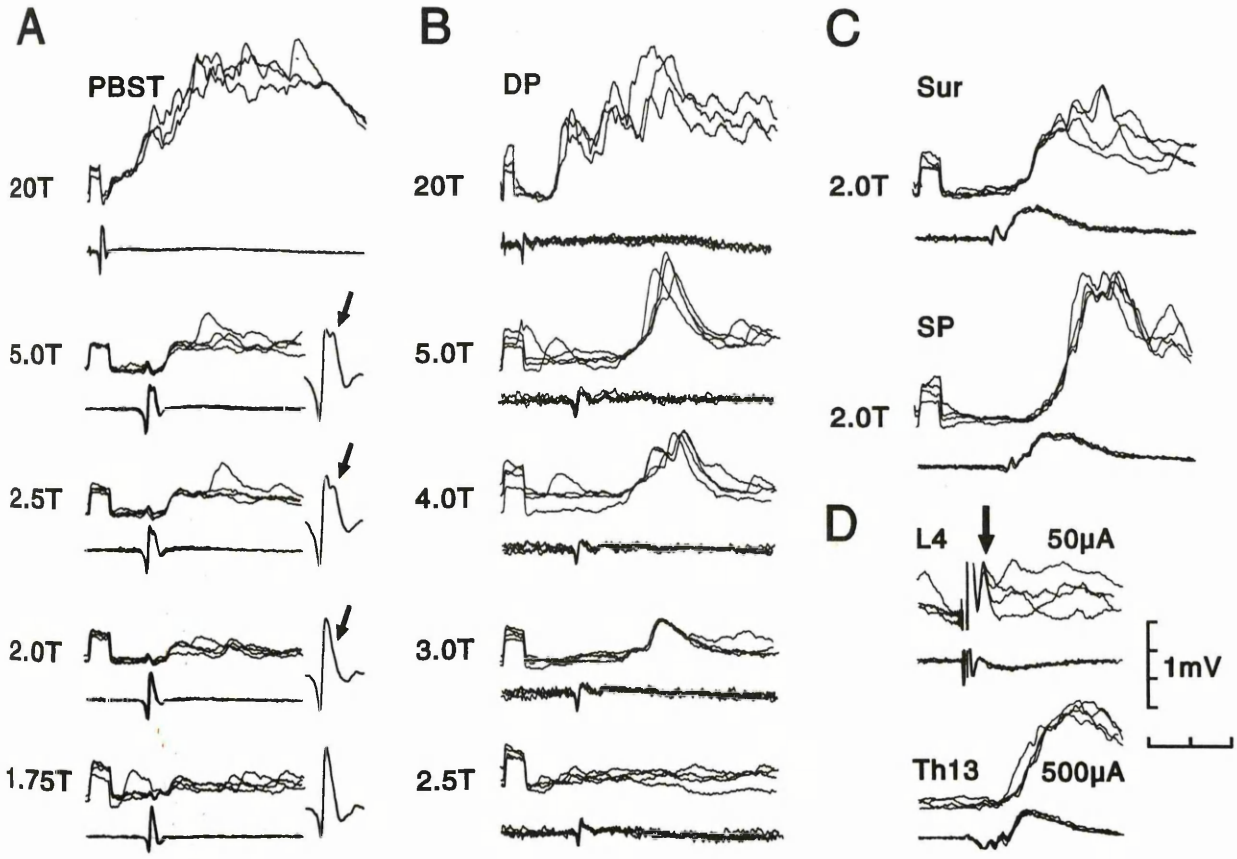

A
PBST
20T
5.0T
2.5T
2.0T
1.75T
B
DP
20T
5.0T
4.0T
3.0T
2.5T
C
Sur
2.0T
SP
2.0T
D
L4
50μA
1mV
Th13
500μA

Fig. 47. Intracellular recordings from an non - projecting interneurone with excitatory input from group II muscle afferents. Upper traces of each pair are intracellular records from the interneurone and lower traces are records from the surface of the spinal cord (four superimposed sweeps). A - C, effects of electrical stimulation of muscle nerves at different stimulus strengths. D, EPSPs evoked by joint and interosseal afferents. E, PSPs evoked by cutaneous afferents. Note that stimulation of both Q and DP at 20T produced additional PSPs attributable to group III afferents. Recording location; caudal L6. Time calibration; 4ms for upper records in A and B, 2ms all other records.

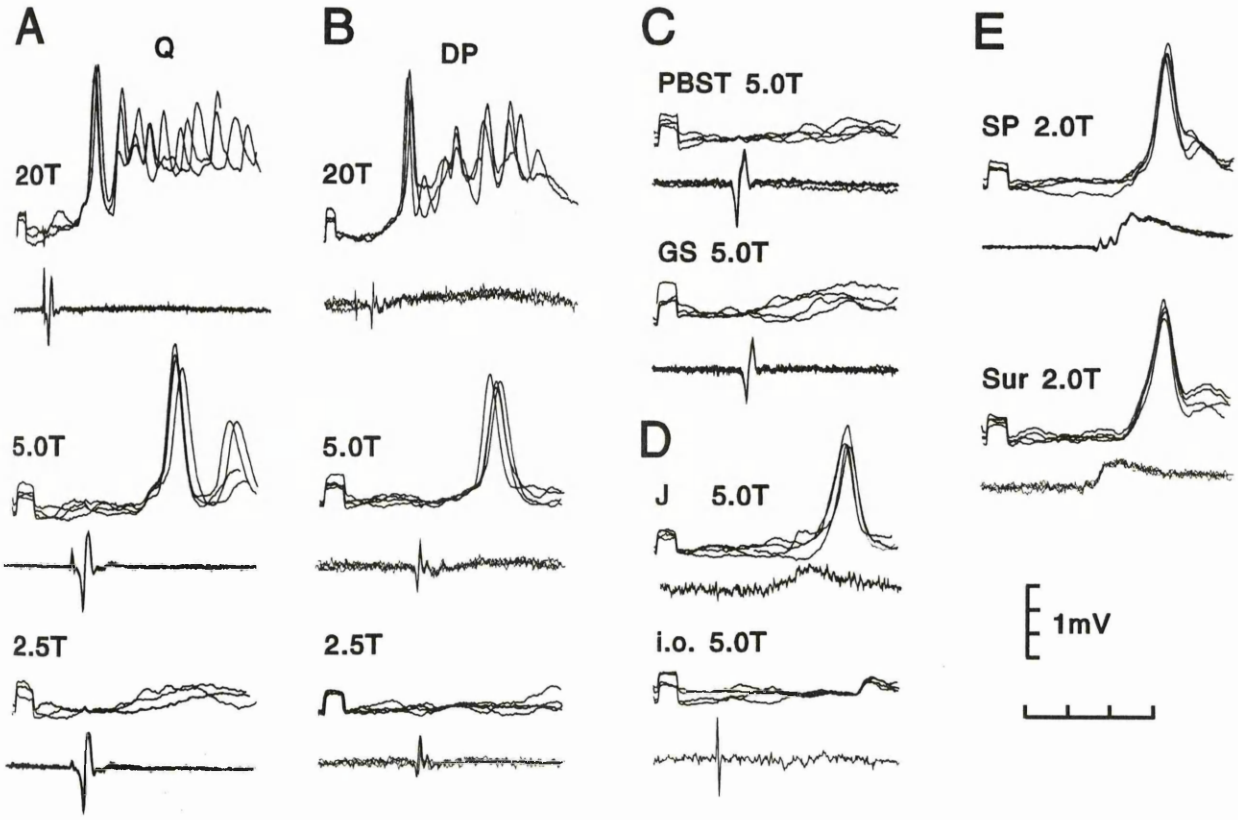

A
Q
20T
5.0T
2.5T
B
DP
20T
5.0T
2.5T
C
PBST 5.0T
GS 5.0T
D
J 5.0T
i.o. 5.0T
E
SP 2.0T
Sur 2.0T
1mV

Fig. 48. Intracellular recordings from a non - projecting interneurone. Upper traces of each pair are intracellular records from the interneurone and lower traces are records from the surface of the spinal cord (four superimposed sweeps). A - D, EPSPs evoked by electrical stimulation of muscle nerves. E, PSPs evoked by cutaneous afferents. Note large EPSPs evoked by group II afferents of Q and PBST. Note also complete absence of group I input and lack of evidence for IPSPs. Recording location; caudal L7.

