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STUDIES ON THE MAMMALIAN MUSCLE SPINDLE

Robert William Banks

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Papers submitted in candidature for the degree of D.Sc.

University of Durham

1994

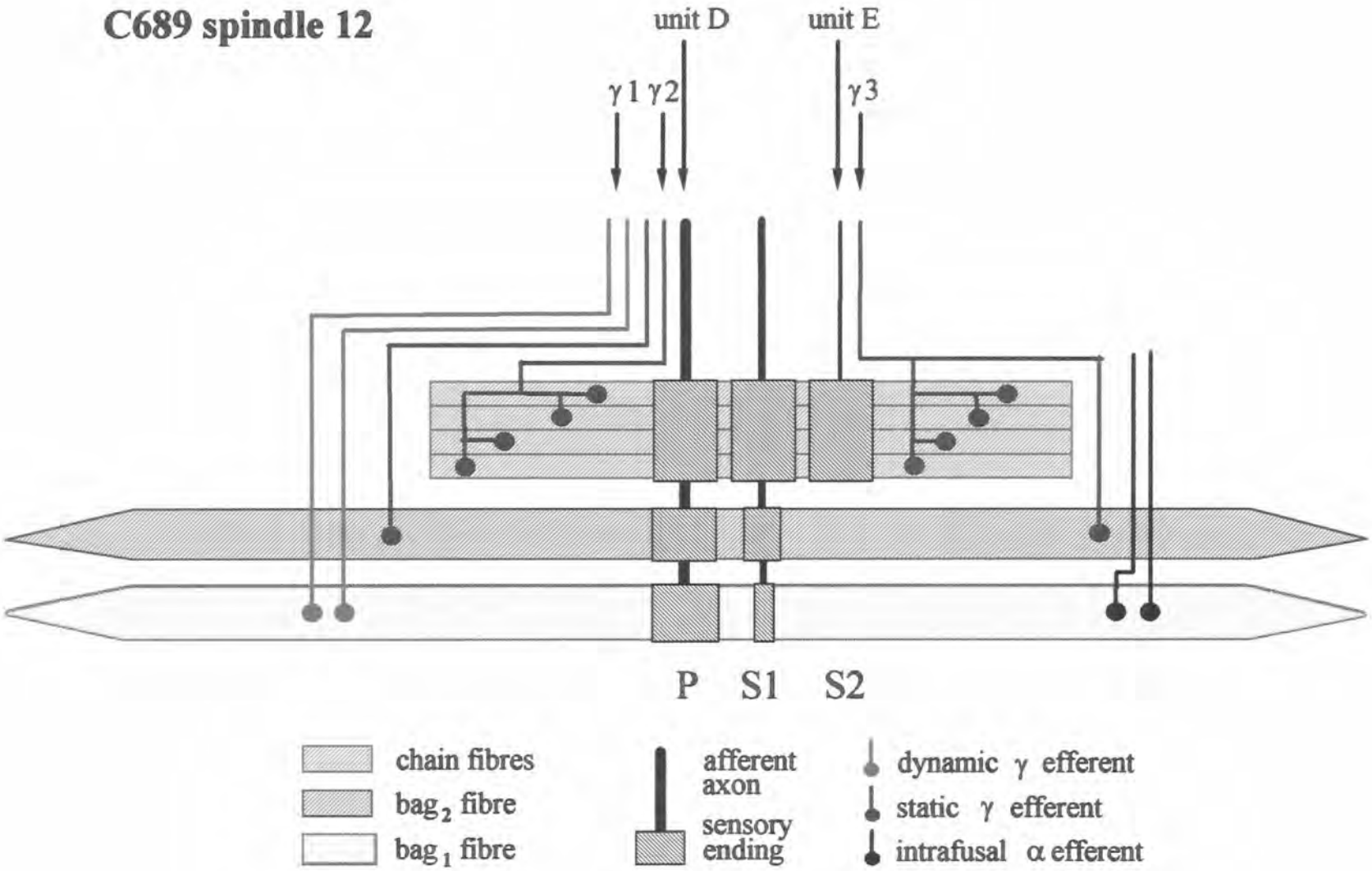


21 DEC 1995

Dedication

for my wife, my children, and my parents

C689 spindle 12



Frontispiece

A schematic diagram showing the innervation of a muscle spindle from the tenuissimus muscle of the cat. See Banks (1991) The distribution of static γ-axons in the tenuissimus muscle of the cat. *Journal of Physiology* **442**, 489-512 (paper 42).

Robert William Banks, B. Sc., Ph. D.

Studies on the Mammalian Muscle Spindle

D.Sc. 1994

The subjects of these studies are the major components of the mammalian muscle spindle, which is an encapsulated proprioceptor serving to monitor skeletal muscle length and length change. Those components are: specialized intrafusal muscle fibres; sensory nerve endings that form intimate contacts with the intrafusal fibres; and motor nerve fibres by means of which the central nervous system can exercise control over the sensitivity of the spindle.

My first important contribution was to establish the number of types of intrafusal fibre (1-8, 11). Their different mechanical properties help to shape the responses of the sensory endings in characteristic ways (papers 15 and 42). Detailed reconstructions of sensory endings revealed recognizable features of the primary ending that were consistently associated with the different intrafusal fibres (10, 13, 18, 20, 33). The sites of nerve impulse generation and coding are being studied in relation to the branching pattern of the sensory nerve fibres (45, 50, 55).

Analysis of the innervation of individual spindles has revealed the interplay of random and deterministic factors in spindle construction (20, 36, 37, 40, 41, 44, 48, 52, 53). As yet it is unknown how the differences that exist between muscles in this respect are related to their specific roles in motor control or kinaesthesia. However, reflex activity appears to be grossly disturbed in muscles that have been reinnervated following nerve section, since functional endings may be formed in inappropriate locations (22, 25, 28-31, 34, 38, 39, 43, 46).

The motor innervation of the spindle was for long controversial, especially concerning the distribution of the different functional categories of axon. I have pursued histophysiological and probabilistic approaches to this problem, about which there now appears to be a large measure of agreement in favour of my conclusions (9, 12, 19, 21, 23, 26, 27, 35, 41, 42, 44, 48, 49).

Papers 1-6 in the following list are based on work that originally formed part of a thesis presented in candidature for the degree of Ph. D. in the Faculty of Medicine, University of Sheffield. For each full paper of which I am a co-author an estimate of my contribution to the overall effort is given as a percentage in the list.

1. R.W. Banks (1971) Histochemical studies on rabbit intrafusal fibres. J.Anat. 108; 613-614.

2. R.W. Banks & N.T. James (1971) The fine structure of the guinea-pig muscle spindle. J. Anat. 110; 161-162.

3. R.W. Banks & N.T. James (1973) The blood supply of rabbit muscle spindles. J. Anat. 114; 7-12. (70%)

4. R.W. Banks & N.T. James (1973) The fine structure of the guinea-pig muscle spindle. Z. Zellforsch. 140; 357-368. (99%)

5. R.W. Banks & N.T. James (1974) Quantitative studies on the distribution of myofilaments in intrafusal muscle fibres. Cell Tiss. Res. 150; 521-527. (5%)

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51. R. W. Banks, L. Decorte, F. Emonet-Dénand, M. H. Gladden & F. Sutherland (in press) The exceptional structure of muscle spindles in superficial lumbrical muscles of the cat hind limb. In A. Taylor & M. H. Gladden (Eds.) Alpha and Gamma Motor Systems Plenum Press, New York. (10%)

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Paper 1.

R.W. Banks (1971)
Histochemical studies on rabbit intrafusal fibres.
J.Anat. 108; 613-614.

Fig. 14

44. Histochemical studies on rabbit intrafusal fibres. By R. W. BANKS (introduced by N. T. JAMES). *Department of Human Biology and Anatomy, University of Sheffield* (Fig. 15)

Muscle spindles of mammals so far studied contain two types of intrafusal fibre. These are identified by the distribution of their nuclei and are termed nuclear bag fibres and nuclear chain fibres. It is generally accepted that the rabbit is an exception and contains only nuclear bag fibres (Barker & Hunt, *Nature, Lond.* **203**, 1964). The enzyme histochemical profiles of intrafusal fibres were studied in serial cryostat sections of several hind limb muscles of the rabbit. Twenty-seven muscle spindles were examined and three types of intrafusal fibre were clearly distinguished. These are clearly visible in sections stained for phosphorylase activity followed by treatment with iodine (Fig. 15). Type I fibres stained strongly for myosin ATPase, succinic dehydrogenase and phosphorylase activities. The phosphorylase reaction product stained purple with iodine. Type II fibres showed intermediate staining for myosin ATPase and succinic dehydrogenase activities but weak staining for phosphorylase. Type III fibres did not stain for myosin ATPase activity. They showed weak staining for succinic dehydrogenase activity but stained strongly for phosphorylase, the reaction product staining brown with iodine. In the majority of spindles all three types of intrafusal fibre were present, as in the figure. Type I fibres are shorter than either type II or type III fibres. The histochemical profile of type I fibres is similar to that of nuclear chain fibres in other species. Similarly type II and type III fibres correspond with the two types of nuclear bag fibres present in the rat (Yellin, *Am. J. Anat.* **125**, 1969).



Fig. 15

Paper 2.

R.W. Banks & N.T. James (1971)
The fine structure of the guinea-pig muscle spindle.
J. Anat. 110; 161-162.

Fig. 10*b*

47. **The fine structure of the guinea-pig muscle spindle.** By R. W. BANKS and N. T. JAMES. *Department of Human Biology and Anatomy, University of Sheffield* (Fig. 11)

Muscle spindles are complex sensory organs found in skeletal muscles. The fine structure of those in guinea-pig lumbrical muscles has not previously been described. Serial longitudinal and transverse sections of 10 muscle spindles were examined.

Two types of intrafusal muscle fibre may easily be distinguished in both longitudinal and transverse sections. Those with a prominent M line contain large mitochondria and an extensive sarcoplasmic reticulum. Those without an M line contain smaller mitochondria (Fig. 11 A) and have a less extensive sarcoplasmic reticulum. These characteristics do not vary in different regions of a fibre. Only those fibres without an M line regularly extend beyond the capsule of perineural epithelium.

In the equatorial sensory regions of the muscle fibres there are myofibrils surrounding the central nuclei, and these are continuous with myofibrils in the polar regions. Sensory nerve endings either assume an irregular form or occur as small annulo-spiral endings.

Twenty-five motor nerve endings were examined in the polar regions of muscle spindles. They are associated with varying degrees of post-junctional folding. Some endings lie directly on the muscle fibre without folding of the post-junctional membrane (Fig. 11 B). Other endings are

associated with moderate folding of the post-junctional membrane (Fig. 11C). In addition there are endplates associated with marked folding of the post-junctional membrane which overlie greater amounts of sarcoplasm (Fig. 11D).

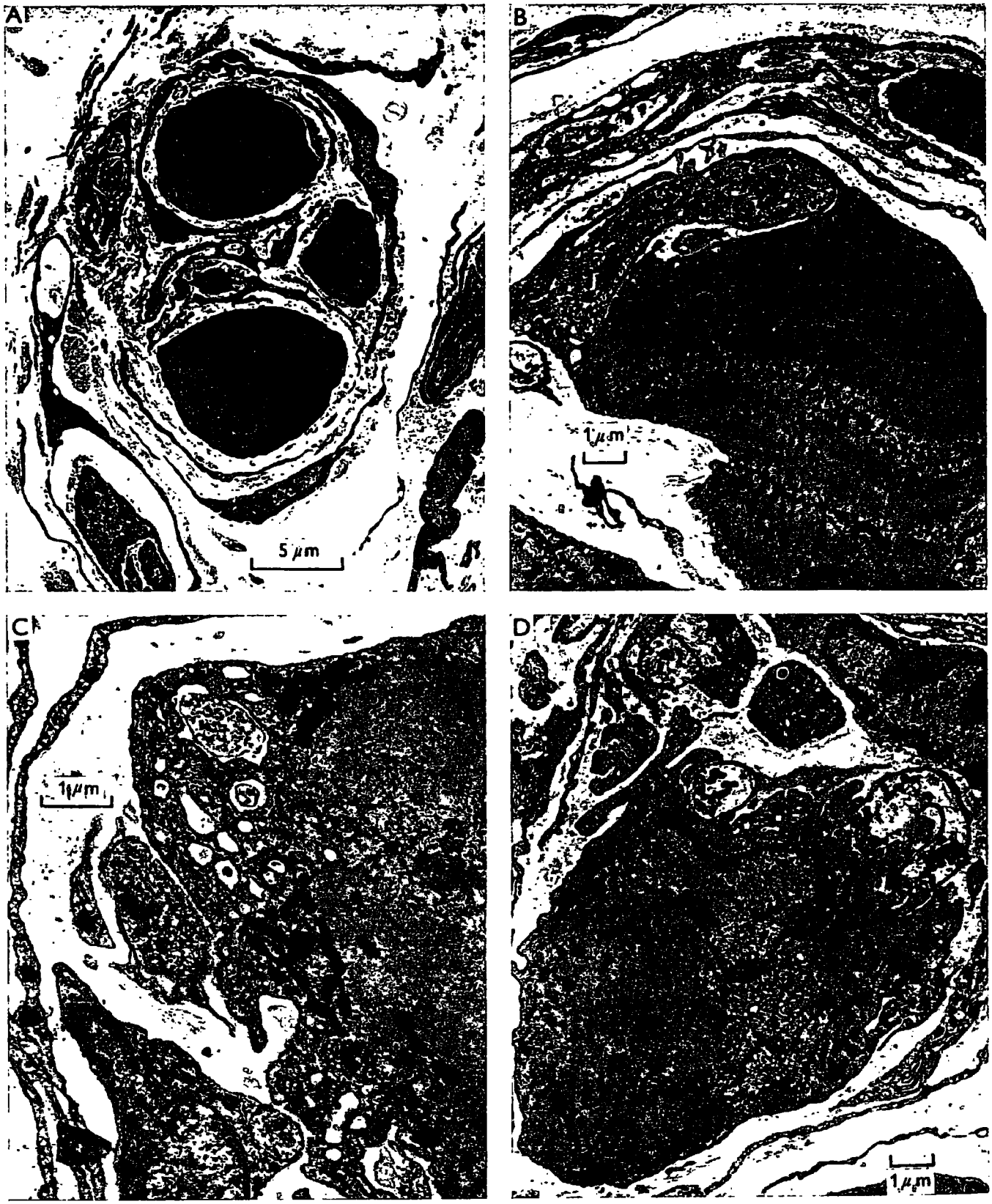


Fig. 11

Paper 3.

R.W. Banks & N.T. James (1973)
The blood supply of rabbit muscle spindles.
J. Anat. 114; 7-12.

J. Anat. (1973). 114. 1, pp. 7–12
With 5 figures
Printed in Great Britain

The blood supply of rabbit muscle spindles

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(*Accepted* 3 *November* 1972)

INTRODUCTION

Recent physiological investigations have indicated that depolarizing agents may enter muscle spindles by means of blood vessels which penetrate the spindle capsule (Kidd, Kucera & Vaillant, 1972). Although capillaries can be seen with the light microscope in the periaxial spaces of human spindles (Cooper, 1960; Cazzato & Walton, 1968), in rat and cat spindles examined with the electron microscope (Merrillees, 1960; Landon, 1966; Corvaja, Marinozzi & Pompeiano, 1969) they have been seen only in the spindle capsules. A recent electron microscopical study of 12 rat spindles (James & Meek, 1971) revealed that only one of the spindles, the largest, contained periaxial capillaries. In order to obtain more information on the possible occurrence of periaxial capillaries in a common laboratory animal, detailed studies were carried out on rabbit spindles.

MATERIALS AND METHODS

Six adult male New Zealand rabbits of average weight 2·5 kg were anaesthetized with 60 mg of Nembutal per kg body weight. A single hind limb lumbrical muscle was exposed, using a single incision, in each of three rabbits. A tenuissimus muscle was similarly exposed in each of the other three rabbits. Each muscle was covered, *in situ*, with a 3% solution of glutaraldehyde in 0·1 M phosphate buffer at pH 7·3 for 5 minutes. During this stage of fixation either the digits were dorsiflexed or the hind limb was rigidly fixed, as appropriate, to prevent muscle shortening.

Each muscle was removed from the rabbit and placed in fresh buffered glutaraldehyde for 18 hours at 4 °C. The muscle was then transferred to 0·1 M phosphate buffer at pH 7·3 to which sucrose had previously been added to a final concentration of 10%. This solution was maintained at 4 °C and was changed 3 times at 2 hourly intervals. The muscles were then placed in a 2% aqueous solution of osmium tetroxide for 1 hour at 4 °C, dehydrated in a graded series of ethanol solutions and infiltrated with Araldite. Each muscle was then cut transversely into numerous small pieces and the Araldite was polymerized. Semi-thin transverse sections, each approximately 0·5 μm thick, were cut from each block of muscle, stained on a hot plate with 1% toluidine blue in a 1% aqueous solution of borax (Pease, 1964), and examined for their spindle content.

Semi-thin and thin transverse sections were cut at regular intervals of 20–30 μm from blocks seen to contain muscle spindles. Thin sections were placed on collodion-

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Fig. 1. Transverse section through the equatorial region of a spindle containing seven intrafusal muscle fibres. Part of the periaxial space (PS) is visible, in which are two capillaries (C). Note the multilayered capsule (cap) and the inner capsule cells (i.c.) which surround the capillaries and the intrafusal muscle fibres. ×2780.

coated grids and stained for 5 minutes with an aqueous solution of lead citrate (Reynolds, 1963). The sections were examined with a Philips EM 200 electron microscope at an accelerating voltage of 60 kV.

RESULTS

Nineteen muscle spindles were examined. At least one capillary was visible in each spindle equatorial region (Fig. 1). Such capillaries have been termed intrafusal capillaries. They possessed continuous endothelia devoid of fenestrations (Fig. 2), lay freely within the periaxial space, and were longitudinally orientated for most of their lengths. Branching and anastomosis of the intrafusal capillaries were frequently observed.

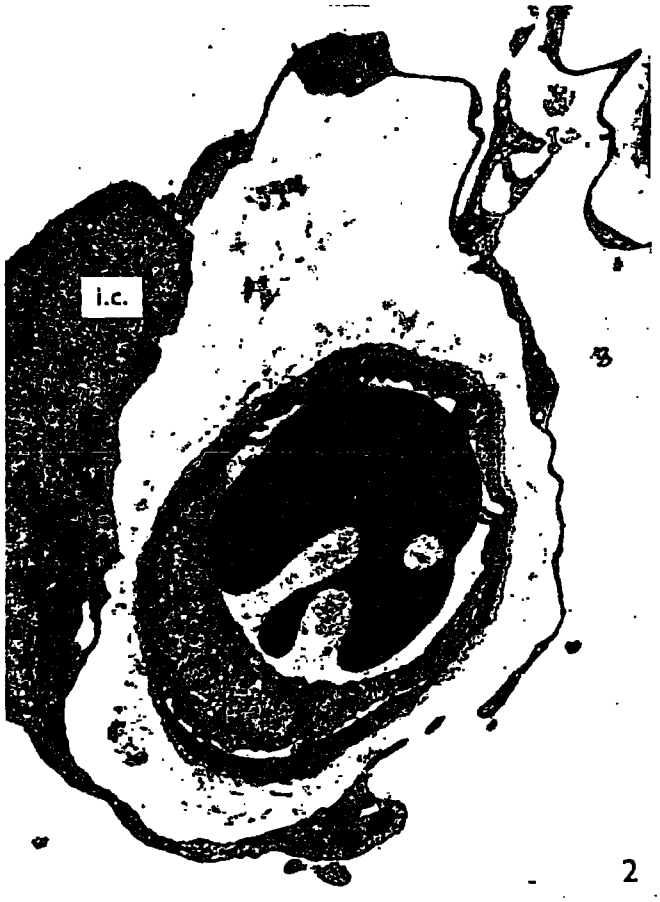
In 70% of spindles each intrafusal capillary was surrounded by a single layer of inner capsule cells (Figs. 1, 2). Direct contacts between capillaries and intrafusal muscle fibres, similar to those of extrafusal musculature, were never seen. The inner capsule cells further separated the capillaries from the intrafusal fibres and the sensory nerve endings. Only in 30% of cases were capillaries seen to approach intrafusal fibres without a complete surrounding layer of inner capsule cells (Fig. 3).

The intrafusal capillaries were continuous with those of the spindle polar regions. In the polar regions of 16 spindles the capillaries were found only between the layers of the capsule (Fig. 4). In only three spindles were capillaries found lying close to the polar regions of the intrafusal fibres (Fig. 5).

DISCUSSION

The present observations clearly indicate that rabbit spindles are highly vascular compared with those of other species so far examined. Capillaries rarely penetrate the capsules of rat spindles (James & Meek, 1971) and have not been seen in the periaxial spaces of guinea-pig spindles (Banks & James, 1972). The presence of intrafusal capillaries in only some species may be related to the oxygen and metabolic demands of the intrafusal fibres. Rat spindles contain fewer intrafusal fibres than rabbit spindles. The spindles of larger animals, which possess numerous intrafusal fibres, might be expected to contain intrafusal capillaries, whilst those of small animals, which contain few intrafusal fibres, might be expected to lack capillaries. Except for human spindles (Cooper, 1960; Cazzato & Walton, 1968) we have been unable to find any references to capillaries in the periaxial spaces of muscle spindles.

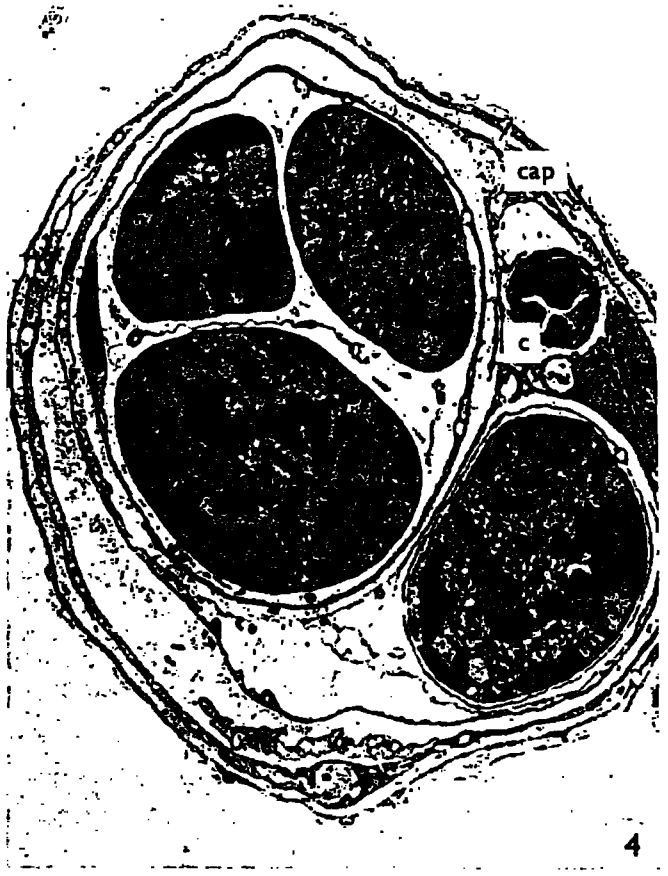
The presence of capillaries within spindles may be related to the myoglobin content of intrafusal fibres. It is well known that myoglobin either acts as an oxygen store or accelerates the diffusion of oxygen in regions where demand would otherwise exceed supply (James, 1971*a*, 1972). In rat and guinea-pig spindles the large nuclear bag fibres contain higher concentrations of myoglobin than the smaller nuclear chain fibres. This may be due to the lack of intrafusal capillaries and consequently to a relatively poor oxygen supply (James, 1971*b*). In a preliminary histochemical study of myoglobin in rabbit spindles (James, 1968) most of the intrafusal fibres showed identical staining reactions despite the known wide range of their metabolic activities (Barker & Stacey, 1970; Banks, 1971). It is possible that the presence of intrafusal



2



3



4



5

capillaries in the rabbit ensures an adequate oxygen supply and removes the necessity for a high concentration of myoglobin in the larger intrafusal fibres.

The spindle capsule is formed from the perineural epithelium of its nerve supply (Shantha, Golarz & Bourne, 1968) and serves as a highly active diffusion barrier (Waggener, Bunn & Beggs, 1965; Olsson & Reese, 1971). Curarization of animals – for example, using Flaxedil (gallamine triethiodide) – blocks intrafusal neuromuscular junctions much later than extrafusal neuromuscular junctions, and their recovery is similarly delayed (Emonet-Denand & Laporte, 1966; Carli, Diette-Spiff & Pompeiano, 1967). It is possible that the delay in blocking is due to the perineural epithelial cells which usually intervene between the capillaries and the spindle contents (James & Meek, 1971; Banks & James, 1972). If the rabbit is found to be the only common laboratory animal which possesses intrafusal capillaries then it could be a most useful animal for studying the pharmacology of muscle spindles.

SUMMARY

Intrafusal capillaries occur regularly in the periaxial spaces of rabbit muscle spindles. They have previously been noted only in the human.

The occurrence and distribution of intrafusal capillaries in the rabbit have been studied using a combination of light and electron microscopy. Typically a single capillary was present between the capsule layers of each spindle polar region. In the equatorial region the capillary entered the periaxial space to lie approximately parallel to the intrafusal muscle fibres.

The role of the blood supply in relation to oxygen uptake and myoglobin content of the intrafusal muscle fibres is discussed. The similarity of the blood supply of rabbit and human muscle spindles may be of considerable value in comparative pharmacological and physiological studies.

This work was supported by grants from the Medical Research Council and Science Research Council of Great Britain. R. W. Banks acknowledges an M.R.C. Studentship.

Fig. 2. Transverse section of an intrafusal capillary showing the continuous endothelium. The capillary is surrounded by inner capsule cells (i.c.). × 9000.

Fig. 3. Transverse section through the juxta-equatorial region of a spindle showing a capillary close to two intrafusal fibres. The inner capsule cells surrounding the capillary do not completely separate it from the periaxial space (PS). × 3600.

Fig. 4. Transverse section through the polar region of a spindle showing a capillary (c) in the spindle capsule (cap). This is the typical condition in a polar region. × 1880.

Fig. 5. Transverse section through the polar region of a spindle in which a capillary (c) is present within the periaxial space. This condition is less common than that shown in Fig. 4. × 1390.

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Paper 4.

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The Fine Structure of the Guinea-Pig Muscle Spindle

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Summary. Nuclear bag and nuclear chain intrafusal fibres are present in guinea-pig muscle spindles. Unlike muscle spindles in other species two types of nuclear chain fibre seem to be present. The electron microscopical appearance of one type of nuclear chain fibre is similar to that of nuclear bag fibres.

It is suggested that under tension the nuclei of small nuclear bag fibres become sufficiently displaced to form nuclear chain-like fibres. The frequent occurrence of fibres which combine some of the properties of both nuclear bag and nuclear chain fibres indicates the possible occurrence of a third type of intrafusal fibre.

The sensory innervation of guinea-pig muscle spindles is similar to that of the cat and the rat. Three types of motor nerve ending which could be classified according to the complexity of their subneural apparatus were seen.

Key words: Muscle spindle — Guinea-pig — Electron microscopy.

Introduction

Mammalian muscle spindles are complex sensory organs which occur exclusively in skeletal muscles. Each contains a number of narrow striated intrafusal muscle fibres, which are enclosed within a capsule of perineurial epithelium. In the equatorial region of the muscle spindle, the capsule is separated from the intrafusal fibres by a dilated periaxial space. It is generally accepted that two types of intrafusal fibre can be distinguished according to the distribution of their equatorial nuclei (Boyd, 1962). Nuclear bag fibres contain a central group of nuclei in their equatorial regions. Nuclear chain fibres contain a single central longitudinal row of nuclei in their equatorial regions.

Usually each spindle is innervated by several motor and sensory nerves. Primary sensory nerve endings usually occur as annulo-spirals wound around the equatorial regions of the intrafusal muscle fibres. Usually a single primary sensory nerve innervates each spindle. Secondary sensory nerve endings, where present, are more irregular in form. The polar regions of the intrafusal muscle fibres are innervated by small diameter fusimotor fibres.

Several descriptions of the fine structure of other mammalian muscle spindles are available, e.g. in the cat (Adal, 1969; Corvaja, Marinozzi and Pompeiano, 1969; Scalzi and Price, 1971), the rat (Hennig, 1969; Landon, 1966; Merrillees,

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1960; Ovalle, 1971, 1972; Rumpelt and Schmalbruch, 1969) and the dog (Banker and Girvin, 1967). Some information is also available for the rabbit (Corvaja and Pompeiano, 1970), the tree-shrew (During and Andres, 1969) and man (Gruner, 1961; Rumpelt and Schmalbruch, 1969).

Materials and Methods

Young male Hartley strain guinea-pigs, of average weight 350 g were kept in cages 50 × 38 cm for two weeks prior to examination of their spindles. Five guinea-pigs were kept in each cage.

Anaesthesia was induced and maintained in each guinea-pig with chloroform and a single lumbrical muscle of one hind-limb was exposed. Each muscle was covered, in situ, with a 3% solution of glutaraldehyde in 0.1 M phosphate buffer at pH 7.3 for 5 minutes. During this stage of fixation the digits were dorsi-flexed at an angle of 45° to prevent shortening of the sarcomeres.

Each lumbrical muscle was then removed from the foot and placed in fresh glutaraldehyde fixative for 18 hours at 4°C. The muscle was then transferred to 0.1 M phosphate buffer at pH 7.3, to which sucrose was added to a final concentration of 10%. This solution was maintained at 4°C and was changed 3 times at two hourly intervals. The muscle was then placed in a 2% aqueous solution of osmium tetroxide for one hour at 4°C, dehydraded with a graded series of ethanol and infiltrated with Araldite.

Each muscle was then cut transversely into four parts and the Araldite was polymerized. Each resulting block was examined separately, and semi-thin transverse sections, approximately 0.5 μm thick, were cut. The sections were stained with 1% toluidine blue in a 1% aqueous solution of borax on a hot-plate (Pease, 1964) and examined for their spindle content.

Semi-thin and thin transverse sections were cut at intervals of 10 μm or less from blocks containing spindles. Some of the blocks were reorientated and thin longitudinal sections were placed on collodion coated grids and stained for five minutes with an aqueous solution of lead citrate (Reynolds, 1963). The sections were examined with a Philips EM 200 electron microscope at an accelerating voltage of 60 kV.

Results

Capsule and Periaxial Space

Fifteen muscle spindles were examined. Typically the capsule was composed of several alternating layers of perineurial epithelial cells and collagen fibres (Fig. 1). Each perineurial epithelial cell was sheet-like, approximately 0.2 μm thick but locally thickened by mitochondria and a flattened nucleus, approximately 8 × 1 μm. The edges of adjacent cells abutted directly or overlapped to a

Fig. 1. Transverse section through a capsule in the equatorial region of a spindle. A blood capillary (*bc*) may be seen in the capsule wall, which consists of perineurial epithelium (*pne*) and collagen. Within the capsule is a periaxial space (*ps*) containing a flocculent precipitate (×7200)

Fig. 2. Transverse-section through the polar region of a spindle. Large mitochondria are present in the smallest intrafusal fibre. The capsule consists of a single layer of perineurial epithelium (*pne*). (×2860)

Fig. 3. Transverse section through the equatorial region of a spindle containing a prominent periaxial space (*ps*). Four intrafusal fibres are surrounded by individual sheaths of inner capsule cells (*ic*). In one fibre part of the nerve ending is completely surrounded by sarcoplasmic folds. A sensory cross terminal (*sct*) lies in contact with two fibres. (×1670)



Fig. 4. Longitudinal section through the equatorial region of a nuclear bag fibre which possesses a small nuclear bag. Several cross sections of the primary sensory ending are visible. The sensory nerve endings are enclosed within the basement membrane (*bm*) of the muscle fibre. (×2860)

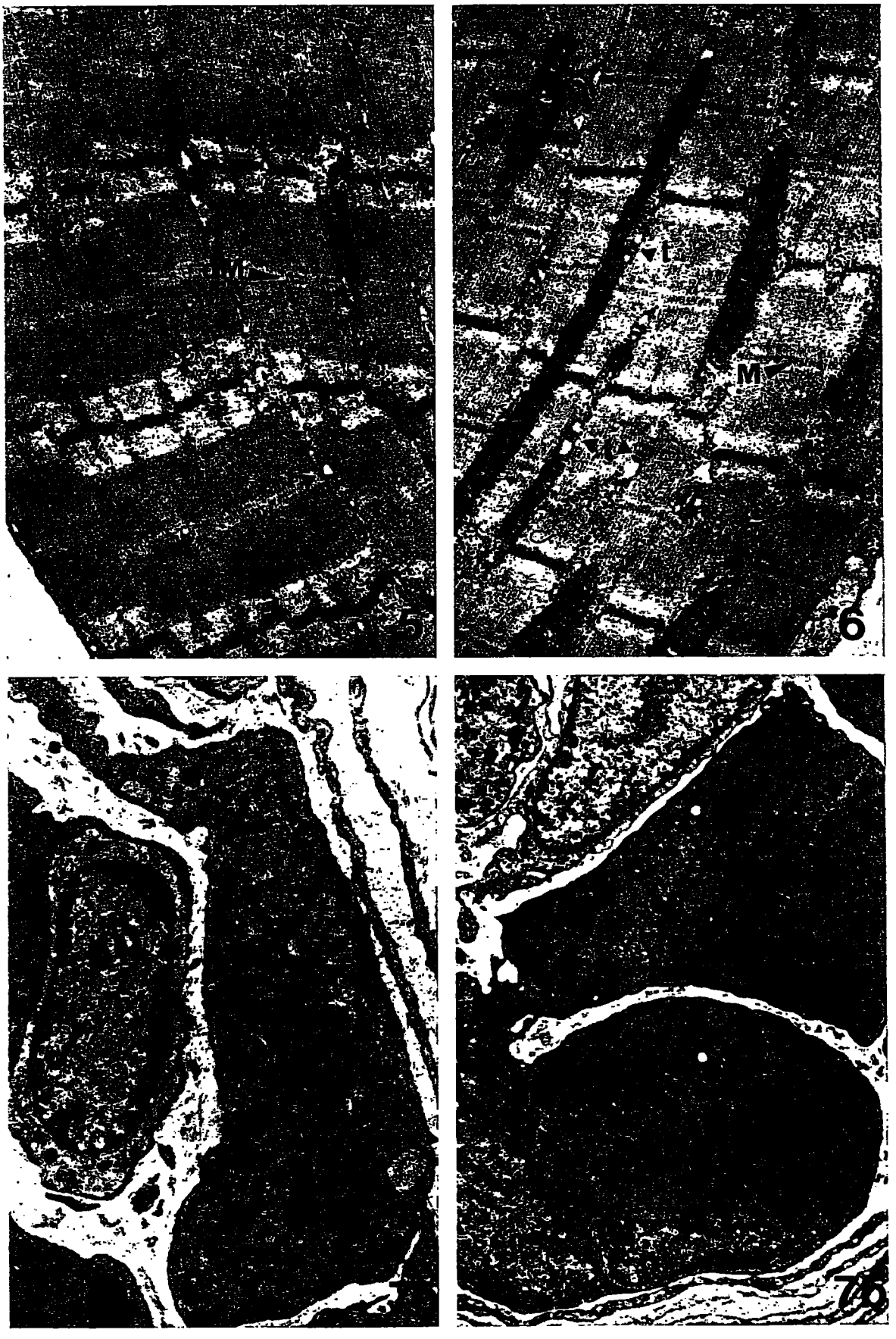


Fig. 5. Longitudinal section in the polar region of an intrafusal muscle fibre which possesses faint double M-lines (*dM*) and small mitochondria. Few triads (*t*) are visible. (×15000)

variable extent. Basement membrane was present on the surface of these cells. The longer intrafusal muscle fibres passed through the ends of the capsule and were inserted into extrafusal perimysium. Single layers of perineurial epithelium were present in the polar regions of the capsule (Fig. 2), whilst up to ten layers were present in the equatorial regions of the spindle. Individual perineurial epithelial cells extended only partly around the equatorial capsule, thus in a transverse section through the equatorial region the number of layers of perineurial epithelium varied in different regions around the circumference of a capsule. The average number of layers in the spindle equatorial region was approximately six.

Capillaries were present between the layers of perineurial epithelium (Fig. 1). Usually a single capillary was visible in each transverse section. Each capillary was flattened transversely, measured approximately 10×4 μm and possessed a continuous endothelium, typical of muscle capillaries. The capillaries in the capsule were always separated from the periaxial space by at least one layer of perineurial epithelium. Capillaries were not seen within the periaxial space.

In the equatorial region of the spindle, the capsule was separated from the intrafusal fibres by a prominent periaxial space of approximately 70 μm average maximum diameter (Figs. 3 and 4). The periaxial space contained a moderately electron-dense, flocculent material (Fig. 1).

Inner capsule cells surrounded the intrafusal muscle fibres in the equatorial region of the spindle, separating the muscle fibres from the periaxial space and from each other (Fig. 3). The surface of the inner capsule cell was not covered by basement membrane. Each cell possessed an approximately oval nucleus 8×2 μm in diameter, surrounded by a thin rim of cytoplasm. The cytoplasm was extended to form several sheet-like processes. These connected with similar processes of the same or other cells to form a single complete layer surrounding each intrafusal muscle fibre. Occasionally the layer was incomplete due to the presence of a nerve fibre passing from one compartment to another (Fig. 3).

Intrafusal Muscle Fibres

The spindles contained an average of 4.8 intrafusal muscle fibres, with a minimum of three fibres. Nuclear bag and nuclear chain fibres could clearly be differentiated.

Small nuclear bags (Fig. 4) were seen in the largest fibres. No more than two nuclear profiles could be seen in single transverse sections. These nuclei were irregularly shaped, approximately 5 μm in diameter and the nuclear bag extended 20–30 μm along the intrafusal fibre. At each end of the nuclear bag was a myotube consisting of a central row of cylindrical nuclei approximately 3 μm in diameter

Fig. 6. Longitudinal section in the polar region of an intrafusal muscle fibre which possesses single prominentM-lines (*M*) and large mitochondria. Numerous triads (*t*) can be seen. ($\times$ 15000)

Fig. 7a and b. Transverse sections of satellite cells possessing prominent nuclei (*scn*). In (7a) dilated terminal cisternae of sarcoplasmic reticulum (arrowed) are present in a nuclear chain fibre possessing single M-lines ($\times$7500). In (7b) a satellite cell lies in contact with two intrafusal fibres. ($\times$4500)

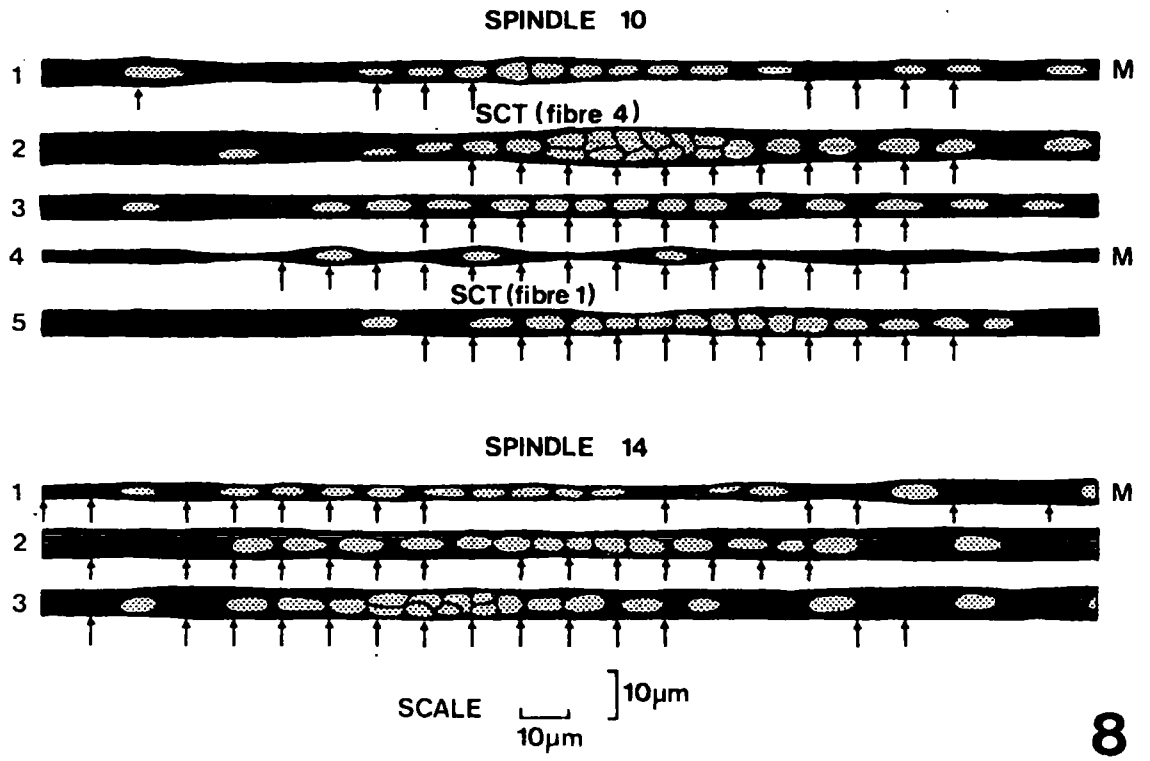


Fig. 8. Reconstructions of the equatorial regions of the intrafusal fibres of two spindles. These show the distribution of nuclei and sensory nerve endings (arrowed) visible in semi-thin sections. Fibres numbered 1 and 4 in spindle 10 share a sensory cross terminal (*SCT*). *M* indicates those fibres which possess single prominent M-lines. The other fibres possess faint double M-lines

and up to 10 μm in length. Faint double M-lines were present in the A-bands of the nuclear bag fibres (Fig. 5). Mitochondria were small, rarely exceeding 2 μm in length. They occurred predominantly in the A-band regions. Myofibrils were poorly delineated due to the small amount of sarcoplasmic reticulum present. A few triads and pentads occured within the A-band regions (Fig. 5). The average diameter of this type of fibre, measured at 10 μm intervals over 200 μm lengths of the equatorial regions, was 9 μm.

The equatorial nuclei of the nuclear chain fibres were cylindrical in shape, approximately 3 μm in diameter and up to 10 μm long. In the juxta-equatorial regions of the nuclear chain fibres the nuclei became more widely separated by intervening sarcoplasm with numerous mitochondria. The nuclear chain fibres could be differentiated into two types according to their fine structure. The structure of the first type was similar to that of the nuclear bag fibres with faint double M-lines. The second type possessed prominent single M-lines (Fig. 6). Mitochondria were long, often extending up to 6 μm in length. The sarcoplasmic reticulum formed conspicuous networks in the regions of the Z-lines. Triads and pentads were much more numerous than in the nuclear bag fibres and the nuclear chain fibres with faint double M-lines. They usually occurred within the A-band regions. Glycogen was abundant in sarcoplasm and was often associated with the sarcoplasmic reticulum. The average diameter of the nuclear chain fibres, measured at 10 μm intervals over 200 μm lengths of the equatorial regions, was 6 μm.



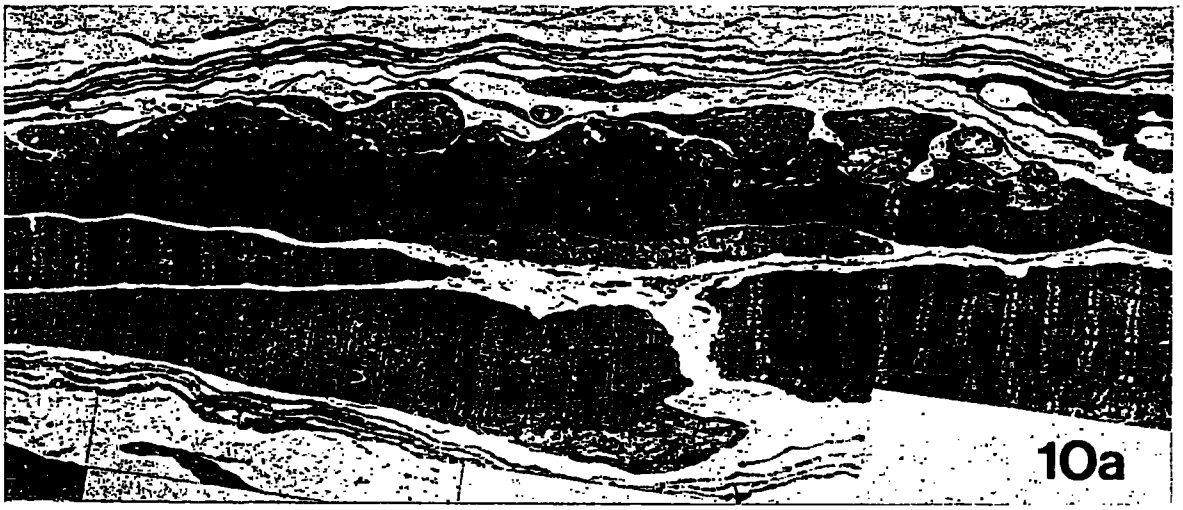
Fig. 9. Transverse section of a motor nerve ending. A prominent nucleated (*nsa*) sole-plate is present. There is marked folding in the post-junction membrane. The nerve terminals (arrowed) are covered by Schwann cells (*S*). (×9000)

Myofilaments were present between the equatorial nuclei and the plasmalemma of each intrafusal fibre (Fig. 4). In transverse section they could be seen as an annular sheet surrounding a central core of nuclei. They were continuous with the polar myofilaments but were not grouped into distinct myofibrils, due to the small amount of sarcoplasmic reticulum present.