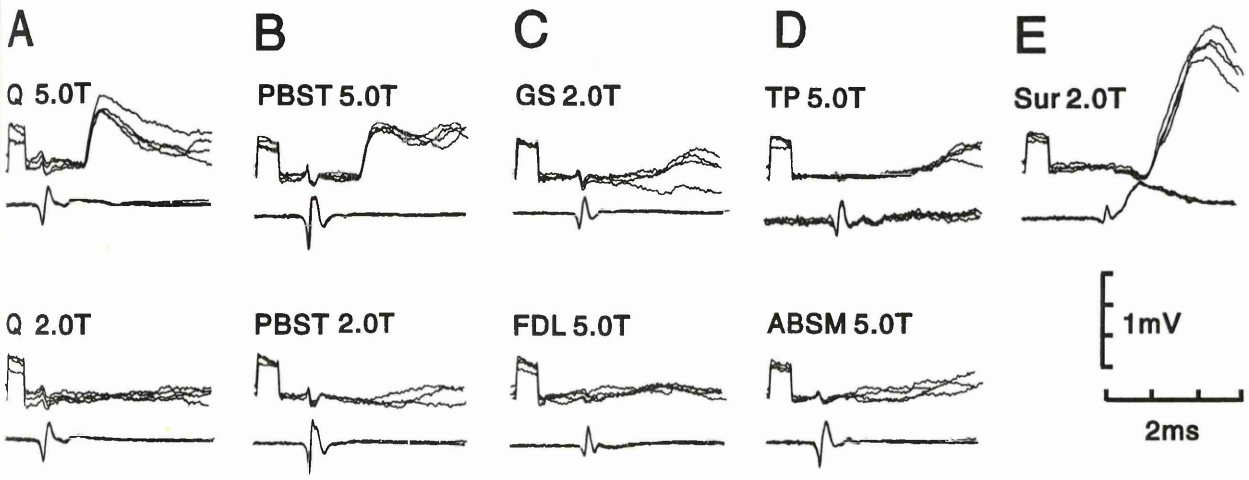

A
Q 5.0T
Q 2.0T
B
PBST 5.0T
PBST 2.0T
C
GS 2.0T
FDL 5.0T
D
TP 5.0T
ABSM 5.0T
E
Sur 2.0T
1mV
2ms

Fig. 49. Intracellular recordings from a non - projecting interneurone. Upper traces of each pair are intracellular records from the interneurone and lower traces are records from the surface of the spinal cord (four superimposed sweeps). A - D, EPSPs evoked by electrical stimulation of muscle nerves. E, PSPs evoked by cutaneous afferents. Note large EPSPs evoked by group II afferents of Q and DP and small EPSPs evoked by group I afferents of Q and ABSM. Note also probable IPSPs cutting short early EPSPs evoked by Sur in particular but also Q and DP. Recording location; caudal L6.

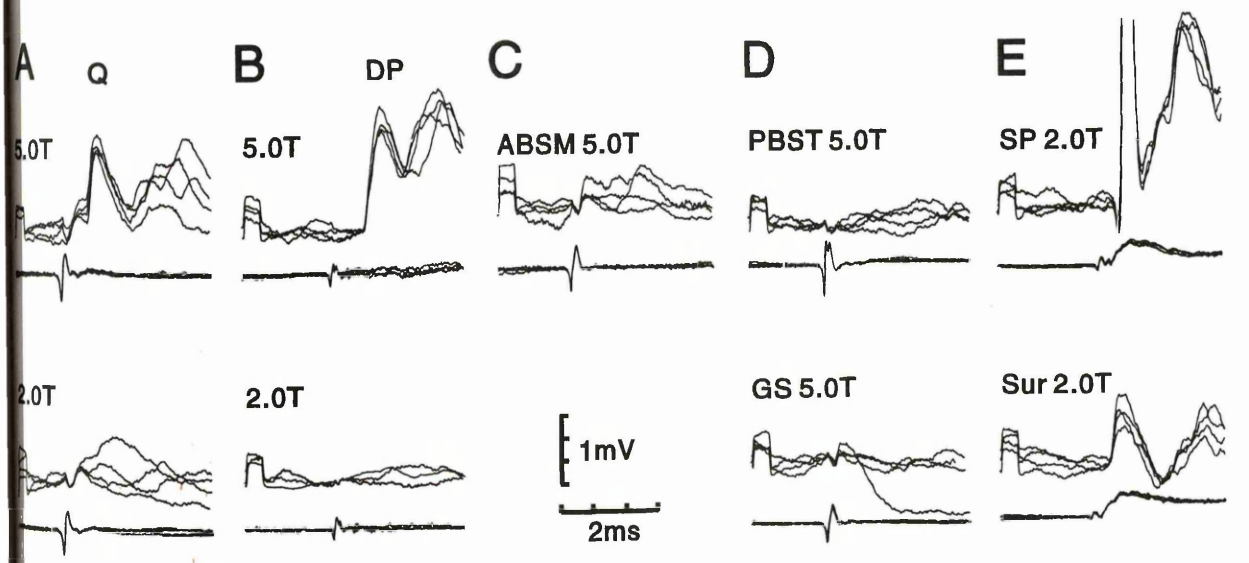

A
Q
5.0T
2.0T
B
DP
5.0T
2.0T
C
ABSM 5.0T
1mV
2ms
D
PBST 5.0T
GS 5.0T
E
SP 2.0T
Sur 2.0T

Fig. 50. Pattern of excitatory peripheral input to the sample of interneurones. A and B, histograms showing the proportion of interneurones in which extracellularly recorded discharges (A) or intracellularly recorded EPSPs (B) were evoked by group II muscle afferents of the range of muscle nerves tested and by cutaneous afferents of the sural and superficial peroneal nerves. In B, the shaded portions of the histograms indicate the proportion of neurones in which EPSPs were evoked by group I muscle afferents of each of the muscle nerves tested. The nerves are identified above the histograms and the numbers of neurones tested are shown beneath.

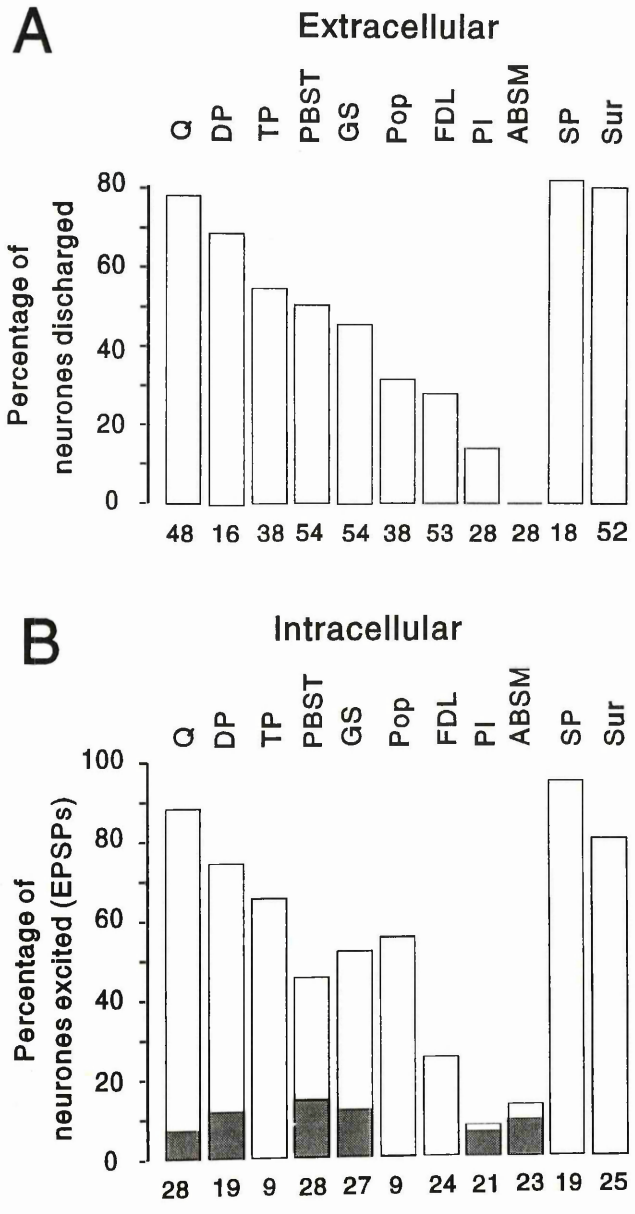

A
Extracellular
Q
DP
TP
PBST
GS
Pop
FDL
PI
ABSM
SP
Sur
Percentage of neurones discharged
80
60
40
20
0
48 16 38 54 54 38 53 28 28 18 52
B
Intracellular
Q
DP
TP
PBST
GS
Pop
FDL
PI
ABSM
SP
Sur
Percentage of neurones excited (EPSPs)
100
80
60
40
20
0
28 19 9 28 27 9 24 21 23 19 25

Fig. 51. Latencies of discharges and EPSPs evoked in the sample of interneurones by afferents of various nerves. The histograms show the latencies of discharges (histograms headed *Extracellular*) and EPSPs (histograms headed *Intracellular*) measured from the onset (i.e. peak of the first positive deflection) of volleys in group I muscle afferents or cutaneous afferents. The latencies of discharges and EPSPs evoked from muscle nerves include only those evoked by group II afferents. The solid filled bars in the intracellular histograms indicates those EPSPs that are considered likely to have been evoked monosynaptically by the very fastest conducting group II afferents of muscle nerves or the fastest conducting cutaneous afferents (see text for further details). The latencies are grouped in 0.2 ms bins. The grey shaded bars indicate EPSPs evoked by popliteus group II afferents. The black bars under the histograms for muscle nerves indicate the range of latencies of EPSPs that could be consistent with the monosynaptic actions of the full range of group II muscle afferents (i.e. with conduction velocities of 70 to 35 m/s). Note, however, that at the longer end of these ranges monosynaptic actions of slowly conducting afferents can not be distinguished from di - or tri - synaptic actions of faster conducting afferents. A few discharges and EPSPs which occurred at latencies between 6 and 8 ms are not shown.

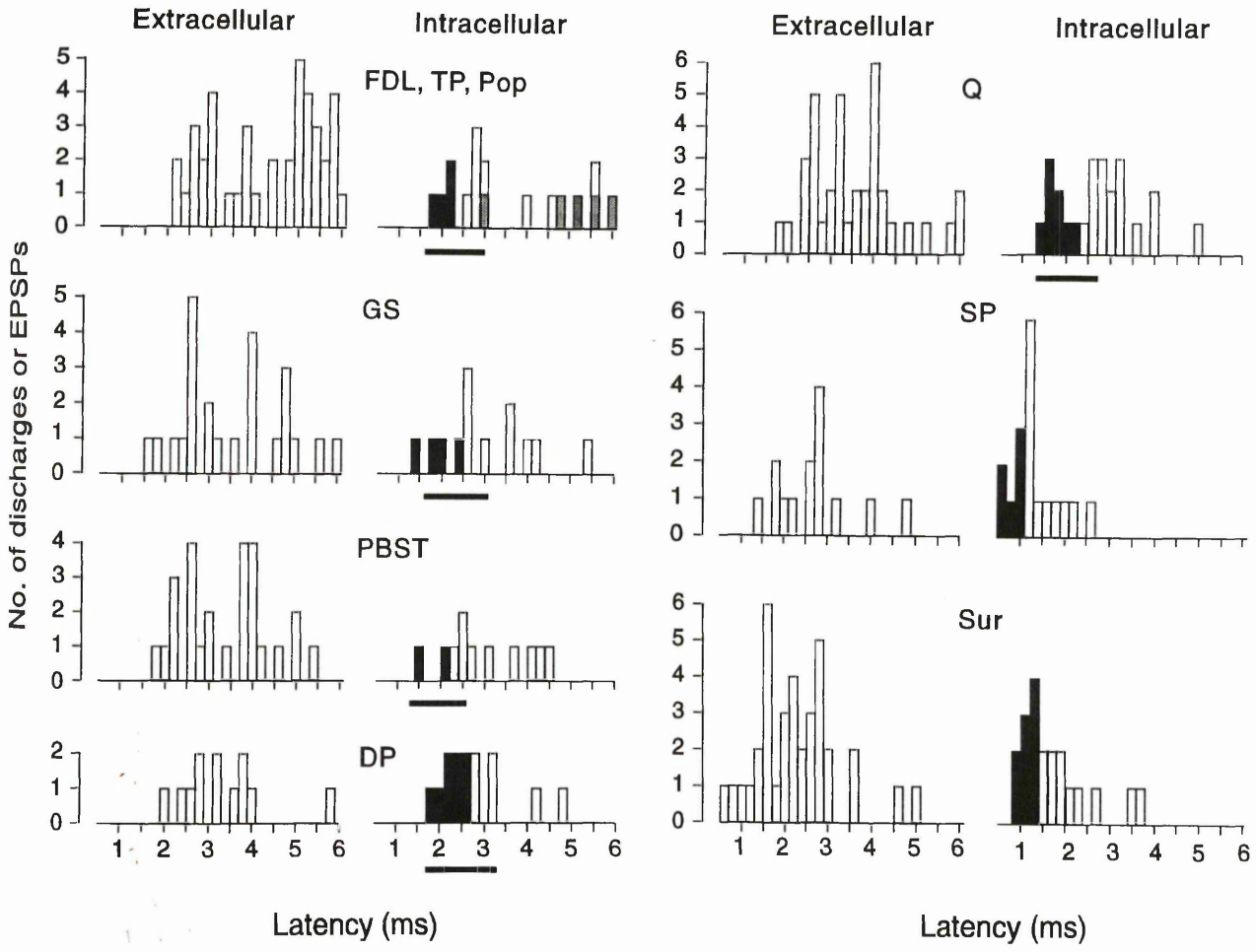

Extracellular
Intracellular
FDL, TP, Pop
GS
PBST
DP
Q
SP
Sur
No. of discharges or EPSPs
Latency (ms)
Latency (ms)

Fig. 52. Convergence from group II muscle afferents to individual interneurones. A, histograms showing the proportion of neurones discharged by group II afferents of two or more muscle nerves (left), three or more muscle nerves (middle), and in which EPSPs were evoked by group II afferents of 3 or more muscle nerves (right). The solid bars represent neurones in both the L6 and L7 segments, the shaded bars neurones in the L7 segment and the open bars neurones in the L6 segment. Note the greater convergence in L7 than L6. B, histograms showing a comparison of the proportions of interneurones in the L7 and L6 segments in which EPSPs were evoked by group II afferents of different nerves. The shaded bars represent neurones recorded in the L7 segment and the open bars represent neurones recorded in the L6 segment. Note that group II afferents of PBST, GS & FDL excited a larger proportion of neurones in the L7 segment than in L6 while afferents of Q, DP & TP were similarly effective in both segments.

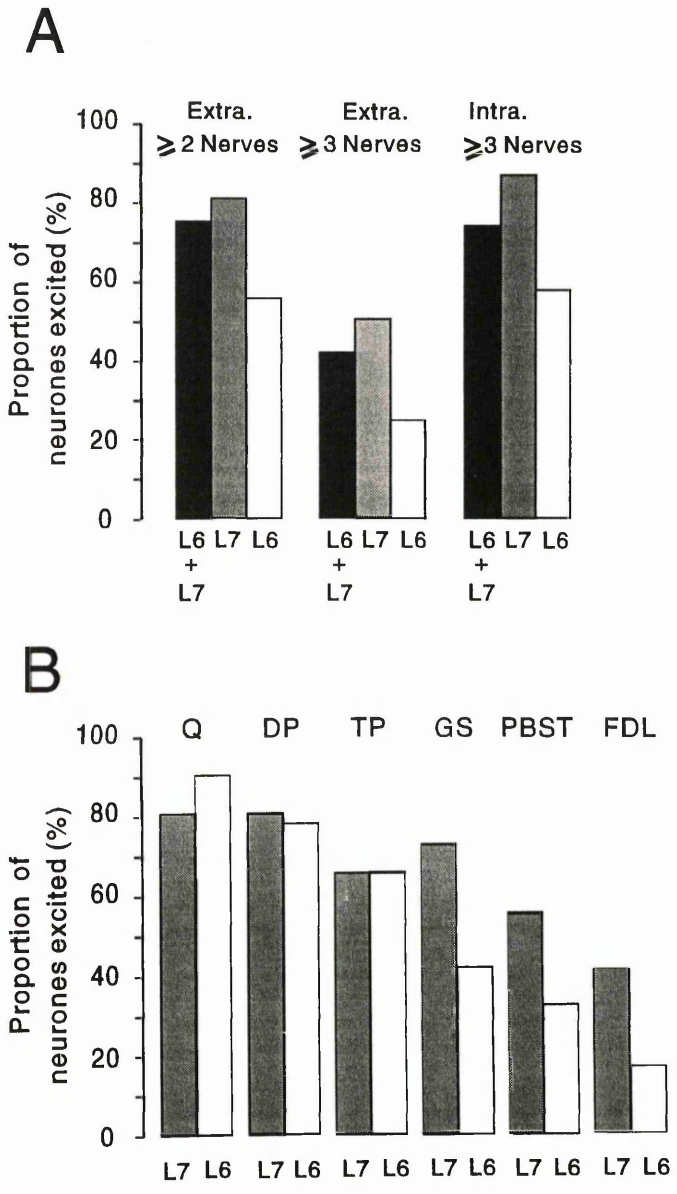
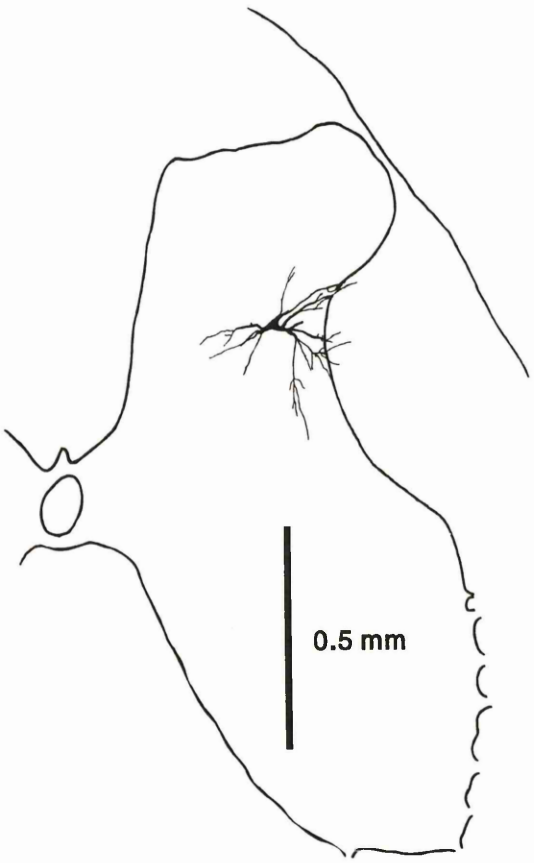