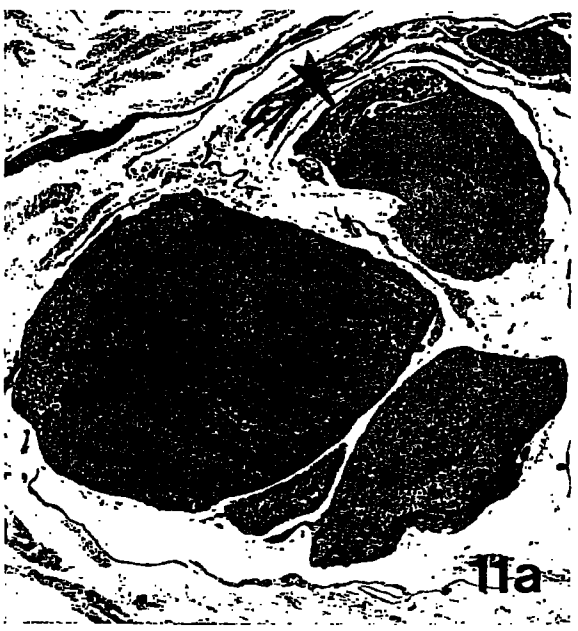
Dilated terminal cisternae of the sarcoplasmic reticulum occurred in each type of fibre (Fig. 7). They occurred most frequently in the nuclear chain fibres with prominent single M-lines. Dilated terminal cisternae have also been described in rat intrafusal fibres (James and Meek, 1970; Ovalle, 1971).

In each spindle at least one nuclear chain fibre possessing prominent single M-lines was present, while at least one nuclear chain fibre possessing faint double M-lines and usually one nuclear bag fibre were also present (Fig. 8).

Satellite cells were present between the plasmalemma and basement membrane of each type of intrafusal muscle fibre (Figs. 7a and b and 10b). They were approximately 3 μm in diameter and 10 μm long and their long axes lay parallel to the axes of the parent fibres. They contained a small amount of cytoplasm with









Figs. 10 and 11

abundant ribosomes and rough endoplasmic reticulum and were found in all regions of the spindle. Occasionally two intrafusal muscle fibres shared a single satellite cell, with a continuous basement membrane surrounding all three cells (Fig. 7b).

Innervation

Nerve endings were seen in the equatorial regions of the spindles (Figs. 3 and 4). The membranes of the nerve endings and the underlying intrafusal muscle fibres were separated by a gap approximately 20 nm wide, without an intervening basement membrane. However, the free surfaces of the nerve endings were covered by basement membrane continuous with that of the intrafusal muscle fibres. These nerve endings were assumed to be sensory. They often occupied small troughs in the surfaces of the intrafusal muscle fibres (Fig. 4). Occasionally parts of the nerve endings were seen to be completely surrounded by folds of the intrafusal fibres (Fig. 3).

The form of the nerve endings was determined by reconstructing serial 0.5 μm sections. Regular spirals of endings overlay the nuclear bags and the mid-regions of the nuclear chains (Figs. 3 and 4), while more irregular branched endings, often with terminations lying parallel to the axis of the intrafusal fibre, occured on either side of the spirals (Fig. 3). Four examples of sensory cross terminals, which cross-link two intrafusal fibres (Adal, 1969) were found (e.g. Fig. 3). Three occurred between pairs of nuclear chain fibres with prominent single M-lines. The fourth occurred between a nuclear bag fibre and a nuclear chain fibre with single M-lines. Fig. 8 is a diagram of the equatorial regions of spindles reconstructed from 0.5 μm toluidine blue stained sections taken at intervals of 10 μm. Regions of sensory nerve endings and their positions relative to the equatorial nuclei of the intrafusal muscle fibres are shown.

Nerve endings seen in the polar regions of the spindles differed from those of the equatorial regions. They contained membrane-bounded vesicles about 35 nm in diameter (Fig. 11b). The pre-junctional and post-junctional membranes were at least 60 nm apart. Intervening basement membrane was clearly visible and continuous with that of the intrafusal muscle fibres. These nerve endings were assumed to be motor. Twentyfive such endings were observed on the polar regions of twentytwo intrafusal muscle fibres. There was a wide variation in the morphology of the motor nerve endings.

Fig. 10. Longitudinal section of a motor nerve ending lying on a nuclear chain fibre which possesses single M-lines. A nucleated sole-plate is present but is less prominent than that in Fig. 9. The degree of folding of the post-junctional membrane is variable, some regions show no folding (×1100). Detail of Fig. 10a illustrating a satellite cell (*scn*) and a Schwann cell (*S*). (×5760)

Fig. 11. Transverse section of a motor nerve ending (arrowed) lying on an intrafusal fibre which possesses faint double M-lines. (×9880). Detail of Fig. 11a. There is no sole-plate. A small motor termination (arrowed) lies beneath the main termination of the ending. Where the nerve terminals do not make synaptic contact with the muscle fibre they are invested with Schwann cells (*S*). The nerve ending is entirely enclosed by a thin extension of the intrafusal sarcoplasm (*sif*). (×16000)

Three types of nerve endings could be differentiated according to the degree of folding of the post-junctional membranes and upon the presence or absence of an intrafusal sole-plate.

Nerve endings of the first type (12% of the total) possessed prominent nucleated sole-plates, typically projecting about 4 μm above the surface of the intrafusal muscle fibre (Fig. 9) and 6 μm in diameter. The post-junctional membranes were folded into clefts 60 nm wide and up to 1 μm deep. These nerve endings were all found on nuclear bag fibres or nuclear chain fibres with faint double M-lines. The second type (72% of the total) possessed nucleated or non-nucleated sole-plates, projecting about 2 μm above the surface of the intrafusal muscle fibres and extending up to 60 μm along the fibres (Figs. 10a and b). The post-junctional membranes were usually folded into clefts 0.3 μm wide and 0.5 μm deep. These nerve endings were found on nuclear bag fibres and nuclear chain fibres of both types. Nerve endings of the third type (16% of the total) did not possess a sole-plate (Figs 11a and b). The post-juncional membrane was rarely folded. This type of nerve ending was also found on each of the three types of intrafusal muscle fibre.

The regions of sensory and motor innervation were separated by a non-innervated region at least 200 μm in length.

Discussion

The ultrastructural features of the guinea-pig muscle spindle are similar to those of other mammals described, e.g. the cat (Adal, 1969; Corvaja, Marinozzi and Pompeiano, 1969) and rat (Landon, 1966; Merrillees, 1960).

Three types of intrafusal fibre in the guinea-pig spindle have been described here. The nuclear bag fibres possessing faint double M-lines are similar to those described in the cat (Adal, 1969; Corvaja, Marinozzi and Pompeiano, 1969), rat (Landon, 1966; Ovalle, 1971) and the rabbit (Barker and Stacey, 1970; Corvaja and Pompeiano, 1970). The nuclear chain fibres possessing prominent single M-lines are similar to the nuclear chain fibres of those species. However, nuclear chain fibres possessing faint double M-lines have not previously been described. These may represent an intermediate type of fibre such as those described in the cat (Barker and Gidumal, 1961) and rabbit (Barker and Stacey, 1970). Recent enzyme histochemical studies in the rat (James, 1971) and rabbit (Banks, 1971; Barker and Stacey, 1970) also indicate that three types of intrafusal fibre exist.

The possible effects of stretching the intrafusal fibres during fixation may be relevant. Stretching of a nuclear bag fibre or contraction of its polar regions may cause the equatorial nuclei to line up as a nuclear chain. The nuclear chain fibres, if allowed to shorten under reduced tension could form small nuclear bags with two, or occasionally three, nuclei visible in a transverse section. This effect could be particularly noticeable if the difference in diameter of the bag and chain fibres is small. Karlsson, Hooker and Bendeich (1971) have described similar changes in the distribution of the equatorial nuclei of frog intrafusal muscle fibres after stretching.

The fine structural properties of the intrafusal fibres may reflect differences in the nature of their contractile properties. Smith (1966) reported that in the rat spindle smaller intrafusal fibres, presumably possessing single M-lines (Landon,

1966), contracted faster than the larger fibres. Boyd (1966) observed rapidly contracting nuclear chain fibres and slowly contracting nuclear bag fibres in isolated preparations of cat spindles. Also extrafusal fibres with prominent M-lines are said to contract more rapidly than those with faint M-lines (Hess, 1967).

Primary and secondary sensory nerve endings (Ruffini, 1898) could not be differentiated on the basis of their fine structural properties. However, the primary ending usually has a more regular annulo-spiral form than the secondary endings. In the guinea-pig the more regular spiral nerve endings, situated centrally are likely to be primary, while those irregularly branched nerve endings occurring in the juxta-equatorial regions are likely to be secondary. Similar observations have been reported in the cat spindle by Corvaja, Marinozzi and Pompeiano (1969).

The three types of motor nerve ending seen in the guinea-pig spindles are similar to those of the cat (Adal and Barker, 1967; Barker, Stacey and Adal, 1970) and the rat (Hennig, 1969). The first type is similar to P_1 plates (Adal and Barker, 1967; Barker, Stacey and Adal, 1970; Hennig, 1969). These possess prominent nucleated sole-plates with marked post-junctional folding and are similar to the endplates of extrafusal twitch fibres. In the cat this type of intrafusal endplate may be innervated by branches of skeletomotor nerve fibres (Bessou, Emonet-Denand and Laporte, 1965). However, no such information is available for the guinea-pig. The second type is similar to P_2 plates (Adal and Barker, 1967; Barker, Stacey and Adal, 1970; Hennig, 1969). These have a less prominent, but more extensive, sole-plate with less folding of the post-junctional membrane than the P_1 plates. The third type is similar to trail endings (Adal and Barker, 1967; Barker, Stacey and Adal, 1970; Hennig, 1969). These do not possess sole-plates and there is little or no folding of the post-junctional membrane. Although three distinct types of motor nerve endings seem to be present, it is possible that there is a continuous morphological spectrum of neuromuscular junctions.

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Paper 5.

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Quantitative Studies on the Distribution* of Myofilaments in Intrafusal Muscle Fibres

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Summary. Muscle spindles contain two types of intrafusal muscle fibre, nuclear bag fibres and nuclear chain fibres. The intrafusal fibres of rabbit and guinea pig spindles have been studied using quantitative stereological techniques at the ultrastructural level. The cross-sectional areas occupied by myofilaments have been measured in the polar and equatorial regions of both types of intrafusal fibre. There are considerably fewer myofilaments in the equatorial regions of *both* types of fibre compared with their polar regions.

Key words: Muscle spindle — Intrafusal fibres — Myofilaments — Electron microscopy — Quantitative stereology.

Introduction

Muscle spindles are complex sensory organs found exclusively in skeletal muscles. It is generally accepted that they contain two major types of intrafusal muscle fibre each of which receives a rich motor and sensory innervation. The two types of intrafusal fibre, nuclear bag fibres and nuclear chain fibres, are differentiated according to the distribution of their nuclei. Nuclear bag fibres contain a central equatorial bag-like collection of nuclei and are often relatively broad in diameter. Nuclear chain fibres contain a longitudinally orientated central row of nuclei in their equatorial regions and tend to be relatively narrow in diameter. Sensory nerve endings surround the equatorial region of each fibre.

When examined by light microscopical techniques the polar regions of nuclear bag fibres seem well striated whilst their equatorial regions seem poorly striated. It is generally believed that such differences in staining intensities reflect a paucity of myofibrils in nuclear bag equatorial regions compared with their polar regions (Barker, 1948; Boyd, 1962; Matthews 1972). Electron microscopical studied have confirmed that there are relatively few myofilaments in the equatorial regions of nuclear bag fibres (Corvaja, Marinozzi and Pompeiano, 1969; Landon, 1966; Merrillees, 1960).

Since equatorial regions contain few myofibrils they are thought to be more elastic than the polar regions. Consequently, any externally applied stretch of a spindle rapidly and preferentially elongates the equatorial elastic region and deforms the overlying sensory nerve endings. This deformation increases their sensory discharge.

Attempts have previously been made to measure the reduction of equatorial myofibrils by estimating the cross-sectional areas of myosin ATPase reactive

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Paper 6.

R.W. Banks & N.T. James (1975)
Rabbit intrafusal muscle fibres
J. Anat. 119; 193.

12. **Rabbit intrafusal muscle fibres.** By R. W. BANKS and N. T. JAMES. *Department of Human Biology and Anatomy, University of Sheffield*

Early studies on rabbit muscle spindles indicated that they contain only one type of intrafusal muscle fibre according to their morphology (Barker, *Quart. J. micr. Sci.* **89**, 1948; Barker & Hunt, *Nature, Lond.* **203**, 1964) and their myoglobin content (James, *Nature, Lond.* **219**, 1968). Subsequent enzyme histochemical and ultrastructural studies indicated that either two (Spiro & Beilin, *J. Histochem. Cytochem.* **17**, 1969; Corvaja & Pompeiano, *Pflügers Arch. ges. Physiol.* **317**, 1970) or three types (Barker & Stacey, *J. Physiol.* **210**, 1970; Barker *et al.*, *Research in Muscle Development and the Muscle Spindle*, Excerpta Medica, 1972) are present. Confirmatory evidence is presented here which supports the view that there are three types of rabbit intrafusal fibre.

Spindles from several hindlimb muscles were found to contain 4–7 intrafusal fibres. Two types could be identified according to their ultrastructure. (*a*) Fibres which possessed prominent M lines. These contained relatively large mitochondrial volume fractions when analysed using sterological techniques. (*b*) Fibres which did not possess M lines. These contained a significantly smaller mitochondrial volume fraction ($\sim 35\%$ less, $P < 0.001$).

In each spindle examined the fibre with the largest equatorial diameter was always a nuclear bag fibre which did not possess M lines. The remaining fibres in each spindle always possessed M lines, but some of these were nuclear-bag fibres and some were nuclear-chain fibres.

The equatorial diameters of the muscle fibres of a spindle, when expressed relative to the diameter of the largest fibre, form a trimodal distribution. Fibres of intermediate equatorial diameter were nuclear-bag fibres and the smallest diameter fibres were nuclear-chain fibres. Relative polar diameters formed a bimodal distribution.

Rabbit intrafusal fibres have previously been classified according to their enzyme histochemical properties as type 1, type 2 or type 3 (Banks, *J. Anat.* **108**, 1971).The relatively short and thin type 1 fibres correspond to the nuclear-chain fibres; type 2 fibres probably correspond to the nuclear-bag fibres which possess M lines. The singly-occurring large type 3 fibre corresponds to the large nuclear-bag fibre devoid of M lines. The type 2 fibres were of greater polar diameter than either of the other types ($P < 0.01$ in each case).

Paper 7.

R.W. Banks, D. Barker, D.W. Harker & M.J. Stacey (1975)
Correlation between ultrastructure and histochemistry of mammalian intrafusal muscle fibres.
J. Physiol. 252; 16-17P.

[*From the Proceedings of the Physiological Society*, 18–19 *July* 1975
Journal of Physiology, **252**, 16–17*P*]

Correlation between ultrastructure and histochemistry of mammalian intrafusal muscle fibres

By R. W. Banks, D. Barker, D. W. Harker and M. J. Stacey. *Zoology Department, Durham University, Durham*

We have devised a technique that allows for adjacent sections of the same muscle spindle to be prepared for either histochemical or ultrastructural study. Muscle is frozen in iso-pentane cooled to −160° C and serial transverse sections cut in batches at about 15 μm alternating with much thicker ones at about 60 μm. Various histochemical techniques are then applied to the thin sections, while the thick sections are processed for the observation of ultrastructure in both transverse and longitudinal section. We have sectioned cat and rabbit peroneus longus, peroneus digiti quinti and tenuissimus muscles, and the same peroneal and soleus muscles of the rat, studying one spindle from each muscle. Histochemical profiles of intrafusal muscle fibres were determined with respect to acto-

Table 1. Correlation of ultrastructure and histochemistry in cat, rabbit and rat intrafusal muscle fibres at two levels in the spindle: A, that adjacent to the area of equatorial nucleation; B, that part of the juxta-equatorial region lying nearest to the equator. Bag fibres designated 'bag_1' and 'bag_2' on the basis of their ATPase reactions following Ovalle & Smith (1972). Staining reactions: +, low; + +, medium; + + +, high. Condition of M line: 0, absent; M, present, dM, two faint parallel lines.

	Level	Fibre	Diameter	alk ATPase	P'ase	Glycogen	M line
Cat	A	Bag_1	Medium	+	+	+/+ +	0/dM
		Bag_2	Large	+ +	+ +	+ +	0/dM
		Chain	Small	+ + +	+ + +	+ + +	M
	B	Bag_1	Medium	+	+	+	0/dM
		Bag_2	Large	+ +	+ +	+ +	M
		Chain	Medium	+ + +	+ + +	+ + +	M
Rabbit	A	Bag_1	Medium/large	+	+	+ +	0/dM
		Bag_2	Large	+ +	+ +	+ +	0/dM
		Chain	Small	+ +	+ +	+ +	M
	B	Bag_1	Medium/large	+	+/+ +	+ +	0/dM
		Bag_2	Large	+ + +	+ +	+	M
		Chain	Medium	+ + +	+ + +	+ +	M
Rat	A	Bag_1	Medium	+	+	+	0/dM
		Bag_2	Large	+ +	+ +	+	0/dM
		Chain	Small	+ +	+ + +	+ +	M
	B	Bag_1	Medium	+	+/+ +	+ +	0/dM
		Bag_2	Large	+ +	+ +/+ + +	+ +	M
		Chain	Medium	+ + +	+ + +	+ +/+ + +	M

[P.T.O.

myosin ATPase after alkali pre-incubation (alk ATPase; Guth & Samaha, 1971), phosphorylase (P'ase; Eränkö & Palkama, 1961), and glycogen (PAS method). Ultrastructural observations have so far been restricted to noting the M-line conditions.

The results (Table 1) show that there may be variations in histochemical profile along the length of all types of intrafusal muscle fibre, and that the bag_2 fibres also show regional differences in ultrastructure.

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Paper 8.

D. Barker, R.W. Banks, D.W. Harker, M.J. Stacey & A. Milburn (1976)
Studies of the histochemistry, ultrastructure, motor innervation and regeneration of mammalian intrafusal muscle fibres
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Studies of the Histochemistry, Ultrastructure, Motor Innervation, and Regeneration of Mammalian Intrafusal Muscle Fibres

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INTRODUCTION

One of the main findings recognized during the Durham Symposium on Muscle Spindles (April 1974) was that mammalian limb spindles possess two types of nuclear bag muscle fibre distinguished by differences in length, diameter, distribution of associated elastic fibres, histochemical profile, ultrastructure, and development. There was some doubt as to how to classify the fibres into two types, and how they should be named, since the histochemical and ultrastructural observations reported by various workers (see review by Barker, 1974) had been made on separate preparations of different spindles, so that to some extent their correlation was a matter for conjecture. It was agreed that a final decision on naming the two types should await the correlation of histochemical and ultrastructural characteristics in one and the same spindle, preferably in a number of different species (Barker and Laporte, 1975).

We begin by summarizing the progress we have made towards this end, and then give some account of other work that we have been engaged in. This includes work on cat fusimotor innervation, in collaboration with our colleagues in Paris and Toulouse, and a study of the degeneration and regeneration of rat spindles following the administration of the local anaesthetic bupivacaine.

HISTOCHEMICAL/ULTRASTRUCTURAL CORRELATIONS

We found that a direct comparison between the histochemical profile and ultrastructure of an intrafusal muscle fibre could be made by cutting frozen serial transverse sections in batches at about 15 μm alternating with much thicker ones at about 60 μm. The thin sections could be used for the application of various histochemical techniques, while the thick ones were processed for the observation of ultrastructure in both transverse and longitudinal section. By sectioning according to this sequence, the histochemical and ultrastructural characteristics of each type of intrafusal muscle fibre can be correlated at all levels from equator to extreme pole as it is traced through the spindle. Our observations using this technique have so far been made on 34 spindles sampled from various hindlimb muscles of the cat, rabbit and rat. The histochemical

profiles examined were those of myofibrillar ATPase following alkaline preincubation (Guth and Samaha, 1970), phosphorylase (Eränkö and Palkama, 1961), and glycogen (PAS method). The results show that (i) in addition to chain fibres, all the spindles contain two types of bag fibre, usually one of each type; (ii) histochemical profiles vary along the length of individual intrafusal muscle fibres; (iii) there are regional differences in ultrastructure in bag fibres; and (iv) mistakes have been made in some previous indirect histochemical/ultrastructural correlations concerning bag fibres such that ultrastructural and morphological properties have been ascribed to the wrong histochemical type (Banks et al., 1975, 1976a).

In this study we found it convenient to distinguish three regions between the equator and the insertion or origin of a spindle pole, namely, *region A*, that part of the equatorial region lying between the equator and the equatorial end of the periaxial space; *region B*, that part of the pole extending from the equatorial end of the periaxial space to the end of the capsule; and *region C*, the extracapsular part of the pole (see Fig. 1). Since the equatorial length of the periaxial space and the length of the capsule vary according to the number of sensory endings present, it follows that regions A and B are shortest in those spindles that receive a primary ending only (these also have the shortest overall length). Thus in the 8 poles of 4 such spindles from cat tenuissimus the mean distance of the equatorial end of the periaxial space (level A/B) from the equator was 238 μm (range 150—290 μm), and the mean distance of the end of the capsule (level B/C) from the equator was 1278 μm (range 1100—1850 μm). Comparable mean distances from the equator for 4 spindle poles in which region A included two secondary endings were 826 μm (range 655—990 μm) for level A/B and 2165 μm (range 1950—2470 μm) for level B/C (data from Barker, 1974, Fig. 3). Analyses of the sensory innervation of spindles in various cat hindlimb muscles (Barker, 1962; Boyd, 1962) indicate that the most common types of spindle pole are those that receive one secondary ending and those that receive none. The length of such poles is usually 4.0—4.5 mm, with region A occupying up to 0.5 mm, region B 1.5 mm, and region C 2.0—2.5 mm.

Ovalle and Smith (1972) distinguished two types of bag fibre in cat and monkey spindles on the basis of their ATPase staining reactions and called them "bag_1" and "bag_2" fibres. We have adopted these terms, abbreviating them, as convenient, to b_1 and b_2. A difference of alkaline ATPase staining intensities between bag_1 (low) and bag_2 (medium or medium/high) obtains in cat, rabbit and rat spindles, being most marked in regions A and B (see Fig. 2). In region C it is less obvious owing to an increase in staining intensity of the bag_1 fibres. In cat spindles the bag_2 fibres are medium and the chain fibres high, but in rabbit and rat spindles the ATPase profiles of the bag_2 and chain fibres are very similar over most of their lengths. With the phosphorylase reaction a profile pattern in rabbit spindles of low (bag_1), medium (bag_2), high (chains) is most evident in region B, but in region C it is lost as the staining intensity of the bag_1 fibres rises to the same medium level as shown by the bag_2 fibres. In cat and rat spindles the two types of bag fibre are not clearly differentiated by this reaction. The glycogen levels of the bag_1 fibres are generally higher than those of the bag_2 fibres in rabbit and rat spindles, but in the cat the reverse is true except in region C where they are the same. Further information about the

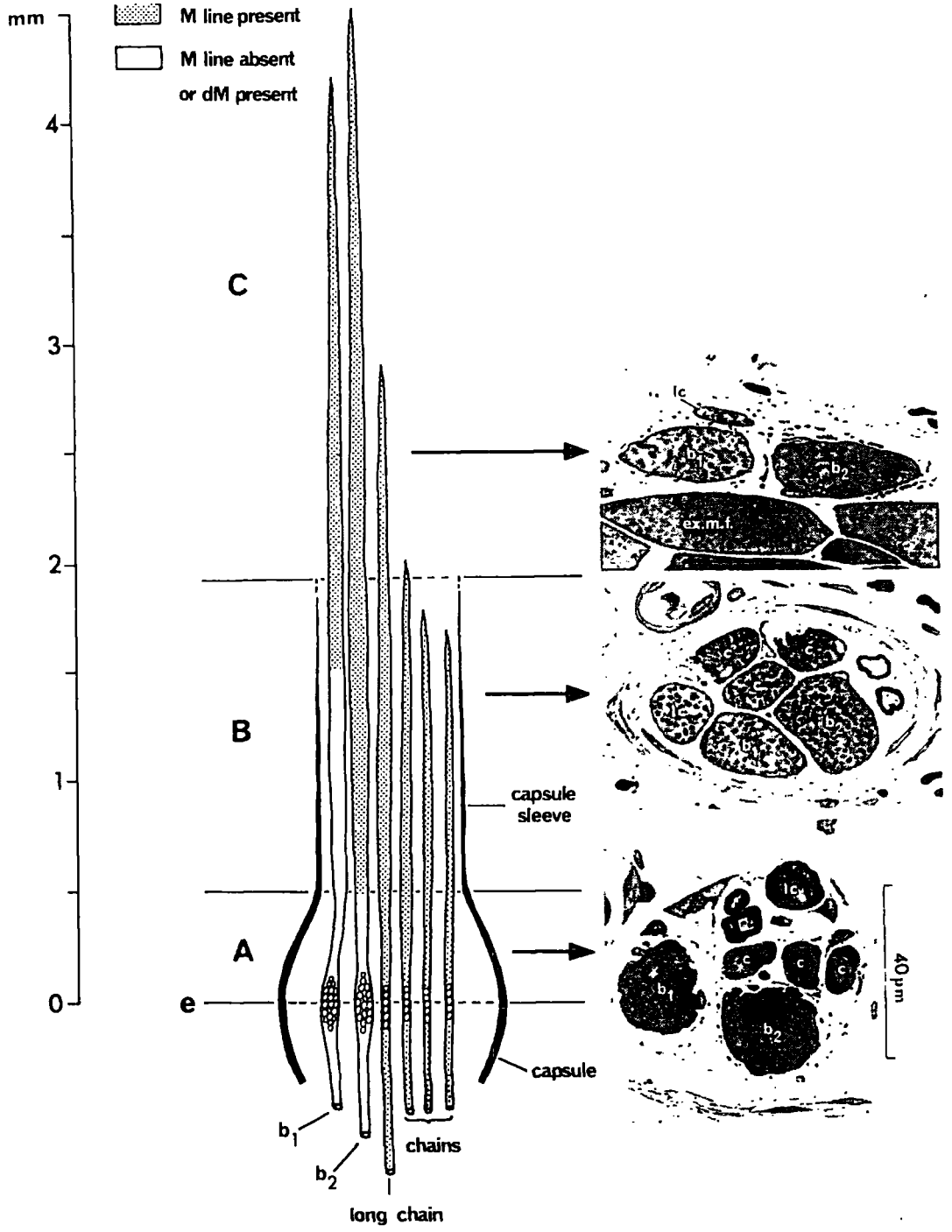


Fig. 1. On the left of the figure the equatorial region and pole of a typical cat spindle are schematically represented (muscle fibre width scale x 3 that used for length). The axial bundle consists of one bag_1 (b_1) fibre, one bag_2 (b_2) fibre and 4 chain fibres (one long). Their M line condition is indicated from equator (e) to polar tip in the regions A, B, and C. Representative transverse sections of a cat tenuissimus spindle through the three regions are shown on the right of the figure. Note dissociation of bag_1 fibre from the rest of the axial bundle in region A, and presence of long chain (lc) fibre in region C. ex.m.f., extrafusal muscle fibre; c, chain fibre.

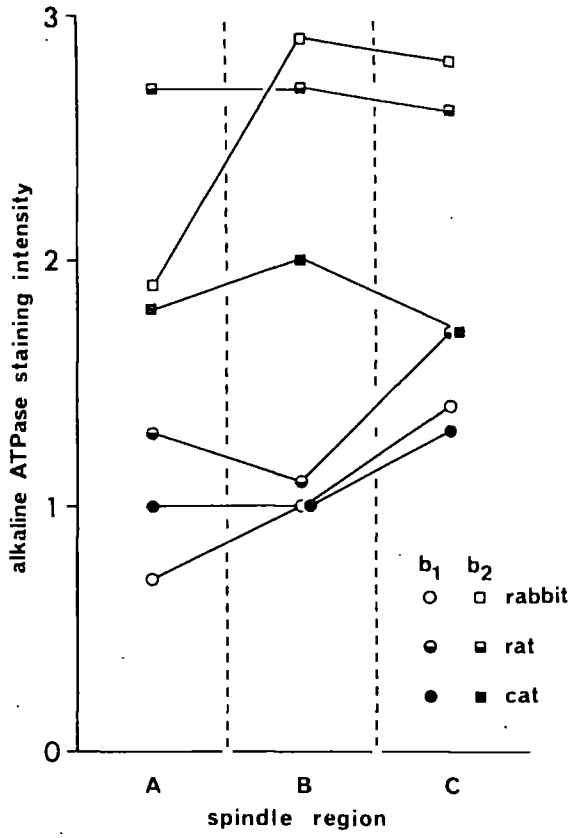


Fig. 2. Alkaline ATPase staining intensity in bag_1 (b_1) and bag_2 (b_2) fibres of rabbit, rat and cat spindles (indicated by symbols as shown in key) in regions A, B and C. Values are average numbers of points awarded on a scale 0 (absent), 1 (low), 2 (medium), 3 (high) in examining transverse sections sampled from a number of levels in each region.

glycogen profiles of cat intrafusal fibres is given below in connexion with the glycogen depletion work (see p. 75); for full details of the histochemical profiles obtained with all three methods see Banks et al. (1976a).

It was possible to form a fairly rapid assessment of the variation in histochemical profile along the length of an intrafusal fibre pole from the serial batches of 15 μm transverse sections. Preparing and processing the thick 60 μm sections for ultrastructural observation of course takes much longer, and our present data have been gathered from regions A, B and C sampled at various levels in the three species studied. We have also confined our observations at this stage mainly to variation in condition of the M line. This may be present as a single prominent line, a condition we have designated as "M". Alternatively the M line may be absent, or present as two faint parallel lines. Since a non-M line sarcomere and one with a faint double M line may be adjacent in the same muscle fibre, both conditions are included under the designation "dM".

The chain fibres show the M condition at all levels sampled in all three species. The condition in the bag_2 fibres of all spindles is dM for most of region A switching to M as they approach level A/B. The condition in cat bag_1 fibres is dM in region A switching to M towards the polar end of region B; in rabbit bag_1 fibres a similar switch occurs in the middle of region C; and in rat bag_1

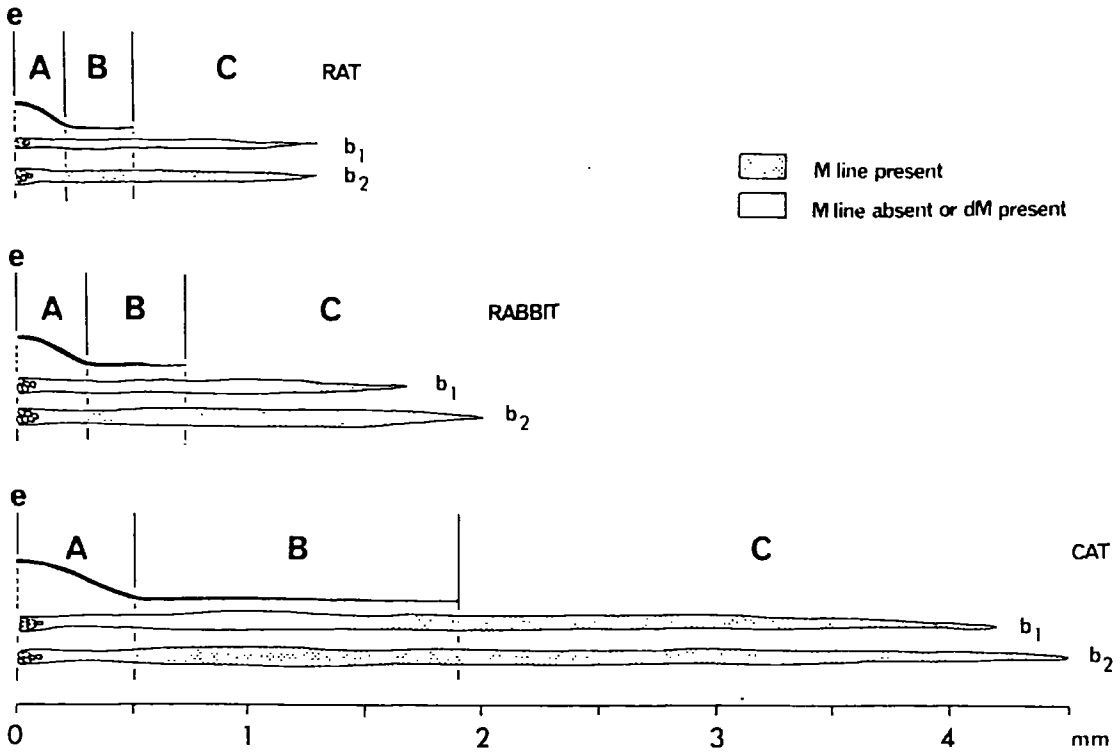


Fig. 3. Variation in M line condition of bag_1 (b_1) and bag_2 (b_2) fibres in regions A, B and C of the poles of typical rat, rabbit and cat spindles (muscle fibre width scale × 3 that used for length). e, equator.

fibres the condition is dM throughout (see Fig. 3). We have not yet obtained preparations that reveal the nature of the transition from a dM to an M condition. The fact that the transition zone in the bag_1 fibres of cat and rabbit spindles occurs at a similar distance from the equator (1.0—1.5 mm) suggests that this may be a standard length, determined perhaps by developmental factors associated with the primary afferent. A situation thus occurs in which, owing to the differences in average spindle length between the three species, the switch from the dM to the M condition is intracapsular in cat spindles (region B), extracapsular in rabbit spindles, and absent in rat spindles.

Micrographs illustrating the ultrastructure of cat bag_1 and bag_2 fibres at levels sampled from regions A, B and C are shown in Fig. 4a—f. When the thick sections are fixed for electron microscopy during the application of our technique, contraction cannot be opposed and the sarcomeres of the muscle fibres are greatly shortened. It is interesting to note, however, that the sarcomeres of bag_1 fibres in all three species always remain longer under these conditions than those of bag_2 fibres (compare Fig. 4c and d), chain fibres, and extrafusal fibres. Apart from this, in regions where fibre types have the same M line condition, e.g., as in the case of b_1 and b_2 fibres in part of region A, or all three fibre types in cat region C, there is no obvious difference in their ultrastructure. At this stage of the work our impression is that the change of M line condition in a bag fibre is accompanied by a change of other ultrastructural features. In the transition from dM to M the mitochondria appear to change from being small and

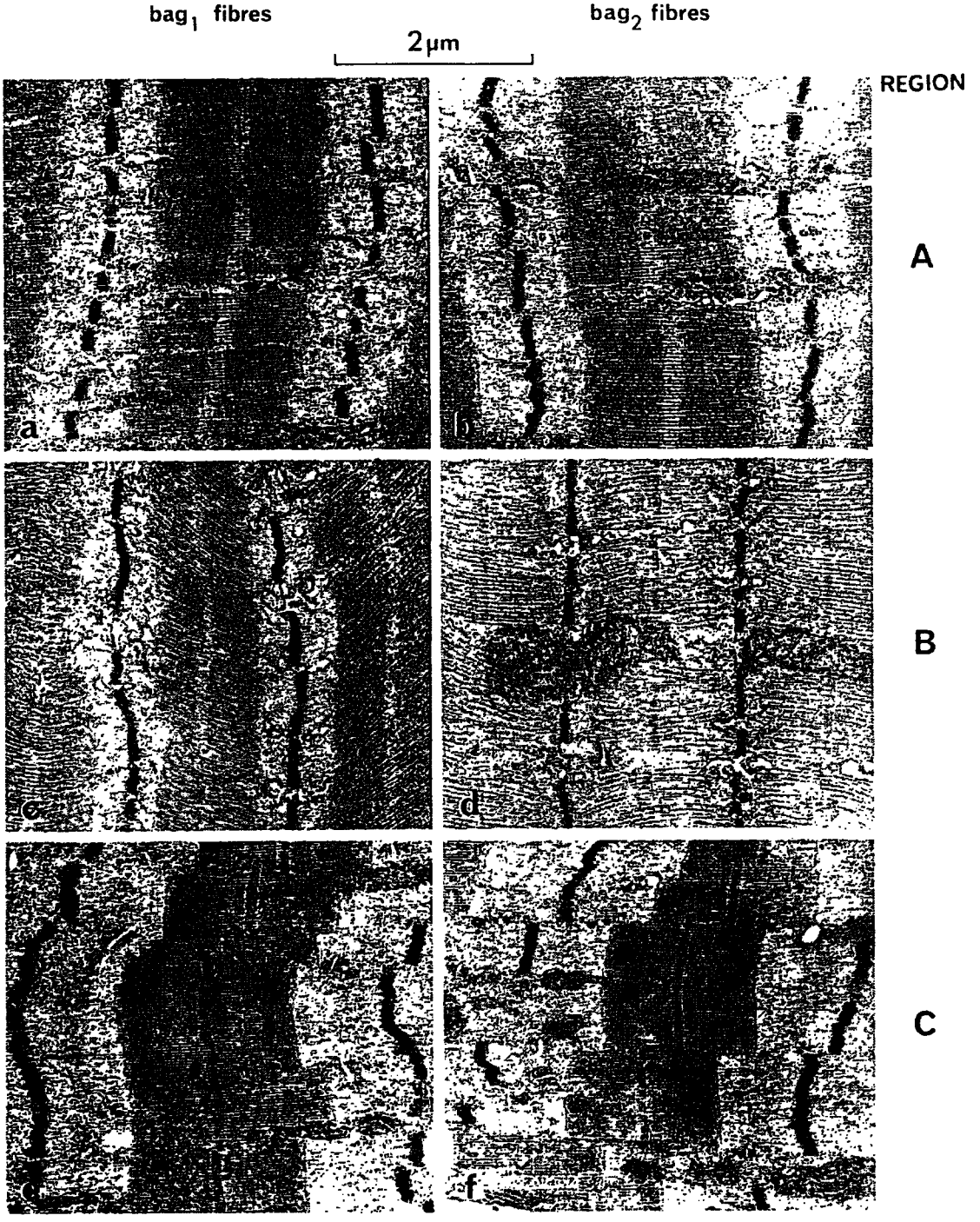


Fig. 4. Electron micrographs of representative longitudinal sections of bag fibres from regions A, B and C of cat tenuissimus spindles. In $A_{a,b}$ the condition of the M line is dM in both types of fibre; in $B_{c,d}$ the condition is dM in the bag_1 fibre, but M in the bag_2 fibre; in $C_{e,f}$ it is M in both types. In c and d the electron micrographs illustrate sections obtained using the combined histochemical/ultrastructural technique of Banks et al. (1976a); the sections illustrated in a, b, e and f are from muscle fixed in the normal way for electron microscopy.

scarce to being larger and more numerous; the amount of interfibrillar sarcoplasm increases; and the sarcotubular system becomes better developed. Variation in histochemical profile along the length of an intrafusal fibre in the reactions that we have examined does not appear to be correlated with the condition of the ultrastructure.

Since there is regional variation in the histochemical profiles of intrafusal muscle fibres, and in the ultrastructure of bag fibres, it is scarcely surprising that some investigators (e.g., Arendt and Asmussen, 1974) have recognized more than three histochemical types, and that others (e.g., Barker et al., 1972b) have made erroneous indirect histochemical/ultrastructural correlations. In their ultrastructural study of dog spindles Banker and Girvin (1971) did not distinguish between two types of bag fibre, but they were on the right track when they observed that the bag fibres had M lines in the poles and lost them in the equatorial region.

Though, generally speaking, it may be said that there is a hierarchy of length and diameter among the three types of fibre in the sequence bag_2—bag_1—chain, these characteristics are not an entirely reliable guide as to fibre type. In cat spindles bag_2 fibres are usually longer than bag_1 fibres, but in rabbit and rat spindles they are usually about the same length. Also in some cat spindles the length of one of the chain fibres may be similar to, or even longer than, the bag_1 fibre (see p. 75). Bag_2 fibres are thicker than bag_1 fibres, and both types of bag fibre are thicker than chain fibres except in rabbit spindle poles where the diameters of bag_1 fibres and chain fibres are not significantly different (Banks and James, 1975).

A feature that is useful in helping to identify bag fibres in cat spindles is that in their course through the equatorial region the bag_1 fibre generally lies somewhat apart from the rest of the axial bundle, whereas the bag_2 fibre is closely associated with the chain fibres (see Figs. 1, 8). This may be true of all mammalian spindles that have four or more chain fibres. During rat development the three types of intrafusal fibre arise sequentially in the order bag_2, bag_1, chain. Bag_1 and chain fibres each develop in association with the older bag_2 fibre, presumably separating from it in cat spindles to a greater (bag_1) or lesser (chains) extent after the fusion of their constituent myoblasts.

The main characteristics of the three types of intrafusal muscle fibre in cat, rabbit and rat spindles may be summarized as follows.

Bag_1 fibres

Diameter: medium, similar to chains in rabbit, thicker than chains in cat, rat.

Length: usually shorter than b_2 fibres in cat, usually same length as b_2 fibres in rabbit, rat.

Development: second fibre formed, usually dissociates from b_2 fibres and chains equatorially in cat.

Alkaline ATPase profile: low.

M line condition: rat, dM; rabbit, dM switching to M in extracapsular pole (region C); cat, dM switching to M in capsule sleeve (region B).

Bag_2 fibres

Diameter: always the thickest.

Length: usually longest in cat, usually same length as b_1 fibres in rabbit, rat.

Development: first fibre formed, remains closely associated with chain fibres equatorially in cat.

Alkaline ATPase profile: medium in cat, medium/high similar to chains in rabbit, rat.

M line condition: dM for short stretch adjacent to nuclear bag, otherwise M for rest of length.

Chain fibres

Diameter: thinnest in cat, rat, similar to b_1 fibres in rabbit.

Length: shortest, but some chains in cat may be as long as b_1 fibres or longer.

Development: last fibres to be formed, remain closely associated with b_2 fibre equatorially in cat.

Alkaline ATPase profile: high.

M line condition: M line present throughout length.

CAT FUSIMOTOR INNERVATION

In collaboration with Laporte and his colleagues we have analysed the distribution of static and dynamic γ axons to cat tenuissimus spindles using Edström and Kugelberg's (1968) glycogen depletion technique (Barker et al., 1974, 1976). Our study differs in a number of respects from a similar one made by Brown and Butler (1973): the muscle portions containing the activated spindles were quick-frozen and then fixed in absolute ethanol during freeze-substitution in order to avoid the "streaming" of glycogen granules; sampling of γ static axons was not restricted to those of relatively fast conduction velocity; and the two types of bag fibre were taken into account in the analysis. In each experiment a single γ axon supplying tenuissimus spindles was prepared and its function, static or dynamic, determined by measuring the dynamic index after 2—3 mm ramp stretches applied during repetitive stimulation at 100/sec. Glycogen depletion in the muscle fibres innervated by the axon was obtained by repetitively stimulating it during several periods of blood occlusion. The activated spindles were located to within 1—3 mm by gently pulling on the connective tissue near the edge of the stretched muscle and observing the change in frequency of the primary ending discharge. After freeze-substitution the muscle portions were embedded in Paramat and serial transverse 10 μm sections cut, stained for glycogen (PAS method), and examined for depletion. Serial reconstructions were made of each experimental spindle in order to ascertain the levels of glycogen and zones of glycogen depletion in each intrafusal muscle fibre.

The glycogen levels of cat tenuissimus intrafusal muscle fibres show some regional variation. In bag_1 fibres the level is medium in regions A and B, though it may drop to medium/low or low over short stretches. In region C the level is medium rising to medium/high, or occasionally high, towards the polar extremity. The level in bag_2 fibres is mainly medium/high; there may be short medium

stretches in regions A and B, and there is usually a rise to a high level towards the polar extremity. The chain fibres have the most glycogen, the level being generally high throughout, though there may be occasional stretches where the level drops to medium/high or medium/low. Long chain fibres are particularly liable to show this variation. There is a gradation of length among cat tenuissimus chain fibres such that the origins or insertions of some lie inside the capsule whereas those of others lie outside. In a sample of 369 chain fibres measured in this study 40.6% of the origins/insertions were intracapsular, 59.3% extracapsular (see Fig. 5). The percentages of origins/insertions lying 0.5 mm or more and 1.0 mm or more beyond the end of the capsule were 30.3 and 7.9, respectively. Long chain fibres beginning or ending 1.0 mm or more beyond the end of the capsule were present in 25 (29.4%) of 85 spindle poles; usually only one was present, occasionally two, rarely three.

Control muscles were examined in order to ascertain whether the glycogen profiles were affected by a regime of blood occlusion without nerve stimulation, or by the standard experimental procedure from which both blood occlusion and nerve stimulation had been omitted. The controls were normal; they lacked any blanched zones such as occur with glycogen depletion.

The stimulation of 8 single static γ axons (conduction velocity range 19.0—45.0 m/sec) produced zones of glycogen depletion in 27 whole spindles and 5 half spindles (i.e., spindles cut in two when the muscle portion was excised so as to leave only one complete pole). The stimulation of 4 dynamic γ axons (conduction velocity range 23—46 m/sec) produced zones of glycogen depletion in 16 whole spindles and one half spindle. The following results emerged.

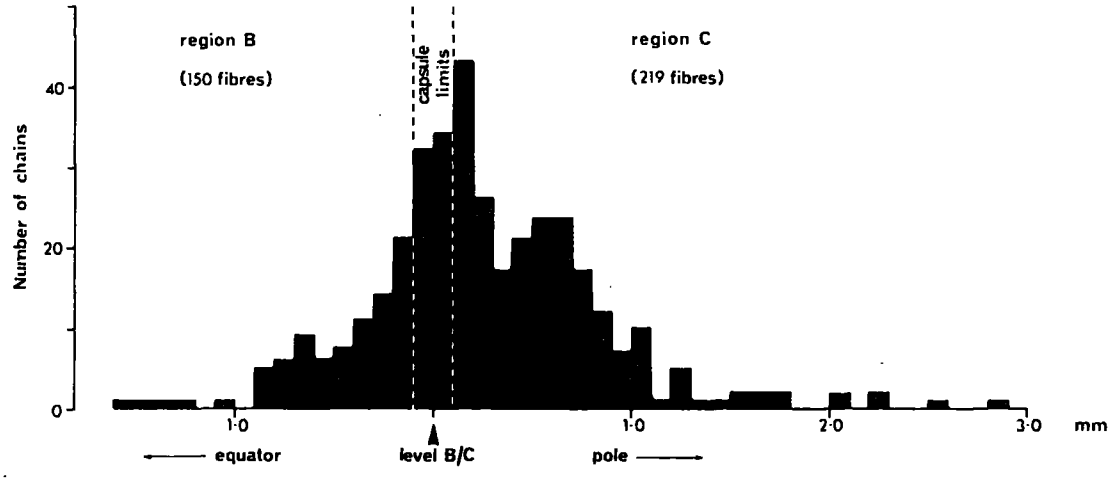


Fig. 5. Histogram of the origin/insertion points of 369 chain fibre poles belonging to cat tenuissimus spindles (35 complete spindles, 13 complete half spindles) reconstructed from serial 10 μm transverse paraffin sections stained for glycogen (PAS method). Transition between region B (intracapsular) and region C (extracapsular) at level B/C indicated by arrowhead.

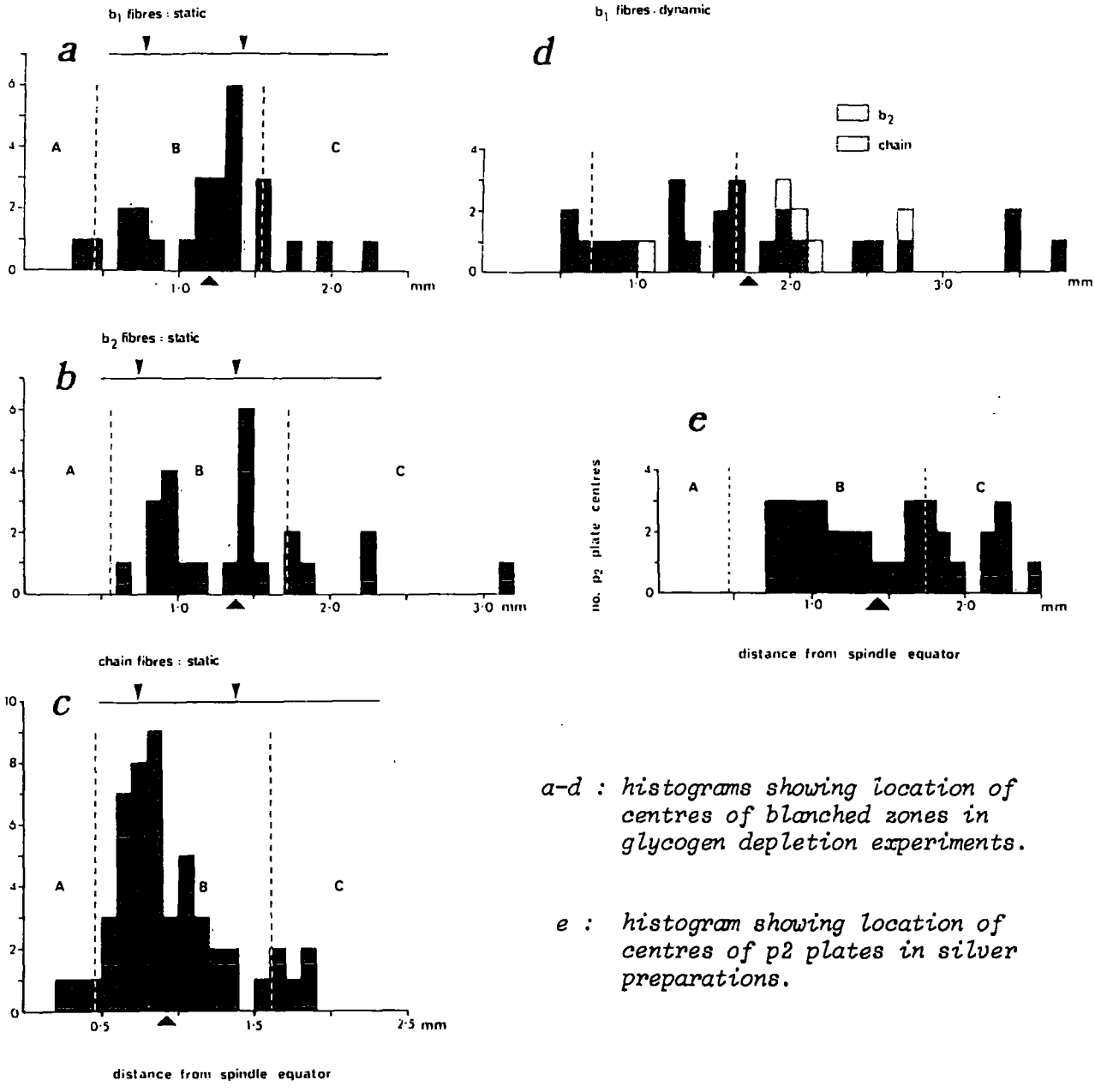
Static γ axons. (i) Analyses of 27 spindles showed that 13 (48.2%) had bag and chain depletions, about twice as many as those in which depletion was restricted to bag fibres only (8, or 29.6%) or to chain fibres only (6, or 22.2%). (ii) Almost as many bag fibres were depleted as chains, and among the bag fibres, both types were equally involved. (iii) Of the 8 spindles in which depletion was restricted to bag fibres, three involved bag_1 fibres only, one involved a bag_2 fibre only, and 4 involved both types. It must be noted, however, that in 5 of these spindles (including all those with bag_1 only depletions) there were patches in some of the chain fibres where the glycogen level dropped from high to medium. Although this is a feature of the normal profile the possibility cannot be excluded that some of these patches represent zones of partial depletion. (iv) The same static axon usually differed in its pattern of distribution to each of the spindles it supplied. (v) Bag-fibre involvement was restricted to bag_1 fibres on stimulating axons with conduction velocities slower than 25 m/sec.

Dynamic γ axons. Analyses of 16 spindles showed that depletion was almost exclusively restricted to bag_1 fibres. In only 3 spindles were other types of fibre involved; in one this was a bag_2 fibre, in two it was a long chain fibre. Of the 21 spindle poles in which depletion occurred, the fibre types involved were bag_1 in 20, bag_2 fibres in 2, and long chain fibres in 2.

The results obtained in the experiments with static γ axons agree well with those obtained from a study of the distribution of static γ axons to tenuissimus spindles in which all other motor axons had degenerated (Barker et al., 1973). In that study silver staining was used and a detailed histological analysis made of 30 spindles innervated by 6 static γ axons (conduction velocity range 35—48 m/sec). In terms of trail endings supplied to bag (b, type not specified) and chain (c) fibres the static axons innervated 37 spindle poles as follows: bc poles, 48.7% (19 bc, 1 bbc); b only poles, 24.3% (8 b, 1 bb); c only poles, 27.0% (10). This compares with the distribution of blanched zones among the bag and chain fibres of the 44 spindle poles activated by static axons in the glycogen depletion experiments, as follows: bc poles, 41% (15 bc, 3 bbc); b only poles, 34% (12 b, 3 bb); c only poles, 25% (11).

The lengths of the zones depleted of glycogen by static γ axons varied from 0.1 mm to about 1.5 mm in all three fibre types, the mean length being around 0.5 mm. In the majority of instances the depletion of a fibre was restricted to a single zone in one pole. Histograms of the distances of the centres of the depleted zones from the equator for each fibre type are shown in Fig. 6a—c in relation to mean distances of levels A/B and B/C (broken vertical lines) from the equator in each sample of spindle poles concerned. In each fibre type most of the zone centres are seen to lie in region B, the mean distance for all 26 bag_1 fibre zones being 1548 μm, all 24 bag_2 fibre zones 1395 μm, and all 51 chain zones 931 μm; for all fibres the mean was 1111 μm. The histograms compare well with the location of trail endings as seen in silver preparations. In 21 silver-stained tenuissimus spindle poles the mean distance of the centre of trail-ending areas lay 1101 μm from the equator, the nearest and furthest limits of the areas being at mean distances of 753 μm and 1391 μm, respectively (range 490—2330 μm) (data from Barker, 1974, Fig. 3; see also Barker et al., 1970).

A similar histogram of the distances from the equator of the centres of the 25 zones depleted of glycogen in bag_1 fibres by dynamic γ axons is shown in



a–d : histograms showing location of centres of blanched zones in glycogen depletion experiments.

e : histogram showing location of centres of p2 plates in silver preparations.

Fig. 6. Histograms of the distances from the spindle equator of the centres of the zones depleted of glycogen in each type of intrafusal muscle fibre on stimulating single static γ axons (a—c) and single dynamic γ axons (d) (data from Barker et al., 1976). In a—c the horizontal line above each histogram represents the range of the limits of trail-ending areas as measured in 21 silver-stained spindle poles from cat tenuissimus; arrowheads above the line indicate the mean distances from the equator of the nearest and furthest limits of the trail-ending areas (data from Barker, 1974, Fig. 3). Broad arrowhead below the abscissa of each histogram in a—d indicates the mean distance from the equator of the centres of the depleted zones in the fibre type concerned (excluding the bag_2 and chain fibres in d). Histogram e shows the distances from the equator of the centres of 35 p_2 plates as measured in 19 silver-stained spindle poles from cat tenuissimus (data from Barker, 1974, Fig. 3); broad arrowhead below the abscissa indicates mean distance. Spindle pole regions A, B and C are shown in each histogram with the levels A/B and B/C indicated by broken vertical lines at their mean distance from the equator for each sample of fibre poles (a—d) or spindle poles (e) concerned.

Fig. 6d; the 5 zone centres in the depleted bag_2 and long chain fibres are included, distinguished by different shading. It will be noted that the zone centres do not lie mostly in region B, as in the case of the static bag_1 depletions (Fig. 6a), but are about equally distributed through regions B and C and extend over a greater polar length. Their mean distance from the equator also lies further along the pole being extracapsular at 1732 μm. This distribution compares well with that of p_2 plates as seen in silver preparations. The characteristic location of these is over a polar length of about 1 mm that includes the transition from regions B and C. Some may occur towards the extreme end of the pole, and others may lie closer in towards the equator, though they do not encroach into region A as do trail endings. Some of these points are illustrated in Fig. 6e, a histogram of the distances from the equator of 35 p_2 plates as measured in 19 silver-stained tenuissimus spindle poles (data from Barker, 1974, Fig. 3). Owing to the hazards of teasing, the majority of the spindle poles were cut at a level 2.5—3.0 mm from the equator so that some p_2 plates with an extreme polar location may have been lost. This probably accounts for the mean distance of the p_2 plate centres being extracapsular at 1435 μm, i.e., slightly nearer to the equator than the equivalent mean of the zone centres of dynamically depleted bag_1 fibres.

In a sample of 50 p_2 plates examined in silver preparations of spindles teased from cat peroneal muscles Barker et al. (1970) found that "90% were located on bag fibres, 10% on chain. In two instances a p_2 fibre was seen to branch so as to supply one plate to a bag fibre and one to a chain fibre. One p_2 plate was seen to terminate on a bag fibre as well as on an adjacent chain fibre; another spread its terminals over two bag fibres" (p. 331). These observations, taken in conjunction with the distribution and location of the zones depleted of glycogen by dynamic γ axons, strongly indicate the probability that such axons terminate in p_2 plates.

Further evidence of this has come from experiments in which a local contraction in a bag fibre produced by stimulating a dynamic γ axon is observed, photographed, and precisely located prior to processing for examination with electron microscopy (Banks et al., 1976b). The ultrastructure of the terminal present in the activated region is then compared with the ultrastructure of p_2 plates previously located and photographed in spindles stained with methylene blue (attempts to stain the dynamically activated spindle itself with methylene blue have so far failed). Longitudinal sections through motor endings found at the site of the observed local contraction produced by stimulating a dynamic γ axon show terminals whose length and ultrastructure are very similar to those of p_2 plates previously stained with methylene blue (see Fig. 7). The postsynaptic membrane in such plates is mostly smooth and not thrown into wide, shallow folds as described by Barker et al. (1970). Observations of the ultrastructure of γ fusimotor endings that we have made since then make it clear that the presence or absence of postsynaptic folding is not a reliable criterion for distinguishing between the terminals of trail endings and p_2 plates. It may be that postsynaptic folding is related to muscle fibre type and distance from the equator.

By observing the regional M line condition, diameter, and the equatorial relationship with chain fibres, type of bag fibre can confidently be identified in

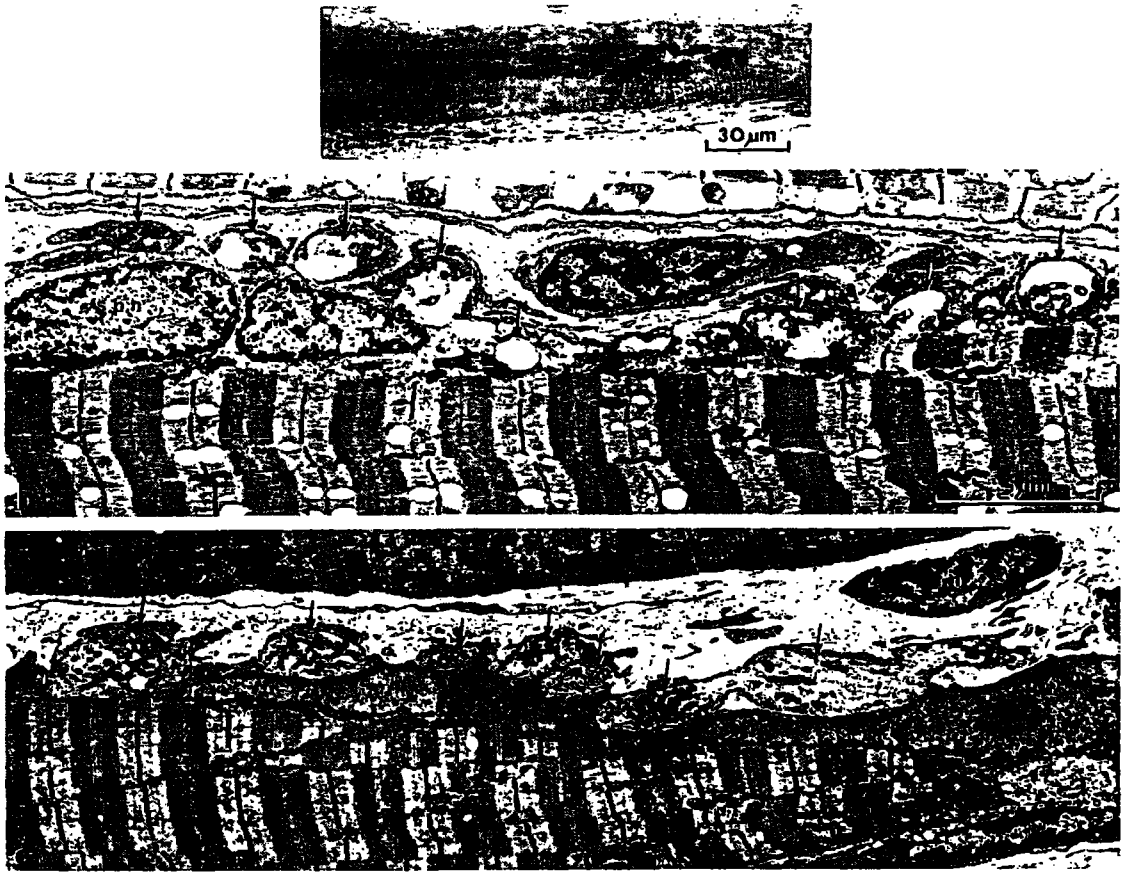


Fig. 7. a—c: light and electron micrographs of p_2 plates in cat tenuissimus spindles. a: methylene blue preparation of a p_2 plate in region B. b: electron micrograph of a longitudinal section through part of the p_2 plate shown in a. Vacuolated appearance of the axon terminals (indicated by arrows) and the bag_1 muscle fibre is due to damage caused by methylene blue staining. c: electron micrograph of an oblique longitudinal section through part of a motor ending located on a bag fibre (probably bag_1 type) at the site of observed local contraction (2.05 mm from the equator) produced by stimulating a dynamic γ axon. Position, length and ultrastructure of this ending suggest that it is a p_2 plate. f.n., fibroblast nucleus; s.p.n., sole-plate nucleus. (From work in progress by Barker, Bessou, Pagès and Stacey.)

ultrastructural preparations providing all three criteria are satisfied. On this basis we have identified two p_2 plates stained with methylene blue, and subsequently examined ultrastructurally, as being positively located on a bag_1 fibre in one case, and doubtfully so in another (two criteria only satisfied). Of two motor endings located at the sites of local contractions produced by stimulating dynamic γ axons and identified ultrastructurally as p_2 plates, one was positively identified as being situated on a bag_1 fibre, the other doubtfully considered to be on a bag_1 fibre.

Using these criteria we have checked on bag fibre type in other work in which muscle fibres were marked by the electrophoretic injection of the fluorescent dye Procion yellow and examined ultrastructurally after recording their membrane responses during fusimotor activation (Barker et al., 1972a, 1975a). In 6 experiments in which dynamic γ axons were stimulated a bag_1 fibre was

marked in 5 instances, and a chain fibre in a sixth. The fibres marked in these experiments are, of course, not necessarily the only ones activated by the fusimotor stimulation.

There is thus strong evidence that the dynamic response to γ fusimotor stimulation in cat spindles is mainly produced by γ axons terminating in p_2 plates on bag_1 fibres. However, the p_2 innervation is not exclusively restricted to bag_1 fibres, and the evidence from glycogen depletion experiments shows that, though a bag_1 fibre is always involved, a bag_2 or a chain fibre may also occasionally participate. The situation with respect to dynamic β axons is very similar. Histological evidence indicates that the fusimotor collaterals of β axons terminate as p_1 plates on bag fibres (75%) and chain fibres (25%) (Barker et al., 1970). Glycogen depletion experiments in which the sites of termination of 3 dynamic β axons were studied in 12 cat tenuissimus spindles (Barker et al., 1975b) showed that depletion was restricted to bag_1 fibres in 9 spindles, but that in two spindles a bag_2 fibre was also depleted, and in one spindle depletion also occurred in one bag_2 fibre and three chain fibres.

Boyd et al. (1975) maintain that all cat spindles contain two functionally distinct types of bag fibre; that those controlled by dynamic axons are never operated by static axons, and vice versa; and that chain fibres are always operated by static axons (see also Boyd and Ward, 1975). They therefore propose that the two bag fibre types be designated "dynamic" nuclear bag fibres and "static" nuclear bag fibres. It is difficult to accept this concept in view of our own findings. We cannot regard bag_1 fibres as being operated solely by dynamic axons since the evidence from glycogen depletion experiments shows that they are activated by static axons as often as bag_2 fibres. Moreover bag fibre involvement is restricted to bag_1 fibres on stimulating static γ axons with conduction velocities slower than 25 m/sec. Nor can we regard bag_2 fibres or chain fibres as being activated solely by static axons, since on occasion either or both these fibre types may be activated by dynamic axons, γ or β.

Nevertheless we must take account of an observation made by Bessou and Pagès (1975), which appears to support the view that one type of bag fibre is selectively operated by dynamic axons. They found that all dynamic γ axons activate bag fibres, but not the same bag fibres that are activated by static γ axons. Thus when a dynamic γ axon and a static γ axon supplying the same spindle were stimulated, the dynamic axon was observed to activate one of the bag fibres where the static axon activated the other, often together with one or more chain fibres. While acknowledging that some spindles may well have this pattern of motor innervation, we feel that the activation of bag_1 fibres by static γ axons cannot be ruled out on the basis of this observation for the following reasons. (i) The results of the static glycogen depletion experiments show that most of the centres of the depleted zones in bag_1 fibres lie in regions A and B; 28% are located less than 1 mm from the equator, 76% less than 1.5 mm. This intracapsular area is the least favourable for observing weak local contractions of the type that occur in bag fibres. Bessou and Pagès (1975) acknowledge that such contractions may be missed if chain fibres are strongly contracting simultaneously in the same pole. Furthermore in region A, and for most of region B (i.e., over a length of about 1.5 mm from the equator) the ultrastructure of bag_1 fibres is different (dM condition) from that found over

the rest of its polar length (M condition), and, except for part of region A, different from that of bag_2 fibres. It is not yet known whether these differences are associated with any differences in contractile properties. (ii) The conditions for observing weak local contractions in bag fibres are much better in region C (extracapsular) than in region B (intracapsular). In our collaborative work most of the contractions produced by stimulating dynamic γ axons are located in region C, 3.0—4.0 mm from the equator. However, the centres of zones in bag_1 fibres depleted of glycogen by dynamic γ activation (Fig. 6d) are distributed to regions B and C about equally. (iii) Identification of type of intrafusal muscle fibre by Bessou and Pagès (1975) was made on the basis of length and diameter. In our experience this can give rise to error, e.g., long chain fibres can be mistaken for bag fibres and vice versa.

We are thus left, as usual, with a piece of the jigsaw that just won't fit. This time it is the bag_1 fibre. Is it possible that this fibre, depending on the way in which it is activated, can give either a dynamic or a static effect? Bag fibres activated by dynamic axons have not so far been observed to be also activated by static axons, but that may be due to some of the reasons outlined in the preceding paragraph. Though much more information is required, the evidence at present available to us from glycogen depletion experiments (Fig. 6a, d), ultrastructural observation, and silver preparations (Fig. 8) suggest that bag_1 fibres can be innervated by either trail endings or p_2 plates. We do not know whether this may be true of one and the same bag_1 fibre, though we have observed instances of p_2 and trail innervation being supplied to a bag fibre whose type cannot be reliably ascertained (see Barker et al., 1970, Fig. 41). In this connexion the glycogen depletion experiments show that the average distances from the equator of the centres of zones depleted by static axons in bag_1 and bag_2 fibres are respectively 30.4% and 30.5% of their total polar lengths, whereas the comparable figure for zones depleted by dynamic axons in bag_1 fibres is 46.9%.

There is now a considerable amount of evidence indicating that of the two kinds of γ motor endings, trail endings are involved in the static response and p_2 plates in the dynamic response. That is not to deny that either ending may on occasion be involved in the contrary response, for we must certainly allow for the probability that it is the type of muscle fibre activated that is the relevant factor rather than the type of ending. Regional differences may also be of crucial importance. All intrafusal contractions are local; even those associated with propagated action potentials that sometimes occur in chain fibres involve only one pole (Bessou and Laporte, 1965; Bessou and Pagès, 1972, 1975). Hence perhaps consideration should go beyond fibre type and concentrate on the regional characteristics of the contracting zone.

In some tandem spindles it occasionally happens that a bag muscle fibre in one of the spindle units will continue through to the next encapsulation as a chain fibre (Barker and Cope, 1962; Eldred, as cited by Barker, 1962). From what is known of spindle development (Barker and Milburn, 1972; Landon, 1972; Milburn, 1973) it seems likely that tandem spindles with two linked capsules form as the result of two primary sensory axons making contact with a primary generation myotube (the future bag_2 fibre) at two points some distance apart. If one of the primary axons were to arrive somewhat later than the



Fig. 8. a: the equatorial region and part of one pole (region B) of a spindle from cat peroneus brevis. The innervation consists of a primary ending (P) in the equatorial region and a trail-ending area (tr.e.a.) in the pole. The bag_1 fibre (b_1) is dissociated equatorially from the bag_2 (b_2) and chain (c) fibres and is individuated by annulospiral terminals. Its course through the pole towards the trail-ending area is plainly visible. b: part of the trail-ending area in a, photographed at higher magnification. Arrowhead indicates a trail ramification terminating on the bag_1 fibre. Teased, silver preparation (method of Barker and Ip, 1963).

other, the sequential development of successive generations of myotubes that it engendered would lag behind such development initiated by its earlier partner. In that event the bag_1 fibre in the former spindle unit would be starting to develop at a time when the first chain fibre was already forming in the latter. In the subsequent polar fusion of myoblasts in the intercapsular region fusion could occur between the polar extremities of the bag_1 fibre and a chain fibre, especially if this were a long chain fibre. It is unlikely that bag_2 fibres engage in such fusions since they are already well developed in the muscle primordium before the primary axons reach them.

However this may be, we encountered a tandem spindle with a bag_1/long chain compound fibre in one of the static glycogen depletion experiments. The proximal pole of a large spindle unit (A) consisted of a bag_1 fibre, a bag_2 fibre, and five chain fibres, one of them a long chain. This was linked to a smaller unit (B) whose distal pole consisted of a bag_1 fibre, a bag_2 fibre, and four chains. The poles of the two units in the intercapsular region were linked by the bag_2 fibre, which was continuous, and a compound fibre formed by fusion between the long chain fibre of the large unit and the bag_1 fibre of the small. In the region of fusion of chain with bag, fibre diameter increased and glycogen level dropped from high to medium. The static γ axon activated four muscle fibres in this tandem spindle producing zones of glycogen depletion as follows. *Unit A.* Bag_1 fibre, one zone in region B (200 μm long), proximal pole; one in region C (810 μm), distal (intercapsular) pole. *Unit B.* Compound fibre, bag_1 portion; two intercapsular zones, one in region C (470 μm), one in region B (420 μm); and one in region B (200 μm), distal pole. Chain fibres, distal pole; one zone 330 μm long in one chain fibre, one 230 μm long in another. The total length of muscle fibre of bag_1 type activated was thus 2.1 mm as against a total chain length of 0.56 mm.

Because of its anomalous nature the data from this spindle, activated by a static γ axon with a conduction velocity of 28 m/sec, are omitted from Fig. 6. Did the type of response from its two primary endings depend on the type of motor ending involved? (In this case these were presumably all trail endings.) Or did it depend on the type of muscle fibres activated? If we accept that the latter is more probable and agree with the hypothesis that contractions of bag_1 fibres are exclusively associated with dynamic responses, and those of chain fibres with static responses, we find ourselves in a dilemma. In view of the large amount of bag_1 activation we would presumably have to ignore the relatively slight activation of chain fibres and conclude that, had the two primary responses been individually monitored, both would have been dynamic. But in two other spindles activated by this axon only chain fibres were depleted, and we would have to regard the responses from these as static. However, this would lead to a situation where the same axon was producing different types of response from different spindles, and all previous reports (Crowe and Matthews, 1964; Brown et al., 1965; Bessou et al., 1966) are to the effect that a fusimotor axon has the same action on the spindles that it supplies.

It could be argued that in those spindles where a fusimotor axon innervates different types of fibre (i.e., in terms of the selective hypothesis, dynamic bag fibres and static bag fibres, or dynamic bag fibres and chain fibres), similarity of action is achieved by the effect of contraction in one fibre type overriding or

eliminating the effect of that in another. An explanation on these lines has been put forward to account for the action of those γ axons which produce a dynamic response when stimulated at low frequency, and a static response when the rate of stimulation is increased (Emonet-Dénand et al., 1972). But it is difficult to see how such overriding or elimination could operate at the same frequency of stimulation. In view of these uncertainties and contradictions it seems to us that little further progress can be made until the function of a fusimotor axon is correlated with primary response and pattern of motor innervation in each spindle that it supplies.

It would be helpful for all concerned if there could be a restatement of the functional properties of static and dynamic fusimotor axons. We need clearly defined criteria for classifying primary responses as static or dynamic, which take full account of their behaviour during different frequencies of motor stimulation and different methods of applying stretch. If there are different kinds of static and dynamic axons, let these differences be specified in precise terms. Without such a restatement it will be difficult to provide satisfying answers to the sort of questions posed by some of the recent histological findings. For example, are there any differences between the dynamic responses of cat, rabbit, and rat spindles that might be correlated with the differences in ultrastructure of their bag_1 fibres? Is the nature of the primary response affected in any way if it is produced by the activation of more than one type of intrafusal muscle fibre? For example, is there any difference in the dynamic response from a cat spindle in which fusimotor activation is confined to a bag_1 fibre, to a response from one in which a bag_2 fibre, or a long chain fibre, is activated in addition?

EXPERIMENTS WITH BUPIVACAINE

If the long-lasting local anaesthetic bupivacaine (Marcain) is applied to the surface of a muscle, the superficial muscle fibres undergo rapid degeneration followed by complete regeneration (Sokoll et al., 1968; Benoit and Belt, 1970; Libelius et al., 1970). The action of the drug is specifically myotoxic and does not affect the motor innervation. If bupivacaine combined with hyaluronidase is injected into a muscle, the whole muscle degenerates and then regenerates (Hall-Craggs, 1974). It was not clear what happened to the muscle spindles in such circumstances, and it seemed worthwhile to investigate since, if the intrafusal muscle fibres were similarly affected, it would provide an experimental model for studying their development in the adult. The results of such an investigation have justified this hope and have unexpectedly provided a possible opportunity for using the model to discover the function of the nuclear bags and chains (Milburn, 1976a, b).

The muscle used was adult rat peroneus longus. It was injected with 0.5 ml of bupivacaine prepared in sterile 0.9% saline solution containing 15 IU of hyaluronidase, and then processed for electron microscopy at postoperative intervals varying from 4 hr to 21 days. Degeneration of the intrafusal muscle fibres begins within 4 hr and is advanced after 2 days. The equatorial nuclei become disorganized and pyknotic and finally disappear so that after 2 days the

bags and chains in most spindles are absent. The muscle fibres ultimately become reduced to tubes of often thickened basement membrane containing varying amounts of necrotic, filamentous and granular material. The satellite cells, however, survive this phase, and may become the myoblasts that participate in the subsequent regeneration. The spindle nerve supply is affected differently according to whether it is sensory or motor. The motor innervation is little affected; the terminals simply withdraw from the degenerating muscle fibres and become invested by Schwann cells. But the sensory endings degenerate and the axon branches supplying them also show signs of necrosis. The capsule and the periaxial space remain normal.

Three days after injection phagocytes have infiltrated the degenerating muscle fibres to remove debris, and myoblasts have appeared at their periphery within the basement membrane. Muscle fibre regeneration proceeds as the myoblasts fuse to become myotubes. By the end of the third week three types of muscle fibre have been fully restored in the axial bundle with normal differences in size and ultrastructure, but lacking equatorial nucleation. Instead of bags or chains there is simply an occasional central nucleus lying in a thin bed of sarcoplasm. The motor innervation is restored, but the sensory endings that regenerate lack spirals and differ in ultrastructure from normal terminals.

Thus the ultimate effect of bupivacaine injection is to produce "enucleated" spindles. The regenerating primary axon appears to lack the morphogenetic influence it possessed during development so that nuclear aggregations do not form at the site of reinnervation. The absence of nuclear bags and chains may in turn account for the failure of the regenerated terminals to develop annulospiral configurations. By reducing the strength of the bupivacaine/hyaluronidase injection it may be possible to produce enucleation with minimal disturbance to the sensory innervation. Such spindles could provide an experimental model for the study of the part played by the nuclear bags and chains in the production of responses from primary and secondary endings.

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DISCUSSION

BOYD: Naturally I'm very interested in your glycogen depletion work, which seems to be the zone in which we are least well in accord. You mentioned that static gamma axons could deplete both types of nuclear bag fibre. It is important to know whether one type is depleted in one spindle and the other type in another spindle. How often are both types of bag fibre depleted?

BARKER: We have two spindles where both are depleted.

BOYD: And only two bag fibres in those spindles, not three?

BARKER: That's right.

BOYD: It was not clear to me how you identify bag_1 and bag_2 in your glycogen preparations.

BARKER: There are two main ways. One is by the profile in non-depleted areas compared with normal controls. Secondly, by bonus points like dissociation in the equatorial region, which certainly distinguishes the bag_1 very nicely, and also length and diameter, though we would never rely on length and diameter alone.

BOYD: Then I understand you cannot directly correlate your bag_1—bag_2 classification with electron microscopy?

BARKER: That's precisely what we've done. Once you've got your index, once you've correlated diameter with histochemical profiles and with ultrastructure, then you just subtract ultrastructure and you've got the other features there. You can confidently identify the bag fibres.

BOYD: I won't pursue that further, but this is an area which we feel is debatable.

MATTHEWS: How confident are you about the glycogen depletion technique being a method of uniform sensitivity in detecting activity of intrafusal muscle fibres? The bag_1 fibre, by having very little glycogen to begin with, may be an unduly sensitive indicator of a very small amount of bag_1 contraction. Are you perhaps sharpening a small amount of motor innervation of bag_1 into apparently a rather large effect?

BARKER: There is regional variation in all the enzyme activities we've looked at, not just with glycogen. In the region where bag_1 has the dM structure you do get occasional low stretches in the normal glycogen profile, but we are confident from our controls, particularly in fresh-frozen material, that there is a difference between blanching from activation and the paleness of these normal low stretches.

Paper 9.

.R.W. Banks, D. Barker, P. Bessou, B. Pagès & M.J. Stacey (1976)
Serial-section analysis of cat muscle spindles following observations of the effects of stimulating dynamic fusimotor axons.
J. Physiol. 263; 180-181P.

[*From the Proceedings of the Physiological Society*, 2–3 *July* 1976
Journal of Physiology, **263**, 180–181*P*]

Serial-section analysis of cat muscle spindles following observation of the effects of stimulating dynamic fusimotor axons

By R. W. Banks, D. Barker, P. Bessou, B. Pagès and M. J. Stacey.
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Tenuissimus spindles were prepared in Toulouse as for cinematographic analysis (Bessou & Pagès, 1975) in order to detect the sites of focal contraction elicited by the repetitive stimulation of single dynamic γ axons. Eleven contraction sites were located in seven spindles 2·05–4·10 mm from the equator. Each contraction occurred as a focus of sarcomere shortening in the extracapsular region of a nuclear-bag fibre. Its position was marked on a photograph of the living spindle preparation.

After fixation in glutaraldehyde the preparations were sent to Durham for histological analysis. Serial 1 μm transverse sections were cut for light microscopy, and, at selected intervals, thin sections for electron microscopy. Muscle-fibre types were identified on the basis of length, diameter, equatorial nucleation and position (Banks, Harker & Stacey, 1976), and distribution of associated elastic fibres (Gladden, 1976). Motor terminals were identified as p_2 plates on the basis of similarity in ultrastructure with that described for p_2 plates previously stained with methylene blue (Barker, Banks, Harker, Milburn & Stacey, 1976).

Surprisingly, only 2 of the 11 contraction sites coincided with the location of a p_2 plate; these were situated on bag_1 fibres. Five other sites were 'plateless', but in these spindles p_2 plates were located on bag_1 fibres 1·04–2·55 mm nearer the equator; 3 of these plateless sites coincided with an adhesion between the bag fibres and a blood vessel. No motor endings were found at the remaining 4 contraction sites; sectioning 0·4–2·5 mm (mean 1·43 mm) on either side has so far failed to reveal any. The absence of endings from some contraction sites may possibly be associated with the presence of adhesions. If sarcomeres are shorter in adhesion regions than elsewhere, the electrotonic spread of potential, or, eventually, the propagation of action potentials towards the poles, would result in their preferential shortening (Huxley & Peachey, 1961), thus imitating a focal contraction occurring beneath a p_2 plate.

Incidental observations have been made on the distribution of secondary endings. Six secondaries were distributed as follows (equatorial location indicated according to Boyd, 1962): three S_1 endings innervated all three muscle-fibre types; one S_2 ending innervated bag_1 and chain fibres, another chains only; one S^3 ending innervated chains only.

[P.T.O.

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Paper 10.

R.W. Banks, D. Barker & M.J. Stacey (1977)
Intrafusal branching and distribution of primary and secondary afferents.
J. Physiol. 272; 66-67P.

[*From the Proceedings of the Physiological Society*, 8–9 *July* 1977
Journal of Physiology, 272, 66–67*P*]

Intrafusal branching and distribution of primary and secondary afferents

By R. W. Banks, D. Barker and M. J. Stacey. *Department of Zoology, University of Durham, Durham DH1 3LE*

The intrafusal branching and distribution of three primary and four secondary afferents have been ascertained from serial 1 μm transverse

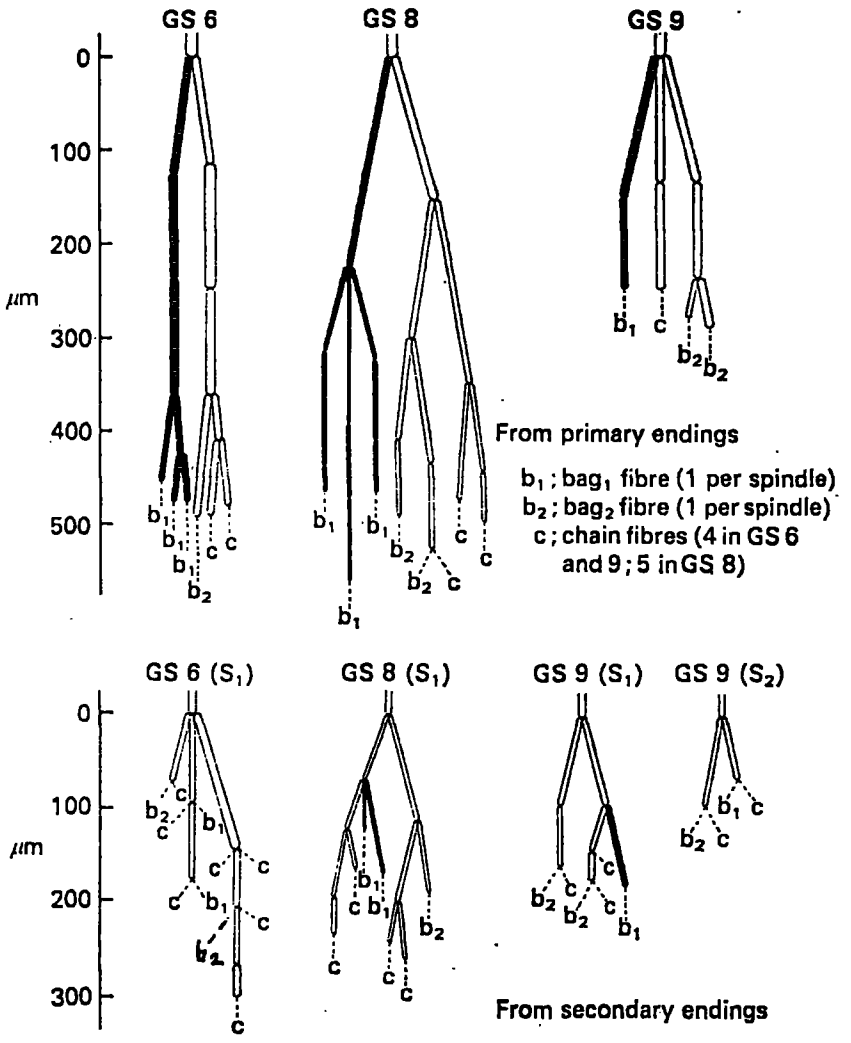


Fig. 1. Branching and distribution of primary and secondary afferents supplying cat tenuissimus spindles. Black branches supply bag_1 fibres exclusively.

sections of cat tenuissimus muscle spindles (Fig. 1). The branching patterns of the primary afferents provide an anatomical basis for the suggestion (Hulliger, Matthews & Noth, 1977) that there are two or more com-

[P.T.O.

petitively interacting pace-makers controlling the static and dynamic components of the primary-ending response. These could be located in each first-order branch so that a primary ending would possess one dynamic and one or two static pace-makers. By comparison a secondary ending would usually possess only static pace-makers, with the possibility of some modulation by dynamic input.

Each afferent illustrated supplied terminals to every muscle fibre. Of three further secondary afferents, two were distributed to chain fibres (GS 8, S_2; GS 9, S_3), one to bag_1 and chains (GS 8, S_1). In the primary endings unmyelinated preterminal branches were distributed to each bag fibre, but whereas some chain fibres received them, others were supplied by sensory cross-terminals only. Contrary to the assumptions made by Rudjord & Rommetvedt (1970), there were no obvious differences between the terminals on bag fibres and those on chains.

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Paper 11.

R.W. Banks, D.W. Harker & M.J. Stacey (1977)
A study of mammalian intrafusal muscle fibres using a combined histochemical and ultrastructural technique
J. Anat. 123; 783-796.

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With 13 *figures*
Printed in Great Britain

A study of mammalian intrafusal muscle fibres using a combined histochemical and ultrastructural technique

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INTRODUCTION

The presence of two types of nuclear-bag muscle fibre in mammalian muscle spindles is suggested by recent evidence from histochemical and ultrastructural studies, reviewed by Barker (1974). However, some doubt remains as to the exact properties of the nuclear-bag fibre population, since the various techniques used in these studies have not been applied to the same spindle (Barker & Laporte, 1975).

In this study we have established the histochemical profiles of intrafusal muscle fibres from various hind limb muscles of the cat, rabbit and rat and correlated these with the ultrastructure of the same fibres. The technique involved the collection of groups of serial frozen sections for histochemistry, alternating with single, much thicker sections for electron microscopy.

Preliminary observations (Banks, Barker, Harker & Stacey, 1975) have shown that two types of nuclear-bag fibre occur in the spindles of all the muscles studied, usually one of each type. These have been designated 'bag_1' and 'bag_2' on the basis of their ATPase staining reactions, following the nomenclature of Ovalle & Smith (1972). Variations in histochemical profile along the length of individual intrafusal muscle fibres were found, and the bag fibres also showed regional differences in ultrastructure.

MATERIALS AND METHODS

Material was obtained from the tenuissimus (TEN), peroneus longus (PL) and peroneus digiti quinti (PDQ) muscles of an adult cat and an adult rabbit, and from the PL, PDQ and soleus (SOL) muscles of an adult rat.

Thirty four spindles were examined, all of the spindles being incomplete to a greater or lesser extent. The sample was distributed as follows: cat, 6 spindles (2 TEN, 2 PL, 2 PDQ), mean number of intrafusal fibres 6·8 (range 6–8); rabbit, 13 spindles (2 TEN, 1 PL, 10 PDQ), mean number of intrafusal fibres 4·4 (range 4–5); rat, 15 spindles (5 SOL, 6 PL, 4 PDQ), mean number of intrafusal fibres 4·1 (range 3–6).