A
Extra.
≥2 Nerves
Extra.
≥3 Nerves
Intra.
≥3 Nerves
Proportion of neurones excited (%)
100
80
60
40
20
0
L6 L7 L6
+
L7
B
Q
DP
TP
GS
PBST
FDL
L7 L6

Fig. 53. Location of an ascending tract neurone with group II input within the spinal grey matter. Partial reconstruction of one neurone intracellularly labelled with biocytin is shown on an outline of the grey matter. This ascending tract neurone was located in the ventrolateral part of the dorsal horn at the L6 - L7 border.



L6-L7 border

Fig. 54. Extracellular recordings from an ascending tract neurone. A & B, peripheral input to the ascending tract neurone. Upper pairs of records show the effects of single stimuli applied to muscle nerves at 5T and to Sur and interosseous at 2T. Lower pairs of records show the lack of response to 2 shocks applied to muscle nerves at 2T. C - E, tests of projection. C, records show an antidromic impulse (indicated by arrow) produced by stimulation within the lateral funiculus at L4. D, shows action potentials following faithfully a train of stimuli applied at Th13. E, the top set of records show the orthodromic impulse produced by stimulation of peripheral nerve and antidromic impulse produced by stimulation at Th13. The bottom set of records show the result of collision of the antidromic impulse with orthodromic impulses occurring within the critical interval. The arrow indicates where an antidromic impulse would have been seen had collision not occurred. Recording location; middle L7.

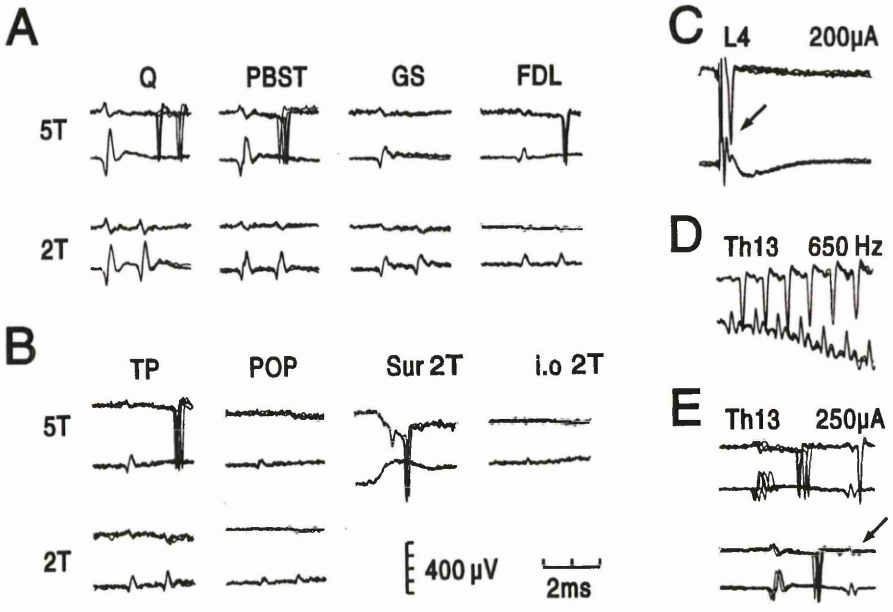

A
Q
PBST
GS
FDL
5T
2T
B
TP
POP
Sur 2T
i.o 2T
5T
2T
400 µV
2ms
C
L4
200µA
D
Th13
650 Hz
E
Th13
250µA

Fig. 55. Extracellular and intracellular recordings from an ascending tract neurone. A, B, C & D, peripheral input from various muscle nerves, cutaneous and joint nerves. Each panel of records shows responses to stimulation of the peripheral nerve indicated. The upper most record of each panel is an extracellular recording of the discharge produced by a stimulus of 5T (muscle nerve) and 2T (cutaneous and joint nerves). The remaining pairs of records show intracellular recordings following stimuli applied to the indicated nerve at different stimulus strengths. A, stimulation of Q and DP evoked EPSPs which grew in amplitude between 3T and 5T and are therefore attributable to group II afferents. B & C, stimulation of ABSM, PBST, GS and PI at 20T produced PSPs attributable to group III afferents. D, EPSPs produced by stimulation of cutaneous and joint nerves at 2T. E, antidromic response (indicated by arrow, extracellular recording) produced by stimulation at Th13. Each trace is composed of four superimposed sweeps. Calibration scale shows sensitivity for extracellular (e.c.) and intracellular (i.c.). Recording location; rostral L7.