The muscles were removed immediately post mortem and frozen in isopentane cooled to − 160 °C with liquid nitrogen. Serial frozen sections were cut on a cryostat from portions of each muscle. Groups of about ten 15 μm thick sections for the application of histochemical techniques were collected alternately with single 60 μm

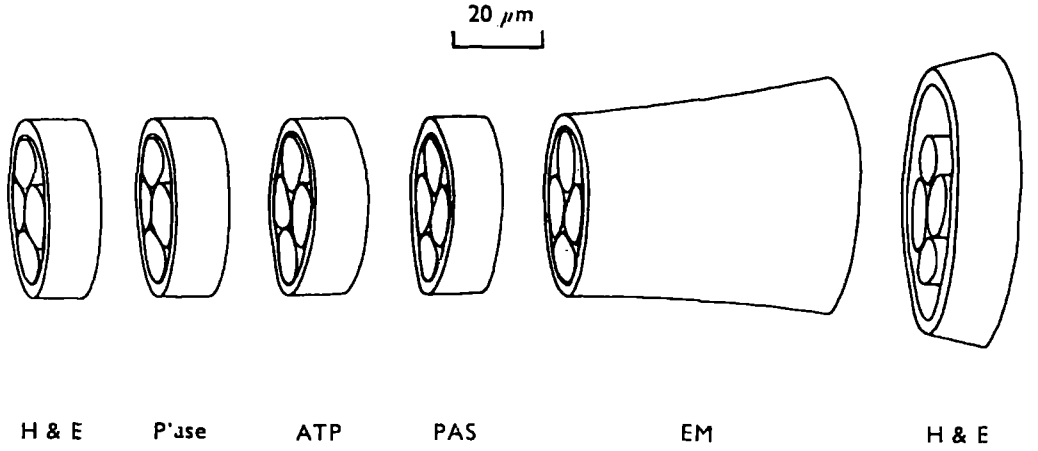


Fig. 1. Diagrammatic illustration of the sectioning technique. Groups of 15 μm thick sections were cut for histological and histochemical processing. The histological sections were stained with haematoxylin and eosin (H & E) to monitor the progress of sectioning. The histochemical sections were processed for (i) phosphorylase (P'ase), (ii) ATPase after alkaline preincubation (ATP), (iii) periodic acid–Schiff (PAS). Alternating with these sections a 60 μm thick section was cut for electron microscopy (EM).

thick sections for ultrastructural study (Pierobon Bormioli & Schiaffino, 1974) as shown in Figure 1.

Histochemistry

The histochemical profiles of the intrafusal muscle fibres were established using three staining techniques: myofibrillar ATPase following alkaline pre-incubation (Guth & Samaha, 1970); phosphorylase (Eränkö & Palkama, 1961); and glycogen as shown by the PAS technique.

In addition some sections from each group of 15 μm thick sections were stained with haematoxylin and eosin in order to follow the progress of sectioning.

Electron microscopy

Each 60 μm thick frozen section was fixed for 18 hours in 5% glutaraldehyde in 0·1 M sodium cacodylate buffered at pH 7·2. The sections were washed in the buffer solution, post-fixed for 2 hours in 1 % osmium tetroxide buffered at pH 7·2 in 0·1 M sodium cacodylate, washed in buffer, dehydrated through a graded series of ethanols, immersed in propylene oxide and finally embedded in Epon.

Sections were cut on an LKB Ultrotome or a Reichert OMU3, stained with uranyl acetate and lead citrate and examined with an AEI EM801 electron microscope at an accelerating voltage of 80 kV.

Reconstructions

Several spindles were reconstructed diagrammatically using data obtained from the histochemically stained sections. Cross sectional areas of intrafusal fibres were measured on calibrated micrographs using a planimeter. From each of these values the diameter of a circle having the same area was calculated and the diameters thus obtained were plotted against the positions of the corresponding sections.

Table 1. *Histochemical staining reactions in three regions of intrafusal muscle fibres classified into bag_1, bag_2 and chain types*

(Values are average numbers of points awarded on a scale 0 (absent), 1 (low), 2 (medium), 3 (high). The number of regions sampled is given in parentheses after each value.)

	A		B		C	
ATPase						
Rabbit						
Bag_1	0·7		1·0		1·4	(12)
Bag_2	1·9	(17)	2·9	(19)	2·8	(13)
Chain	2·5		3·0		2·7	
Rat						
Bag_1	1·3		1·1		1·7	(6)
Bag_2	2·7	(9)	2·7	(19)	2·6	(5)
Chain	2·7		2·9		3·0	(2)
Cat						
Bag_1	1·0		1·0		1·3	
Bag_2	1·8	(6)	2·0	(7)	1·7	(3)
Chain	2·8		3·0		2·7	
P'ase						
Rabbit						
Bag_1	0·9		1·3		2·0	(13)
Bag_2	1·6	(16)	2·1	(19)	2·0	(14)
Chain	1·9		3·0		2·9	
Rat						
Bag_1	1·4		2·1		2·1	(10)
Bag_2	1·4	(18)	1·8	(17)	1·6	
Chain	1·5		3·0		2·6	(5)
Cat						
Bag_1	1·4		1·8		1·7	
Bag_2	1·6	(5)	1·8	(5)	2·0	(3)
Chain	1·8		3·0		3·0	
PAS						
Rabbit						
Bag_1	1·2		2·2		2·1	(11)
Bag_2	1·0	(16)	1·4	(17)	1·2	
Chain	1·2		2·9		2·9	(10)
Rat						
Bag_1	1·7		1·9		2·3	(3)
Bag_2	1·9	(7)	1·5	(13)	1·7	
Chain	1·7		1·9		2·0	(1)
Cat						
Bag_1	1·0		1·3		1·7	
Bag_2	1·7	(3)	1·5	(6)	1·7	(3)
Chain	2·0		3·0		3·0	

RESULTS

Histochemistry

In order to facilitate the description of regional variations the muscle spindle was considered as comprising three regions arbitrarily defined by the equatorial nucleation and by the condition of the capsule, namely A, the level from the equator to the equatorial end of the periaxial space; B, that part of the polar region enclosed by the

capsule; C, the extracapsular part of the polar region. Some results from regions A and B have been reported previously (Banks *et al.* 1975). Each section was assigned to its appropriate region and the staining intensities of the intrafusal fibres were estimated on a scale of 0 (absent), 1 (low), 2 (medium), and 3 (high). This is similar to the grading system used before (Banks *et al.* 1975) of 0, +, + + and + + +, but has the advantage that the results from a number of spindles can be easily pooled, thus taking account of any variations without making the presentation of results too cumbersome.

In every spindle examined two types of nuclear-bag fibre (one of each type) were distinguished, whereas the nuclear-chain fibres, usually more than one per spindle, formed a homogeneous group.

The histochemical profiles of muscle spindles from different muscles of the same animal did not show any consistent differences and the results for each region from all the muscles were therefore combined. The results for each of the three species are given in detail in Table 1.

Comparison of the results from different species showed that the ATPase staining reactions were the most consistent. The nuclear-bag fibres were therefore classified on the basis of this reaction as bag_1 fibres (those with relatively low ATPase activity) and bag_2 fibres (those with relatively high ATPase activity) (Ovalle & Smith, 1972).

The following general observations apply to all three species unless otherwise stated. In each type of intrafusal fibre the staining intensities produced by each histochemical method vary along the length of the fibre. Staining intensities in region A are usually lower than those of the polar regions B and C.

With the ATPase method, in rabbit and rat spindles, bag_2 fibres are indistinguishable from chains over most of their lengths (Fig. 3). In cat spindles bag fibres almost always show a lower ATPase intensity than chains (Fig. 11). In all three species the differences between the ATPase intensities of bag_1 and bag_2 are most marked in regions A and B. The greater similarity in region C is due mainly to increase in the intensities of bag_1 fibres.

With the phosphorylase reaction, differences between the three types of intrafusal fibre were most marked in rabbit spindles (Fig. 2). Bag_1 and bag_2 fibres were not clearly differentiated in rat and cat spindles with this method. Nuclear-chain fibres usually stained purple and nuclear-bag fibres brown, indicating that nuclear-bag fibres possess a branching enzyme not present in nuclear-chain fibres (Swanson, 1948).

Figs. 2–5. Photomicrographs of a series of closely adjacent transverse sections through region B of a rabbit tenuissimus spindle. These should be compared with the diagrammatic illustration of the sectioning technique shown in Fig. 1. Note the relatively large diameters of the chain fibres.

Fig. 2. Phosphorylase staining intensity of the bag_1 fibre (b_1) is low, of the bag_2 fibre (b_2) is medium and of the chains (c) is high.

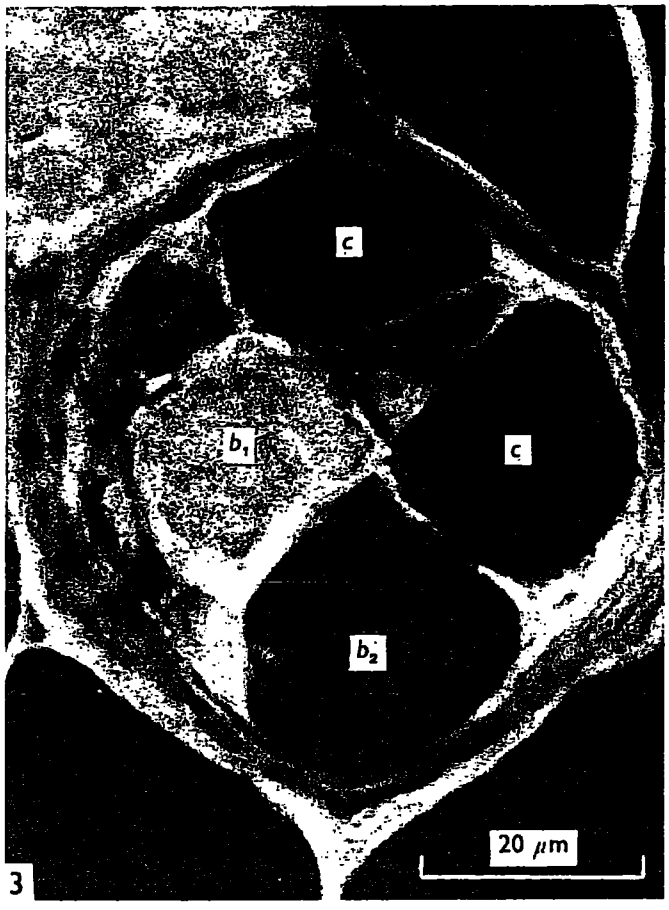
Fig. 3. Staining intensity of myofibrillar ATPase following alkaline preincubation is low in bag_1 and high in bag_2 and chains.

Fig. 4. PAS staining intensity of the bag_1 fibre is medium, of the bag_2 fibre is low and of the chains is high.

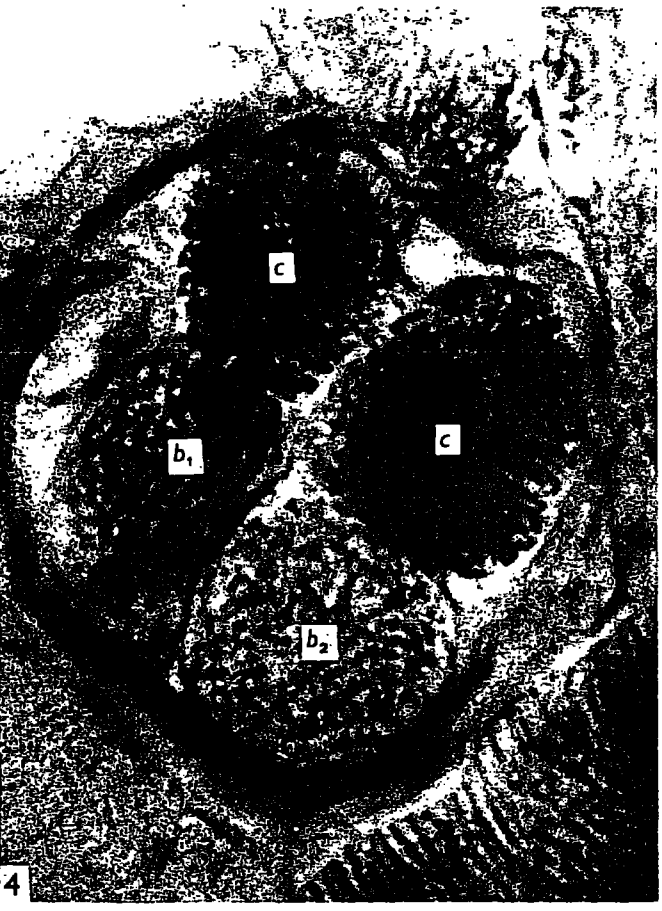
Fig. 5. A 60 μm thick section embedded in Epon showing the intrafusal fibres identified from the adjacent histochemically stained sections.



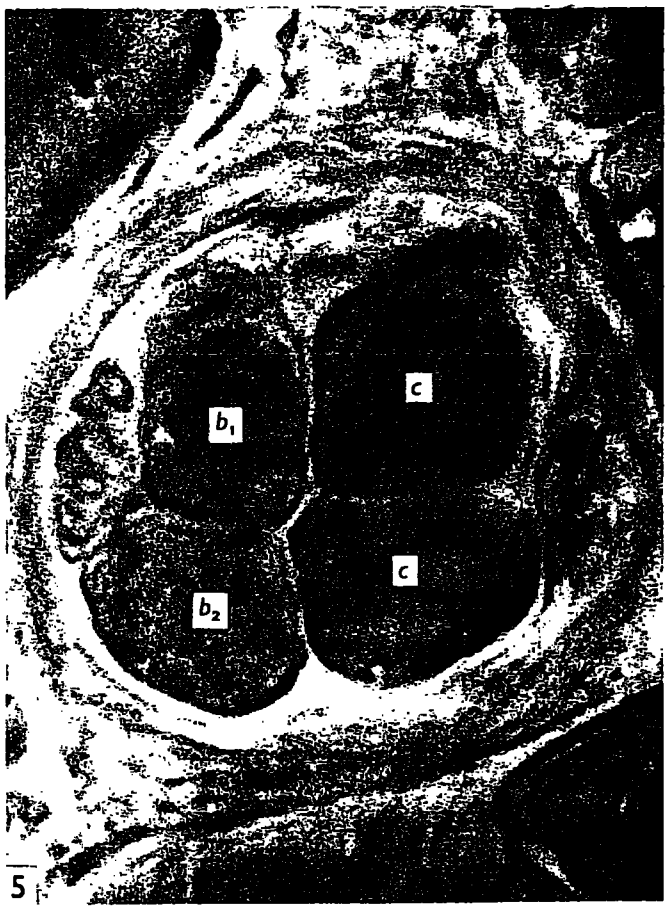
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5

In rabbit and rat spindles bag_2 fibres usually contained less glycogen than bag_1 fibres (Fig. 4), the difference being more apparent in rabbit spindles. In rabbit and cat spindles the chain fibres contained more glycogen than either bag_1 or bag_2 fibres.

Electron microscopy

Comparison of the thick, Epon-embedded sections with the adjacent histochemically stained sections enabled each intrafusal fibre in the thick sections to be assigned with certainty to its appropriate fibre type (Fig. 5). Conditions for the preservation of fine structure were not optimal. Membrane systems, particularly sarcoplasmic reticulum, seemed the most susceptible to damage and observations were restricted to regions where these were intact. During fixation contraction could not be opposed and the sarcomeres of intrafusal and extrafusal fibres were greatly shortened. However, the sarcomeres of bag_1 fibres were consistently longer than those of bag_2 fibres, chain fibres and extrafusal fibres (Figs. 6–9; Fig. 10). In all three types of intrafusal fibre the A bands were 1·5 μm wide and the Z lines 75 nm thick, which is comparable to the thickness of the Z lines of extrafusal intermediate fibres of the rat (Schiaffino, Hanzlikova & Pierobon, 1970).

The condition of the M lines provided conspicuous differences between the intrafusal fibre types. Two major conditions were recognized. Firstly, sarcomeres possessed an M line that appeared as a prominent single structure in low power micrographs, but with higher power a substructure of five parallel faint lines was seen. This condition is designated M. The M lines of extrafusal fibres were of this type.

Secondly, sarcomeres possessed an M line consisting of two faint parallel lines. This condition has been designated dM. Sarcomeres in which no M line was visible were included in this type since they always occurred close to sarcomeres possessing faint double M lines (Fig. 8). It is probable that the faint double line is only visible in suitably orientated myofibrils with straight sarcomeres (Ovalle, 1971). Both dM and M conditions were present in different regions of some intrafusal fibres, but the nature of the transition is unknown. Bag_1 fibres (Figs. 6, 8) were of dM type throughout their lengths in rat spindles, whereas in rabbit and cat spindles they were of dM type in the equatorial region and much of the polar region, but M type in region C. Bag_2 fibres were always of dM type in the equatorial region and M type in the poles (Figs. 7, 9). The transition region corresponded approximately with the level A/B

Figs. 6–9. Electron micrographs of longitudinal sections of nuclear bag muscle fibres, showing the variation in structure in different regions, Figs. 6–8 illustrate the dM condition and Fig. 9 the M condition (see text).

Note the varying degree of shortening of the sarcomeres produced by the fixation technique.

Fig. 6. Rat: b_1, region A. No M line is visible in the pseudo-H zone (arrowhead).

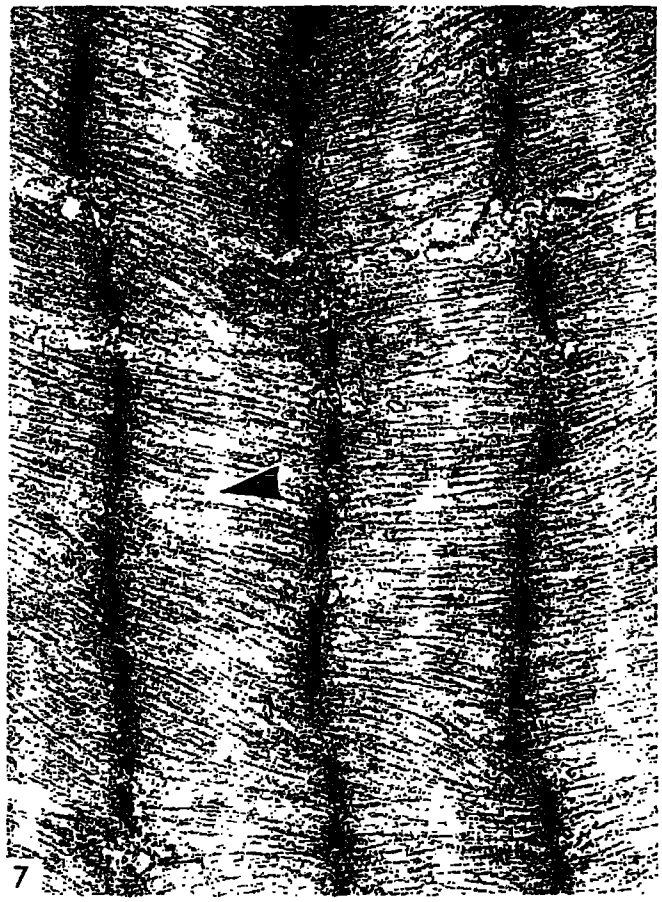
Fig. 7. Rat: b_2, region A, As in the b_1 fibre, no M line is present in the pseudo-H zone (arrowhead).

Fig. 8. Cat: b_1, region B. A double M line is present in some sarcomeres (arrowed) but not in others (arrowhead).

Fig. 9. Cat: b_2, region B. An M line is present in each sarcomere (arrowed).



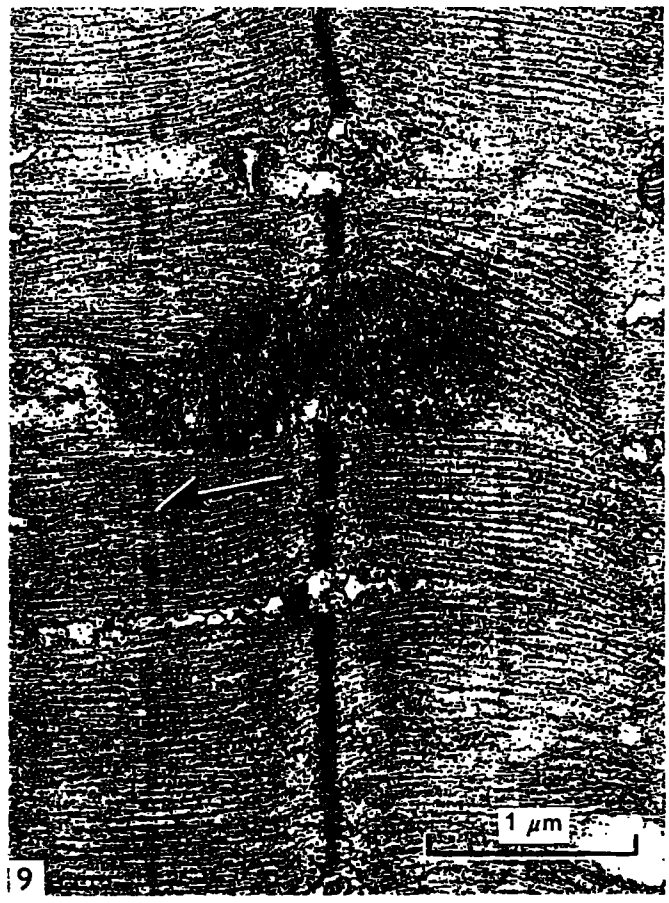
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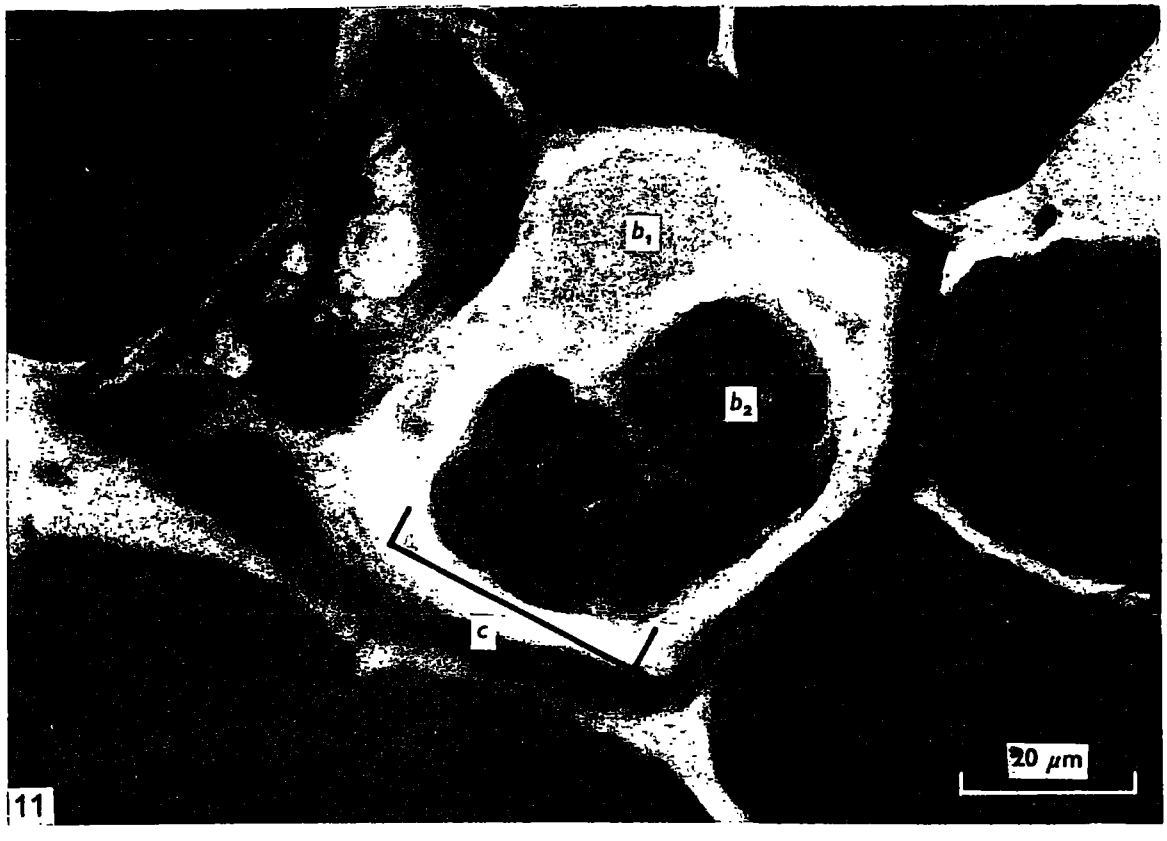


Fig. 10. Electron micrograph of longitudinal section of rat nuclear chain muscle fibre, showing the presence of an M line (arrow).

Fig. 11. Photomicrograph of a transverse section through region A of a cat tenuissimus spindle, showing myofibrillar ATPase staining intensities: b_1 is low; b_2 medium and the chains (c) high. Note that b_2 is closely associated with the chains, whereas b_1 is somewhat dissociated from the other fibres.

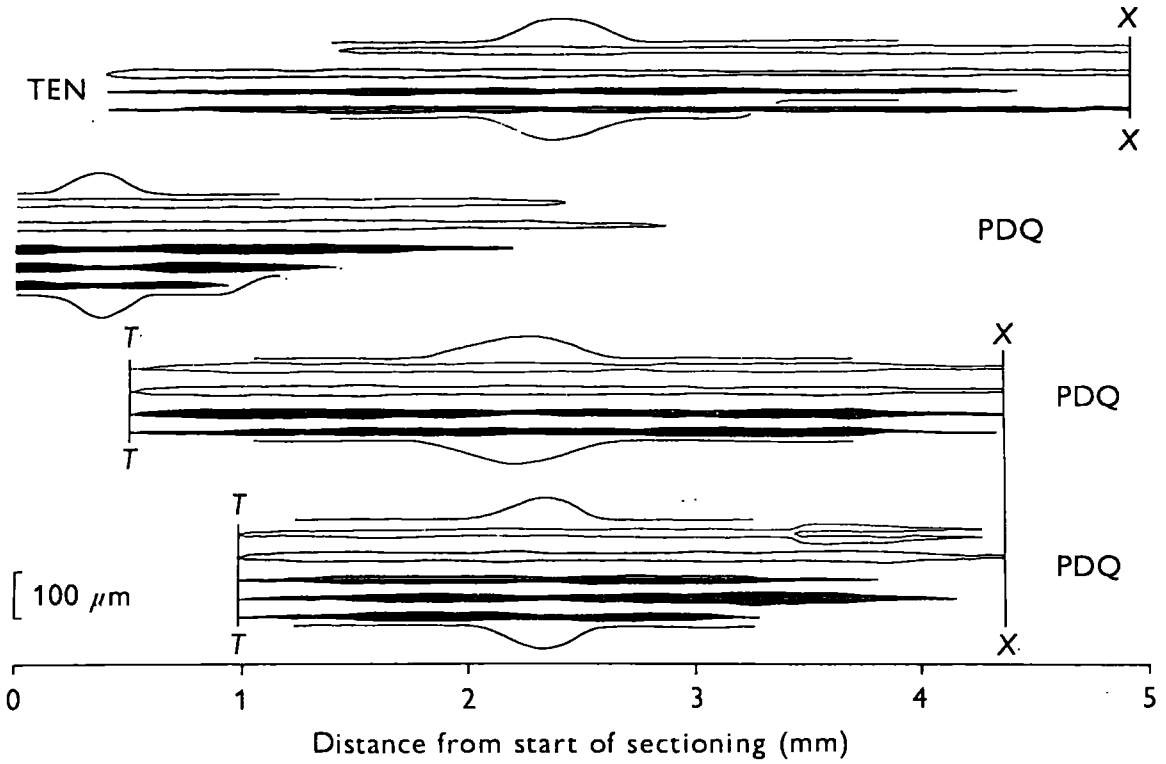


Fig. 12. Reconstructions of four rabbit spindles showing the relative dimensions of bag_1 fibres (white), bag_2 fibres (stippled) and chain fibres (black). Note that the vertical scale is twice the horizontal. PDQ, peroneus digiti quinti; TEN, tenuissimus; *T*, tendinous insertion of spindle; *X*, end of sectioning.

division. Chain fibres were of M type throughout the whole of their lengths (Fig. 10). Using this criterion they were, therefore, indistinguishable from bag_2 fibres in the spindle poles.

Reconstructions

Rabbit and rat spindles reconstructed as described above are shown in Figs. 12 and 13. The diameter measurements used to prepare the reconstructions are summarized in Table 2.

Bag_1 and bag_2 fibres are usually about the same length, but bag_2 fibres are only of significantly greater diameter than bag_1 fibres in the rat. Chain fibres are usually shorter than bag_1 or bag_2 fibres and are usually of significantly smaller diameter. In the polar regions, however, the diameters of bag_1 fibres and chain fibres are not significantly different (Figs. 2–5). Similar results have been obtained for rabbit spindle poles by Banks & James (1975). All types of intrafusal fibre taper over long distances of the polar regions before ending. This has the effect that the mean polar diameters are usually smaller than the corresponding mean equatorial diameters, despite the fact that the maximum diameters are often found in the poles. This is particularly apparent in rabbit chain fibres.

In the equatorial regions of cat spindles the chain fibres are often closely associated with the bag_2 fibres whereas bag_1 fibres are clearly separated from the two other types (Fig. 11).

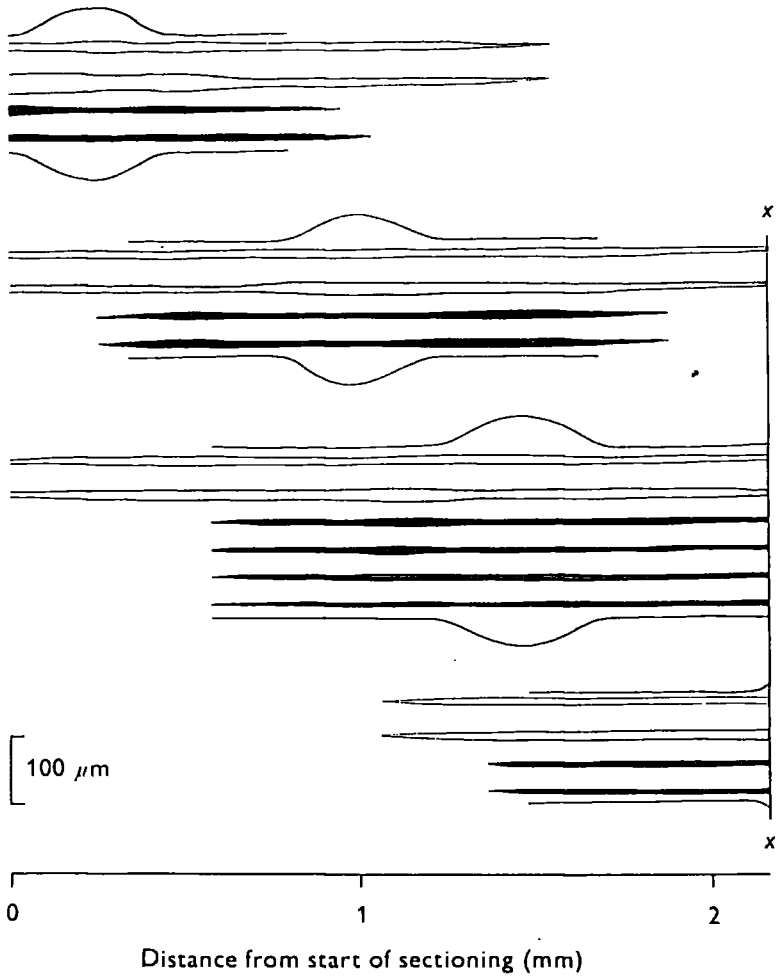


Fig. 13. Reconstructions of four rat spindles. For explanation see Fig. 12. All spindles from PDQ.

DISCUSSION

The present work clearly establishes the presence of three types of intrafusal fibre in mammalian muscle spindles. However, the three types cannot always be differentiated on the basis of any single technique, whether morphological, histochemical or ultrastructural. Application of any one technique to the muscle spindle usually results in the inclusion of two of the types within a single group. It is perhaps for this reason that the view that there are only two types of intrafusal fibre has persisted for so long (Matthews, 1972). The implications of the new classification are far-reaching and earlier results may need to be re-interpreted, particularly with regard to the innervation of the muscle spindle.

Previous attempts to correlate the histochemical and ultrastructural properties of intrafusal fibres have all involved certain assumptions since the different techniques have been applied to different spindles. It is clear that in many cases the assumptions made were incorrect, so that ultrastructural and morphological properties were sometimes ascribed to the wrong histochemical type. In the present work no such assumptions have been necessary. Earlier classifications of intrafusal fibres and attempts to correlate their histochemical and ultrastructural properties are summarized in Table 3 and are compared with the present classification.

Table 2. *Mean diameters (± standard error of the mean) of intrafusal muscle fibres from the equators and poles of rabbit and rat muscle spindles*

(All measurements in μm. Values of *P* for the null hypotheses (mean diameter fibre *x*) = (mean diameter fibre *y*) are given.)

	Equator	Pole
	RABBIT	
Bag_1	18·0±0·62	15·1±0·42
Bag_2	18·6±0·75	16·7±0·54
Chain	14·5±0·40	14·4±0·48
Ho	*P*	*P*
$Bag_1 = bag_2$	N.S.	N.S.
Bag_1 = chain	< 0·001	N.S.
Bag_2 = chain	< 0·001	< 0·01
	RAT	
Bag_1	12·3±0·56	11·3±0·59
Bag_2	18·7±2·30	14·4±0·69
Chain	9·1±0·61	9·4±0·38
Ho	*P*	*P*
$Bag_1 = bag_2$	< 0·05	< 0·01
Bag_1 = chain	< 0·01	N.S.
Bag_2 = chain	< 0·01	< 0·001

It is possible that those classifications which involve more than three types of fibre have arisen because of the occurrence of regional variations in intrafusal fibres. Variations in both histochemical and ultrastructural properties of intrafusal fibres are clear from the present work. Also Yellin (1974) has noted variations in ATPase activity and Banker & Girvin (1971) found that nuclear-bag fibres, which had M lines in the polar regions, lost them in the equatorial region. Other changes in the contractile apparatus reported by Banker & Girvin (1971) have not been seen in this work. Harriman, Parker & Elliott (1975) have found acid-stable ATPase activity restricted to the polar regions of nuclear-chain fibres, but present throughout one type of nuclear-bag fibre in human spindles.

In addition to those listed in Table 3, several authors have described more than two types of intrafusal fibre. The evidence was histochemical (Ogata & Mori, 1962, 1964, mouse, rat, cat and human spindles; Wirsen & Larsson, 1964, mouse spindles; James, 1971*b*, rat and guinea-pig spindles) and morphological (Cuajunco, 1927, 1940, pig and human spindles; Barker & Gidumal, 1961, cat spindles; Ogata & Mori, 1962, 1964; James, 1971*b*; Maynard & Tipton, 1971, rat spindles). Also Banks & James (1973) described three types of intrafusal fibre from guinea-pig lumbrical spindles on the basis of their diameters and ultrastructure in the equatorial region. There was a large fibre of type dM and two types of small fibre, one of type dM and one of type M. On the basis of the present work, these may be equated with bag_2, bag_1, and chain types respectively. It is interesting that the equivalent of the bag_2 and bag_1 fibre types described by Banks & James (1973) did not possess a well-developed nuclear bag.

Table 3. *Classifications of intrafusal muscle fibre types compared with the present classification*

Author, type of study and experimental animal	Original classification	Probable equivalent
Boyd (1962), morphology, cat	Nuclear bag	Bag_1 / Bag_2
	Nuclear chain	Chain
Yellin (1969), histochemistry, rat	A	Bag_2
	B	Bag_1
	C	Chain
	(One other type)	Bag_1
Barker & Stacey (1970), histochemistry and morphology, rabbit	Nuclear bag	Bag_2
	Nuclear chain	Chain
	Intermediate	Bag_1
EM	Nuclear bag	Bag_1
	Nuclear chain	Chain
	Intermediate	Bag_2
Barker, Harker, Stacey & Smith (1972), histochemistry and EM, rabbit	Nuclear bag	Bag_1
	Nuclear chain	Chain
	Intermediate	Bag_2
Morphology	Nuclear bag	Bag_2
	Nuclear chain	Chain
	Intermediate	Bag_1
James (1971*a*), histochemistry, rat; Banks (1971), histochemistry, rabbit	1	Chain
	2	Bag_2
	3	Bag_1
Ovalle & Smith (1972), histochemistry, monkey and cat	Bag_1	Bag_1
	Bag_2	Bag_2
	Chain	Chain
Milburn (1973), histochemistry and morphology rat	Typical bag	Bag_2
	Intermediate bag	Bag_1
	Chain	Chain
EM	Typical bag	Bag_1
	Intermediate bag	Bag_2
	Chain	Chain
Arendt & Asmussen (1974), histochemistry, rat, rabbit, cat, guinea-pig	1 / 2	Bag_1
	3 / 4	Bag_2
	5 / 6	Chain
Banks & James (1975), histochemistry, EM and morphology, rabbit	1	Chain
	2	Bag_2
	3	Bag_1

The two types of ultrastructure present in intrafusal fibres have been described by a number of workers (see Barker, 1974, for review). Nuclear-bag fibres have usually been correlated with the dM type and nuclear-chain fibres with the M type. It is clear from the present work that this is only true in the equatorial region and that in the polar regions the bag fibres change from dM to M except the rat bag_1 fibre. Banks & James (1975) have shown that in the polar regions of rabbit spindles the dM type

(i.e. bag_1) contains a significantly smaller volume proportion of mitochondria than the M type. No attempt was made to subdivide the M type into bag_2 and chain fibres.

The three types of intrafusal fibre have been found to arise sequentially during development in the order bag_2, bag_1, chain (Milburn, 1973 and personal communication). Bag_1 and chain fibres each develop in association with the older bag_2 fibre and separate from this fibre after the fusion of their constituent myoblasts. In the equatorial region of adult cat spindles the chains often continue to be quite closely associated with bag_2. The development of intrafusal fibres involving three generations of myotubes is very similar to the pattern of extrafusal development (Milburn, 1973), as is strikingly suggested by comparison of the neonatal cat spindle (Scalzi & Price, 1971, Fig. 11) with developing rat extrafusal fibres (Kelly & Schotland, 1972, Fig. 3).

SUMMARY

A direct correlation of the histochemical and ultrastructural properties of intrafusal muscle fibres has been achieved by cutting frozen serial sections for histochemical applications (15 μm thick sections) and for electron microscopy (60 μm thick sections) in a repeating sequence.

Three types of intrafusal fibre were recognized, including two types of nuclear-bag fibre, designated bag_1 and bag_2. In addition to histochemical and ultrastructural differences, the three types of fibre differed in length and diameter. Regional variations of histochemical and ultrastructural properties were found.

The results are compared with previous attempts to correlate histochemical and ultrastructural properties of intrafusal muscle fibres based on indirect methods.

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Paper 12.

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HISTOLOGICAL ANALYSIS OF CAT MUSCLE SPINDLES FOLLOWING DIRECT OBSERVATION OF THE EFFECTS OF STIMULATING DYNAMIC AND STATIC MOTOR AXONS

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SUMMARY

1. Eleven cat tenuissimus spindles have been analysed mainly by cutting serial, transverse, 1 μm thick sections following direct observation of the effects of dynamic motor (γ or β) stimulation.

2. Histological results from these spindles were also used to interpret the effects of static fusimotor stimulation of other spindles.

3. Dynamic motor stimulation usually produced contractions seen as convergent movements of sarcomeres in single bag fibres, identified as bag_1 fibres for reasons given in the text.

4. In one spindle a single dynamic axon produced a translational movement in one pole of a bag_1 fibre and a convergent movement in each pole of a bag_2 fibre, together with movements in other unidentified (presumably chain) fibres. Subsequent analysis showed that besides innervating both bag fibres the axon also supplied two chain fibres.

5. Contrary to expectation, motor endings on the bag_1 fibres seldom occurred at the sites of convergent movement. Only two cases of coincidence occurred among sixteen foci and twenty-one motor endings; otherwise focus and nearest ending were separated by distances of 0·85–2·5 mm.

6. Most of the convergent movements of sarcomeres observed in bag_1 fibres occurred in a region of the pole that is ultrastructurally distinct from the region where most of the motor endings were located. The possible relevance of this to the production of contractions in the bag_1 fibre is discussed.

7. Convergent movement foci in bag_2 fibres produced by the stimulation of static axons occurred largely within the same regions of the pole as the motor endings were located, though, whereas foci were observed in both intra- and extracapsular regions, most of the endings were intracapsular.

INTRODUCTION

Repetitive stimulation of single dynamic γ axons elicits junctional potentials in intrafusal muscle fibres (Bessou & Pagès, 1972), and is seen to produce one or several foci of sarcomere shortening in nuclear-bag muscle fibres (Boyd, Gladden,

McWilliam & Ward, 1973; Bessou & Pagès, 1975). Bessou & Pagès (1975) suggested that such foci represent local contractions, pointing out that the region in the spindle pole where they are observed is largely coincident with that from which junctional potentials have been recorded.

The main purpose of the present study was to examine the ultrastructure of the sites of observed contraction produced by dynamic fusimotor stimulation in order to determine the identity of the muscle fibres and motor endings involved. When the experiments began the existence of two types of nuclear-bag fibre in mammalian spindles had been generally recognized, though there was some doubt as to how to classify them into two types. We assumed that they could be identified as 'typical' or 'intermediate' mainly on the basis of the presence or absence of an M line in longitudinal section (Barker, Harker, Stacey & Smith, 1972). However, the results of early experiments proved this to be untenable, and led to other work in which a technique for the combined histochemical and ultrastructural study of individual spindles (Banks, Barker, Harker & Stacey, 1975) established reliable criteria for the recognition of two types of bag fibres, bag_1 and bag_2, as recently described by Banks, Harker & Stacey (1977). These criteria were accordingly adopted, together with the difference in distribution of elastic fibres associated with the two types, as elucidated by Gladden (1976).

In an initial series of twelve experiments the intention was to observe and examine the sites of contraction produced by stimulating single dynamic γ axons. Barker, Stacey & Adal (1970) suggested that such axons terminated in p_2 plates, which they described as possessing wide and shallow post-synaptic folds. We expected to find such plates present at each contraction site, but, as reported in a preliminary account of the work (Banks, Barker, Bessou, Pagès & Stacey, 1976), it soon became obvious that most sites lacked any kind of motor ending. The histological analysis thus became a matter of relating the contraction site to the motor innervation of the bag fibres in the activated pole. Since bag fibres have been described as receiving all three types of fusimotor ending (Barker *et al.* 1970), the endings supplied by the stimulated axon could only be satisfactorily identified by mapping out the distribution of all the fusimotor axons supplying the spindle pole.

It so happened that the first contraction site sectioned was associated with a motor ending. This occurred in only one other instance among eighteen sites examined. In both cases the post-synaptic membranes were smooth, and the terminals belonged to plates whose ultrastructure was very similar to that of p_2 plates previously located and photographed in spindles stained with methylene blue (Barker, Banks, Harker, Milburn & Stacey, 1976*a*). Though this correlation provided a useful standard, experience proved that the identity of fusimotor terminals could not reliably be ascertained solely on the basis of the presence or absence of post-synaptic folding. This feature could only be evaluated in the context of the full analysis.

In a second series of eight experiments we endeavoured to incorporate methylene-blue staining into the experimental procedure after the observation of intrafusal contractions and before fixation of the spindle for electron microscopy. This proved unsuccessful, but data were obtained on the sites of observed contraction produced by a further two dynamic motor axons, as well as fifteen static γ axons.

METHODS

Experimental procedures. Twenty experiments were performed in Toulouse on adult cats anaesthetized with Nembutal (40 mg/kg given I.P., followed by small amounts injected I.V. as necessary). Each experiment was carried out on a spindle located in the distal half of a tenuissimus muscle, the neuromuscular preparation being the same as that described by Bessou and Pagès (1972). The Ia primary axon, and from one to six single motor axons innervating the spindle, were prepared by splitting L7 and S1 dorsal and ventral roots. The static or dynamic function of the motor axons was determined by observing the effects produced by their repetitive 100/sec stimulation on the response of the primary ending to ramp stretch (Matthews, 1962).

The spindle was prepared in the manner described by Bessou & Pagès (1975) for the observation of intrafusal contractions produced by the repetitive (2–110/sec) stimulation of single fusimotor axons. Identification of β axons, as in the case of the dynamic motor axon isolated in experiment 6, depended on detecting the contractions produced by their stimulation both within the spindle and among neighbouring extrafusal muscle fibres. The possibility that some of the other dynamic motor axons isolated had a skeleto-fusimotor distribution cannot be excluded since (i) some of the extrafusal muscle fibres covering the spindle that were removed in order to facilitate observation may have been innervated by branches of the isolated axon; (ii) branches of the isolated axon may have innervated extrafusal muscle fibres some distance away from the area of dissection and observation, so that their contractions would have escaped detection; and (iii) the conduction velocities of the axons ranged from 26·4 to 39·4 m/sec, and axons within this conduction velocity range have been identified at β (Bessou, Emonet-Dénand & Laporte, 1965). The conduction velocity range of the static motor axons was 27·0–53·0 m/sec; we assume that these axons were γ since no static β axons have been reported as conducting at these speeds, and the indications are that the static β component of fusimotor innervation is restricted to axons with conduction velocities above 85 m/sec (Harker, Jami, Laporte & Petit, 1977).

The position of each convergent movement focus produced by stimulating the isolated motor axons was registered by measuring its distance from the point in the equatorial region where the spindle nerve crosses the periaxial space and reaches the intrafusal bundle. Sarcomere movements are seen throughout the pole converging towards this focus. The positions of prominent nerves and blood vessels were similarly registered. This information was subsequently marked on a photograph of the spindle preparation taken at this stage using a $\times 4$ or $\times 10$ objective. Following photography, unsuccessful attempts were made in experiments 13–20 to stain the spindle with methylene blue in order to observe the type and location of the motor endings on the activated intrafusal muscle fibres.

Histological procedures. The procedures relating to the fixation of the spindle, its despatch to Durham and its preparation there for histological analysis, were as described by Barker, Bessou, Jankowska, Pagès & Stacey (1978), except that post-fixation in osmium tetroxide was included.

Sectioning was restricted to the spindles in experiments 1–10 and 12 and was carried out either on an LKB Ultratome UM1 or a Reichert OMU3 ultramicrotome. The type of sectioning employed in the examination of each spindle (skip-serial transverse, serial transverse or serial longitudinal) is indicated in Fig. 1. Sections approximately 1 μm thick cut for light microscopy were stained with 1·7 % toluidine blue in 1·7 % borax or a saturated solution of *p*-phenylene-diamine in methanol. Serial sections of spindles in experiments 1–4, 5 (in part) 7, 8 and 10 were cut on the LKB Ultratome and collected in batches of about five to twenty on glass slides. The last section of each batch was collected separately since the order of the other sections was not always known. Since every section was collected and examined, and since the smallest motor endings occupied fifteen to twenty transverse sections, or about five longitudinal ones, we are confident that every motor ending present was detected. The thickness of transverse sections was estimated by counting the number of sections cut between a given pair of structures marked on the photograph of the living spindle, thereby obtaining a mean section thickness for that distance relative to the living spindle. No correction for longitudinal shrinkage due to processing was therefore necessary. Serial sections of spindles in experiments 5 (in part), 6, 9 and 12 were cut on the Reichert ultramicrotome and collected on strips of cover-glass in ribbons of ten, five such strips being mounted on each glass slide. Section thickness was assumed to be the nominal 1 μm provided by the graduated knife-advance control on the microtome. Longitudinal shrinkage averaged 8 % as

estimated by comparing distances between pairs of structures in the living preparation with the nominal distances provided by summing section thicknesses. This correction has been applied to all measurements quoted for spindles 5, 6, 9 and 12.

Reconstructions of spindles 6, 9 and 12 were made from photographs (enlarged $\times 4\frac{1}{2}$) of each section taken on 35 mm film with a Zeiss Ultraphot using $\times 40$ or $\times 100$ planapo objectives. Any doubtful points were checked on the original sections. Myelinated axons were traced with ease, often for some distance back into the supplying nerve trunks. Owing to their small diameters non-myelinated axons could not always be located in every section, particularly where they coursed between layers of the capsule.

Sections for electron microscopy were cut at selected sites, stained with uranyl acetate and lead citrate, and examined with an A.E.I. EM801 electron microscope at an accelerating voltage of 80 kV.

RESULTS

Observation of intrafusal contractions

The effects of stimulating thirty motor axons innervating twenty tenuissimus spindles were observed. Sixteen of the axons (conduction velocities 26·4–44·1 m/sec) had a dynamic action; one of these (conduction velocity 44·1 m/sec) was identified as a β axon since it elicited both intrafusal and extrafusal contractions. The remaining axons (conduction velocities 27·0–43·0 m/sec) were static. In thirteen spindles the contractions studied were produced by one motor axon (dynamic in ten experiments, static in three), in five spindles by two axons (dynamic in two experiments, static in three) and in two spindles by both static and dynamic axons.

Sarcomere movements of both convergent and translational types (Bessou & Pagès, 1975) were observed, the latter almost exclusively in chain fibres. Table 1 shows the type of muscle fibre (bag or chain) activated in each spindle, the occurrence of translational movements, and the distance of each convergent movement focus from the equatorial reference point. Movements were usually restricted to one pole of the spindle, whether elicited by static axons (as in eleven of fourteen stimulated) or dynamic ones (as in eleven of sixteen stimulated). In two experiments the effects of stimulating two dynamic axons supplying the same spindle were observed. In each experiment both axons activated the same pole, and the site of the convergent movement focus elicited by each axon was located at the same distance from the equatorial reference point (see Table 1, experiments 5 and 11). In experiment 12 a dynamic axon was seen to activate both bag fibres present in the spindle as well as other unidentified (presumably chain) fibres. In two other experiments (17 and 18) one bag fibre was activated by a dynamic axon, whereas contractions in the other bag fibre present were elicited by either one or two static axons.

A total of twenty-four convergent foci were observed in those experiments in which the stimulation of a dynamic axon activated one bag fibre only (i.e. all except experiment 12). The distances of these foci from the equatorial reference point ranged from 2·05 to 4·34 mm (mean 3·51 mm). The activation of bag fibres by static axons, on the other hand, produced thirteen convergent foci that were mostly located nearer to the equatorial reference point; thus twelve were located at distances of between 1·22 and 2·90 mm (mean 1·94 mm), and only one was observed further away at 4·20 mm. Five foci elicited by static axons in chain fibres were located at distances of 0·8–2·08 mm (mean 1·49 mm).

TABLE 1. Location of intrafusal contractions observed in cat tenuissimus spindles on stimulating single static or dynamic motor axons

The letters A, B are used to indicate the activation of separate bag fibres in a spindle, or the activation of the same bag fibre in both poles. C.v. conduction velocity. tr, translational movements of sarcomeres; numbers following indicate number of chain fibres showing such movements; tr* indicates that such movements were observed in a number of unidentified fibres.

Expt. and spindle no.	C.v. (m/sec) of static (S) or dynamic (D) axon	Occurrence of translational movements; distance (mm) of convergent movement foci from equatorial reference point			
		Proximal pole fibres		Distal pole fibres	
		Bag	Chain	Bag	Chain
1	35·0 D	2·05	—	—	—
2	30·5 D	4·15	—	—	—
3	36·1 D	3·82	—	—	—
4	30·9 D	—	—	3·32	—
5	*a* 36·0 D	A 3·68	—	A 3·90, 4·10	—
	b 34·0 D	A 3·68	—	—	—
6	44·1 D (*β*)	A 3·90	—	A 2·90	—
7	38·1 D	2·28, 3·35	—	—	—
8	33·7 D	A 3·10	—	A 3·64	—
9	39·4 D	—	—	3·82, 4·05	—
10	34·5 D	4·34	—	—	—
11	*a* 26·4 D	—	—	A 3·34	—
	b 30·0 D	A 2·80, 3·40, 3·80	—	A 3·34	—
12	29·1 D	—	tr*	A tr	—
		B 1·00	—	B 1·25	—
13	? S	tr	tr 2–4	2·02	—
14	27·0 S	—	—	—	tr 3
15	*a* 42·0 S	1·80	tr*	—	—
	b 36·2 S	—	—	1·36	tr 2–3
16	*a* ? S	2·70	tr 1–3	—	0·80
	b ? S	—	1·85, tr 2–3	—	—
17	*a* 33·7 D	A 3·60	—	—	—
	b 32·0 S	B 4·20, 1·34	tr 1–2	—	—
18	*a* 28·0 D	—	—	A 3·84	—
	b 33·3 S	—	—	—	2·80, tr 1
	c 43·0 S	—	—	B 2·90, 2·52	—
	d 32·5 S	—	—	—	1·38, tr 1
	e 35·0 S	—	—	B 1·92	—
19	35·5 S	1·22	—	1·33, 2·35	tr 2
20	*a* ? S	—	1·34	—	—
	b ? S	1·78	tr 1–2	—	—

Histological analysis of spindles activated by dynamic motor axons

Numbers of motor endings on bag and chain fibres. Each spindle examined possessed one bag_1 fibre, one bag_2 fibre, and either four or five chain fibres. Analysis of the motor innervation of three spindles (6, 9 and 12) and one half spindle (5) reconstructed from serial transverse sections showed that the poles of chain fibres received fewer endings than those of bag fibres. Two of thirty chain-fibre poles received no motor innervation; among the rest the usual provision was one ending, the maximum

three. The minimum supply to fourteen bag-fibre poles, on the other hand, was two endings, the maximum four (see Table 2).

Location of motor endings on activated intrafusal fibres. Glycogen-depletion experiments have shown that when dynamic γ or β axons activate a single bag fibre in a spindle it is always a bag_1 fibre (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1976*b*, 1977). Of the spindles examined histologically (1–10 and 12) we may therefore assume that the single bag fibres activated in experiments 1–10 were bag_1 fibres. During the experiment on spindle 12 it was observed that the bag fibre activated in the distal pole only (A in Table 1) lay close to an intramuscular nerve trunk coursing alongside, whereas the bag fibre activated in both poles (B in Table 1) lay further away. Thus orientated as we sectioned, we were able positively to identify fibre A as bag_1 and fibre B as bag_2.

TABLE 2. Frequency of different numbers of motor endings per pole supplied to intrafusal muscle fibres in spindles 5, 6, 9 and 12

	Number of motor endings per pole				
Fibre type	0	1	2	3	4
Bag_1	—	—	5	1	1
Bag_2	—	—	4	3	—
Chain	2	20	7	1	—

Total no. of endings: 71

In Fig. 1 the poles of the bag_1 fibres activated in spindles 1–10 and 12 are represented as horizontal bars on which the observed sites of convergent foci and the location of motor endings, as ascertained by sectioning, are indicated according to their distances from the equatorial reference point. In the case of spindle 12 the bag_2 fibre is similarly represented. The positions of sixteen convergent foci and twenty-one motor endings are shown on bag_1 fibres, and it will be seen that focus and ending are coincident (to within 50 μm) in only two instances (in spindles 1 and 7). Among the rest, focus and ending are separated by distances of between 0·85 mm and 2·55 mm; in the case of spindle 5, in which stimulation of two dynamic axons produced the same focus in the proximal pole, two separately innervated endings lay apart from the single focus at distances of 1·75 and 2·45 mm. Moreover, whereas the convergent foci were observed to occur at distances of between 2·05 mm and 4·34 mm from the equatorial reference point, the motor endings were located closer in at distances of between 1·15 and 3·05 mm. This feature is further illustrated

Fig. 1. Schematic representation of nuclear-bag muscle fibres activated in cat tenuissimus spindles by stimulation of single dynamic motor axons. Positions of observed convergent foci (apposed arrows) and histologically located motor endings (filled triangles) are indicated according to their distances from the equatorial reference point. Fibres are of bag_1 type in spindles 1–10 and both bag_1 (b_1) and bag_2 (b_2) in spindle 12. They are represented as horizontal bars in which filled regions indicate serial longitudinal sectioning, open regions serial transverse sectioning (skip serial where lines interrupted). Where ultrastructure has been examined, condition of M line is indicated as *M*, present; *dM*, faint double line or absent; *T*, transitional between *M* and *dM* conditions.

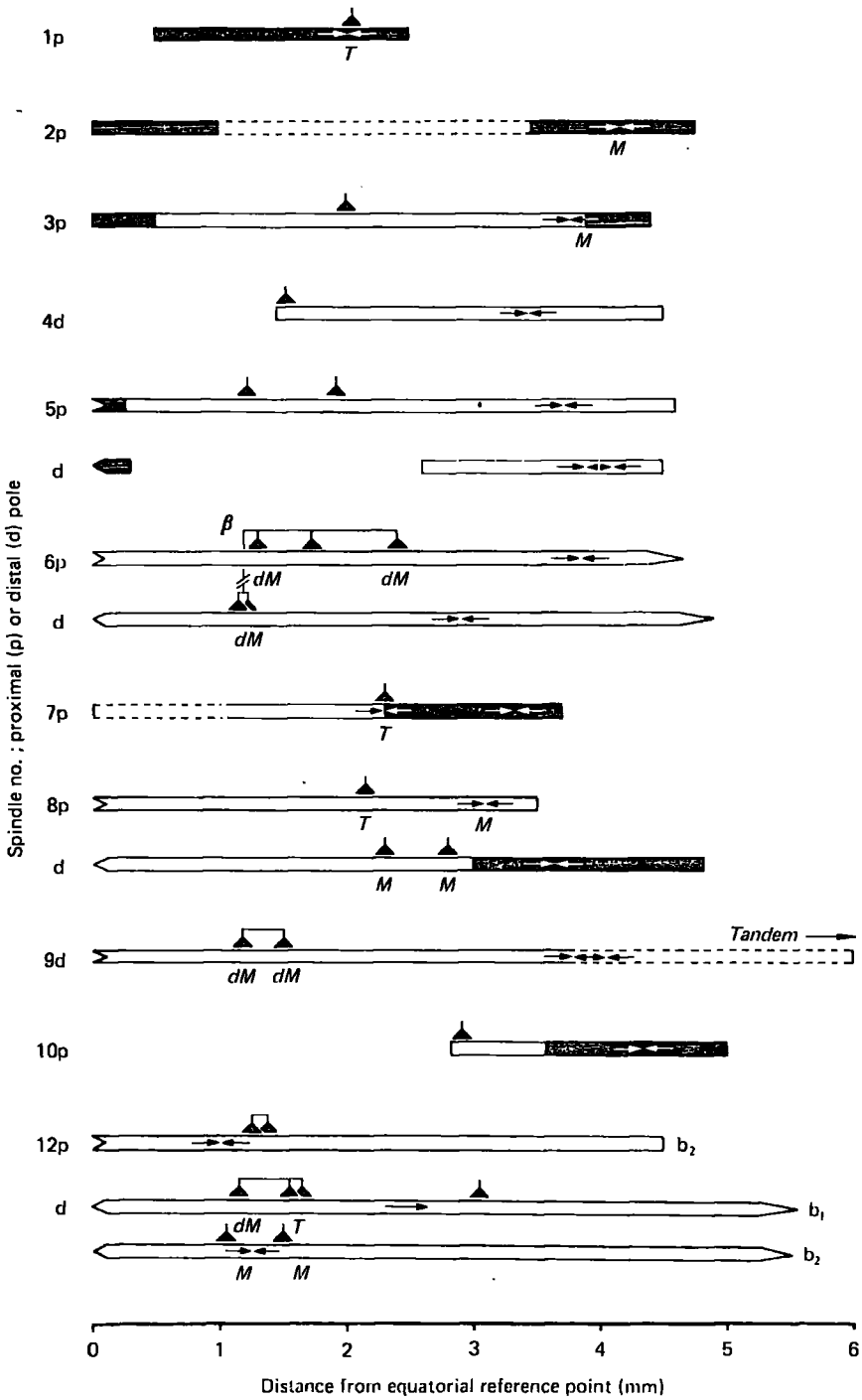


Fig. 1. For legend see facing page.

in Fig. 2*A*, *B* which compares the distances from the equatorial reference point of the sites of all convergent foci observed in bag_1 fibres in spindles 1–12, 17 and 18, with the sites of all motor endings located on the activated poles of bag_1 fibres in spindles 1–10 and 12. In terms of the ultrastructure of bag_1 fibres the significant point to emerge is that, whereas most of the convergent foci are located in the extracapsular region, where the sarcomeres possess M lines (region C, M-type sarcomeres; see Banks *et al.* 1977), most of the motor endings are located in the intracapsular

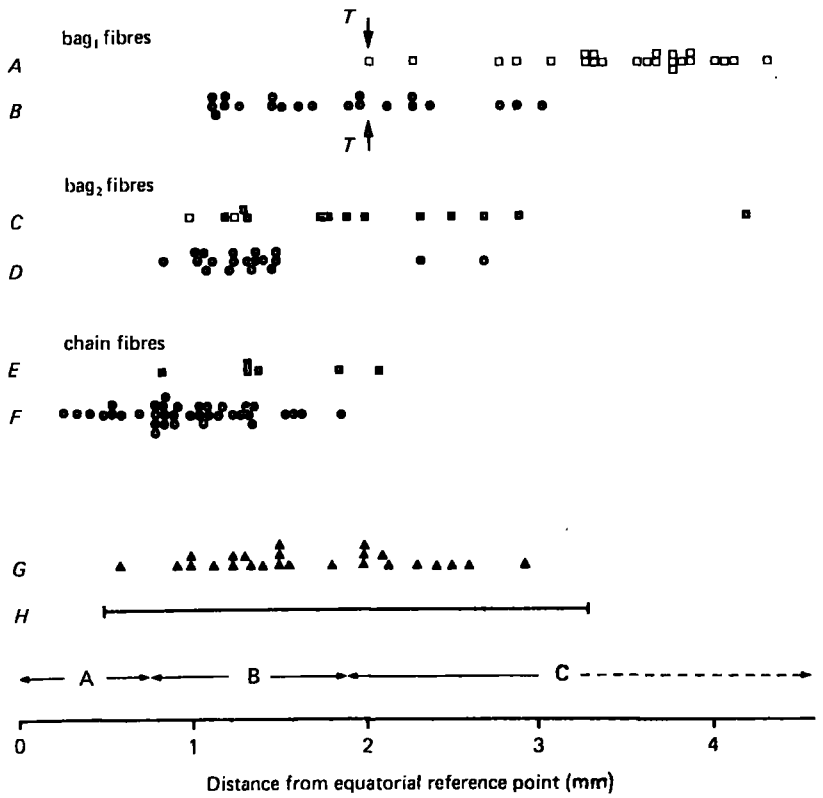


Fig. 2. *A–F*, location of convergent foci observed in cat tenuissimus spindles on stimulating single motor axons, dynamic in *A*, *C* (open squares), static in *C*, *E* (filled squares), compared with motor endings histologically located on bag_1 fibres (*B*), bag_2 fibres (*D*) and chain fibres (*F*). All locations are indicated according to their distances from the equatorial reference point, and may be related to the intracapsular (A, B) and extracapsular (C) regions of the pole; levels A/B and B/C are mean distances calculated from measurements made in spindles 6, 9 and 12. *A*, foci observed in bag_1 fibres in spindles 1–12, 17 and 18. *B*, motor endings located on activated poles of bag_1 fibres in spindles 1–10 and 12. Arrows labelled *T* indicate mean of four locations of transitional dM/M-type sarcomeres in bag_1 fibres. *C*, foci observed in the bag_2 fibre of spindle 12 and in the presumed bag_2 fibres in spindles 13 and 15–20. *D*, motor endings located on bag_2 fibres in spindles 6, 8, 9 and 12. *E*, foci observed in chain fibres in spindles 15, 16, 18 and 20. *F*, motor endings located on chain fibres in spindles 6, 9 and 12. *G*, intracellular recording sites of junctional potentials elicited by stimulation of single dynamic motor axons. *H*, range of location of intracellular recording sites of junctional and spike potentials elicited by stimulation of static motor axons. Data for *G*, *H* from Bessou & Pagès, 1972, Fig. 1, and Barker *et al.* 1978, Table 1.

region where the sarcomeres lack M lines, or possess faint double ones (region B, dM-type sarcomeres). The two convergent foci that occurred nearest to the equatorial reference point (in spindles 1 and 7) were both located in a part of the fibre where the sarcomeres are transitional between dM and M types.

This marked discrepancy in the location of convergent foci relative to motor endings does not occur in bag_2 fibres. Fig. 2*C* plots the location of convergent foci elicited by static axons in bag fibres (presumably bag_2) belonging to spindles 13 and 15–20. The two foci elicited by the dynamic axon in spindle 12 and positively identified as occurring in a bag_2 fibre are also included. For comparison, Fig. 2*D*

TABLE 3. Distribution of motor axons innervating three spindles
Data relevant to the dynamic axon stimulated in each experiment are shown in italics

Expt. and spindle no.	No. static (S) or dynamic (D) axons stimulated in ventral roots	No. axons entering spindle	Muscle-fibre type(s) supplied	Proximal (p) or distal (d) pole supplied
6	1 D (β)	*1*	*b_1*	*d, p*
	2 S	1	b_2	p
		1	c	d
		2	c	p
		2	b_2c	d
9	1 D	*1*	*b_1*	*d*
		1	b_1	p
	4 S	1	b_2	d
		1	c	d
		1	b_2c	d
		2	b_2c	p
12	1 D (?β)	*1*	*b_1*	*d*
		1	*b_2*	*d*
		1	*b_2c*	*p*
		1	b_1	d
	3 S	1	b_2	d
		1	c	d, p
		1	b_1c	p

shows the distances from the equatorial reference point of motor endings located by sectioning on bag_2 fibres in spindles 6, 8, 9 and 12. Foci and endings are seen to occur largely within the same regions (B, C) of the pole, though there is some discrepancy in that most of the endings are located in region B.

Distribution of motor axons innervating three spindles. In experiments 6, 9 and 12 it was possible to compare the number of motor axons, static and dynamic, that activated a spindle on their being stimulated in the ventral roots, with the number of motor axons traced histologically to their entry and distribution within the same spindle. The data are summarized in Table 3, which shows that, whereas the number of ventral-root axons stimulated varied from three to five (total twelve), the number of axons found at spindle entry in each case was seven (total twenty-one). The higher number of axons found at entry may be accounted for partly by intramuscular branching, and partly by the failure to isolate all the supplying axons in the ventral roots.

The distribution of the dynamic axon activated in experiment 12 is seen to involve

all three fibre types. It approached the spindle as three separate branches in an intramuscular nerve trunk that carried axons supplying extrafusal muscle fibres as well as the spindle. The fact that one of the branches contributed extensively to this extrafusal supply before spindle entry suggests the possibility that the stimulated axon may have had a skeleto-fusimotor distribution. The branch that activated the proximal pole of the bag_2 fibre also innervated two chain fibres in the pole.

Of the five axons entering these spindles that belonged to the three dynamic axons stimulated in the ventral roots, four selectively innervated bag fibres (three bag_1 fibres, one bag_2 fibre) and one innervated both bag_2 and chain fibres. Two other axons selectively innervated bag_1 fibres in spindles 9 and 12; presumably they belong to dynamic axons that were not isolated for stimulation during the experiments.

The nine static axons stimulated in the ventral roots provided fourteen at spindle entry, eight axons selectively innervating either bag_2 (three) or chain (five) fibres, and six distributed to both bag and chain fibres (five to bag_2 and chain fibres, one to bag_1 and chain fibres).

Full details of these reconstructed spindles will be published in a separate paper.

Ultrastructure of motor endings. The ultrastructure of sixteen motor endings innervating the activated poles of bag_1 fibres was examined. With one exception, the post-synaptic membranes of all these endings were smooth whether they were located on M, dM, or transitional dM/M-type sarcomeres. The exception was a plate located on M-type sarcomeres in the extracapsular region of the distal pole of the bag_1 fibre in spindle 12. This possessed small junctional folds and an obvious sole plate; these are features which, taken in conjunction with the source of the supplying axon, suggest a p_1 plate supplied by a β axon. By contrast, the five endings located on dM-type sarcomeres supplied by the β axon to the intracapsular region B in both poles of the bag_1 fibre in spindle 6 had smooth post-synaptic membranes and lacked sole plates.

The ultrastructure of the ending supplied to the proximal pole of the bag_1 fibre in spindle 1 has been described and illustrated in longitudinal section by Barker *et al.* (1976*a*). It closely resembles that of similarly sectioned p_2 plates previously located and photographed in spindles stained with methylene blue. The ultrastructure of four endings transversely sectioned in spindles 7 and 8 could be interpreted as that of p_2 plates in the context of the experiments. However, a smooth post-synaptic membrane is not a diagnostic feature of p_2 plates, since it may also be present in the trail terminals of static axons on bag_2 fibres. The myoneural junctions formed by such axons on bag_2 or chain fibres may be either smooth or folded, with the deepest and most regular foldings occurring on chain fibres (cf. Barker *et al.* 1978).

Correlation between dynamic motor innervation and dynamic primary-ending response

Fig. 3 illustrates the responses of the primary endings in spindles 6, 9 and 12 to ramp stretch, with and without stimulation of the isolated dynamic motor axon that supplied each spindle. The greatest increase in the dynamic index is seen to be produced by the β axon in spindle 6, which innervated both poles of the bag_1 fibre. In spindle 9, where only one pole of the bag_1 fibre was activated, the increase in dynamic index is less. The response of the primary ending in spindle 12 contains a smaller

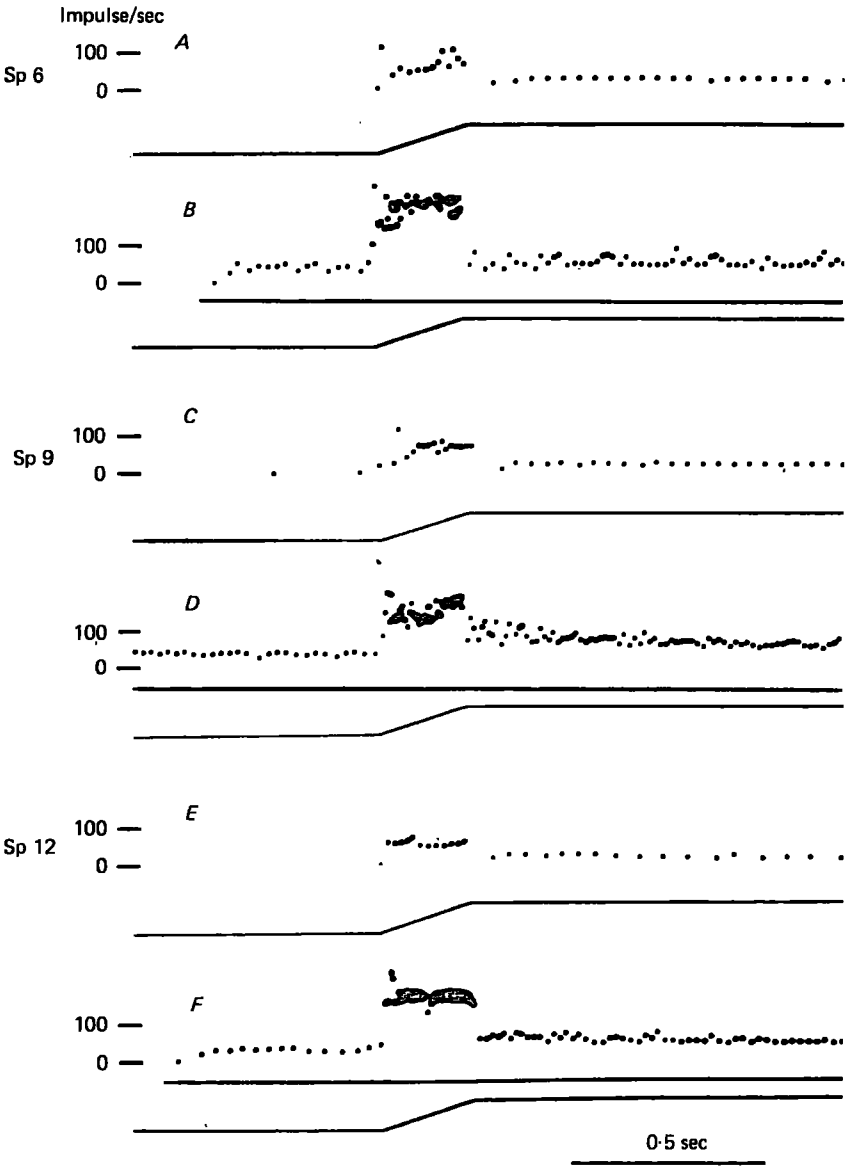


Fig. 3. *A–F*, responses of the primary endings in spindles 6, 9 and 12 to ramp-and-hold stretch without fusimotor stimulation (*A*, *C*, *E*) and during repetitive stimulation, at 100Hz, of single dynamic motor axons (*B*, *D*, *F*). The recordings are of the instantaneous frequency of firing expressed in impulses/sec. Each spot represents one action potential from the primary ending, the vertical displacement being inversely proportional to the time between it and the previous spike. The lower trace shows the stretch of 5 mm amplitude applied at 20 mm/sec to the tenuissimus muscle. In records *B*, *D*, *F*, the horizontal line between the instantaneous frequency and stretch records represents the period of stimulation of the dynamic motor axon. The conduction velocities of these axons and the types of intrafusal fibre they supplied in spindles 6, 9 and 12 may be found in Tables 1 and 3, respectively. The stimulated axon in *B* induced visible contractions of both intra- and extrafusal muscle fibres (skeleto-fusimotor or β axon); in *D* contractions were seen only in an intrafusal muscle fibre (fusimotor or γ axon); and in *F* contractions were seen only in intrafusal muscle fibres, but histological findings suggested that the axon also supplied extrafusal muscle fibres and may have been a β axon.

static component than might be expected in view of the activation of bag_2 and chain fibres. The more regular firing of the primary ending of this spindle during the ramp stretch may be due to the activation of the bag_2 fibre (see Emonet-Dénand, Laporte, Matthews & Petit, 1977).

DISCUSSION

We assumed at the outset of this study that the site of a convergent movement elicited in a nuclear-bag fibre by stimulating a dynamic motor axon would, when sectioned, reveal the motor ending activated. We found, on the contrary, that motor endings were seldom present at such sites but lay a considerable distance apart from them. In bag_1 fibres the convergent movements, with only two exceptions, occurred considerably further from the equator than the motor endings, usually in a region ultrastructurally distinct from that in which the endings were located (Fig. 2*A*, *B*).

Intracellular recording in the region occupied by the motor endings (Fig. 2*G*) has revealed only junctional potentials during dynamic motor stimulation (Bessou & Pagès, 1972; Barker, Bessou, Jankowska, Pagès & Stacey, 1978), and experiments in which the activated muscle fibres have been identified histologically, following injection with Procion Yellow, have shown them to be predominantly bag_1 fibres (Barker *et al.* 1978). How, then, is the excitation transmitted along these fibres from the endings to the sites of convergent movement, sometimes over 2 mm away, in sufficient strength to cause a visible shortening? Koketsu & Nishi (1957) have calculated the length constant (λ) of the frog intrafusal muscle fibre as 0·72 mm; if the value for the cat bag_1 fibre is similar, the depolarization at the convergent movement focus, assuming only junctional potentials to be involved, could be as low as one twentieth that at the motor ending. Arbuthnott, Boyd, Gladden & McWilliam (1977) suppose that the connexion is mechanical and brought about by stretch activation. They suggest that a motor ending in the intracapsular region elicits a convergent movement beneath it, and that the resultant shortening stretches the pole, which responds directly by contracting to produce another, more polar convergent movement at a site where there is no motor ending. Our results do not support this suggestion, since the location of the majority of motor endings (nineteen of twenty-one) in the eleven spindles analysed was not coincident with a convergent movement, and only in four of the nineteen poles of bag_1 fibres activated by dynamic axons was more than one convergent movement observed.

We believe that the ultrastructural heterogeneity of the bag_1 fibre is an important factor affecting the situation. In the region where the motor endings are located the absence of M lines from the sarcomeres and the presence of junctional potentials during dynamic stimulation combine to suggest that this part of the bag_1 fibre is a slow system. However, in the region where the convergent movements occur the possibility that the bag_1 fibre behaves like a twitch fibre is suggested by the presence of M lines (Hess, 1970). As yet no intracellular recordings are available from this region. In the light of these considerations we have suggested (Banks *et al.* 1976) that the membrane of the muscle fibre may be electrically excitable in the extracapsular region where the convergent movements occur, and that it may propagate action potentials if sufficient depolarization is produced by summation of junctional potentials. A convergent movement could then be produced by the preferential

shortening (Huxley & Peachey, 1961) of sarcomeres in a portion of the fibre where they were already shorter than their neighbours. There is also the possibility that the excitation-contraction coupling mechanism is more efficient where the convergent movements occur than where the motor endings are located. Some support for our suggestions is provided by experiment 5 in which the individual stimulation of two dynamic axons activated separate plates on a bag_1 fibre in the intracapsular region, and each produced a convergent movement located at the same extracapsular site.

It is possible that the contraction of bag_2 fibres may be effected in a similar fashion. The sites of the convergent movements produced by static stimulation in our experiments were spread throughout the length of the pole, whereas the location of most of the motor endings was intracapsular (Fig. 2*C*, *D*). This suggests that the sites of convergent movements are seldom coincident with those of endings, though there is no ultrastructural heterogeneity present, as in bag_1 fibres, which could produce a marked displacement in their relative distribution. It has been established by Barker *et al.* (1978) that static axons can elicit both junctional (seven examples) or action (one example) potentials in bag_2 fibres.

Boyd, Gladden, McWilliam & Ward (1977) observed that in 'nuclear bag fibres controlled by dynamic γ axons' (i.e. mostly bag_1 fibres) convergent movement foci occurred between 1·4 and 3·1 mm from the equator. This compares with our range in bag_1 fibres of 2·05–4·34 mm. This discrepancy is difficult to explain, but may arise from the different sample size (eight in their work, fifteen in ours), variation in individual spindle length, and finally the degree of stretch applied to the preparation.

A necessary basis for investigations which have made a cinematographic analysis of intrafusal contractions in living spindles (Bessou & Pagès, 1975; Boyd & Ward, 1975; Boyd, 1976; Boyd *et al.* 1977) has been the assumption that all contractions elicited by fusimotor stimulation produce visible movement open to unambiguous interpretation. That this may not always be so has been discussed by Bessou & Pagès (1975). The limitations of the method and the difficulty of interpretation may explain why Boyd & Ward (1975) reported that in 40 % of the spindles observed by them the two bag fibres shared some innervation in common, though this was later denied by Boyd (1976).

The glycogen-depletion studies of Barker *et al.* (1976*b*, 1977) and the analysis of sub-categories of dynamic and static fusimotor action by Emonet-Dénand, Laporte, Matthews & Petit (1977) have shown that, although dynamic motor axons (γ and β) predominantly innervate bag_1 fibres, they also occasionally innervate bag_2 and chain fibres. Our results confirm this: among the three spindles most extensively analysed (6, 9 and 12) the stimulated dynamic axon was distributed solely to the bag_1 fibre in two, but innervated all three fibre types in the third. We may also note that among the fourteen static axons, or axon branches, entering these spindles, thirteen innervated either bag_2 or chain fibres or both, and one was supplied to bag_1 and chain fibres (see Table 3).

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Paper 13.

R.W. Banks, D. Barker & M.J. Stacey (1979)
Sensory innervation of cat hind-limb muscle spindles.
J. Physiol. 293; 40-41P.

[*From the Proceedings of the Physiological Society*, 20–21 *April* 1979
Journal of Physiology, **293**, 40–41*P*]

Sensory innervation of cat hind-limb muscle spindles

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Reconstructions of primary and secondary endings from serial 1 μm transverse sections of tenuissimus spindles revealed differences in the disposition of terminals on bag$_1$ (b_1) and bag$_2$ (b_2) fibres, which can be recognized in teased, silver preparations. We were therefore able to examine such preparations of spindles from various hind-limb muscles and ascertain the distribution of primary and secondary afferents to b_1, b_2 and chain (c) muscle fibres.

Among 245 primary afferents, 204 had a b_1b_2c distribution, 30 b_2c, 4 b_1c, 6 b_1c, and 1 b_1b_2. Restriction to two fibre types occurred in double primary endings (ten in sample) and in tandem linkages in which the b_2 fibre was continuous from one capsule, where it was accompanied by b_1 and c fibres, to another, in which it was accompanied by c fibres only (usually two) and usually lacked a nuclear bag. The diameter range of the b_2c primary afferents supplying these one-bag-fibre spindles was 2–9 μm (peak 4 μm). Most (82 %) fell within the same diameter range as the b_1b_2c primary afferents (4–12 μm, peak 6 μm); they would thus overlap in conduction velocity, but would presumably lack dynamic sensitivity. The proportion of such afferents supplying peroneus brevis was 25·0 % (9 of 36), tenuissimus 5·4 % (4 of 74), and superficial lumbrical 3·1 % (1 of 32).

Spindles with b_1,b_2, and c fibres may sometimes have more than one b_1 fibre, rarely more than one b_2. Among 204 b_1b_2c primary afferents, 24 were distributed to spindles with two b_1 fibres, 2 to spindles with three b_1 fibres, and 1 to a spindle with two bag fibres of each type. The proportion of spindles with more than one b_1 fibre was 26·7 % in superficial lumbrical (8 of 30), 11·5 % in peroneus brevis (3 of 26), and 6·3 % in tenuissimus (4 of 64).

The distribution of 197 first-order branches of 88 b_1b_2c primary afferents was 'segregated' in 67·6 % (i.e. the branches exclusively supplied either b_1, or b_2 and c, or c fibres), and 'mixed' in 32·4 %, as follows (%): b_1, 23·9; b_2, 4·1; c, 11·7; b_2c, 27·9; b_1b_2c, 16·2; b_1c, 13·2; b_1b_2, 3·0. Mixed distributions occurred more frequently among 22 superficial lumbrical afferents (77·2 %) than among 29 tenuissimus afferents (38 %).

Most secondary afferents terminated on all three muscle-fibre types as endings located in the S_1 position. Of 273 secondary afferents, 73·3 % had a b_1b_2c distribution, 14·0 % b_2c, 8·0 % b_1c, and 4·7 % c. The diameter range of 193 b_1b_2c secondary afferents was 1–7 μm (peak 3 μm); 40·9 % fell within the same diameter range as the b_1b_2c primary afferents. The maximum area of innervation on b_1 fibres in b_1b_2c secondaries was typically about half that supplied to b_2 fibres.

Paper 14.

R.W. Banks, P. Bessou, M. Joffroy & B. Pagès (1979)
Reflex responses of pools of gastrocnemius lateralis gamma-motoneurones elicited by ipsilateral sural nerve stimulation in the cat.
J. Physiol. 296; 107P.

[*From the Proceedings of the Physiological Society*, 13–14 *July* 1979
Journal of Physiology, **296**, 107*P*]

Reflex responses of pools of gastrocnemius lateralis γ motoneurones elicited by ipsilateral sural nerve stimulation in the cat

By R. W. Banks*, P. Bessou, M. Joffroy and B. Pagès. *Department of Physiology, Faculty of Medicine, 31077 Toulouse Cedex, France*

Reflex responses of intact α and γ motoneurones of gastrocnemius lateralis muscle (LG) to stimulation of the ipsilateral sural nerve, and their consequences on group I and group II afferent discharges from LG, were observed in decerebrate cats. Two electrodes, placed 4 mm apart on a thin branch of the LG nerve, each provided monopolar recording. Action potentials in each class of neurone were segregated electronically according to their direction of propagation and conduction velocity (Joffroy, 1975). The activity of each neuronal group was displayed as the instantaneous mean firing-frequency and was considered to represent that of the homonymous pools contributing to the whole LG nerve.

When present, responses of the γ motoneurones always appeared as increases in their mean firing-frequency. This was brought about by increased activity of tonically firing axons and recruitment of previously silent ones, though simultaneous inhibition of some neurones cannot be excluded. Generally, a single stimulus was effective provided that groups III and IV were stimulated. However in half the experiments repetitive stimulation (2–4 stimuli; 10–50/sec) of group II axons alone evoked a small increase (10–20/sec) of the mean firing-freqency. The amplitude and duration of the γ reflex responses increased successively with the recruitment of groups III and IV, firing rates returning to initial levels some 3–15 sec after cessation of stimulation. Selective stimulation of group III or group IV fibres using anodal blocking of larger diameter axons (Accornero, Bini, Lenzi & Manfredi, 1977) always increased the total γ activity. This is in agreement with the results of Catley & Pascoe (1978) obtained from single fusimotor fibres in the rabbit.

Alpha and γ motoneurones were generally co-activated, though the γ response was earlier. The consequent isometric contractions were accompanied by increases in the mean firing-frequencies of group I and group II afferent fibres in the LG nerve branch. The increase in group I activity probably resulted from the activation of muscle-spindle primary endings and Golgi tendon organs, whereas that of group II can definitely be attributed to muscle-spindle secondary endings. In addition to activation by contractions of intrafusal muscle fibres, primary and secondary endings of spindles may have been stimulated by the asynchronous contractions of asymmetrically located extrafusal motor units.

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Paper 15.

R.W. Banks, P.H. Ellaway & J.J. Scott (1981)
Responses of de-efferented muscle spindles of peroneus brevis and tertius muscles in the cat.
J. Physiol. 310; 53P.

[*From the Proceedings of the Physiological Society*, 12 *September* 1980
Journal of Physiology, **310**, 53*P*]

Responses of de-efferented muscle spindles of peroneus brevis and tertius muscles in the cat

By R. W. Banks, P. H. Ellaway and J. J. Scott*. *Departments of Anatomy and Physiology, University College London, Gower Street, London WC1E 6BT*

In a tandem muscle spindle one capsule may lack the bag_1 intrafusal muscle fibre. Nevertheless, the bag_2 and chain fibres in that capsule do have a primary afferent ending (Banks, Barker & Stacey, 1979). These authors found a high proportion of such spindles in the peroneus brevis (PB) muscle of the cat and suggested that the primary endings lacking terminals on bag_1 fibres should have a low dynamic sensitivity to stretch of the muscle.

We have examined the responses of PB spindle afferents to ramp and hold stretches. The results were compared with those from similar experiments using peroneus tertius (PT), which was chosen because a comparison of its primary and secondary afferents was already available (Jami & Petit, 1979). Experiments were performed on adult cats anaesthetized with pentobarbitone sodium (Sagatal). The left hind limb was denervated extensively except for the appropriate muscle nerve. The muscle tendon was freed from its insertion and attached to an electromagnetic puller. Dorsal and ventral spinal roots L7 and S1 were cut ipsilaterally, and functionally single afferents were isolated in dorsal root filaments. Ramp stretches of 2–2·5 mm amplitude were applied to the muscle at velocities of 11 or 22 mm sec^{-1}. Maximum physiological length was not exceeded during stretch. Afferent responses were recorded for measurement of dynamic index (difference between peak firing rate towards the end of the ramp and at 0·5 sec after its completion) and static response (difference between firing rate before the ramp and that 0·5 sec after its completion).

When dynamic index was plotted against conduction velocity of the afferent axon, spindle afferents could not, for either muscle, be separated into two groups corresponding to primary and secondary endings. As was found by Jami & Petit (1979) for PT, plotting the ratio of dynamic index to static response against conduction velocity did produce more of a separation with units conducting above 70 m/sec having the higher ratios.

Further separation of the afferents was revealed when dynamic index was plotted against static response. No correlation was evident for the complete population but, for a given static response, an afferent conducting at more than 70 m/sec almost invariably had a greater dynamic index than a more slowly conducting afferent.

In none of these plots for either muscle was a third group of afferents evident which might have indicated the presence of a particular group of primary endings with low dynamic sensitivity corresponding to the b_2/chain endings of Banks, Barker & Stacy (1979).

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* S.R.C. Scholar.

Paper 16.

R.W. Banks (1981)
On the form of the Z-disk in skeletal muscle of the rat.
J. Anat. 133; 157-159.

[*From the Proceedings of the Anatomical Society of Great Britain and Ireland,* 1–3 *April* 1981. J. Anat., (1981), **133**, 1, p. 157–159]

D.18. On the form of the Z-disc in skeletal muscle of the rat. By R. W. BANKS. *Department of Anatomy and Embryology, University College, London*

It has recently been shown that the networks of T-tubules in adjacent sarcomeres of frog muscle fibres are interconnected in a helicoidal manner (Peachey & Eisenberg, *Biophys. J.* **22**, 1978). This presumably reflects a similar geometry of the sarcomeres themselves. I have examined the accessorius muscle of the hind foot of the rat to see if corresponding sarcomeric arrangements occur in mammalian skeletal muscle. Serial, 1 μm thick plastic-embedded sections were cut longitudinally from muscles fixed under tension. The sections were stained with toluidine blue, and micrographs were used to make reconstructions of the Z-discs of portions of two muscle fibres, each containing about 13 sarcomeres.

Deviations from a simple planar form of the Z-disc occurred by longitudinal displacement o-corresponding parts of several adjacent discs. The complementary parts of the Z-discs defined sarcomeres of slightly different lengths, so that, when the number of sarcomeres in the myofibrils adjacent to the displacement differed by one, the complementary parts were again in register. The visual effect was similar to that of a vernier measuring scale.

Such 'vernier figures' were very commonly seen in any longitudinal section of the whole muscle; however, only in the reconstruction could the full complexity of the form of the Z-discs be realized. Thus, in some cases, corresponding parts of adjacent Z-discs were interconnected by the displaced complementary parts, producing helicoids with pitches of one or two sarcomeres. Both vernier figures and helicoids reflect the same structural feature, that is local variation in relative sarcomere length. If there is any functional significance in the present observations it is probably to be sought in that variation.

Paper 17.

R.W. Banks (1981)
The number and distribution of satellite cells of intrafusal muscle fibres in a muscle spindle of the cat.
J. Anat. 133; 694.