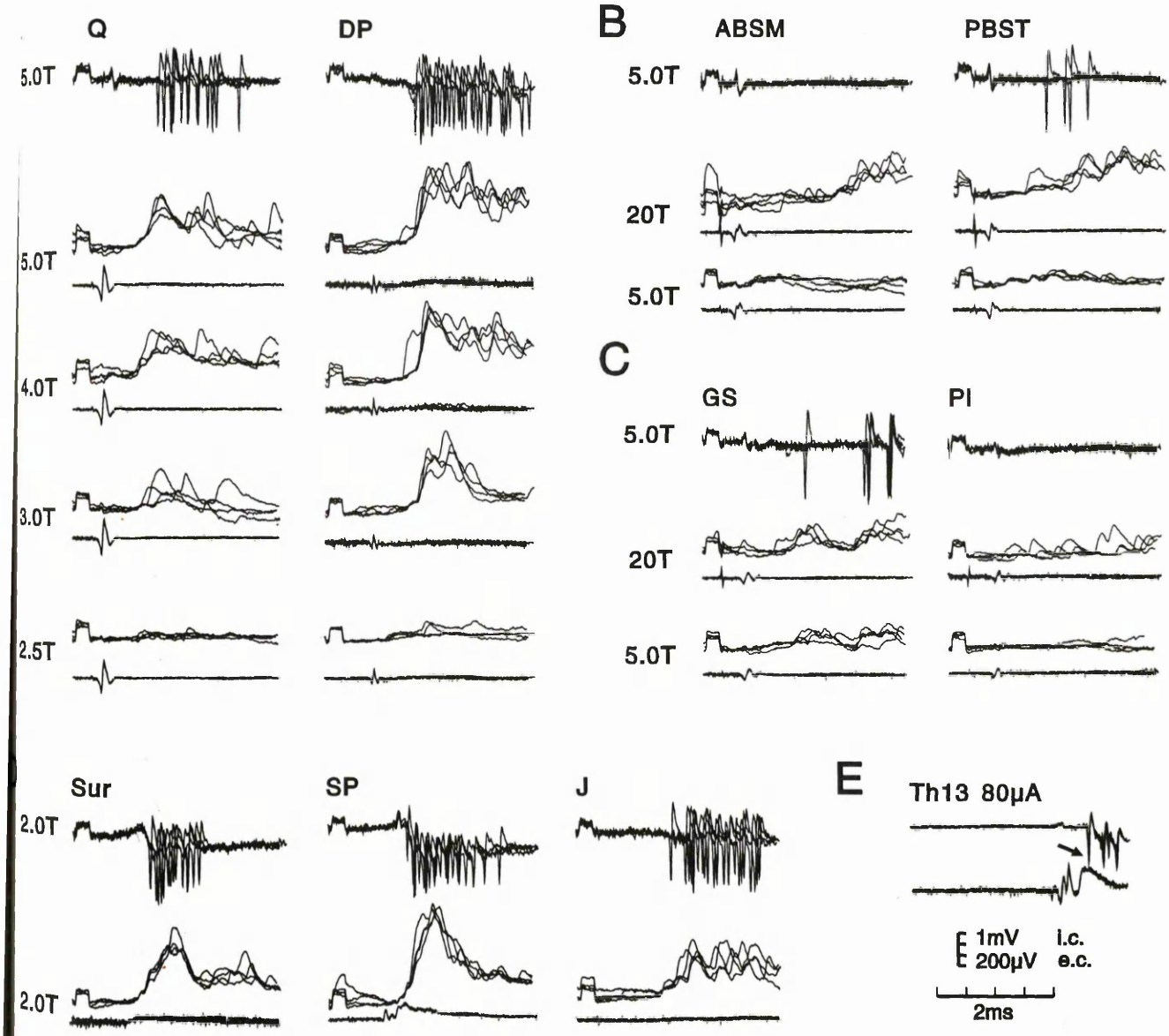

Q
DP
5.0T
5.0T
4.0T
3.0T
2.5T
B
ABSM
PBST
5.0T
20T
5.0T
C
GS
PI
5.0T
20T
5.0T
Sur
SP
J
2.0T
2.0T
E
Th13 80μA
1mV
200μV
i.c.
e.c.
2ms

Table 4. Latencies of field potentials recorded in lower - lumbar segments. Means and ranges of latencies of field potentials evoked by group I and group II afferents of each of the nerves investigated are shown. The values represent the shortest latencies observed during each transverse mapping of the grey matter. Table A shows latencies measured with respect to the group I afferent volley and table B latencies measured with respect to the stimulus artefact. The group I field potentials were recorded simultaneously with group II potentials at the same locations.

A. Latencies from group I volley

Nerve	Group I field pot. (ms)			Dorsal horn group II field pot. (ms)			Intermediate group II field pot. (ms)		
	mean	range	n	mean	range	n	mean	range	n
PBST	0.80	0.57-0.97	8	1.50	1.10-2.45	5	2.40	2.10-2.95	8
GS	0.63	0.42-1.22	8	1.55	1.32-2.05	4	3.04	2.40-3.35	8
Q	0.95	0.70-1.21	10	-	-	-	2.64	1.90-3.37	13
FDL	0.49	0.40-0.57	8	-	-	-	2.10	1.37-2.95	8
TP	0.56	0.55-0.57	3	1.43	1.02-1.82	8	2.30	1.60-2.92	7
Pop	-	-	-	2.07	1.42-2.80	5	-	-	-

B. Latencies from stimulus

Nerve	Group I field pot. (ms)			Dorsal horn group II field pot. (ms)			Intermediate group II field pot. (ms)		
	mean	range	n	mean	range	n	mean	range	n
PBST	1.84	1.65-1.95	8	2.40	2.10-2.95	5	3.44	3.10 - 4.02	8
GS	2.23	2.00-2.75	8	3.30	2.0 - 2.75	4	2.23	3.07-3.70	8
Q	1.59	1.35-1.82	10	-	-	-	3.34	2.52 - 4.15	13
FDL	2.38	2.20-2.55	8	-	-	-	3.98	3.20 - 4.95	8
TP	2.37	2.35-2.40	3	3.32	2.97-3.77	8	4.13	3.45 - 4.70	7
Pop	-	-	-	3.95	3.17-4.7	5	-	-	-

3.0.4. Discussion

3.0.4. Discussion

Relationship between surface potentials and potentials in the dorsal horn

Electrical activation of group II afferents produces distinct cord dorsum potentials at the surface of midlumbar and sacral segments of the spinal cord. These cord dorsum potentials reflect activity at synapses between group II afferents and neurones within the underlying grey matter, primarily within the dorsal horn, where large field potentials (up to 1 mV) with a similar time course to the surface potentials can be recorded (Edgley & Jankowska, 1987a; Jankowska & Riddell, 1993).

In contrast, at the lower - lumbar level, group II afferents of most of the muscle nerves which have been investigated (Q, PBST, ABSM, GS, FDL and Pop) produce only very small cord dorsum potentials. Correspondingly, the actions of group II afferents within the dorsal horn of the lower - lumbar segments has been found to be relatively weak. Some nerves (e.g. Q & FDL) produced dorsal group II potentials rarely if at all and even those nerves which were effective produced potentials which were much smaller in amplitude (usually less than 200μV), and more restricted in their distribution than the potentials produced by group II afferents projecting to either end of the lumbosacral enlargement (midlumbar and sacral segments).

The actions of group II afferents of TP were an exception to the general findings in that they showed evidence of a more powerful projection to the dorsal horn than afferents of other nerves. Group II afferents of TP

produced distinct cord dorsum potentials, the average amplitudes of which were only slightly smaller than those produced by group II afferents terminating in the sacral segments. Correspondingly, the dorsal group II field potentials evoked by afferents of TP were of larger amplitude (mean maximum amplitude 220 μV) and were observed more consistently, throughout both the L6 and L7 segments, than those of other nerves.

Distribution of field potentials in the grey matter

The present study has largely confirmed the previous observations of Fu et al. (1974) on the actions of group II afferent of GS in the lower - lumbar segments. In addition, investigations have been extended to the actions of group II afferents of a range of muscle nerves in order to assess the extent to which processing of information from group II muscle afferents occurs within neuronal circuits in the lower - lumbar segments and to obtain information on the organisation and likely location of interneurones in reflex pathways from group II afferents of distal hind - limb muscles (See below, section on interneurones).

Since field potentials reflect the flow of current at synapses formed by afferent fibres with their target spinal neurones (see Eccles, Fatt, Landgren & Winsbury, 1954; Coombs, Curtis & Landgren, 1956), the relatively small field potentials recorded within the dorsal horn of the lower - lumbar segments indicate a less powerful projection from group II muscle afferents to this region than to the equivalent regions of the midlumbar and sacral segments, where much the strongest actions of group II afferents are

on neurones located in the dorsal horn (Edgley & Jankowska, 1987a,b; Jankowska & Riddell, 1993,1994). This in turn implies that there are differences in the functional organisation of neurones processing information from group II muscle afferents in the dorsal horn of different segments of the lumbosacral enlargement. Previous observations on the sources of input to ascending tract neurones located in the dorsal horn provides evidence consistent with this. Ascending tract neurones in the lower - lumbar segments, including neurones contributing to the spinocervical tract (Harrison & Jankowska, 1984), neurones of the postsynaptic dorsal column system (Jankowska, Rastad & Zarzeki, 1979) and neurones of the dorsal spinocerebellar tract (Aoyama, Hongo & Kudo, 1988), appear to receive little if any input from group II muscle afferents. Some spinothalamic tract neurones in the lumbosacral enlargement of the monkey are reported to be excited by group II afferents (Foreman, Applebaum, Beall, Trevino & Willis, 1974; Foreman, Schmidt & Willis, 1977; Foreman, Kenshalo, Schmidt & Willis, 1979) but this has yet to be confirmed for the cat in which the spinothalamic tract is less well developed than the primate (Carstens & Trevino, 1978; Jones, Apkarian, Stevens & Hodge, 1987; Craig, Linington & Kniffki, 1989). It could be argued that previous studies may not have sampled the most lateral aspects of the grey matter where group II field potentials were frequently located in the present study. However, the lack of ascending tract neurones receiving input from group II muscle afferents in the lower - lumbar segments is supported by recordings made of neurones

in the lateral grey matter. Only 7 of 81 neurones (8%) excited by group II afferents which were encountered during this study had axons which projected rostral to the lumbar segments (See below, section on Th13 projecting neurones). In contrast, neurones in the dorsal horn of the midlumbar and sacral segments appear to provide an important route for the transmission of information signalled by group II muscle afferents to supraspinal structures. Neurones contributing to the spinocervical tract in both the midlumbar and sacral segments are strongly excited by group II muscle afferents in addition to their powerful input from cutaneous afferents (Hammer et al. 1994; Riddell et al. 1994), as are neurones of the dorsal spinocerebellar tract in the midlumbar segments (Edgley & Jankowska, 1988).

In addition to neurones of ascending tracts, the dorsal horn of the midlumbar and sacral segments contain a functionally heterogeneous population of group II interneurones, some of which appear to be excitatory and others inhibitory (Edgley & Jankowska, 1987b; Bras et al. 1989; Jankowska & Riddell, 1994; Jankowska et al. 1993b; Maxwell, Kerr, Jankowska & Riddell, 1997). These interneurones have mainly local actions within the segment containing or adjacent to the cell body and there is evidence that some are involved in the presynaptic inhibition of group II muscle afferents (Riddell, Jankowska & Huber, 1995; Jankowska & Riddell, 1995). The results (Results, Section on interneurones) shows that some interneurones excited by group II muscle afferents are located within the dorsal horn of the lower - lumbar segments but it remains to be determined

whether any of these interneurones perform functions analogous to the dorsal horn neurones studied in the midlumbar and sacral segments.

Intermediate group II potentials in the lower - lumbar segments were if anything larger (maximally up to 400μV) than those recorded in the dorsal horn and although potentials evoked by a given nerve tended to be patchy in their distribution, group II afferents of one or more of the muscle nerves tested evoked potentials at virtually all of the sites where electrode tracks were made. The intermediate potentials are therefore of a comparable amplitude to those seen in the intermediate region of the midlumbar segments (Edgley & Jankowska, 1987a) and much larger and more widespread than those seen in the sacral segments (see Fig. 4 of Jankowska & Riddell, 1993). Indeed, since the later occurred only close to the L7 - S1 border it is probable that they represent the caudal extent of the field potentials described here.

Preliminary reports that group II muscle afferents in branches of the tibial nerve arising just below the popliteal fossa are among those with synaptic actions within the lower - lumbar segments were based on observations of the effects of combined stimulation of the nerves to FDL, TP and Pop together with a small branch innervating the interosseal membrane (FDL et al.; Harrison & Riddell, 1989; Harrison et al. 1994). In the present experiments these nerve branches were stimulated individually in order to assess the actions of each and the nerve to the interosseous membrane was removed in order that the actions of group II afferents innervating Pacinian corpuscles in the interosseal membrane could be excluded (Hunt,

1961; Boyd & Davey, 1968). The results confirm that group II afferents of these muscle branches have synaptic actions in the lower - lumbar segments and show that the most effective of these are group II afferents of TP which evoke field potentials in both the dorsal horn and intermediate area throughout both the L6 and L7 segments. Group II afferents of FDL also project to the lower - lumbar segments but their actions are restricted mainly to the intermediate region of the L7 segment.

The actions of group II afferents of Q have been extensively investigate in the midlumbar segments where they produce a powerful excitation of spinal neurones. However, field potentials produced by these afferents have been reported to decline rapidly in amplitude caudal of mid - L5 (Edgley & Jankowska, 1987a, see their Fig. 5). In the present study, although stimulation of Q failed to evoke field potentials in the dorsal horn of the L6 - L7 segments, distinct group II potentials were frequently observed in the intermediate region. These observations are in agreement with a report that the reflex actions of Q group II muscle afferents on motoneurones (at least, excitation of flexor motoneurones) are mediated in part by interneurones located caudal of the L5/L6 border (Cavallari & Pettersson, 1991).

Distribution of field potentials in relation to morphological studies of group II muscle afferents.

There have been several morphological studies of the pattern of termination of functionally identified group II muscle spindle afferents (mainly

of ankle extensors) terminating in the lower - lumbar segments (Fyffe, 1979, see also Brown, 1981; Mannen et al. 1981; Ishizuka et al. 1984; Hongo, 1992). In addition, Fu & Schomburg (1974) have used intraspinal stimulation of the central branches of group II muscle spindle afferents of GS to map their area of termination. Both the morphological and electrophysiological results show that group II muscle spindle afferents terminate in the dorsal horn and in the intermediate region of the lower - lumbar segments where group II field potentials were recorded in the present study. The intra - axonal staining of group II muscle afferent fibres has shown in addition that the same group II afferents often give rise to axon collaterals that terminate in both the dorsal and intermediate regions and that some afferents also terminate within the motonuclei which were not explored in the present investigation.

The present results show that group II afferents have a less powerful action in the dorsal horn of lower - lumbar segments than in sacral segments but a stronger action in the intermediate zone of the lower - lumbar segments than sacral segments (Jankowska & Riddell, 1993,1994). These observations are entirely consistent with morphological observations, unfortunately never published in full, on the pattern of termination of intra - axonally stained group II afferent fibres. These provided evidence that the pattern of termination of collaterals from the same group II afferent fibre varies depending upon its location relative to the homonymous motor column (Ishizuka et al. 1984). At the level of the motor column, each collateral gives rise to about 150 boutons which are distributed in the

intermediate grey matter (laminae V - VII), while caudal to the motor column the number of boutons per collateral increases dramatically (to 350 - 750) and the great majority of boutons are distributed within the dorsal horn (laminae IV and V). For the afferents which were the subject of this study (medial gastrocnemius and flexor digitorum brevis), the caudal end of the homonymous motor column occurs close to the border between the L7 and S1 segments (Romanes, 1951). The change in the pattern of termination of the afferent fibres therefore correlates with the transition from the large amplitude group II field potentials seen in the dorsal horn of the sacral segments to the smaller more ventrally distributed potentials seen in the lower - lumbar segments.

Other aspects of the morphology of group II afferent fibres may explain the more patchy and restricted nature of field potentials recorded in the lower - lumbar segments compared to the dorsal horn of sacral segments. Although axon collaterals originate at similar intervals (800 - 1000 μm) along the length of the ascending and descending branches of group II fibres, at the level of the homonymous motor column (L6 and L7) these collaterals show a very limited development in the rostro - caudal plane (about 200 μm), whereas beyond the motor column (L7/S1) the dorsal horn collaterals of the same group II fibre are highly developed in the rostro - caudally plane, extending for 500 - 1000 μm (Fyffe, 1979, see Brown 1981; Mannen et al. 1981)

Receptor origin of the group II afferents.

In muscle nerves, afferents classified as group II on the basis of diameter or conduction velocity originate in large part from the secondary ending of the muscle spindle. However, a smaller and variable proportion of group II afferents may innervate non - spindle receptors or form free nerve endings within the muscle, while others may innervate joint or other non - muscular tissue (for reviews see Chapter 1.0. General introduction). Electrical stimulation of muscle nerves activates all types of group II afferents indiscriminately so that there must always be some doubt as to which are responsible for the synaptic actions which result. Nevertheless, a number of arguments favour the proposal that muscle spindle group II afferents are likely to be responsible, perhaps in large part, for the group II potentials recorded in the lower - lumbar segments. Firstly, the limited information currently available on the properties of non - spindle group II muscle afferents studied electrophysiologically shows them to conduct mainly towards the lower end of the group II range of conduction velocities (Rymer, Houk & Crago, 1979; Cleland, Hayward, & Rymer, 1990). In contrast, at least the earliest components of the field potentials evoked in the lower - lumbar segments are mediated by the fastest conducting group II muscle afferents. Secondly, there is direct evidence from the electrophysiological and morphological studies of the termination of group II afferents described above, that functionally identified group II muscle spindle afferents project to the lower - lumbar segments and terminate within those regions of the grey matter where group II field potentials are recorded (Fu &

Schomburg, 1974; Fyffe, 1979, see also Brown, 1981; Mannen et al. 1981; Ishizuka et al. 1984; Hongo, 1992).

Interneurones

Most previous studies of interneurones in the lower - lumbar segments have concentrated on the medial region of lamina V - VI where neurones strongly excited by group I afferents but with little or no input from group II muscle afferents are located (e.g. Eccles et al. 1960; Hongo et al. 1966; Harrison & Jankowska, 1985). In contrast, the present investigation shows that interneurones located in the regions dorsal and lateral to this area are strongly excited by group II muscle afferents but only weakly if at all by group I afferents. The mainly lateral distribution of these neurones may partly explain why group II interneurones in the lower - lumbar segments have been relatively infrequently encountered and have not previously been studied in detail (Fukishima & Kato, 1975; Lundberg et al. 1987b see Jankowska, 1992, for review).

Previous studies of lower - lumbar interneurones and their axons

Lundberg et al. (1987b, see also Harrison et al. 1994) have recorded intracellularly from a sample of 15 interneurones which were monosynaptically excited by group II muscle afferents. Most of these were excited by group II afferents of several muscle nerves, including those providing input to the present population of neurones. In addition, evidence of convergent excitation from cutaneous, joint and group III muscle afferents was observed. Recordings have also previously been made from axons in the lateral funiculus belonging to interneurones activated by group II muscle afferents (Harrison & Riddell, 1989). These recordings were made after

lesioning the dorsal columns (at the L5/L6 border) to interrupt primary afferent input to midlumbar interneurones and so the axons must have originated from neurones with cell bodies in the lower - lumbar or sacral segments. Since subsequent studies of group II interneurones in sacral segments have revealed that these do not project to the L4 level (Jankowska & Riddell, 1994) the axons almost certainly originate from those neurones of the present population with an ascending projection to the L4 level.