[*From the Proceedings of the Anatomical Society of Great Britain and Ireland.*
July 1981. J. Anat., (1981), **133**, 4, p. 694]

D.9. The number and distribution of satellite cells of intrafusal muscle fibres in a muscle spindle of the cat. By R. W. BANKS. *Department of Anatomy and Embryology, University College London* (Fig. 14)

The numbers of satellite cells and their distribution on the three types of intrafusal muscle fibre (Banks *et al. J. Anat.* **123**, 1977) have been determined in one pole of a cat tenuissimus spindle serially sectioned at 1 μm intervals and stained with toluidine blue. Observations in selected regions of other spindles indicate that the results are generally applicable. Satellite cells were distinguishable from subsarcolemmal myonuclei by the presence around their nuclei of thin rims of palely stained cytoplasm, that continued beyond the ends of each nucleus as a gradually tapering, small eminence on the surface of the muscle fibre. Occasionally it was possible to confirm electron microscopically that these profiles were satellite cells. Mean length of the cells was 17·0 μm (range 5–40 μm, n = 121) though the true length was probably greater than this since the finely tapered ends of the cells may not always have been visible.

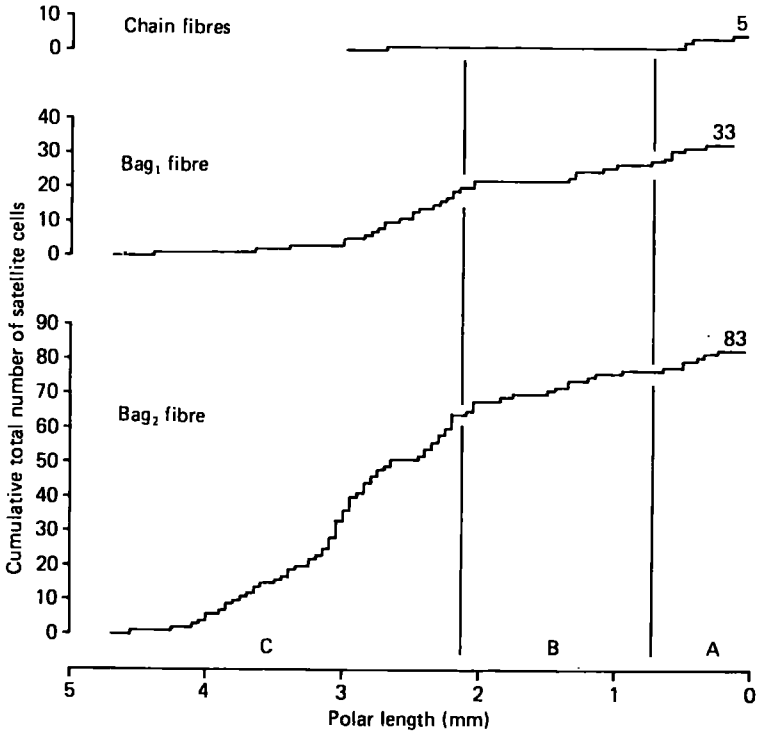


Fig. 14

The Figure illustrates the cumulative total numbers of satellite cells for each of the three types of intrafusal muscle fibre. In this spindle there were four chain fibres, one bag_1 and one bag_2 fibre. Letters A, B, C refer to the periaxial space, the capsular and the extracapsular poles respectively. The length scale is approximately calibrated in mm and counts from the primary sensory ending. Note that the great majority of satellite cells occur on the bag_2 fibre, with over half the total on its extracapsular portion alone. There is a minimum density of cells on each bag fibre towards the end of the capsule as revealed by the slope of the curves. Of the five cells on the chain fibres three were each common to two muscle fibres. It is noteworthy that the order bag_2, bag_1, chain is the same both for developmental sequence (Milburn, *J. Cell Sci.* **12**, 1973) and for decreasing numbers of satellite cells.

Paper 18.

R.W. Banks, D. Barker & M.J. Stacey (1981)
Structural aspects of fusimotor effects on spindle sensitivity.
In A. Taylor and A. Prochazka (Eds.) "Muscle Receptors and Movement" pp.5-16.
Macmillan, London and Basingstoke.

Structural aspects of fusimotor effects on spindle sensitivity

R. W. BANKS*, D. BARKER† AND M. J. STACEY†

SUMMARY

The distribution of motor and sensory axons to the three types of intrafusal muscle fibre have been determined using reconstructions of serially sectioned spindles and teased, silver-impregnated, whole spindles.

The results obtained from the analysis of fusimotor axons indicate that there is very little trail innervation, and a correspondingly low static input, distributed to bag_1 fibres.

Whereas the S_1 secondary ending is predominantly distributed to chain fibres, it almost invariably also innervates both types of bag fibre.

The variability of some histological features of primary and secondary innervation from four hindlimb muscles is described, and the significance of these findings discussed in relation to spindle sensitivity.

INTRODUCTION

The sensitivity of the sensory nerve endings in mammalian muscle spindles is controlled by the central nervous system via motor (γ and β) activation of intrafusal muscle fibres. It is now known that three types of intrafusal muscle fibre are normally present in each muscle spindle (Banks, Harker and Stacey, 1977), and it is generally agreed that their different mechanical properties are important in determining the characteristics of the sensory response. Thus, the bag_1 fibre mediates the dynamic responsiveness of the primary ending, whereas the bag_2 and chain fibres mediate its static responsiveness. Also, the secondary ending, which usually shows comparatively little dynamic sensitivity, predominantly innervates chain fibres. Finally, dynamic fusimotor

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neurons innervate bag_1 fibres whereas static fusimotor neurons innervate bag_2 and chain fibres.

There remains one major area of disagreement and that is whether or not static fusimotor axons frequently innervate bag_1 fibres. Studies using the glycogen-depletion technique suggest that they do (Brown and Butler, 1973; Barker *et al.*, 1976; Emonet-Dénand *et al.*, 1980), but observation of living spindles during fusimotor stimulation indicates that bag fibres activated by dynamic axons (presumably bag_1) are not also activated by static axons (Bessou and Pagès, 1975; Boyd *et al.*, 1977).

This paper summarises recent results obtained from reconstructions of serially sectioned muscle spindles and from analysis of silver-impregnated, teased whole spindles. We discuss their relevance to spindle sensitivity and its fusimotor control. The results fall into two parts: first, the distribution of fusimotor axons to the three types of intrafusal muscle fibre, and second, some details of the histology of primary and secondary sensory endings. The preparative methods used in serial sectioning and reconstructions, and in the silver impregnation, are fully described elsewhere (Banks *et al.*, 1978; Banks, in preparation; Barker *et al.*, 1970) and only relevant details will be given here. All results were obtained from cat hindlimb muscles. The serial sectioning was performed on tenuissimus muscles only.

THE INTRAFUSAL DISTRIBUTION OF FUSIMOTOR AXONS

Serial, 1 μm thick transverse sections of three almost complete spindles and one half-spindle were used to make schematic reconstructions of their somatic motor innervation. The results of the half-spindle were typical, and will be described here.

Intrafusal muscle fibres were identified as nuclear-bag and nuclear-chain types by their lengths, diameters and equatorial nucleation. The bag fibres were further subdivided into bag_1 and bag_2 types by the dissociation of the bag_1 fibre from the others in the equatorial region and by the association of elastic fibres with the polar regions of the bag_2 fibre (figure 1). Neuromuscular junctions and their supplying axons, whether myelinated or unmyelinated, were easily recognisable (figure 1) and readily traceable through successive sections.

The reconstructed half-spindle was the proximal pole of spindle 5 in the study of Banks, *et al.* (1978). In this pole a bag fibre, presumably bag_1, was activated by two dynamic γ axons. Correspondingly, the bag_1 fibre possessed two motor endings, and each was supplied by an axon with no other intrafusal terminals in the pole (figure 1). Three other axons entered the pole, one ending in the periaxial space at some distance from the muscle fibres. Each of the remaining two axons supplied motor endings to the bag_2 fibre and to two of the four chain fibres (figure 1). They were presumably static axons and may

be correlated with the trail innervation seen in silver-impregnated preparations (Barker *et al.*, 1973). In the example of this half-spindle, therefore, there was no common innervation of bag_1 with either bag_2 or chain fibres. In the three reconstructed whole spindles only one axon was found to supply a bag_1 fibre and another type of fibre (in this case a chain), whereas eight axons supplied endings to both bag_2 and chain fibres (Banks, in preparation). The distribution of all 26 axons traced is given in table 1, and is illustrated schematically in figure 2.

Table 1. Intrafusal distribution of fusimotor axons in three spindles and one half spindle (cat tenuissimus).

Muscle-fibre type	*Number of axons supplied to* one pole	both poles	*Totals*
bag_1	6	1	7
bag_2	5	—	5
chain(s)	4	1	5
bag_1 and chain	1	—	1
bag_2 and chain(s)	8	—	8
			26

If the glycogen-depletion results are an accurate reflection of the innervation of the bag_1 fibre, virtually each one should be innervated by at least one static axon in addition to any dynamic axons. This is because, on average, each static axon depletes three out of five bag_1 fibres (Barker *et al.*, 1976) and, since there are about three static axons to each spindle, the probability of at least one static axon innervating the bag_1 fibres is 0.94. Despite the small sample involved, our results clearly contradict this inference. It might be argued that some of the axons innervating bag_1 fibres in the reconstructed spindles also innervate bag_2 or chain fibres in other spindles; however, this complication is not suggested by the glycogen-depletion results, and it is known that most fusimotor axons have the same action on different primary endings (Emonet-Dénand *et al.*, 1977). It might also be argued that the axons from bag_1 and other fibres have not been traced sufficiently far back to reach their common origin; however, many such examples have been found for the bag_2/chain system, and this is in complete accord with both glycogen-depletion and direct observation of living spindles.

In the recent classification of sub-categories of static and dynamic fusimotor action (Emonet-Dénand *et al.*, 1977) the intermediate categories II to V were interpreted as arising from various degrees of common innervation of bag_1 with bag_2 or chain fibres. Since about 30% of the responses fell in these categories a fairly high proportion of such common innervation would be expected, and this would be more or less consistent with the glycogen-depletion results. However, in the light of our present findings we believe that

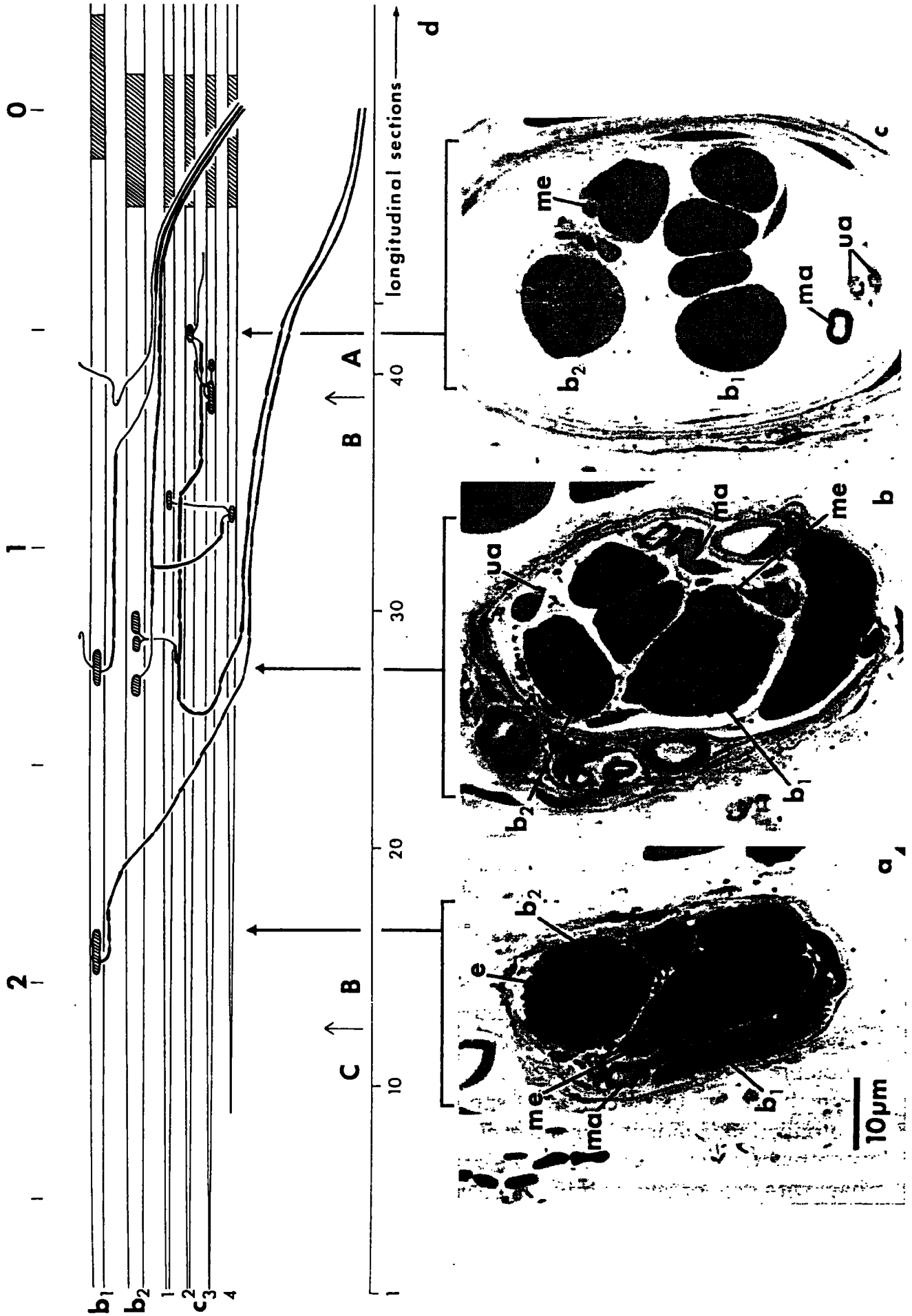

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Figure 1 (opposite) (a)–(c). Representative 1μm thick transverse sections of the half-spindle described in the text. Bag_1 (b_1), bag_2 (b_2) and four chain fibres are present in all the sections. In (a) note the elastic fibres (e) associated with the bag_2 fibre, and the motor ending (me) on the bag_1 fibre. The myelinated axon (ma) supplying this ending is visible nearby. In (b) note the motor ending on bag_1. The section passes through the preterminal heminode of the supplying axon. An unmyelinated axon (ua) with its associated Schwann-cell nucleus is close to the bag_2 fibre, on which it terminated. This is a branch of the myelinated axon present in section (c). Other axons may be identified by reference to the reconstruction (d). In (c) note the motor ending on chain 2 and the three axons (one myelinated, two unmyelinated) within the periaxial space. The two remaining axons were present in the section but are not included in this field of view.

(d) Schematic reconstruction of the half-spindle described in the text. The locations of sections (a)–(c) are indicated by connecting lines. Only longitudinal dimensions are accurate, and are calibrated in slide numbers (lower scale), each slide containing 50 1 μm sections. The upper scale shows the approximate corresponding lengths (in mm) in the living spindle, allowing for 8% shrinkage. Vertical arrows indicate the limits of A, the periaxial space; B, the capsular pole; and C, the extracapsular pole. Muscle fibres are identified to the left of the drawing. The approximate location of the primary ending is indicated by the hatched rectangles to the right. Motor endings are indicated by hatched ovals. In the fusimotor axons unmyelinated regions and nodes of Ranvier are shown as thin lines, myelinated internodes as thick lines. One axon ends freely in the periaxial space, but perhaps had branches innervating muscle fibres in the other pole. Two other axons had freely ending ultraterminal branches.

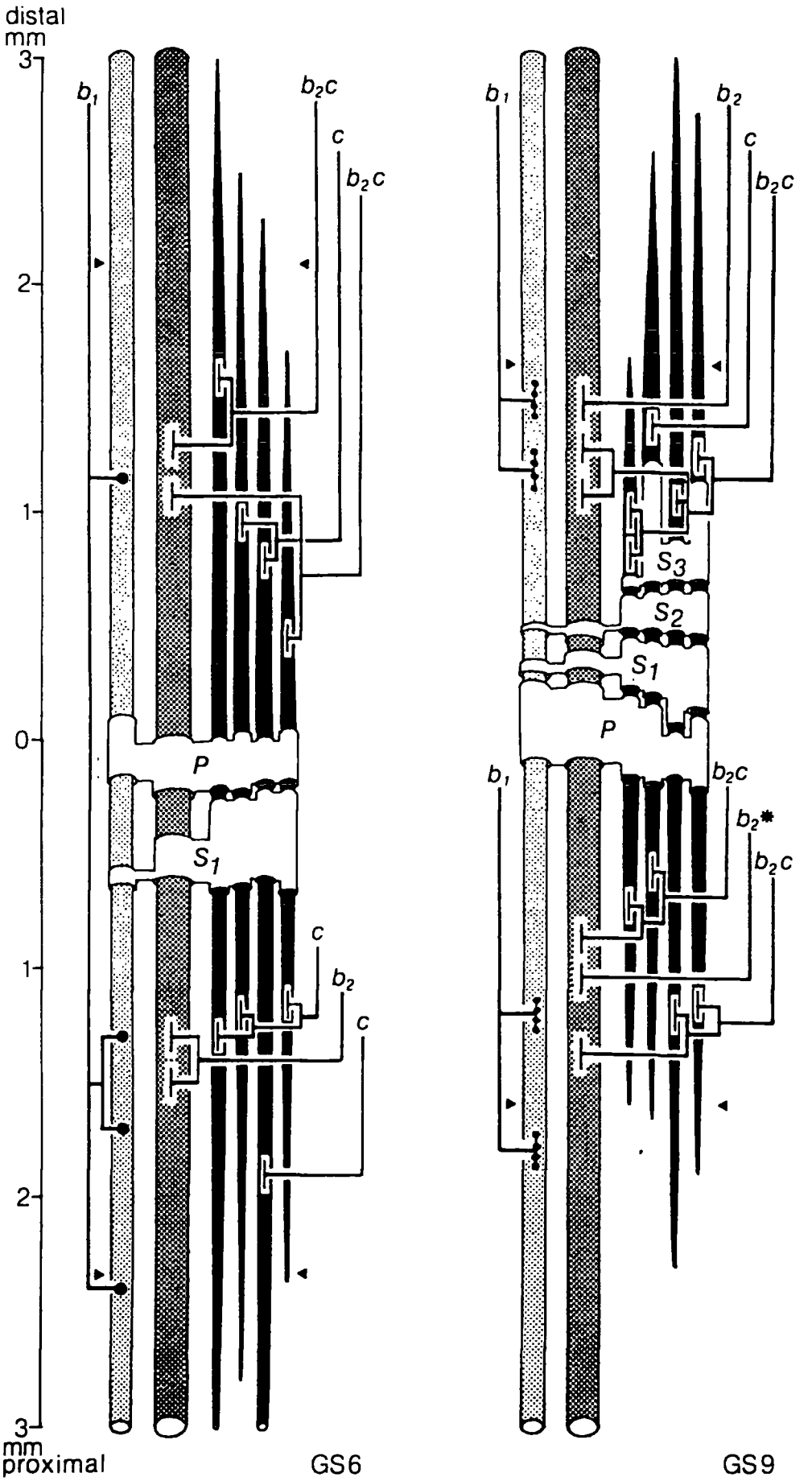
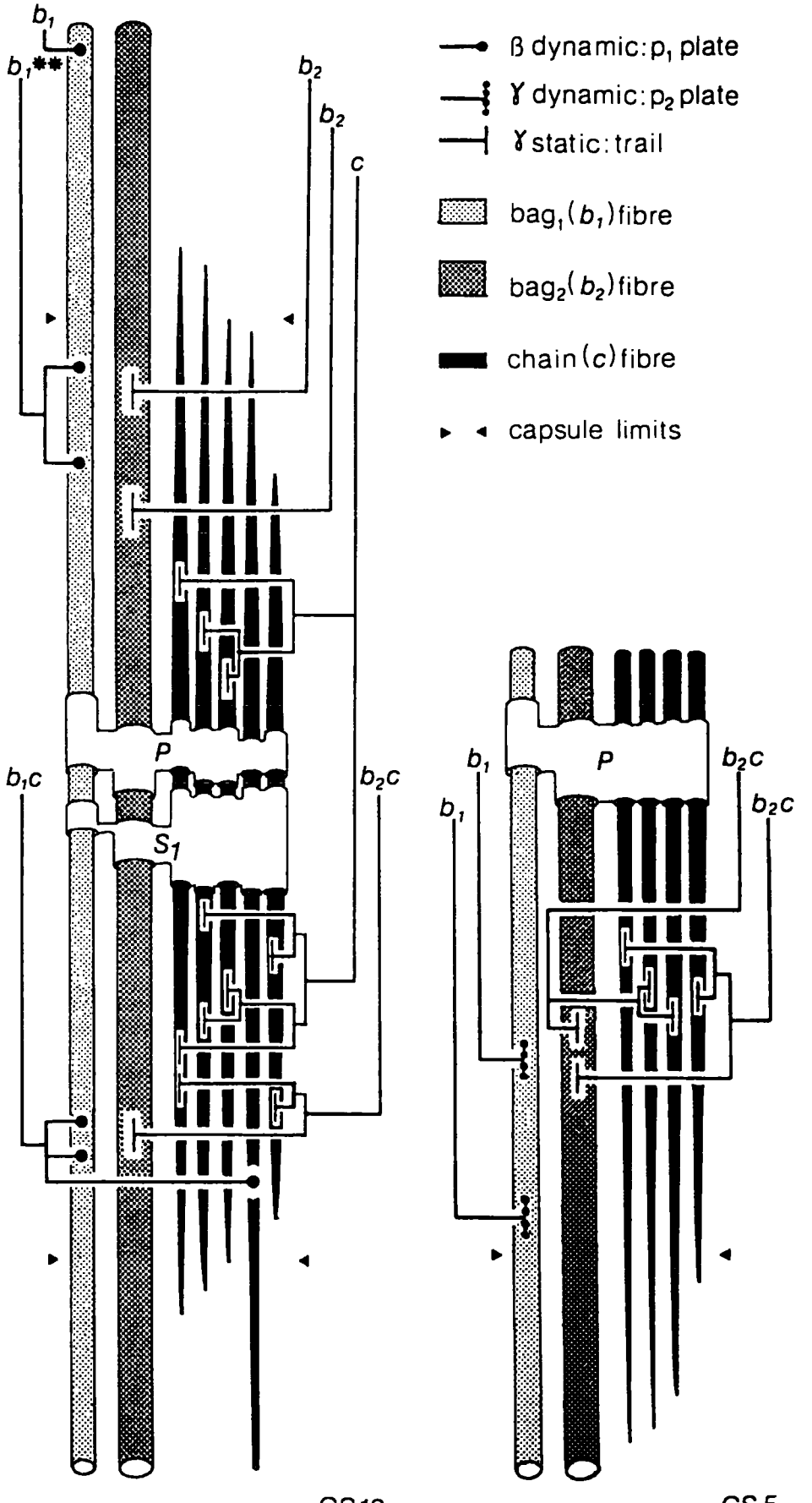


Figure 2 (continued opposite). Schematic representations of the innervation of the reconstructed cat tenuissimus spindles referred to in the text. Single asterisk in GS 9



indicates uncertain identification of this motor axon and ending; double asterisk in GS 12 indicates possibility that this axon may be γ dynamic rather than β dynamic as shown.

only the category III responses of Emonet-Dénand *et al.* (1977) truly represent this occurrence, and if so this would indicate that about 5% of fusimotor axons have terminals on bag_1 as well as bag_2 or chain fibres. It seems likely that these axons are either predominantly dynamic or static when taking their total distribution into account (Emonet-Dénand *et al.*, 1977).

We conclude, therefore, that the incidence of static innervation (presumably via trail terminals) on bag_1 fibres is rather low, at least in tenuissimus muscle spindles. We are at present analysing the motor innervation of whole silver-impregnated spindles from a variety of hindlimb muscles in order to increase our sample size and to test the generality of the conclusion. It is our impression, at this preliminary stage, that trail endings rarely occur on bag_1 fibres.

THE SENSORY INNERVATION

Two primary endings and one S_1 secondary ending were reconstructed isometrically from serial, 1 μm thick transverse sections. Detailed descriptions of these together with the reconstructions themselves will be published elsewhere (Banks, Barker and Stacey, in preparation). The two primary endings were strikingly similar in several respects, namely the areas of contact between sensory terminals and each type of intrafusal muscle fibre; the form of the terminals, particularly the differences between those on bag_1 and bag_2 fibres; and the branching pattern of the afferent axons (Banks, Barker and Stacey, 1977). We then examined silver-impregnated, teased, whole spindles to see if these features were recognisable and, if so, how consistent they were. Spindles from four hindlimb muscles—peroneus brevis (PB), peroneus tertius (PT), superficial lumbrical (SL) and tenuissimus (T)—were used. The form of the terminals was the most consistent feature, and was readily interpretable in terms of that seen in the reconstructions. We were therefore able to identify bag_1 and bag_2 fibres in the silver preparations, relying on a constant relationship of sensory terminal form to muscle fibre type as one of several criteria.

We have previously reported that in four primary afferent axons from tenuissimus spindles the first-order branches supplied bag_1 fibres separately from bag_2 and chain fibres (Banks, Barker and Stacey, 1977). The branching pattern of many primary axons could be ascertained from silver-impregnated spindles and we were able to compare this feature in a number of muscles (table 2). Various degrees of segregation were present, and the proportion of spindles in which complete segregation occurred varied between the different muscles. Also shown in table 2 are the proportions of spindles that contained two bag_1 fibres in addition to a bag_2 fibre. Again this varied in the different muscles investigated.

All these features of the primary innervation are likely to have functional

Table 2. Primary endings in cat hindlimb muscles.

	Percentage with segregated distribution to bag_1 fibres		*Percentage innervating two bag_1 fibres (in spindles with three bag fibres)*	
Muscle	% segregated	total counted	% on two bag_1 fibres	total counted
Peroneus brevis	64	14	14	29
Peroneus tertius	54	11	5	22
Superficial lumbrical	30	23	29	28
Tenuissimus	80	30	7	62

correlates, but as yet the only one for which the relationship seems clear is that of the branching pattern of the axons and the occurrence of separate static and dynamic pacemakers (Hulliger and Noth, 1979).

The reconstructed S_1 secondary ending possessed terminals on all three types of intrafusal muscle fibre, though those on the four chain fibres dominated its appearance. Even so, 25% of the contact area between sensory terminal and muscle fibre occurred on the bag fibres (17% on bag_2 and 8% on bag_1). The branching pattern and distribution of four other S_1 secondary afferents were reconstructed and in each case the bag_1 fibre was included.

When we were able to identify bag_1 and bag_2 fibres confidently in silver-impregnated material, we began an analysis of the secondary endings in whole spindles from four hindlimb muscles (PB, PT, SL, T). In terms of the distribution to bag and chain fibres our results are similar to those of Boyd (1962). Most of the terminals of secondary endings were distributed to chain fibres, largely in the form of loose, widely spaced spirals and incomplete loops (figure 3(a)). On bag fibres the terminals were usually in the form of sprays (figure 3(a) and (b)) or claw-like configurations (figure 3(b) and (c)).

Of all the secondary endings, only those in the S_1 position consistently innervated one or other bag fibre in addition to chains. Thus among 144 S_1 endings, 130 had terminals on all three types of muscle fibre, 11 supplied bag_2 and chain fibres and three supplied bag_1 and chain fibres. It was apparent that the area of contact between sensory terminals and muscle fibres was very variable in different endings. As a simple estimate of this variability, we counted the numbers of terminal branches on the bag fibres. The counting was restricted to 72 of the best silver-impregnated endings from 58 spindles. The sample included 79 bag_1 fibres and 70 bag_2 fibres. In each case the majority of bag fibres had between eight and 12 S_1-terminal branches distributed to them. The variability between muscles is illustrated in table 3 where the proportions of bag_1 and bag_2 fibres possessing eight or more S_1-terminal branches are given for four hindlimb muscles (PB, PT, SL, T). The significance of these results is difficult to assess in terms of the proportions of S_1

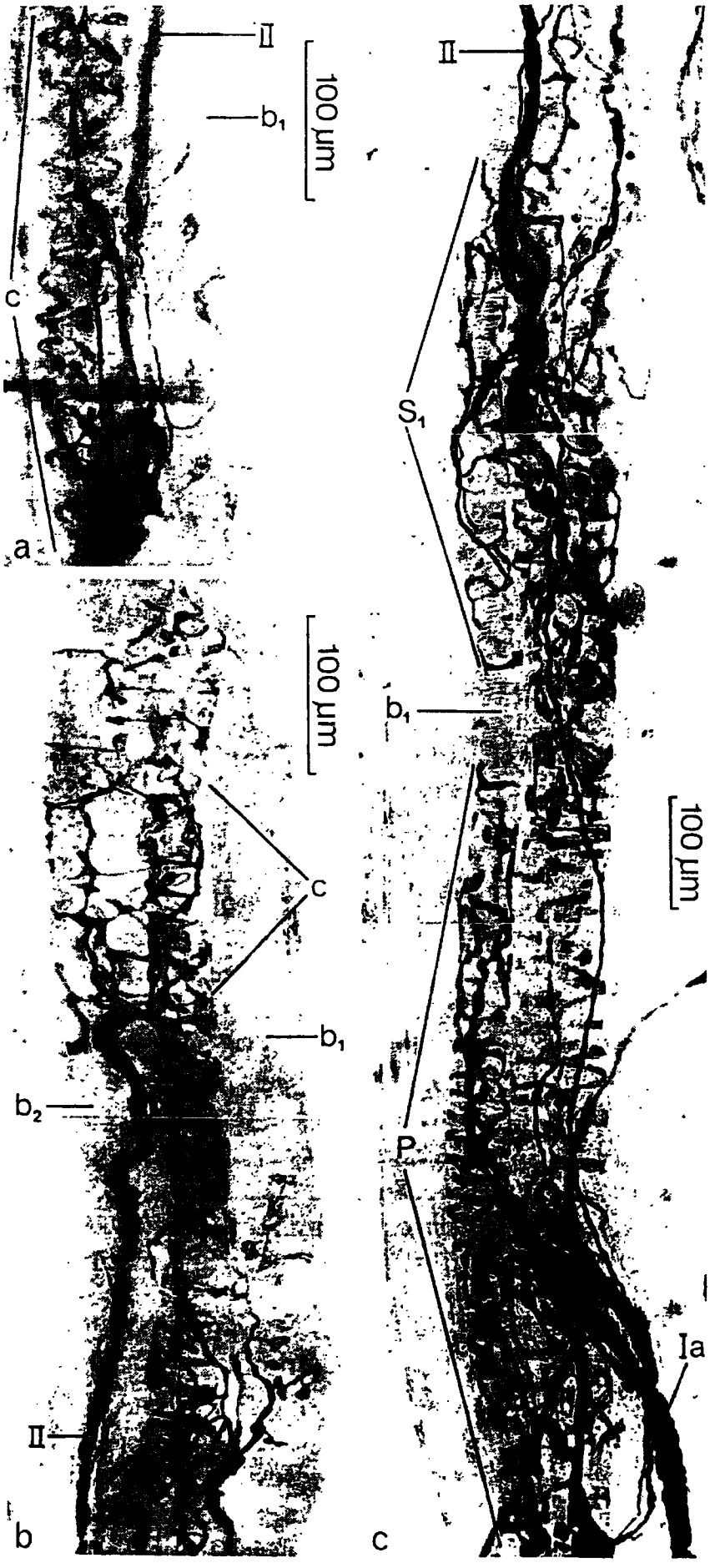

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Table 3. Percentage of bag fibres receiving eight or more terminal branches from S_1 secondary endings.

Muscle	% bag_1 fibres	total counted	% bag_2 fibres	total counted
Peroneus brevis	53	19	100	15
Peroneus tertius	44	16	44	16
Superficial lumbrical	69	16	92	13
Tenuissimus	75	28	65	26

secondary endings supplied to bag fibres, since a similar count of terminal branches was not possible for chain fibres. However, as an approximate guide we may note that in the reconstructed ending described above there were about eight terminals on the bag_1 fibre and 18 on the bag_2 fibre. It seems, therefore, that in many S_1 secondary endings the bag_1 fibre alone may receive about 10% of the contact area of the ending.

It is tempting to see in these results the structural substrate for the variable dynamic component in the responses of secondary endings. If this is so it is at first surprising that dynamic fusimotor stimulation has so little effect on secondaries (Appelberg *et al.*, 1966). However, secondary terminals on bag fibres occur in regions of the muscle fibres that possess far more myofilaments than in the equatorial regions below the primary terminals. Dynamic fusimotor activation of bag fibres may not, therefore, have the same effect on secondary endings as on primary endings. This suggestion is supported by recent results of Jami and Petit (personal communication, 1980), who have found some secondary endings to be activated by dynamic axons, leading to an increase of static firing but without an increase in their dynamic indices.

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Figure 3 (opposite). Photographs of teased, silver preparations (method of Barker and Ip, 1963) of cat hindlimb muscle spindles illustrating features of sensory innervation.

(a) Part of an S_1 secondary ending supplied to a tenuissimus spindle showing spray of terminals supplied to the bag_1 (b_1) fibre on the right and loose spirals supplied to the chain fibres (c) on the left. II, secondary afferent.

(b) Secondary afferent (II) supplies an S_1 secondary ending to a superficial lumbrical spindle. The terminals are distributed to the bag_2 (b_2) fibre on the left, the bag_1 (b_1) fibre on the right, and the chain fibres (c) in the middle.

(c) Equatorial region of a superficial lumbrical spindle supplied with an S_1 secondary ending (upper half) and primary ending (lower half). Focus adjusted so as to pick out bag_1 fibre (b_1) and its primary (P) and secondary (S_1) terminals. Ia and II, primary and secondary afferents.

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A histological study of the motor innervation of the cat's muscle spindle

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INTRODUCTION

Mammalian muscle spindles contain three types of intrafusal muscle fibre, identifiable histologically (Banks, Harker & Stacey, 1977) and physiologically (Boyd, 1976). Each muscle spindle is usually innervated by a single primary sensory ending that has terminals on all the intrafusal muscle fibres. The response of the ending has static and dynamic components, sensitive to muscle length and rate of change of muscle length, respectively. These two components are under largely independent control by the central nervous system via the fusimotor neurons, most of which are identifiable as 'static' or 'dynamic' according to their action on the primary response (Emonet-Dénand, Laporte, Matthews & Petit, 1977). The pattern of innervation of intrafusal muscle fibres by fusimotor axons is of considerable interest in understanding how this control is effected.

There is now general agreement that dynamic axons innervate bag_1 fibres almost exclusively, whereas static axons innervate bag_2 and chain fibres, either separately or together (Barker *et al.* 1976*b*; Boyd, Gladden, McWilliam & Ward, 1977; Barker *et al.* 1978). However, due to conflicting evidence from the different methods of investigation, there is still disagreement about the extent or occurrence of static fusimotor input to bag_1 fibres (Barker *et al.* 1976*b*; Boyd *et al.* 1977).

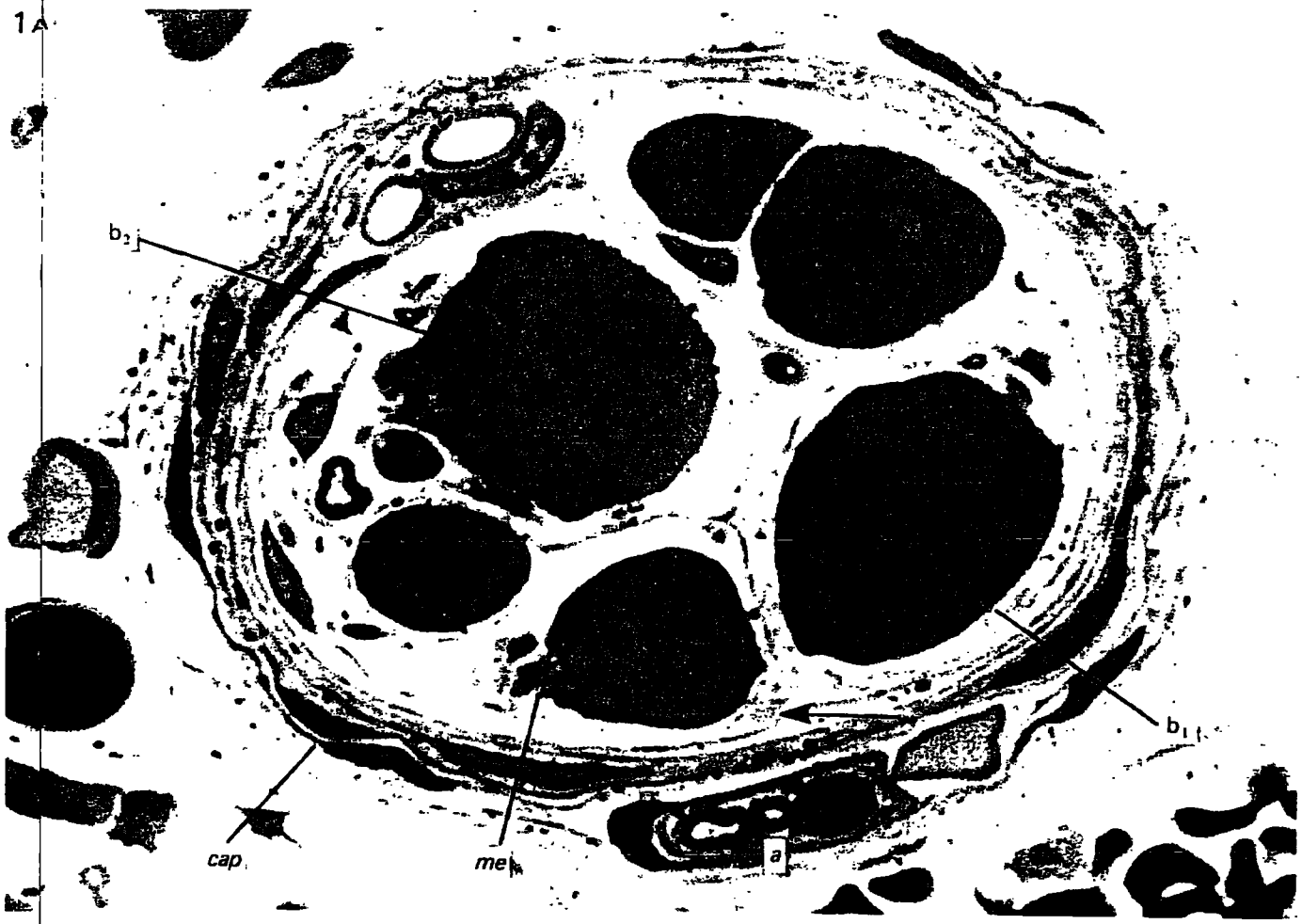
The present study provides direct histological evidence that the bag_1 fibre is only rarely innervated by static axons. A detailed description of one spindle-pole, together with a summary of the other results and the conclusions, has been published previously (Banks, Barker & Stacey, 1981).

MATERIALS AND METHODS

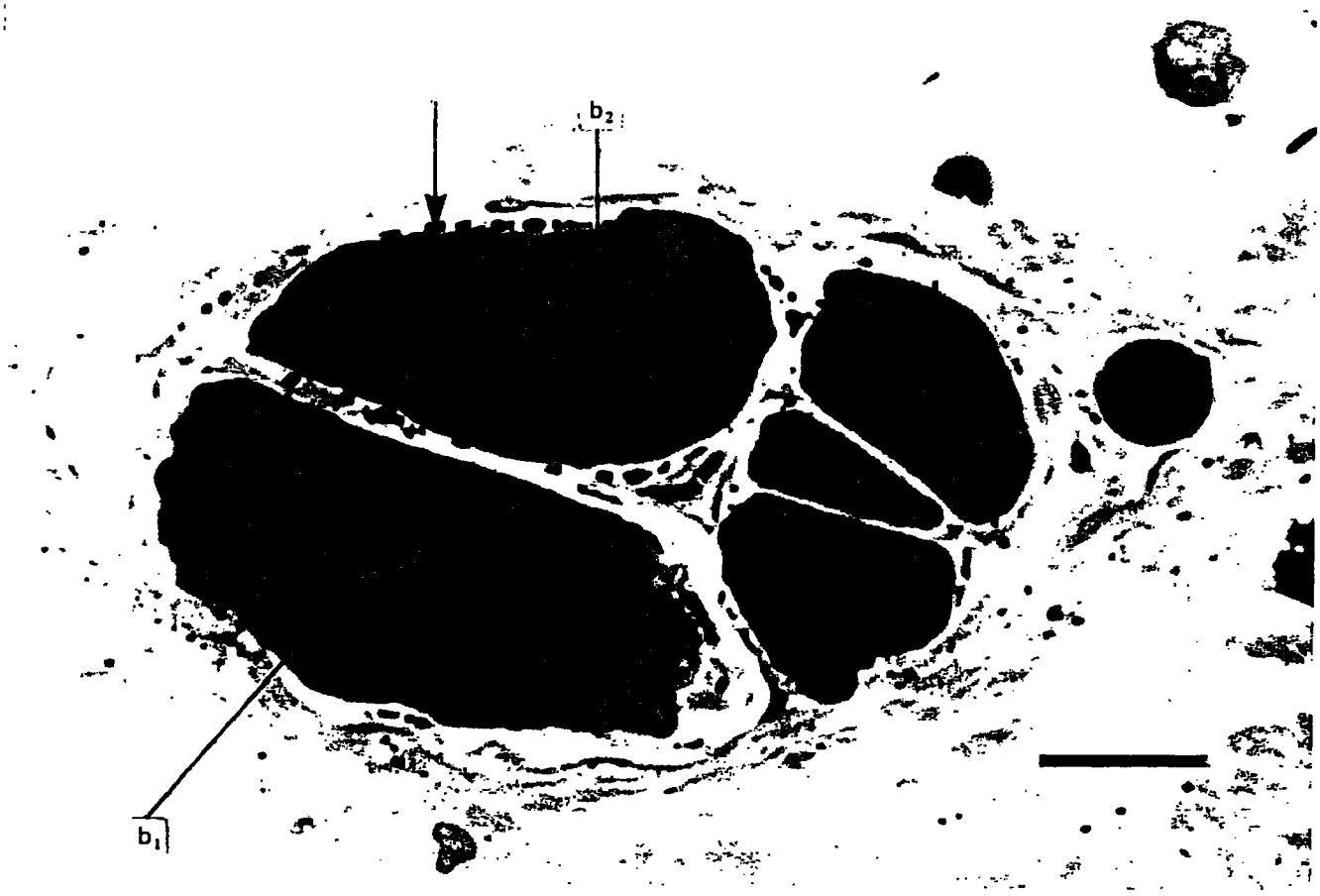
Tenuissimus muscles of four adult cats were used. The results to be described were obtained from four spindles (numbers 5, 6, 9 and 12) that formed part of a series of combined physiological and histological experiments (Banks *et al.* 1978) where details of the methods of fixation, embedding and sectioning can be found.

Serial, transverse, 1 μm thick sections were cut, except in the region of the sensory endings in spindle 5, which was cut longitudinally. Every section containing part of a nerve terminal, sensory or motor, was photographed on 35 mm film with a Zeiss Ultraphot using $\times 40$ or $\times 100$ planapo objectives (Fig. 1). At least every tenth section was photographed where other features of special interest occurred, namely sensory and motor axons and insertion of muscle fibres, and throughout three poles as far as the sectioning continued. Photomicrographs were enlarged $\times 4{\cdot}5$ from the negatives and were used to make two types of reconstruction:

1A



1B



(1) Schematic diagrams showing the branching of sensory and motor axons and their myelination, the location of sensory and motor terminals, lengths of intrafusal muscle fibres, limits of capsule and periaxial space, and locations of contraction foci, blood vessels, etc. recorded in the living preparations. Slightly simplified versions of these are given in Figures 2–4.

(2) Semi-diagrammatic, isometric reconstructions of the motor nerve terminals. These were made by tracing the relevant features of each photomicrograph onto acetate film, using a coloured ink code to distinguish the various components. Adjacent drawings were placed along an inclined line to produce the required amount of spatial resolution. Since no external reference was available, the simplifying assumption was made that one part of the reconstruction always fell on this line. For the motor endings, the sarcolemma diametrically opposite the ending was assumed to intersect the line, since usually only one muscle fibre was involved and the total length was relatively small. The reconstructions were built up by drawing the envelopes of each group of ten tracings using the same colour code and finally making India ink tracings of the envelopes.