The projection of lower - lumbar group II interneurones to L4 is a feature that they share with identified interneurones in pathways of group I non - reciprocal inhibition (Ib inhibitory interneurones) which are also located in the lower - lumbar segments (Hongo et al, 1983a; Brink et al. 1983). However, the later are located in a deeper and more medial region of the intermediate zone than the group II interneurones and are strongly excited by group I muscle afferents (both Ia and Ib). Very few Ib interneurones (less than 10%) are excited by group II muscle afferents and although most are excited by cutaneous afferents, the linkage in this pathway is not monosynaptic as in most group II interneurones. There are therefore several clear differences between Ib inhibitory interneurones and group II interneurones with an ascending projection to the L4 level which indicate that the two types of neurones will be activated by quite different sensory input and contribute to quite different reflex pathways (see below).

Comparison with group II interneurones in midlumbar and sacral segments

The distribution of lower - lumbar group II interneurones investigated in the present study differs from that of group II interneurones located in other segments of the lumbosacral enlargement. The midlumbar segments contain two distinct populations of group II interneurones; one located in the dorsal horn and the other in the intermediate and ventral grey matter, while in sacral segments group II interneurones are present only in the dorsal horn (Edgley & Jankowska, 1987b; Jankowska & Riddell, 1994). The location of group II interneurones in the lower - lumbar segments overlaps with that of both the dorsal horn and intermediate type interneurones but their distribution is restricted to a smaller area; being less prevalent at the most dorsal (lamina III & IV) and most ventral (in lamina VII) areas where group II interneurones are found in the midlumbar and sacral segments.

As for group II interneurones located in other segments, the effects of stimuli of up to five times threshold applied to muscle nerves were dominated by the actions of group II muscle afferents. Group II inputs to lower - lumbar interneurones were drawn from a uniquely wide field of muscle origin. As in midlumbar interneurones (Edgley & Jankowska, 1987b), group II afferents of Q and DP provided the most effective source of group II input, but group II afferents of several other nerves which have negligible effects on midlumbar interneurones (PBST, GS and to a lesser extent FDL) also provided appreciable excitatory input to the lower - lumbar interneurones. The excitation of lower - lumbar interneurones by group II afferents of Q is consistent with evidence obtained after selectively interrupting transmission through either mid - lumbar or lower - lumbar

interneurones, which shows that the reflex actions of these afferents are mediated by interneurones in both these regions (Cavallari & Pettersson, 1991). It remains to be determined whether the actions of Q in the midlumbar and lower - lumbar regions originate from the same or different heads of the muscle.

Although a proportion of the group II EPSPs evoked by group II muscle afferents in lower - lumbar interneurones were monosynaptic, the longer latencies of some of the EPSPs and discharges evoked by group II afferents suggest that the interneurones also receive di - and oligo - synaptic input from group II afferents. If so, then part of this excitation, especially that produced by group II afferents of GS and PBST, might be mediated by group II interneurones in the dorsal horn of sacral segments (Jankowska & Riddell, 1994). These interneurones are strongly excited by group II afferents of PBST and GS in particular and some have axons that ascend for a short distance (perhaps a few mm) in the lateral funiculus and give rise to axon collaterals which arbourize within the intermediate grey matter (Jankowska et al. 1993b). There is also electrophysiological evidence that some sacral dorsal horn interneurones are interposed in oligo - synaptic pathways to motoneurones (Jankowska & Riddell, 1994).

In addition to excitation from group II muscle afferents, last - order group II interneurones in the intermediate region of the midlumbar segments frequently receive convergent excitation from group I muscle afferents (62% of neurones) which is sufficient to discharge 15 % of cells. Although some of the group II interneurones in the lower - lumbar segments were also excited

by group I muscle afferents the incidence of this convergence was much lower (29% of neurones) and it was generally insufficient to discharge the neurones even when attempts were made to produce temporal summation. The general weakness of the convergent input from group I and group II muscle afferents in lower - lumbar group II interneurones is in agreement with the low degree of spatial facilitation of transmission in group II reflex pathways that is produced by group I muscle afferents (Jankowska, Perfilieva & Riddell, 1996).

Convergence from cutaneous, joint, interosseous and group III muscle afferents is a feature of group II interneurones at all of the locations at which they have been studied. However, in terms of the proportion of neurones excited and proportion of monosynaptic connections, the influence of cutaneous afferents on the lower - lumbar interneurones more closely resembles their action on dorsal horn group II neurones than on group II intermediate zone interneurones in midlumbar segments. This presumably reflects the more dorsal distribution of the lower - lumbar interneurones in comparison to the midlumbar intermediate interneurones.

In each of the other populations of group II interneurone so far studied IPSPs are regularly evoked by stimulation of both muscle and cutaneous nerves. In contrast, IPSPs were only rarely observed in the lower - lumbar interneurones, a feature which has also been commented on by Lundberg et al. (1987b). This suggests that there is little inhibitory interaction amongst lower - lumbar group II interneurones or indeed between lower - lumbar interneurones and interneurones located elsewhere (but see below).

Are there two functionally different populations of lower - lumbar group II interneurones?

Lower - lumbar group II interneurones of the present sample fell evenly into two main categories; those that were, and those that were not, antidromically activated from the L4 level. Though it is possible that a few interneurones projecting to L4 failed to be activated by the stimuli applied in the lateral funiculus, since most ascending axons were activated at strengths well below the maximum stimuli applied, it is most unlikely that significant numbers of interneurones would have been mis - identified. There would appear, therefore, to be at least two functionally different populations of group II interneurones in the lower - lumbar segments raising the question of what their respective roles might be.

One possibility is that the non - projecting interneurones might be functionally analogous to the dorsal horn neurones of the midlumbar and sacral segments while the L4 - projecting interneurones are functionally analogous to the intermediate zone interneurones. However, this seems unlikely to be the case since both sets of interneurones were distributed within a broadly similar area and received a similar pattern of peripheral input. In particular, group I excitation which characterises intermediate zone but not dorsal horn interneurones was seen in the same proportion of L4 - projecting and non - projecting interneurones. Another possibility which is considered below, is that both sets of neurones may be interposed in pathways to motoneurones but that one group is inhibitory and the other excitatory.

Are the lower - lumbar interneurones last - order pre - motor interneurones?

As already noted, lower - lumbar group II interneurones receive inputs from group II afferents of a wide range of nerves. Group II afferents of some of these nerves (PBST, GS, FDL) are virtually without effect on group II last - order interneurones in midlumbar segments (Edgley & Jankowska, 1987b) and yet are known to evoke postsynaptic potentials in motoneurones (e.g. Lundberg et al. 1987a). The only other population of neurones with appropriate group II inputs (in the sacral segments) appear not to project directly to motoneurones (Jankowska & Riddell, 1994, Jankowska et al. 1993b). This evidence suggests that some of the present population of interneurones might be the last - order interneurones in reflex pathways from group II muscle afferents of these muscle nerves.

Since the lower - lumbar group II interneurones and the hind - limb motoneurones which are their potential targets lie within the same or nearby segments, the latencies of discharges of interneurones evoked by group II muscle afferents should be similar (within about 1.0 ms) to the latencies of group II PSPs in motoneurones for which they may be responsible. Comparison of the latency data for interneuronal discharges presented in Fig. 51 with the latencies of group II EPSPs and IPSPs of various nerve origin recorded in motoneurones (see Fig. 6 of Lundberg et al. 1987a), shows that the interneurones could theoretically be responsible for even the earliest EPSPs evoked in flexor motoneurones and that the range of

latencies of interneuronal discharges span the ranges of latencies of both EPSPs and IPSPs in motoneurones.

The location of the group II lower - lumbar interneurones is also consistent with the possibility that some may be last - order interneurones projecting to motoneurones. The interneurones are distributed in those regions of the deep dorsal horn and the intermediate zone (laminae V - VII) where neurones are transneuronally labelled by wheat germ agglutinin - horseradish peroxidase retrogradely loaded into motoneurones of hind - limb muscles (Harrison et al. 1984; Jankowska, 1985).