A number of conventions of line thickness and shading were used in the final drawings. The standard line was that used to indicate the sarcolemma of intrafusal muscle fibres. Nerve terminals, nodes of Ranvier and unmyelinated axonal branches on the near side of the muscle fibres have been shown with the standard line and graded shading. On the far side of the muscle fibres (or on the near side where they were obscured by some other structure) they have been shown with fine lines and even shading. Those on the far side have been shown only in fine outline, when additional structures such as nerve terminals or axons were present on the near side of the muscle fibres. Myonuclei, as seen through sarcolemma, have been drawn with fine outlines and nucleoli have been shown as dots; as seen through the cut ends of muscle fibres they have been drawn with standard outlines. Satellite cells have been drawn with standard or fine outlines according to whether they were on the near or far side of the muscle fibres, respectively. They were usually fusiform in shape. Myelinated internodes have been shown with heavy graded shading where they did not overlie other structures of interest, and in broad outline where they did. Where they were themselves overlain, they have been shown in fine outline or else omitted, if to have included them would have been too confusing. Schwann cell nuclei have usually been shown with nucleoli and with standard or fine outlines according to whether or not they were seen through the axons. Prominent folds, sharp curves and protruberances on the near sides of axons and muscle fibres have been indicated by broad, standard or fine lines, as appropriate.

The structures categorized as nerve terminals in the reconstructions of the motor endings included the flattened cytoplasm of the teloglial cells, which overlaid the terminals and which could not be identified separately in the photomicrographs. The teloglial nuclei and their immediately surrounding cytoplasm have, however, been omitted.

Fig. 1. Transverse, 1 μm thick sections from the proximal pole of spindle 6, stained with toluidine blue. (A) Section 37, slide no. 120. In this mid-polar region bag_1 (b_1), bag_2 (b_2) and the four chain fibres are enclosed by a prominent capsule (*cap*). A motor nerve ending (*me*) is present on chain 1 as well as the two bag fibres (cf. Fig. 2). Three myelinated fusimotor axons (one cut through the Schwann cell nucleus) are present at *a*. The arrow indicates a faint oval profile typical of those found to contain autonomic axons (see text). (B) Section 23, slide no. 140. In this extracapsular polar region bag_2 is closely surrounded by large elastic fibres (arrowed). Chain 4 has ended and a motor nerve ending is present on bag_1 (cf. Fig. 2). Scale bar is 10 μm.

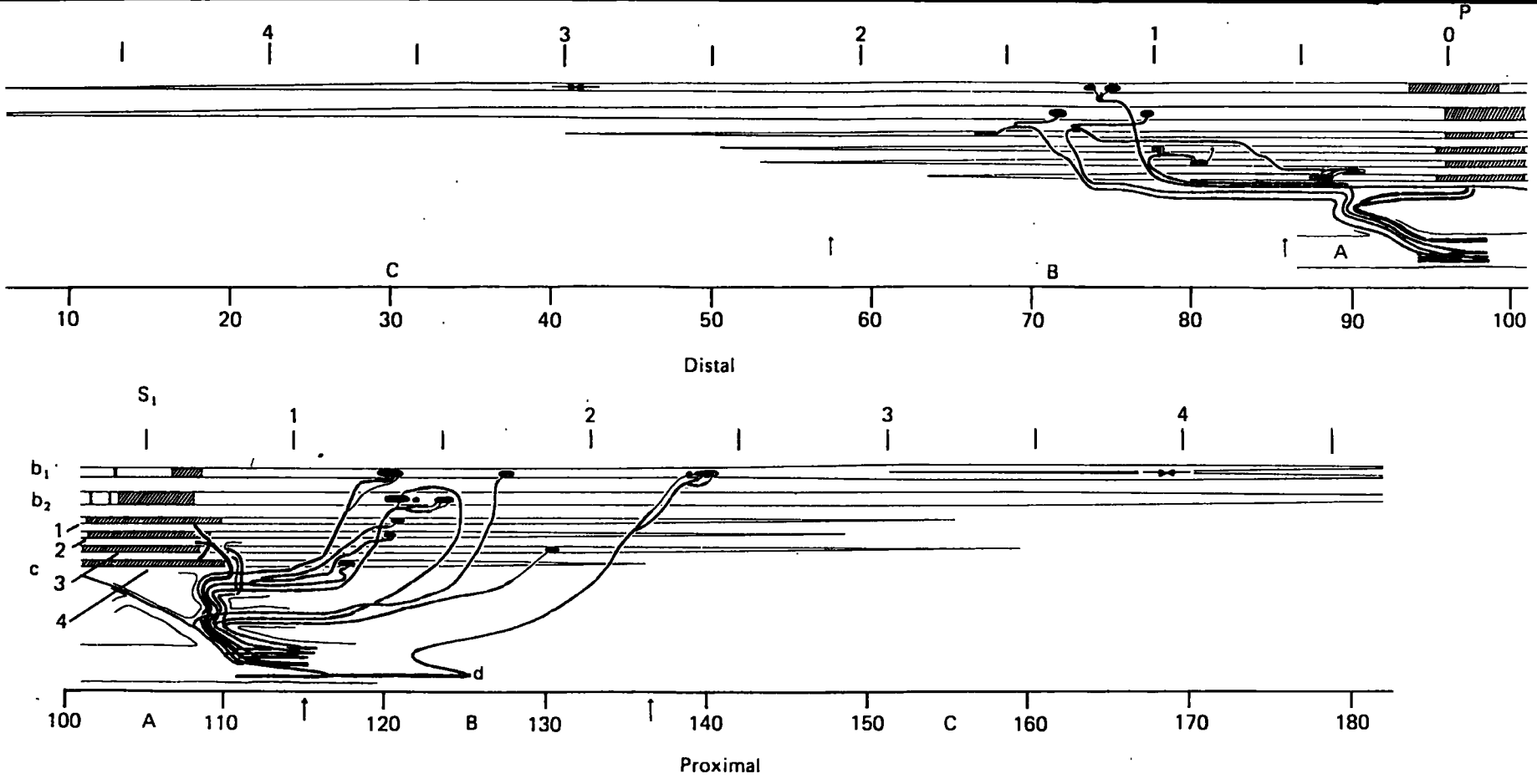


Fig. 2. Schematic reconstruction of spindle 6 divided into distal and proximal parts. Bag_1 (b_1), bag_2 (b_2) and chain (c) muscle fibres are identified to the left of the proximal part; chain fibres are numbered according to their lengths in the distal pole. Longitudinal dimensions only are accurate, with reference to the lower scale. This is marked with slide numbers, each slide containing 50 sections, the total number of sections of this spindle being over 8500. The upper scale gives approximate lengths from the equator (in mm) in the living spindle, allowing for 8% shrinkage during histological processing. Vertical arrows indicate the boundaries between the regions of A, the periaxial space, B, the capsular poles and C, the extracapsular poles. Horizontal arrows indicate movements of sarcomeres that were visible during stimulation of fusimotor axons (in this case a dynamic β axon, see Banks *et al.* 1978). Primary (P) and secondary (S_1) sensory endings shown hatched with their supplying afferents nearby. Detailed reconstructions of these endings will be published elsewhere. Motor nerve endings are represented by filled ovals. Isometric reconstructions are shown as follows: all endings on bag_1 (Fig. 6), proximal endings on bag_2 (Fig. 9A) and chains 2 and 4 (Fig. 10A and B). Myelinated internodes and unmyelinated axons are depicted by thick and thin lines respectively. The motor axons are distributed as follows: 1 axon to both poles of bag_1 fibre, 2 axons to the distal poles of bag_2 and chain fibres, 1 axon to the proximal pole of bag_2 only, and 3 axons to chain fibres only (one distal, two proximal). Axon *d* is referred to in the text. Two axons in the proximal pole each had a branch that appeared to end freely, possibly indicating the occurrence of sprouting. Note that the proximal pole of the bag_1 fibre is partially divided into two.

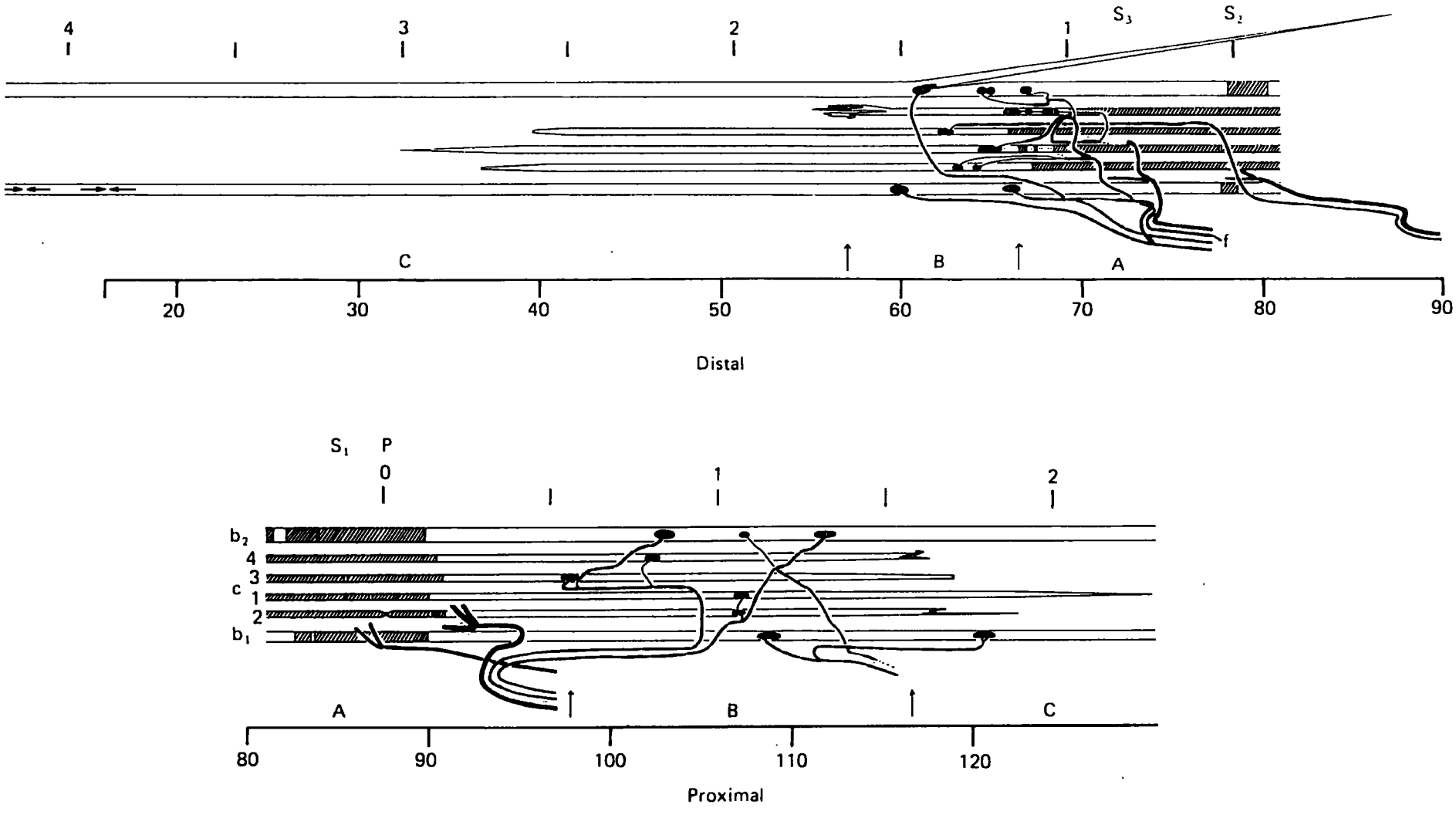


Fig. 3. Schematic reconstruction of spindle 9. See the legend to Fig. 2 for an explanation of the symbols, lettering and scales used. This spindle had three secondary endings (S_1–S_3) on the distal side of the primary; the periaxial space (A) was correspondingly longer and the capsular pole (B) shorter in that part. Isometric reconstructions of the motor endings are shown as follows: all endings on bag_1 (Fig. 7), the distal ending at 1·1 mm on bag_2 (Fig. 9B) and the distal ending on chain 3 (Fig. 10C). The motor axons are distributed as follows: 1 axon to each pole of bag_1 only, 1 axon to each pole of bag_2 only, 1 axon to the distal pole of chain 3 only, and 3 axons to bag_2 and chain fibres (one distal, two proximal). Axon f is referred to in the text. Note that the distal part of bag_2 has a branch that ended outside the spindle's capsule at about the level of the equator.

Gaps in the reconstructions occurred where thin sections were cut for electron microscopy. They have been greatly exaggerated, actually being about 1–2 μm wide. When a sole-plate was present in these regions its junction with the myofibrils has been indicated by a fine line.

RESULTS

Intrafusal muscle fibres

Intrafusal muscle fibres were classified into nuclear bag and nuclear chain types on the basis of length, diameter and equatorial nucleation. Nuclear bag fibres were further subdivided into bag_1 and bag_2 types on the basis of distribution of elastic fibres in the extracapsular region (Gladden, 1976), separation of bag_1 from the other intrafusal fibres in the equatorial region, and the occurrence of sarcomeres lacking characteristic M-lines in a much greater length of bag_1 than bag_2 (Banks *et al.* 1977).

Each muscle spindle possessed one bag_1, one bag_2 and four or five chain fibres. All but the shortest chain fibres extended beyond the polar limits of the capsules and every intrafusal muscle fibre was inserted into perimysium, none being directly inserted onto a capsule. Bag fibres of both types were about 10 mm long, the uncertainty being due to the fact that few bag fibre poles were completely sectioned. Chain fibres were, on average, 4·60 mm long (range 3·15–6·25 mm, $n = 13$).

Branching and distribution of fusimotor axons

Schematic reconstructions of the motor innervation of spindles 6, 9 and 12 are shown in Figures 2–4. The 19 intrafusal muscle fibres were supplied by 22 axons or axonal branches, 7 each to spindles 6 and 12, and 8 to spindle 9.

The six muscle fibres in the proximal pole of spindle 5 were supplied by four fusimotor axons (Banks *et al.* 1981). The distal pole of spindle 5 was not sectioned in the region of the motor innervation.

Each intrafusal muscle fibre was innervated in both poles except for chains 4 and 5 of spindle 12, the distal poles of which were not innervated and, though of apparently normal lengths, were of unusually small diameters. Only two axons were seen to supply both poles of a spindle (axon d of spindle 6 and axon n of spindle 12); though it is possible that more actually did so, the axons concerned not having been traced back to their common source. There is physiological evidence (Banks *et al.* 1978) that one of the axons supplying the bag_1 fibre of spindle 5 innervated both poles.

Most axons entering the spindles arose as branches of parent axons of slightly larger diameter in the intramuscular nerve trunk (11 of 14 in spindles 6 and 12). In each case, the branching node was very close to the origin of the spindle nerve. The parent axon continued in the intramuscular nerve trunk after branching, presumably to innervate other muscle spindles. It is possible that the three axons which did not branch in this way (axons h, k and m of spindle 12) were terminal, rather than lateral branches of parent axons. Also, two of these (axons h and k) possessed branches that entered nerves of apparently exclusively extrafusal distribution. Though these branches were not traced to their termination, it is possible that the axons were skeleto-fusimotor.

On their approach to the spindles, and intrafusally, most of the axons (20 of 26) branched at least once, supplying up to eight neuromuscular junctions each (e.g. axon f of spindle 9). There was a tendency for the preterminal branches to be unmyelinated, the axons becoming small in diameter (less than 1 μm) when this

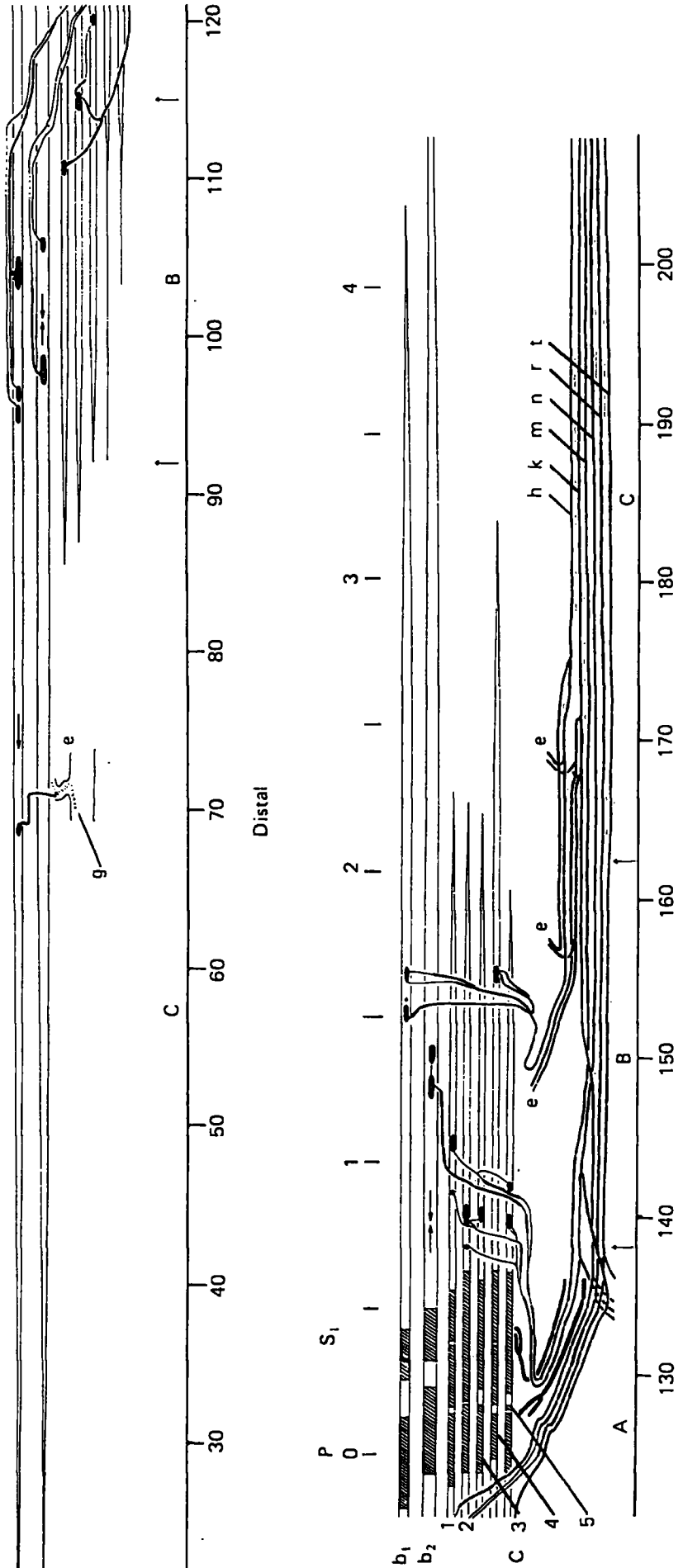


Fig. 4. Schematic reconstruction of spindle 12. See the legend to Fig. 2 for an explanation of the symbols, lettering and scales used. Isometric reconstructions of the motor endings are shown as follows: all endings on bag_1 (Fig. 8), the endings on the distal part of bag_2 (Fig. 9C and D), and the endings on the distal part of chain 2 (Fig. 10D) and the proximal part of chain 4 (Fig. 8D). The motor axons are distributed as follows: 2 axons to the distal pole of bag_1 only, 2 axons to the distal pole of bag_2 only, 1 axon to both poles of several chain fibres, 1 axon to the proximal poles of both bag_1 and chain 4, and 1 axon to the proximal poles of both bag_2 and chain fibres. Apparently extrafusal nerves are indicated by e. Axons g–t are referred to in the text. Note that the distal poles of chains 4 and 5 received no motor innervation and showed abnormally small diameters at distances more than about 0·5 mm from the primary ending.

Table 1. *Frequency of different numbers of motor endings per pole supplied to intrafusal muscle fibres in seven poles*

Fibre type	Number of motor endings per pole				
	0	1	2	3	4
Bag_1	—	—	5	1	1
Bag_2	—	—	4	3	—
Chain	2	20	7	1	—

Total number of endings: 71.

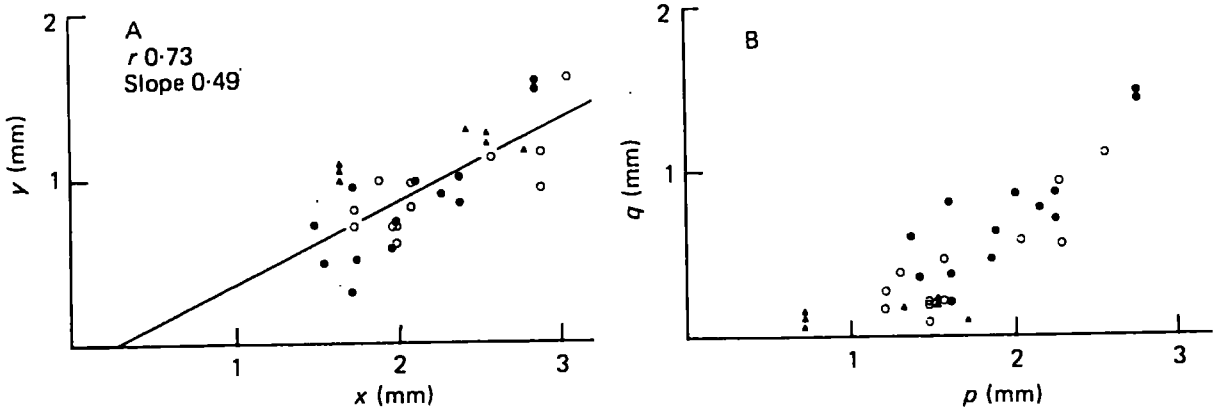


Fig. 5. The location of motor endings on chain fibres in relation to the sensory innervation indicates a determinative role of the primary sensory ending. (A) The distances between the centres of the motor and primary sensory endings (y) plotted against the length of the muscle fibre pole (x), taking the centre of the primary ending as the equatorial limit. Mean values of y for poles without secondary endings (filled circles) with one secondary ending (open circles) or with three secondary endings (triangles) do not differ significantly from that for the whole sample nor from each other. This suggests that the secondary innervation does not noticeably affect the locations of the motor endings (but see below). The intercept of the regression line on x shows that the motor endings are not located at the mid-point of the pole but are biased towards the equator. (B) The distances between the end of all sensory innervation in each muscle fibre pole and the centre of the motor ending (or endings) in that pole (q) plotted against the length of the muscle fibre pole (p), taking the end of the sensory innervation as the equatorial limit. Symbols are as in (A). Mean values of q for poles without secondary endings, with one secondary ending or with three secondary endings differ significantly ($P:S_1$; $S_3:S_1$) or highly significantly ($P:S_3$) from each other. $\bar{q}$ for three secondary endings also differs significantly from the mean value for the whole sample. This confirms that the secondary innervation does not influence the locations of the motor endings, with the possible exception of the occurrence of three secondary endings on a short muscle fibre (the three triangles furthest to the left), when intermingling of the sensory and motor innervation would occur if the usual relationship obtained.

occurred. Of the 26 original axons, 17 were selectively distributed to one type of intrafusal muscle fibre, 8 supplied both bag_2 and chain fibres (there being at least one of this type in each spindle) and only one supplied a bag_1 and a chain fibre (axon h of spindle 12). This was one of those suspected of being skeleto-fusimotor, as described above. However, there was physiological evidence (see Banks *et al.* 1978) that axons g or k, and m and r or t of spindle 12, were all derived from a single parent axon, which would thus have a distribution to all three types of intrafusal muscle fibres in a single spindle.

Fig. 6. Isometric reconstructions of the distal (A) and proximal (B–D) motor endings on the bag$_1$ fibre of spindle 6 (cf. Fig. 2). The endings are the terminals of a dynamic β axon (axon d, Fig. 2) and as such probably represent p_1 plate terminals. (See Materials and Methods for a description of the line and shading conventions used in these and subsequent reconstructions). An electron micrograph of a thin section from the gap indicated by the arrow in A is shown in Fig. 11C. *ma*, myelinated axon; *Scn*, Schwann cell nucleus; *sp*, sole-plate nucleus; *ua*, unmyelinated axon. Scale axes are 10 μm in all isometric reconstructions.

Motor nerve endings

The number of motor nerve endings that occurred in each pole of each fibre type is given in Table 1. Bag fibres always had at least two endings per pole and this was also the most frequent number for both bag_1 and bag_2. However, the multiple endings in each pole of a bag_1 fibre were usually supplied by a single axon, whereas those of a bag_2 fibre were usually supplied by two or three axons, of which at least one also supplied one or more chain fibres.

Chain fibres most commonly possessed a single motor ending per pole, usually derived from an axon supplying other intrafusal muscle fibres, either bag or chain, in the same spindle. When two or, rarely, three endings were present, one was usually very small.

The motor endings of the chain fibres showed a marked tendency to be located close to the mid-point of each pole, taking the equatorial limit to be the mid-point of the part of the primary ending on each intrafusal muscle fibre (Fig. 5). There was a slight tendency for the positions of the endings to be biased towards the equator. The slope of the regression line (0·49) is that which would be expected if each pole were behaving like a single contractile unit, with the neuromuscular junction close to the mid-point, whatever the length of the fibre. This effect was particularly clear in the proximal pole of spindle 12 (Fig. 4) where there was a long chain fibre (i.e. about 1 mm longer then the other chain fibres in that pole). This analysis could not be done for the bag fibres since they were rarely sectioned completely to their ends; however it was clear that in bag fibres, especially the bag_2, there was a much greater equatorial bias in motor ending location than in the chain fibres. (For a detailed description and discussion of the relationships between bag fibre contraction and location of motor endings, see Banks *et al.* 1978).

Twenty four motor endings, including all those on the bag_1 fibres, were reconstructed isometrically (Figs. 6–10). They exhibited a range of form and size and no characteristics were found that would make it possible unequivocally to identify an ending as appropriate to a particular type of intrafusal muscle fibre. For example, only one ending (Fig. 7A) lacked sole-plate nuclei; and, if the axon was myelinated close to the ending, unmyelinated preterminal branches could arise from nodes as well as heminodes in the endings on any type of muscle fibre. However, some features were more commonly associated with the chain fibres than with the bag fibres. Thus, unmyelinated preterminal axons seemed to be more profusely branched to endings on chain fibres, and the muscle fibres themselves usually possessed quite prominent sole-plates, which were generally represented only by nuclei in the bag fibres. Five of the endings, forming the complete supply of the bag_1 fibre in spindle 6, were innervated by an axon known, from physiological evidence, to be skeleto-fusimotor. However, there were no obvious differences between these endings and others on the bag_1 fibres (Figs. 6–8, 11A, C).

Despite the problem of the inclusion of teloglial cytoplasm with the terminals in the reconstructions (see Materials and Methods), it seems likely that the structures shown closely followed the gross form of the terminals since their profiles, adjacent to the gaps where ultrathin sections were taken, were very similar to those of the terminals as seen with the electron microscope (e.g. Figs 7A, 11A, B).

In addition, it was found that the post-junctional membranes were often folded on the chain fibres and occasionally so on the bag_2 fibres (Figs. 12A–C). Only one ending on a bag_1 fibre showed any significant amount of folding and this was also the only bag_1 ending to possess a prominent sole-plate (Figs 8A, 11D). This ending was

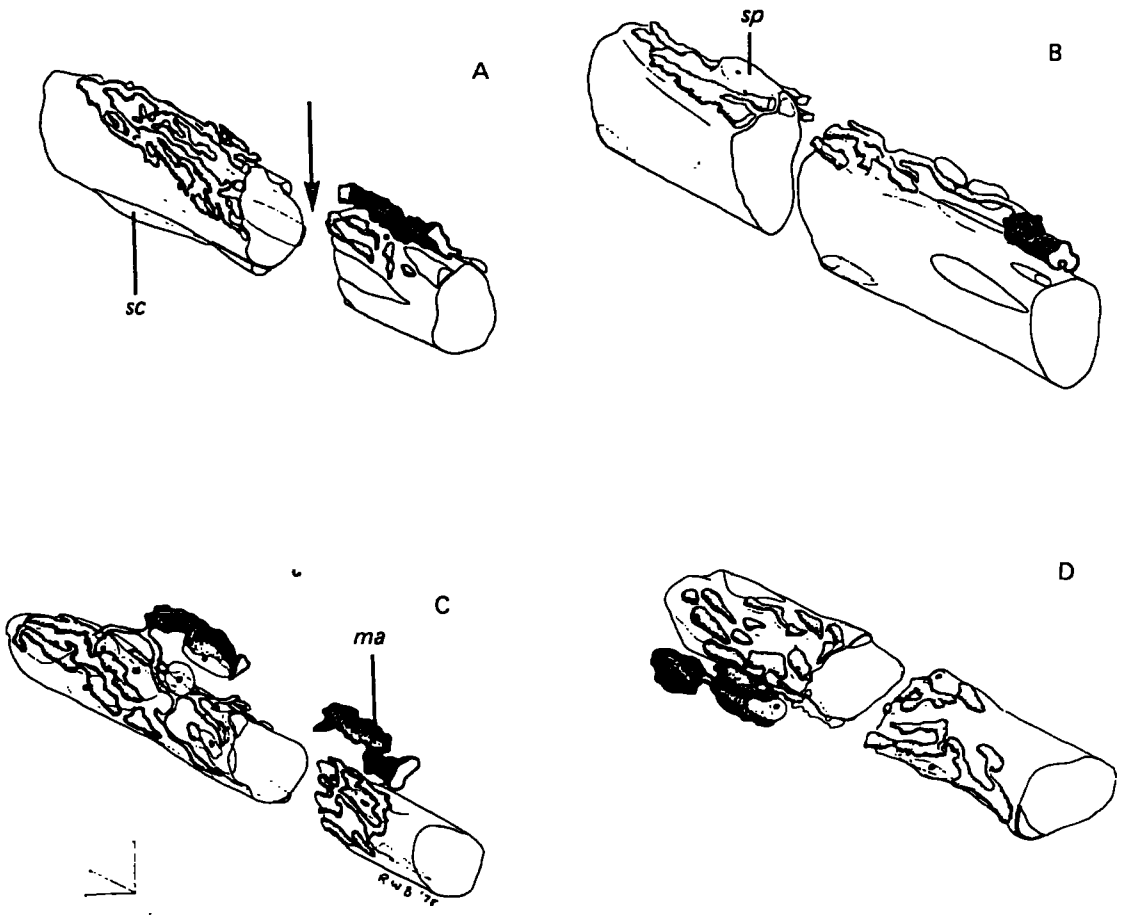


Fig. 7. Isometric reconstructions of the distal (A, B) and proximal (C, D) motor endings on the bag_1 fibre of spindle 9 (cf. Fig. 3). (A) and (B) are the terminals of a dynamic γ axon and therefore probably represent p_2 plates. The nature of the axon supplying (C) and (D) was unknown, but these may also be p_2 plates. An electron micrograph of a thin section from the gap indicated by the arrow in (A) is shown in Fig. 11 A and B. This was the only ending that lacked sole-plate nuclei. *sc*, satellite cell; other lettering as Fig. 6.

exceptional for a bag_1 fibre in that it occurred well within the region of M-type sarcomeres, whereas nearly all other bag_1 endings occurred on dM or transitional type sarcomeres (see Banks *et al.* 1978). The axon supplying the ending approached the spindle in an otherwise extrafusal nerve and it is quite possible that it was skeleto-fusimotor. However, none of the endings supplied by the known skeleto-fusimotor axon in spindle 6 possessed significantly folded post-junctional membranes.

Unmyelinated axons of very small diameters are known to occur in muscle spindles (Santini & Ibata, 1971) in addition to the larger, mostly myelinated axons supplying distinct neuromuscular junctions as described above. They have been identified as autonomic (Santini & Ibata, 1971; Ballard, 1979; Barker & Saito, 1980) and usually occur in groups of two or three embedded in Schwann cells and collagen. When the present work had begun, their significance was unknown as they had not been seen in functional contact with any elements of the muscle spindle (Barker, 1974). In the 1 μm thick transverse sections used in this study, the complex of axons, Schwann cells and collagen could be seen as faint round or oval profiles (Fig. 1 A), though not all such profiles contained unmyelinated axons. Certainly some could be traced for considerable distances during which they sometimes approached and sometimes diverged from the intrafusal muscle fibres.

One such profile was traced into the juxta-equatorial region of spindle 5, which was

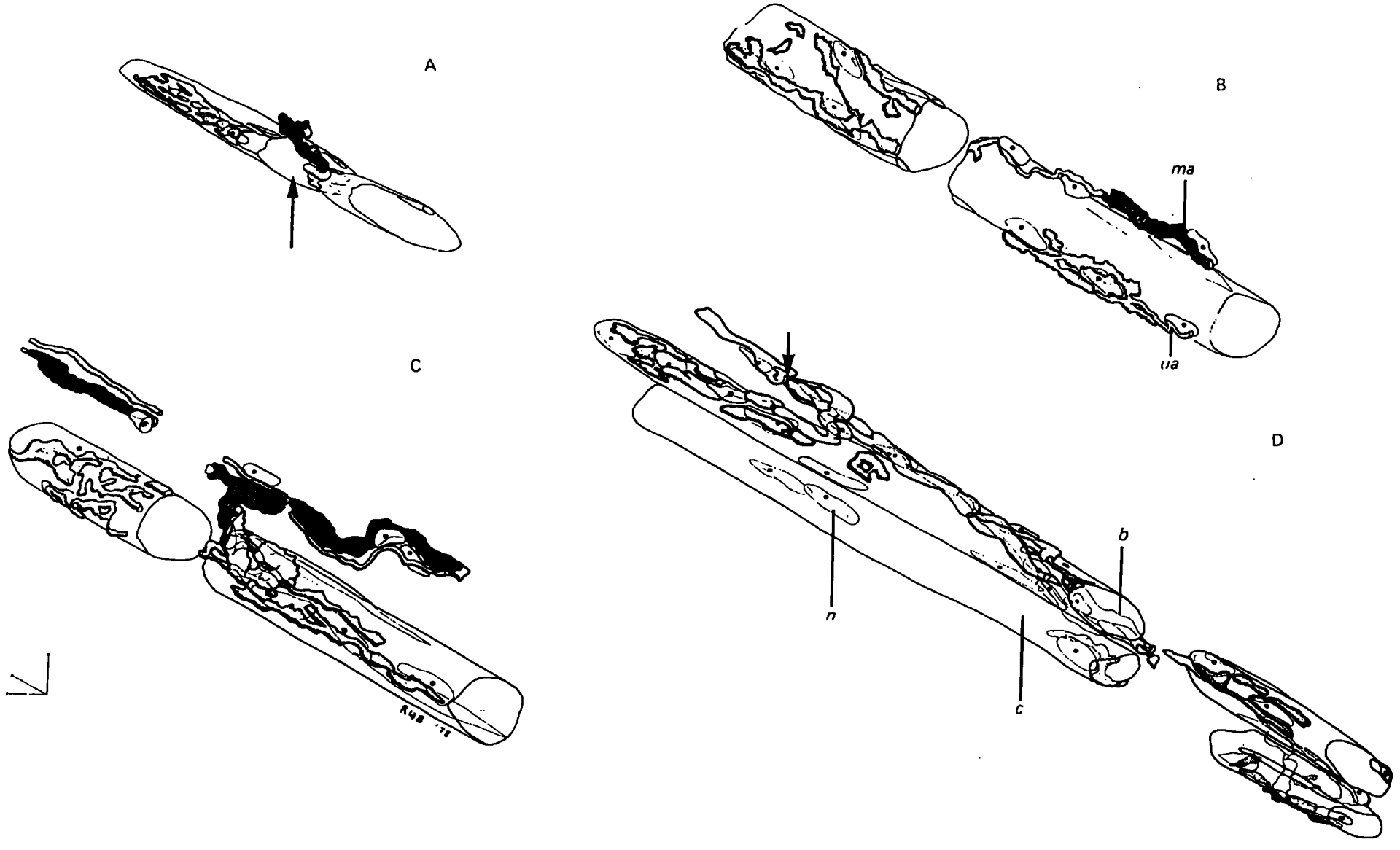


Fig. 8. Isometric reconstructions of the distal (A–C) and proximal (D) motor endings on the bag_1 fibre of spindle 12 (cf. Fig. 4). The identity of these is unknown, but is presumably p_1 or p_2 plates. (A) was the only ending on a bag_1 fibre to have a prominent sole-plate and a noticeable amount of post-junctional folding. An electron micrograph of a thin section from the gap indicated by the arrow is shown in Fig. 11D. The three endings in (B) and (C) were all derived from a single axon. (D) shows the proximal ending on chain 4 in addition to the two endings on bag_1. All three of these endings were derived from a single axon. The last common branching node is indicated by an arrow. This was the only axon innervating a bag_1 fibre that was also found to innervate either bag_2 or chain fibres. *b*, bag fibre; *c*, chain fibre, *n*, myonucleus; other lettering as Fig. 6.

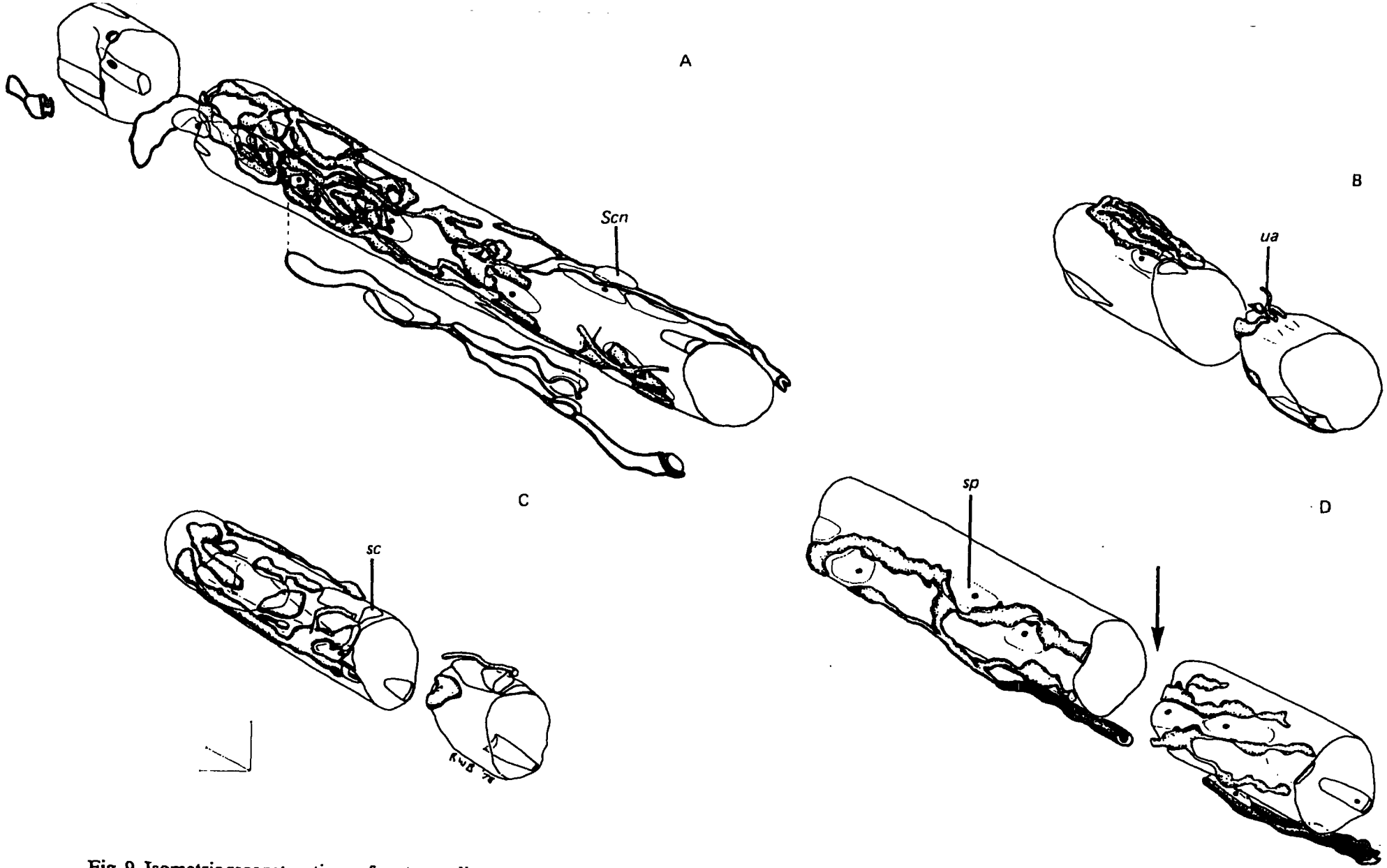


Fig. 9. Isometric reconstructions of motor endings on bag_2 fibres. (A) Spindle 6, proximal at 1·3 mm (cf. Fig. 2). Part of the axon has been displaced vertically downwards. (B) Spindle 9, distal at 1·1 mm (cf. Fig. 3). This is probably a trail terminal and was derived from the same axon (axon f, Fig. 3) that also supplied three chain fibres in the distal pole. (C) Spindle 12, distal at 1 mm (cf. Fig. 4). (D) Spindle 12, distal at 1·5 mm (cf. Fig. 4). An electron micrograph of a thin section from the gap indicated by the arrow is shown in Fig. 12B. The difference in form between (C) and (D) is striking. For lettering see Fig. 6.

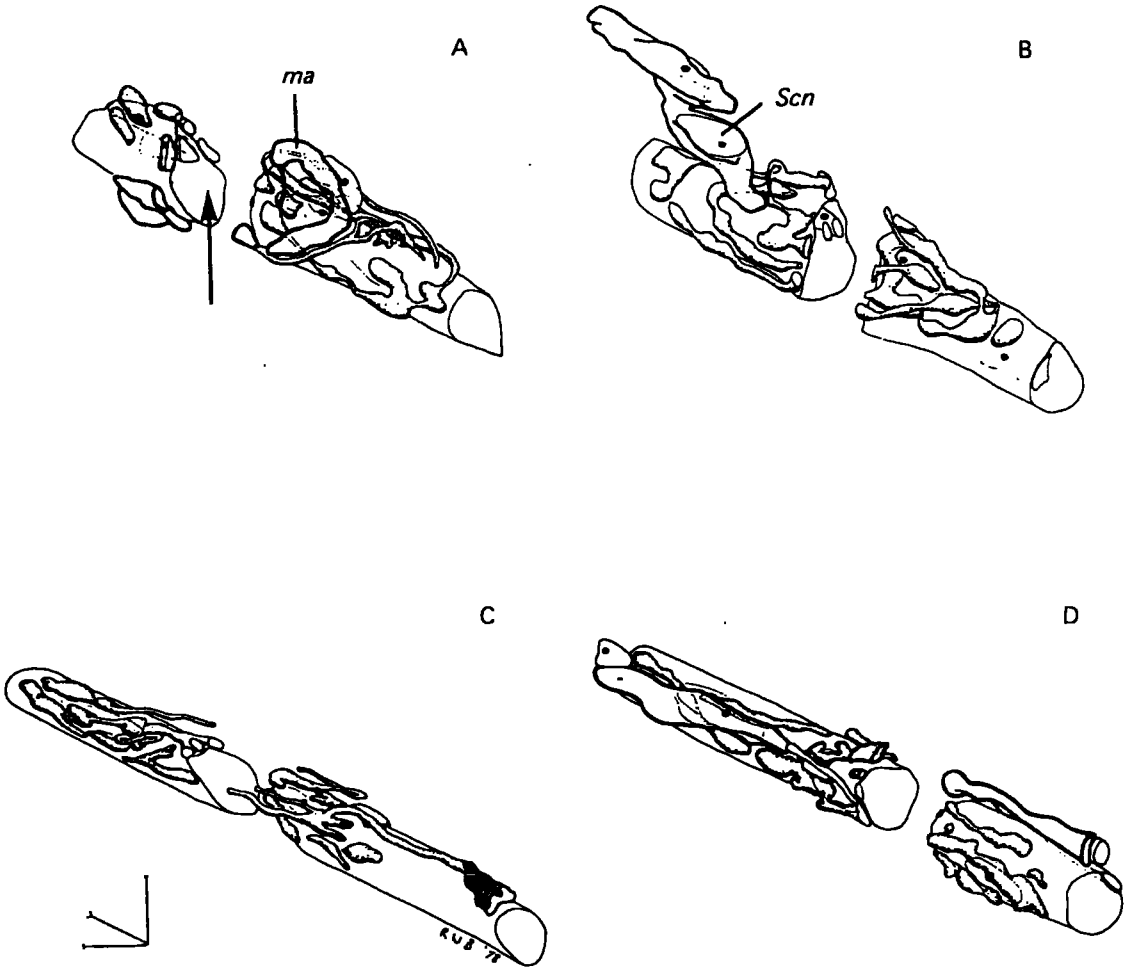


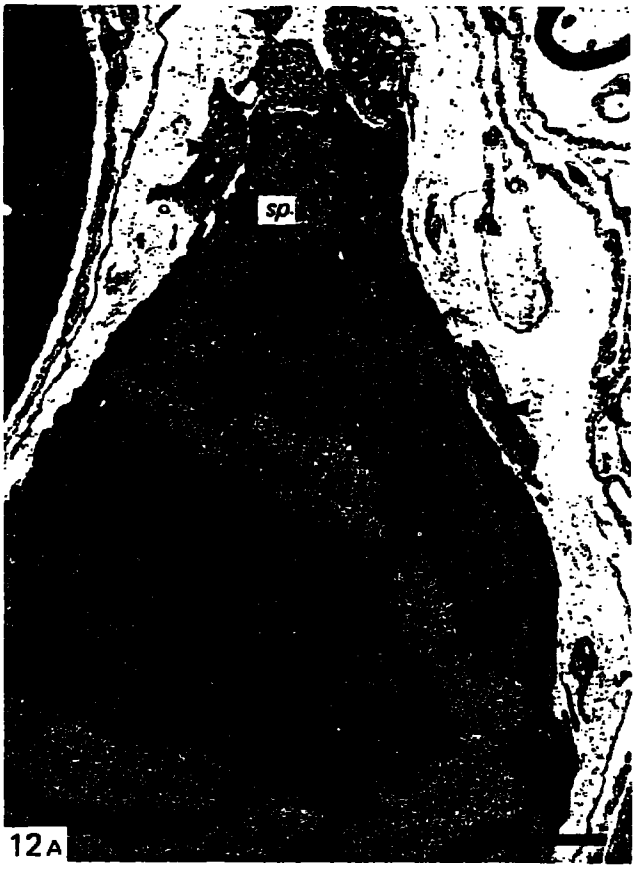
Fig. 10. Isometric reconstructions of motor endings on chain fibres. (A) Spindle 6, chain 2, proximal ending (cf. Fig. 2). An electron micrograph of a thin section from the gap indicated by the arrow is shown in Fig. 12C. (B) Spindle 6, chain 4, proximal ending (cf. Fig. 2). (C) Spindle 9, chain 3, distal ending (cf. Fig. 3). (D) Spindle 12, chain 2, distal ending (cf. Fig. 4). All these endings probably represent trail terminals. (A) and (B) were derived from the same axon. For lettering see Fig. 6.

then turned and cut longitudinally. Using electron microscopy, two groups of terminals were then found approximately 0·5 μm from a nuclear chain fibre, which was the closest possible effector. The nearest blood vessels were separated from the endings by the periaxial space and by cells of the spindle's capsule. The type of axon supplying these endings, their distance from the muscle fibre and their vesicular content together indicate that they are autonomic. Three types of vesicle occurred in them (Fig. 12D): small plain vesicles about 50 nm in diameter, large granular vesicles about 100 nm in diameter and large vesicles containing a moderately electron-dense material and about 150 nm in diameter. In the terminals of the somatic motor endings, the first type of vesicle predominated and there were a few large granular vesicles, but the third type was not found. It is not possible to identify the likely transmitter on the basis of these profiles alone.