There are two sets of preliminary observations relating specifically to the lower - lumbar group II interneurones with an ascending projection to the L4 level which lend support to the idea that these interneurones are interposed in pathways to motoneurones and suggest furthermore, that their actions may be inhibitory. Firstly, in experiments where the dorsal columns were sectioned at L6 to interrupt input from primary afferents to the mid - lumbar segments (see below), electrical stimulation of group II muscle afferents was found to produce IPSPs in midlumbar neurones. These IPSPs were attributed to the actions of lower - lumbar interneurones with an ascending projection passing through the lateral funiculus to the midlumbar level (Hongo, et al. 1983a; Harrison et al. 1993). Secondly, spike triggered averaging of postsynaptic potentials in populations of motoneurones recorded from the ventral roots has been used to examine the actions of a small number of group II - activated neurones which included some with a projection to L4. Discharge activity recorded from the interneurones was found in to be correlated with inhibitory ventral root

potentials, some of which occurred at latencies suggestive of a direct connection with motoneurones (Rudomin et al. 1987). Both sets of preliminary evidence therefore suggests that the group II - activated interneurones with a projection to L4 may be interposed in inhibitory pathways to motoneurones.

If the L4 - projecting group II interneurones were proven to be inhibitory like the Ib interneurones with which they share the common feature of an ascending projection to the L4 level then this would strengthen the possibility that they are in some way functionally related. Group Ib and group II muscle afferents have a similar pattern of reflex actions (excitation of flexors and inhibition of extensors) and could therefore share a similar pattern of projection to motoneurones. Despite being activated by essentially different types of sensory inputs, it is nevertheless possible that these two sets of interneurones work synergistically such that activation of both sets of interneurones during stretch of a loaded muscle produces a more powerful inhibition than activation of either sets of interneurones alone.

There is no evidence relating directly to the actions of the group II interneurones without an ascending projection to L4 but they may well constitute a functionally different population from those with an ascending projection. In a previous study of interneurones with monosynaptic input from group I muscle afferents, all those with a projection to L4 were found to be inhibitory (Ib inhibitory interneurones) while all those lacking an ascending projection were excitatory (Brink et al. 1983). It is possible therefore that a similar organisational principle may apply to the group II interneurones of the lower - lumbar segments and that those lacking the L4 projection may have an

excitatory action on motoneurones. It may be noted that while no obvious differences were seen in the patterns of input to L4 - projecting and non - projecting interneurones, no differences were seen in the small populations of midlumbar interneurones which were identified as having excitatory or inhibitory actions on motoneurones (Cavallari et al. 1987).

Possible function of the projection to L4

The ascending projections of group II lower - lumbar interneurones were tested only by applying stimuli at L4 and Th13 so that those identified as projecting to L4 could in theory have projected to any level between L4 and Th13. However, it is almost certain that the present L4 - projecting neurones are those giving rise to the group II - activated axons recorded from in the lateral funiculus most of which were found to project only as far as the L4 or L3 segments (Harrison & Riddell, 1989). This pattern of projection is very similar to that of the ascending axons of Ib inhibitory interneurones (Fern et al. 1988) which have been shown to contact dorsal spinocerebellar tract (DSCT) neurones in Clarke's column and preliminary observations indicate that lower - lumbar group II interneurones might also project to neurones in Clarke's column and other areas of the L3 and L4 segments (Hongo et al. 1983a; Harrison et al. 1993). Evidence for this was obtained in experiments where recordings were made from neurones in the midlumbar segments after dorsal column lesions had interrupted primary afferent input to midlumbar but not lower - lumbar segments. Any input to midlumbar interneurones is then likely to originate from lower - lumbar

interneurones with an ascending projection in the lateral funiculus to the midlumbar level. In one study of this type activation of group II muscle afferents was reported to produce IPSPs in 70% of unidentified interneurones (Harrison et al. 1993) while in another, a few examples of IPSP were observed in neurones of Clarke's column (see Fig. 2E & F of Hongo et al. 1983a). Other midlumbar neurones which are potential targets of the ascending axons of group II lower - lumbar interneurones are a population of dorsal horn DSCT neurones and neurones of the spinocervical tract, both of which are strongly excited by group II muscle afferents (Edgley & Jankowska, 1988; Hammar et al. 1994). If group II interneurones in the midlumbar segments are among the unidentified neurones that were inhibited by lower - lumbar interneurones (Harrison et al. 1993), then this would imply that when the lower - lumber group II interneurones are active they inhibit the operation of midlumbar group II interneurones. If this is the case then the inhibition does not appear to be reciprocated since only rare examples of IPSPs were seen in the lower - lumbar interneurones.

Th13 projecting neurones

Since the main goal of this study was to investigate interneurones in the lower - lumbar segments, the rostral projections of neurones projecting beyond the lumbar enlargement were tested only up to the Th13 segment. These neurones most likely belong to ascending tracts though it is also possible that they represent examples of propriospinal neurones, for example connecting the spinal enlargement (Hultborn & Illert, 1991). Further

study will be required to clarify the target of these neurones. Nevertheless, the possible identity of these neurones will be discussed briefly.

i) SCT neurones. Harrison & Jankowska (1984) have reported that a small group of SCT neurones in the L6 - L7 segments receive weak input from group II muscle afferent in addition to their strong cutaneous input. All of the Th13 projecting neurones in the present study were excited by both group II muscle afferents and cutaneous inputs and some might therefore be SCT neurones.

ii) Dorsal horn spinocerebellar neurones. Matsushita, Hosoya & Ikeda (1979) and Grant et al. 1982 have provided anatomical evidence that some of the DSCT neurones (are located in the in L6 - L7 segments) retrogradely labelled following injection of HRP into the cerebellum. However, so far electrophysiological studies of DSCT neurones located within the L5 - L6 segments have revealed only input from group I muscle afferents (Aoyama, Hongo & Kudo, 1988). Nevertheless, it is possible that the Th13 projecting neurones of the present study could represent more caudally located examples of the types of group II DSCT neurones which are numerous in the midlumbar segments (Edgley & Jankowska, 1988; Edgley & Gallimore, 1988).

iii) Post synaptic dorsal column neurones. The peripheral input to dorsal column cells is characterised by excitation from low and high threshold cutaneous afferents and low and high threshold muscle and joint afferents (Uddenberg, 1968a,b; Petit, 1972; Noble & Riddell, 1986).

Intracellular recording from post synaptic dorsal column neurones in the L7 - S1 segments has shown that they receive monosynaptic input from group I muscle afferents (in about 50% of the cells tested) but only two out of thirty - two cells were excited by group II muscle afferents (Jankowska, Rastad, Zarzecki, 1979). It therefore seems rather unlikely that the sample of Th13 projecting neurones with group II input in this study constitute part of the post synaptic dorsal column system unless Jankowska et al. (1979) failed to sample neurones in the most lateral part of the dorsal horn. Furthermore, most of the present sample of neurones were excited by stimuli applied to the lateral funiculus strengths less than 300 μA and thus stimulus spread sufficient to excite cells in the dorsal columns is unlikely to have occurred.

iv) Spinothalamic tract neurones. Spinothalamic tract neurones are located in the marginal zone (lamina I), neck of the dorsal horn (laminae IV - V) and ventral horn (VII - X) as shown by retrograde transport of HRP and fluorescent labels from the thalamus (Carstens, Trevino, 1987; Jones, Apkarian, Stevens & Hodge, 1987; Craig, Linington & Kniffki, 1989). The axons of the cells cross the mid line and ascend in the contralateral white matter. The spinothalamic tract in the cat is regarded as a major cutaneous somatosensory pathway (Meyers, Snow, 1982; Holloway, 1981) but some cells are excited by manipulation of muscles (Meyers, Snow, 1982) and there is evidence from electrical stimulation of muscle nerves that muscle afferents (including group I, II and III afferents) activate spinothalamic neurones in monkey (Foreman, Applebaum, Beall, Trevino & Willis, 1974; Foreman, Schmidt & Willis, 1977; Foreman, Kenshalo, Schmidt & Willis,

1979). Since, the Th13 projecting neurones studied of this study projected (with one exception) ipsilaterally, it seems rather unlikely (with the exception of the single contralaterally projecting neurone) that they constitute part of the spinothalamic tract.

Conclusion

The results presented here show that group II afferents of one or more of a range of hind - limb muscles nerves evoke field potentials in both the dorsal horn and intermediate zone at most locations within the L6 and L7 segments. Accordingly, the properties of neurones with input from group II afferents (within the areas where field potentials were recorded) were investigated. This study has shown that interneurones processing signals carried by group II muscle afferents are located throughout the lower - lumbar segments of the cat spinal cord. At least two functionally different types of interneurone appear to exist, one of which is characterised by an ascending axon collateral projecting to the upper lumbar segments. The location and pattern of peripheral input of these interneurones, together with preliminary observations on the population with an ascending projection, strongly suggests that at least some may be last - order interneurones in group II reflex pathways. Further investigations will be required to determine whether these neurones make direct connections with motoneurones.

Chapter 4.0. References

4.0. References

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