Similar results have been reported by Ballard (1978) and the automonic innervation of the spindle has recently been the subject of a detailed study by Barker & Saito (1980), who showed that the majority of the autonomic innervation is typically noradrenergic.



Fig. 11. Transverse sections through motor endings on bag_1 fibres. (A) Spindle 9 (cf. Fig. 7A), Probably a p_2 plate. Note the simple post-junctional structure. (B) Higher power view of (A) showing the thin layer of teloglial cytoplasm covering the free surfaces of the terminals (see Materials and Methods). (C) Spindle 6 (cf. Fig. 6A), probably a p_1 plate. This also has a very simple post-junctional structure. (D) Spindle 12 (cf. Fig. 8A), p_1 or p_2 plate, this was the only ending on a bag_1 fibre to show a well developed post-junctional apparatus with some folding. Some motor terminals are indicated by arrowheads. *sc*, satellite cell; *t*, teloglial cytoplasm. Scale bars (A), (C), (D) 2 μm; (B) 1 μm.



12A



12C



12B



12D

DISCUSSION

Intrafusal distribution of motor axons

The recent demonstration that mammalian muscle spindles contain three types of intrafusal muscle fibre (Banks, Barker, Harker & Stacey, 1975; Gladden, 1976; Banks *et al.* 1977) has led to considerable agreement about their functional significance. Bag_1 fibres are thought to mediate the dynamic response of the primary sensory ending whereas bag_2 and chain fibres mediate its static response (Boyd *et al.* 1977; Barker *et al.* 1978). Furthermore, it is agreed that dynamic fusimotor axons always innervate bag_1 fibres, whereas static fusimotor axons innervate bag_2 or chain fibres or both together (Boyd *et al.* 1977; Barker *et al.* 1978).

However, there is still disagreement about the existence and extent of innervation of bag_1 in common with other types of intrafusal muscle fibre, in particular about the occurrence of static (trail) fusimotor innervation of bag_1 fibres. Direct observation of living spindles (Bessou & Pagès, 1975; Boyd *et al.* 1977) indicated that bag fibres activated by dynamic fusimotor axons (presumably bag_1) are never also activated by static ones. Glycogen-depletion, on the other hand, has indicated that bag_1 and bag_2 fibres are innervated by static axons with about equal frequency (Barker *et al.* 1976*b*; Emonet-Dénand, Jami, Laporte & Tankov, 1980). Accepting for the moment the validity of the results obtained by these two methods, a number of assertions may be made that can be tested using the results of the present work.

(i) Based on direct observations: (*a*) bag_1 fibres are never innervated in common with bag_2 or chain fibres; (*b*) bag_1 fibres are commonly not innervated in one pole (implied in Boyd *et al.* 1977).

(ii) Based on glycogen depletion: bag_1 fibres are frequently innervated in common with bag_2 or chain fibres though not necessarily in the same spindle.

Both (i) (*a*) and (*b*) are evidently not true. Firstly, of the eight axons innervating bag_1 fibres in this study, one also supplied a chain fibre. (The probability that a dynamic axon innervated all three types of muscle fibre (Banks *et al.* 1978) could not be confirmed histologically). Secondly, each bag_1 fibre pole received at least two motor terminals, usually from a single axon. The only intrafusal fibres that lacked motor innervation in one pole, chains 4 and 5 in the distal pole of spindle 12, were of abnormally small diameter in that pole. Should the same be true of any bag fibre that lacked motor innervation, its reduced diameter in the non-innervated pole would be clearly visible in the living spindle.

The occurrence of an axon supplying a bag_1 and chain fibre in common does not, of course, confirm the results of the glycogen-depletion experiments, and some estimate of the frequency of such innervation to be expected must be made in order to assess the validity of (ii). From the results of Barker *et al.* (1976*b*), one can estimate the average frequency that a static axon would be expected to innervate the bag_1 fibre in a spindle as about 3/5. Taking the average number of static axons per

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Fig. 12. (A–C). Transverse sections through probable trail terminals. (A) Spindle 6, bag_2 distal ending at 1 mm (cf. Fig. 2). (B) Spindle 12, bag_2 (cf. Fig. 9D). (C) Spindle 6, chain 2 (cf. Fig. 10A). Some motor terminals are indicated by arrowheads. *sp*, sole-plate nucleus. The chain terminal has a well developed subneural apparatus with a Doyère eminence and post-junctional folding. The bag_2 terminals are intermediate in complexity between the chain type and the simple type usually found on bag_1 fibres. (D) Longitudinal section through an autonomic motor ending close to a nuclear chain fibre in spindle 5. The muscle fibre was the closest possible effector, but showed no obvious post-junctional specialization. The nerve terminal (or varicosity) contains small clear vesicles (*sv*), large dense-cored vesicles (*ldv*) and large opaque vesicles (*lov*). Scale bar (A, B) 2 μm; (C) 1 μm; (D) 0·5 μm.

spindle as 3 (Boyd *et al.* 1977; Banks *et al.* 1978), the probability that a bag_1 fibre will be innervated by at least one static axon is 0·94, or practically certain. This would imply that bag_1 fibres are normally dually innervated by dynamic and static axons, either by multiple innervation of each pole, or by selective innervation of the two poles. Despite the small sample, the present results do not support the possibility of multiple innervation, since each bag_1 fibre pole was usually supplied by a single axon. Selective innervation of the two poles of bag_1 fibres is superficially attractive, since it could account for the apparent non-innervated poles of bag_1 fibres if, during direct observation, their contractions were masked by more powerful ones in bag_2 or chain fibres. However, it seems unlikely that bag_1 contractions during static stimulation could be so systematically missed; nor do the present histological results support the high proportion of shared innervation that would be required. In this context it may be pointed out that the frequent occurrence of bag_2 and chain innervation in common, demonstrated both by direct observation and glycogen-depletion, is reflected in the present work by almost half of the axons to bag_2 or chain fibres supplying both types jointly.

Functional implications

The recent finding of categories of fusimotor action intermediate between the most static and dynamic types (Emonet-Dénand *et al.* 1977) has been interpreted in terms of a considerable amount of common innervation of bag_1 with bag_2 or chain fibres, as indicated by the glycogen-depletion work. Emonet-Dénand *et al.* recognized six categories of action, namely, I, 'pure' dynamic; II, dynamic with suspected static modification; III, unclassifiable; IV, static with suspected dynamic modification; V, static with conceivable dynamic participation; VI, 'pure' static. Category III actions are most likely to be associated with common bag_1 and bag_2 or chain innervation and included in this group are axons whose action varied, depending on the frequency of stimulation or the initial muscle length. Such axons have been observed previously by Emonet-Dénand, Joffroy & Laporte (1972). Their action can be most readily interpreted in terms of the different contractile properties and lengths of bag_1 and chain fibres. However, only about 1 axon in 20 fell into this category. This may be compared with 1 axon from 26 in the present work that was positively shown to innervate bag_1 and either of the other types of intrafusal fibre. The results of recent studies on sub-categories of fusimotor action, although not conclusive, do not support the idea that anything other than category III includes axons supplying bag_1 with bag_2 or chains (Emonet-Dénand & Laporte, 1978; Laporte, 1979).

The highly selective motor innervation of the bag_1 fibre is in marked contrast to the mixed innervation of the bag_2 and chain fibres. Not only do a large proportion of axons branch within a spindle to supply both these types of muscle fibre, but the motor units of most, if not all, static axons contain both chain and bag fibres (Barker *et al.* 1973). Now even 'pure' static axons have heterogeneous effects on the primary response (Emonet-Dénand *et al.* 1977) and it is likely that bag_2 and chain fibre activations affect the primary sensitivity in different ways. Probably the bag_2 fibre mediates a marked position-sensitive response whereas chain fibre contractions lead to a general excitation of the ending with a corresponding reduction of both dynamic and position sensitivities. Some evidence for this can be seen in the results of Emonet-Dénand *et al* (1978). Since bag_2 fibres cannot be activated alone, it seems probable that their major function is to maintain primary afferent position sensitivity during

strong static excitation. Presumably there would be no functional advantages for this position sensitivity to be under separate central control.

Motor endings

The most comprehensive description of the histology of fusimotor innervation is that of Barker, Stacey & Adal (1970), in their categorization of trail and p_1 and p_2 plate types, based on silver impregnation of teased whole spindles. Each of the three types should be represented in the present study since p_1 plates are known (Barker, Emonet-Dénand, Laporte & Stacey, 1980) to be the terminals of β axons (of which there is at least one, in spindle 6), whereas dynamic and static γ axons are thought to end as p_2 plates and trail terminals respectively. The form of the endings, as represented in the isometric reconstructions, does not allow a clear-cut classification into three types, but each can be assigned to p_1, p_2 or trail types on the basis of the above definitions, and the results are consistent with the description of Barker *et al.* (1970).

Kucera (1980*a*, *b*) has described two types of motor ending on the intrafusal muscle fibres of cat spindles, based on reconstruction of serial sections stained to demonstrate cholinesterase activity. The two types differed in size and complexity, the small simple type being called 'rim' endings and the larger, more complex type 'plate' endings. Kucera identified the two types as trail and plate endings, respectively, but the rim endings cannot be trail terminals for the following reasons. In the present work each chain fibre most commonly had a single ending per pole. These were some of the most complex endings in the spindles, with distinct nucleated sole-plates. They can therefore be identified with the plate endings of Kucera, of which there was also only one per pole. In addition, both sets of endings were similarly located on the muscle fibres. Since no other somatic motor endings occurred and trail innervation is almost invariably present on chain fibres (Barker *et al.* 1970), they must be the trail terminals.

The location of the rim endings, their apparently great simplicity and their absence from some muscle fibres (Kucera, 1980*a*, *b*) strongly suggest that they should be identified with the autonomic innervation now known to exist in some spindles (see above and Barker *et al.* 1980). That they possess cholinesterase activity is not surprising since this has been demonstrated in other sites of sympathetic innervation (Eränkö, Reichardt, Eränkö & Cunningham, 1970), and sympathetic neurons have been shown to form cholinergic synapses with skeletal muscle fibres in culture (Nurse & O'Lague, 1975). Kucera, Dorovini-Zis & Engel (1978) have described a 'diffuse' ending in rat spindles, which seems to be homologous to the rim endings of the cat. If so, they also cannot be identified as trail innervation in contrast to the conclusion of Kucera *et al.* (1978).

Barker *et al.* (1970) attempted to correlate the three types of motor ending recognizable in silver preparations with various degrees of ultrastructural complexity. This correlation was subsequently found to be inaccurate (Barker *et al.* 1976*a*; Barker *et al.* 1978) and this has been confirmed in the present work. Complexity, in terms of prominence of sole-plate and degree of post-junctional folding, tends to increase in endings on bag_1, bag_2 and chain fibres respectively. On each type of muscle fibre there may be a tendency for endings closer to the primary sensory ending to be simpler than those further away, though the present sample is too small to allow one to be confident about this. On the other hand, the primary ending definitely seems to influence the location of the motor endings, as might be expected if the arrival of the primary afferent axon triggers the development of the muscle spindle (Landon, 1972; Milburn, 1973).

SUMMARY

The motor innervation of four muscle spindles from the tenuissimus muscle of the cat was demonstrated using reconstructions of 1 μm thick, serial transverse sections. Analysis of the results clearly indicates that the bag_1 intrafusal muscle fibre usually does not receive a static fusimotor input via trail innervation. In contrast to the highly selective innervation of bag_1 fibres, almost half the axons suppying bag_2 or chain fibres branched to terminate on both types of fibre. The significance of these results is discussed in relation to previous studies on fusimotor innervation and to their functional implications.

The presence of autonomic innervation is a further complication that appears to have led to erroneous conclusions concerning the nature of the trail innervation of chain fibres in a recent study of the distribution of cholinesterase activity in the spindle.

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FORM AND DISTRIBUTION OF SENSORY TERMINALS IN CAT HINDLIMB MUSCLE SPINDLES

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The sensory innervation of cat hindlimb muscle spindles was studied by reconstruction, electron microscopy, and examination of teased, silver preparations to ascertain the form of the terminals and their distribution to bag_1 (b_1), bag_2 (b_2), and chain (c) muscle fibres. Reconstructions were made of two primary endings, one S_1 secondary ending, and the branching of four primary and six secondary axons. For the silver analysis spindles were teased from 14 different hindlimb muscles, the largest samples being from tenuissimus, peroneus brevis, p. longus, p. tertius, superficial lumbrical, extensor digitorum longus, and soleus. Among 310 spindles examined, 40 lacked a b_1 fibre. These were all portions of tandem spindles in which the b_2 fibre was continuous from one capsule, where it was accompanied by b_1 and c fibres, to another, in which it was accompanied by c fibres only. These have been designated 'b_2c spindle units' as distinct from 'b_1b_2c spindle units'.

Counts of myonuclei in the primary regions of four b_1b_2c spindle units revealed 52–106 in the nuclear bags, b_1 bags averaging 68, b_2 bags 80. The average number in a myotube region was nine (range 6–12), and in a c fibre 24 (range 11–38). The reconstructions showed a close association between nucleation and innervation, but no constant relation between number of myonuclei and terminal contact area. They also showed that the largest cross-sectional areas of each bag fibre corresponded with the sites of S_1 secondary ending innervation. Approximately 70–90% of the cross-sectional area of each bag fibre was occupied by myonuclei in the bag region, 30–50% in the myotube regions, and 10% in the region of secondary innervation. In b_2c spindle units the equatorial nucleation of the b_2 fibre usually resembled that of a c fibre.

The intramuscular diameter of Ia axons supplying b_1b_2c spindle units (mean 7.5 μm, range 3.4–12.8 μm, $n = 213$) was generally thicker than that of Ia axons supplying b_2c spindle units (mean 5.1 μm, range 2.2–9.0 μm, $n = 37$). The distribution of terminals by the first-order branches (usually two) of Ia axons to b_1, b_2, and c fibres was exclusively from heminodes, and was either segregated (b_1 fibres supplied separately from b_2 and c fibres, thereby resulting in separation of dynamic and static inputs) or mixed. Mixing was restricted most frequently to the dynamic input, and usually resulted from b_1 fibres sharing a supply of terminals with a few c fibres. Distribution of terminals was usually segregated in tenuissimus and mixed in superficial lumbrical muscles, but in most muscles neither type of distribution predominated. Among 270 b_1b_2c spindle units, 32 had more than one b_1 fibre, and 12 had primary endings formed by two Ia axons.

Primary terminal systems supplied to bag fibres consisted of a middle portion, in which the terminals were arranged mainly as regular transverse bands, and portions at each end, in which they were disposed irregularly. In the silver analysis, b_1 terminal systems in 151 b_1b_2c primary endings were distinguished from those supplied to b_2 fibres by having more of their total length occupied by irregular portions (on average 57%, as compared with 33% in b_2 fibres), and more bands per unit length in the middle. In the two reconstructed primaries the b_1 fibres received 33 and 37% of the total terminal contact area, the b_2 fibres 25 and 24%, and the c fibres 5–12% individually, 42 and 39% collectively. Primary endings supplied to b_2c spindle units were mostly irregular in appearance.

The polar position of 351 secondary endings was S_1 253, S_2 79, S_3 15, S_4 3, S_5 1; 67.8% were distributed to b_1b_2c fibres (mostly as S_1 endings), 20.8% to b_2c fibres, 6.3% to c fibres, and 5.1% to b_1c fibres. The intramuscular diameter of II axons terminating as S_1 endings was generally greater (mean 3.9 μm) than that of II axons terminating as S_2–S_5 endings (mean 2.9 μm); 75% of b_1b_2c II axons had diameters that fell within the lower part of the b_1b_2c Ia diameter range. Most II axons had two first-order branches; the distribution of terminals by the first-order branches of b_1b_2c II axons was usually

mixed. Terminals were derived either exclusively from heminodes (as in most S_1 endings) or from both heminodes and penultimate nodes.

The mean length of 313 secondary endings was 348 μm (range 138–716 μm) as compared with a mean length of 359 μm (range 242–608 μm) for 151 primaries. In 83 b_1b_2c S_1 endings the innervated portions of the bag fibres represented, on average, 42% (b_1) and 51% (b_2) of the total length of the ending. In 64% of the endings the b_2 fibre received more terminals than the b_1. In the reconstructed S_1 ending the b_1 fibre received 8% of the total terminal contact area, the b_2 fibre 17%, and the c fibres 16–22% individually, 75% collectively. Some muscles had fewer secondaries than others; the Ia:II ratio ranged from 1:1.2 (superficial lumbrical) to 1:1.8 (peroneus longus).

The constant features of spindle sensory innervation that emerge from this study (e.g. the dense primary innervation of the b_1 fibre) are discussed in the context of spindle development and in terms of their functional significance. The data on the branching of spindle afferents is related to the work of others on pacemakers and the manner in which nerve impulses are generated from the endings and propagated into the axons. We suggest that transduction may occur by a deformation of the sensory terminal owing to increased tension in the basal lamina. It is supposed that the permeability of the Na^+ channels is affected by an intracellular messenger (probably Ca^{2+}) released from a bound state by increase in cytoskeletal tension. Reasons are given as to why the afferent innervating b_2c spindle units should be regarded as primary. The probable functional significance of these units is discussed, and some correlations are made between the function of certain muscles and the characteristics of their spindle populations.

1. Introduction

It is now accepted that mammalian muscle spindles are composed of nuclear-chain fibres and two types of nuclear-bag fibre, bag_1 and bag_2 (Ovalle & Smith 1972; Banks *et al.* 1975, 1977*b*; Gladden 1976); and it is generally agreed that the dynamic responsiveness of the primary ending is mediated by the bag_1 fibre, and its static responsiveness by bag_2 and chain fibres (Barker *et al.* 1976, 1978; Boyd *et al.* 1977). In arriving at these conclusions attention was necessarily focused on the distribution of the motor innervation to the three types of intrafusal muscle fibre. The main purpose of this investigation has been to make a similar enquiry into the distribution of the intrafusal sensory innervation.

The essential features that emerge from previous descriptions of mammalian spindle sensory innervation (mostly cat) may be summarized under the headings of *primary endings*, *secondary endings* and *diameters of spindle afferents*, as follows.

Primary endings. An annulospiral ending (Ruffini 1898) is supplied by branches of a Ia axon (Hunt 1954), which distributes large spirals to the bag fibres and small spirals to the chain fibres (Boyd 1962). A first-order branch of the Ia axon (usually two are produced) may innervate both bag and chain fibres, or exclusively supply either bag or chain fibres (Barker & Cope 1962). The ending terminates on the densely nucleated equatorial parts of the intrafusal muscle fibres (the nuclear bags, myotube regions, and nuclear chains), and occupies a length of about 300 μm. The terminals consist of spirals of up to four or five turns, many half rings, and a few complete rings. They are set closely together around the middle of each nuclear bag, disposed more loosely to either side, and are usually of an irregular clasping form in the myotube regions (Barker 1948). A terminal may end on a single muscle fibre, or on two or three adjacent ones to form an interlocking 'sensory cross-terminal' (Adal 1969). These usually occur between chain fibres, but may also occur between a bag and a chain fibre (Corvaja *et al.* 1969; Banker &

Girvin 1971; Scalzi & Price 1972). Some primary endings in the cat are irregular in appearance and have only a few rings and spirals. Spindles that lack secondaries usually have primaries of this type, as in 'simple' single spindles (Ruffini 1898) and simple spindle units that form part of tandem spindles (Barker & Ip 1961). A primary ending may occasionally be composed of terminals supplied by two axons (Ruffini 1898), and instances of primary endings restricted to bag fibres have been observed (Barker & Cope 1962; Jones 1966).

Secondary endings. Most cat hindlimb spindles are supplied with one secondary ending next to the primary (Barker & Ip 1961), but up to five may occur, three on one side of the primary and two on the other, each occupying lengths of about 400 μm designated S_1, S_2, S_3 according to their position relative to the primary (Boyd 1962). Secondary terminals are also mainly annulospiral (Barker 1948), though some may be in the form of sprays. The spirals are small and restricted to the chain fibres, whereas the sprays terminate on the bag fibres (Boyd 1962). The ending is more dispersed than the primary, and the terminals, some of which interlock around adjacent muscle fibres, are mostly thin and claw-like (Tello 1922).

Diameters of spindle afferents. Measurements of the total internodal diameters of spindle afferents made in the neighbourhood of spindles show that: (i) the total diameter of primary axons is about twice that of secondary axons (Ia, mean 12.4 μm; II, mean 6.0 μm; for cat tenuissimus (Boyd 1962)); (ii) the thinnest primary axons overlap in diameter with the thickest secondaries (Ia–II diameter overlap 8–11 μm (Boyd 1962)), and are mostly supplied to spindles that do not receive secondary endings (Adal & Barker 1962).

These observations were all made before it was realized that bag fibres were of two types, and that the functional differences between them made it necessary to know about their individual sensory innervation. It was this need that motivated the present enquiry.

We began by making reconstructions from serial sections of the sensory innervation of some of the cat tenuissimus spindles whose motor innervation had already been analysed following direct observation of the effects of fusimotor stimulation (Banks *et al.* 1978; Banks 1981*a*). Reconstructions were made of two primary endings, one S_1 secondary ending, and the branching of four primary and six secondary axons. These showed, among other things, that: (i) there were distinct differences in the form of the primary terminals on bag_1 and bag_2 fibres; (ii) the first-order branches of all the Ia axons supplied bag_1 fibres separately from bag_2 and chain fibres (segregated distribution); and (iii) the S_1 secondary axons innervated all three types of muscle fibre.

These features of the reconstructions were easily recognizable in teased, silver preparations of whole spindles, making it possible to identify bag_1 and bag_2 fibres by distinguishing (as one of several criteria) the differences in their sensory innervation. The findings from the small sample of reconstructed tenuissimus spindles could thus be compared with those from a much larger sample of spindles teased from several different hindlimb muscles. This showed, among other things, that the distribution of terminals by first-order branches of Ia axons is not always segregated, and that irregular primary endings are characteristic of spindle units that lack a bag_1 fibre. Preliminary accounts of some of the results have been published (Banks *et al.* 1977*b*, 1979, 1981).

2. Materials and methods

(a) *Reconstructions and electron microscopy*

The sensory endings and axons reconstructed from serial transverse sections belonged to four of the 20 cat tenuissimus spindles studied by Banks *et al.* (1978) in their analysis of intrafusal contractions and motor innervation, namely, spindles 6, 8, 9 and 12 (see their table 1). Additional information was obtained from serial longitudinal sections of spindle 5. Apart from the inclusion of post-fixation with osmium tetroxide, the procedures relating to the fixation of these spindles, their preparation for histological analysis, and the criteria subsequently used for the identification of muscle-fibre type, were as described by Barker *et al.* (1978).

(i) *Microtomy*

Serial transverse sections about 1–2 μm thick, cut for light microscopy, were stained with 1.7 mg/ml toluidine blue in 1.7 mg/ml borax solution, or with a saturated solution of *p*-phenylenediamine in methanol. The sections of spindle 8 were cut on an LKB Ultratome UM1 and collected in batches of about 5–20 on glass slides, the last section being collected separately, since the order of the other sections was not always known. The average thickness of transverse sections relative to the living spindle was estimated by counting the number of sections between a pair of structures, e.g. blood vessels, marked on the photograph of the spindle taken at the conclusion of the experiment (Banks *et al.* 1978) before fixation. No correction for longitudinal shrinkage due to processing was therefore necessary.

Serial transverse sections of spindles 6, 9 and 12 were cut on a Reichert OMU3 ultramicrotome and collected on strips of coverglass in ribbons of ten, five such strips being mounted on each glass slide. The graduated knife-advance control of the microtome provided a nominal section thickness of 1 μm. Longitudinal shrinkage averaged 8% as estimated by comparing distances between pairs of structures in the living preparation with the nominal distances provided by summing section thicknesses. This correction has not been applied to the measurements quoted for these three spindles.

Sections for electron microscopy were cut at selected sites, stained with uranyl acetate and lead citrate, and examined with an A.E.I. EM 801 electron microscope at an accelerating voltage of 80 kV.

(ii) *Photography and reconstructions*

Every section containing part of a sensory axon or terminal was photographed on 35 mm film with a Zeiss Ultraphot with ×40 or ×100 planapo objectives. With ×4.5 enlargements of the photographs, two types of reconstruction were made: schematic diagrams showing the branching and myelination of four primary and six secondary axons supplying the spindles (figure 1); and semi-diagrammatic isometric reconstructions of two primary endings (spindles 6 and 12) and one S_1 secondary ending (spindle 6) (figures 4–6).

The isometric reconstructions were made by tracing the relevant features of each photograph onto acetate film with use of coloured inks to distinguish between the various components. Adjacent tracings were placed along an inclined line to produce the required amount of spatial resolution. Since no external reference was available, the assumption was made that one part of the reconstruction always fell on this line, namely, the centre of the bag_1 fibre. The bag_2

fibre was orientated so that its centre was either vertically above (spindle 6) or horizontally to the right (spindle 12) of this. The bag_1 fibre was thus artificially straightened, while the position of the bag_2 fibre was restricted to a plane that passed through the bag_1 fibre's centre, and the chain fibres were positioned relative to that plane. Examination of longitudinal sections of the equatorial regions of other spindles showed that these constraints produced little distortion. For clarity, the bag fibres were reconstructed separately from the chain fibres. The reconstructions were built up by using the same colours to draw the envelopes of every group of ten tracings, and then by making tracings of the envelopes in indian ink.

(iii) *Measurement of sensory-terminal contact areas*

The photographs used for reconstructing the three sensory endings were also used to estimate the areas of contact between the terminals and the muscle fibres. The length of contact between terminal and fibre, and the length of fibre surface devoid of terminal, were determined in each section of a muscle fibre that included a sensory neuromuscular junction. Measurements were made with a map-measuring wheel, converted into micrometres and summed to give areas in micrometres squared, section thickness being taken as 1 μm. These measurements provided estimates of the total terminal–fibre contact area for each sensory ending, and this could be expressed as a proportion of the total surface area available for contact so as to give an indication of density of innervation. Any error involved in making measurements with the mapping wheel was assessed by using it to determine the lengths of many straight lines of differing lengths, ten measurements being made of each length. A comparison of the means of these measurements with the original terminal–fibre contact lengths showed that the error involved was relatively greater for shorter lengths, and that the probable overall error was an underestimate of about 4%.

(*b*) *Analysis of silver preparations*

(i) *Nature of sample*

The total sample comprised 310 spindle capsules, of which 84 were linked in tandem. They were teased from 72 hindlimb muscles removed from 38 adult cats, as follows.

TABLE 1.

muscle	number of spindle capsules		number of muscles teased	
tenuissimus	70		14	
peroneal muscles				
p. longus	20		4	
p. brevis	61	108	10	23
p. tertius	27		9	
lumbrical muscles				
superficial	34		10	
deep	8	42	1	11
soleus	31		3	
extensor digitorum longus	21		7	
extensor digitorum brevis	7		1	
flexor hallucis longus	11		4	
tibialis anterior	13		5	
tibialis posterior	1		1	
interosseus	5		2	
rectus femoris	1		1	
total . . .	310		72	

The sample of spindles assembled for analysis was selected from the large collection of teased preparations that has accumulated in this laboratory over the years, excellence of staining of sensory innervation being the sole criterion for inclusion. With one exception all the spindles had been stained by de Castro's silver method as modified by Barker & Ip (1963); the exception was a rectus femoris spindle stained with Gairns's (1930) gold chloride method.

Of the 72 muscles, 26 had been the object of experiments involving either de-efferentation (4), de-efferentation and sympathectomy (15), or degeneration of all but one or a few axons in their motor supply (7). Of the total sample of spindle capsules, 83 were teased from these muscles.

(ii) *Diameter and branching of spindle afferents*

The total diameters of 259 Ia and 357 II axons were measured by using a Zeiss micrometer eyepiece and a $\times 40$ objective. The measurements were made as far from spindle entry as possible in order to avoid the length over which diameter increases as the first subdivision before termination is approached. Each measurement recorded was the mean of three internodal readings. Conversion to the equivalent fresh diameter requires multiplication by a factor of 1.5 (Stacey 1969), but this has not been applied to the results.

The branching and distribution of 182 Ia axons was analysed by using a $\times 100$ objective under oil immersion. Each analysis was double-checked; any instance occasioning the slightest doubt resulted in the axon in question being excluded from the final sample.

(iii) *Analysis of sensory endings*

Each primary ending was first examined to distinguish between the terminal systems distributed to the bag_1 and bag_2 fibres on the basis of differences in amount of irregularity. Terminals were regarded as 'regular' when they were set at right angles to the longitudinal axis of the bag fibre, as in the nuclear-bag region, and 'irregular' when they were set diagonally to, or in parallel with, this axis, as in the myotube regions. Confirmation of the identity of the bag fibres was then sought by checking on their length (bag_2 longer); association with (bag_2), or dissociation from (bag_1), chain fibres in the equatorial region; abundance (bag_2) or scarcity (bag_1) of associated elastic fibres in the extracapsular polar region; motor innervation by p_1 and/or p_2 plates (typically supplied to bag_1) and trail endings (typically supplied to bag_2); and sensory innervation by secondary endings (bag_1 usually receives fewer terminals).

Each primary ending was then measured so as to record its total length, the lengths of the regular and irregular portions of the terminal systems on each bag fibre, and the maximum diameter of each terminal system. Finally, the number of terminals encircling each bag fibre in the regular portion of its primary terminal system was counted over a length of 50 μm.

If the spindle under analysis was supplied with one or more secondary endings, the length of each was measured, its position (S_1, S_2, or S_3) was noted, and the supply of terminals to one or both types of bag fibre was observed. In a sample of 83 S_1 secondary endings the number of terminals on each type of bag fibre was counted and the length of the innervated portion of the fibre was measured.

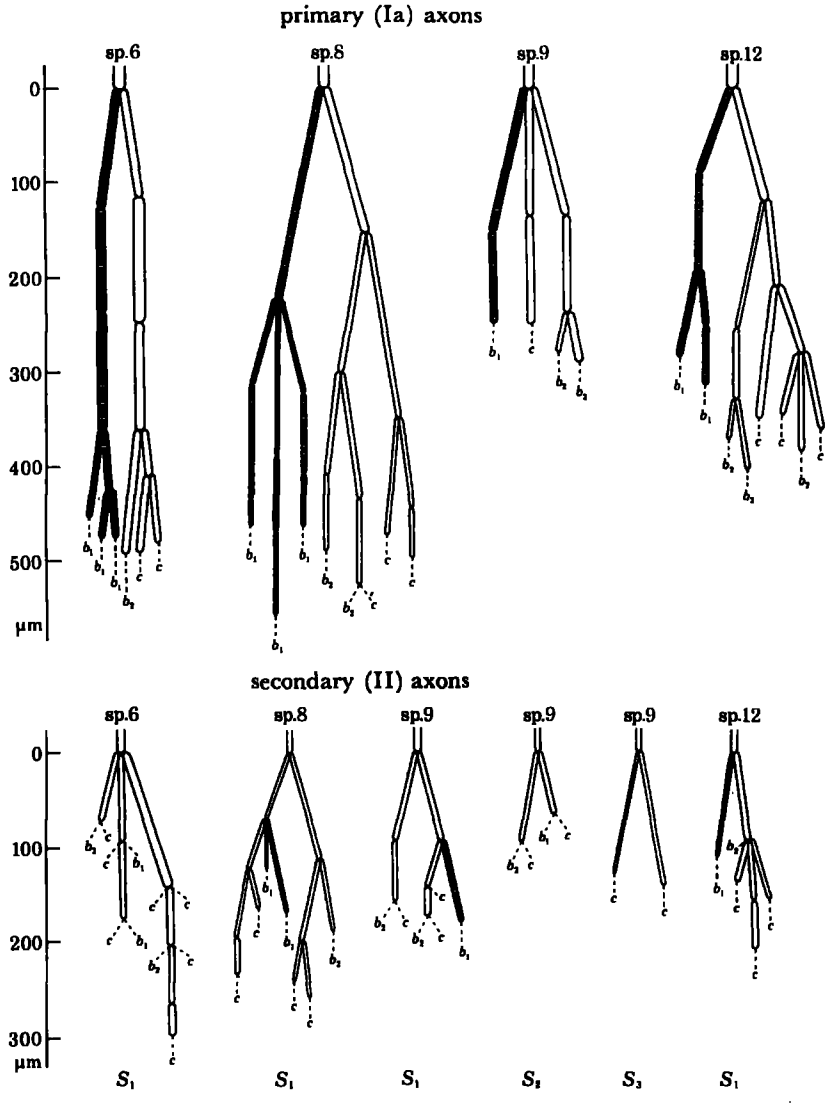


FIGURE 1. Branching of the primary and secondary axons supplying spindles (sp.) 6, 8, 9 and 12, and the distribution of their branches to the three types of intrafusal muscle fibre. Black branches exclusively innervate bag_1 (b_1) fibres; preterminal axons indicated by dotted lines. The primary axon of spindle 12, and the primary and secondary axons of spindle 6, are shown in more detail in the reconstructions reproduced in figures 4, 5 and 6, respectively. Symbols: b_2, bag_2 fibre; c, chain fibre(s); S_{1-3}, secondary endings.

3. RESULTS

(*a*) *Reconstructions and electron microscopy*

(i) *Branching and distribution of spindle afferents*

The sensory innervation of the four spindles studied was: spindle 6, S_1P; 8, $S_1PS_1S_2$; 9, $PS_1S_2S_3$; 12, S_1P (symbols according to Boyd (1962), given in proximal-to-distal order). Figure 1 shows the branching of the four primary axons and six of the secondary axons, and indicates the distribution of the branches to the three types of intrafusal muscle fibre.

The first subdivision of each primary axon produced either two or three first-order branches, one of which was the exclusive distributor of terminals to the bag_1 (b_1) fibre. This branch

usually subdivided further and had two to five internodes. Most of the 18 subdivisions undergone by the four primary axons were dichotomous; only three were trichotomous. One of these was the first subdivision of the axon innervating spindle 9, which resulted in one first-order branch being the exclusive distributor of terminals to each of the three fibre types. In the other three primary axons the bag_2 (b_2) and chain (c) fibres each derived their terminals from the same first-order branch.

The primary axons reached their heminodes after branching over distances of 250–558 μm. The internodes became shorter as the heminodes were approached; the mean lengths of all first to fifth primary internodes in figure 1 are 192, 123, 95, 75 and 58 μm, respectively. When the first subdivision was dichotomous, the first internodes differed in total diameter by about 1.0 μm, the thinner belonging to that which exclusively innervated the b_1 fibre. Total diameter decreased distally by 0.3–6.0 μm (mean 2.6 μm) between first and last internodes, the mean decrease per 100 μm length being 0.4 μm. Myelin thickness was approximately halved as between the last internode of the parent axon and the first internodes of its branches (e.g. from 1.7 to 0.8 μm), and halved again as between first and last internodes (e.g. from 0.6 to 0.3 μm).

The eight secondary axons distributed terminals to the muscle fibres as follows (spindle reference numbers in brackets): S_1 (6), S_1 (8), S_1S_2 (9), S_1 (12) to b_1, b_2 and c fibres; S_1 (8) to b_1 and c fibres; and S_2 (8), S_3 (9) to c fibres only. Reconstructions of the branching of six of the axons (figure 1) showed that the terminals were supplied by preterminal axons that arose, not only from the heminodes, but also from some of the penultimate nodes.

The secondary axons reached their heminodes over distances of 65–300 μm. They did not branch as extensively as primary axons and their internodes were generally shorter; the mean lengths of all first to fourth internodes in figure 1 are 97, 68, 51 and 37 μm, respectively. Trichotomous branching was relatively more frequent (three of 11 subdivisions). Six first-order branches had only one internode; four of these belonged to the dichotomously branching S_2 and S_3 axons innervating spindle 9. There were four instances of first-order branches exclusively supplying one type of muscle fibre, namely, in the S_1 afferents of spindle 6 (to c fibres) and 12 (to the b_1 fibre), and the S_3 afferent of spindle 9 (to c fibres). Total diameter decreased by a mean of 0.4 μm per 100 μm length between first and last internodes, much as in primaries, but there were four instances of increase; these ranged from 0.3 to 1.1 μm. With few exceptions (four in 25) myelin thickness remained the same from the parent axon to the most distal internode, e.g. all 12 internodes in the branching of the S_1 afferent of spindle 8 had a myelin thickness of 0.3 μm.

(ii) *Equatorial nucleation*

The nuclei of intrafusal muscle fibres are located either peripherally underneath the sarcolemma (subsarcolemmal nuclei) or internally among the myofibrils (myonuclei). In teased preparations it is often difficult to distinguish between subsarcolemmal nuclei and those of satellite cells and endomysial fibrocytes. The reconstruction of the distal pole of spindle 6 showed that satellite cells were mostly found in association with the b_2 fibre in the extracapsular region. They were less frequently associated with the b_1 fibre and rarely occurred on c fibres (Banks 1981*b*). Equatorially both satellite cells and subsarcolemmal nuclei were scarce, whereas myonuclei were abundant. The reconstructions produced an opportunity for studying the number and distribution of myonuclei in the area of sensory innervation and the nature of the structural substrate beneath the sensory terminals.

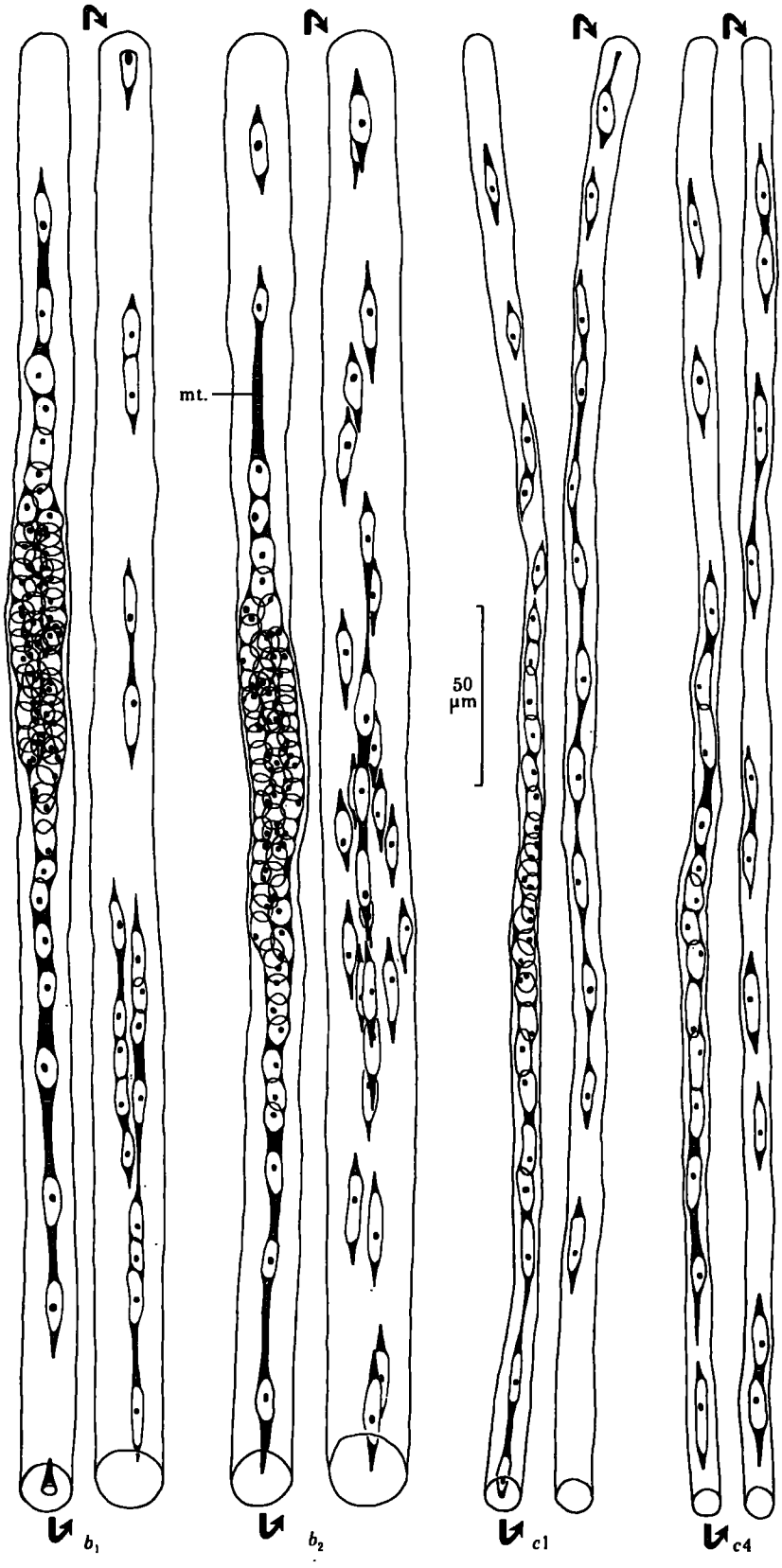


FIGURE 2. For description see opposite.

Figure 2 illustrates the equatorial nucleation of spindle 6 as reconstructed from sections of the primary and S_1 secondary regions of the b_1 and b_2 fibres and the longest and shortest c fibres (see also plates 1 and 2). Each nuclear bag consisted of a sheath of myofibrils that enclosed an aggregation of closely-packed, oval (typically 5 μm × 7 μm) myonuclei. At each end these reduced in number from three to two abreast, and finally to a single row of oblong (typically

TABLE 2. NUMBER AND DISTRIBUTION OF PRIMARY-REGION MYONUCLEI IN FOUR CAT TENUISSIMUS SPINDLES

(Symbols: b_1, bag_1 fibre; b_2, bag_2 fibre. Chain fibres in spindles 6 and 12 numbered in order of decreasing length.†)

spindle no.		bag fibres				chain fibres						combined total
		myotube	bag	myotube	total	1	2	3	4	5	total	
6	b_1	9	52	12	*73*	24	23	22	14		*83*	*228*
	b_2	9	53	10	*72*							
8	b_1	9	52	9	*70*	37	36	23	26	18	*140*	*316*
	b_2	10	88	8	*106*							
10	b_1	6	59	11	*76*	38	30	25			*93*	*256*
	b_2	11	68	8	*87*							
12	b_1	9	34	9	*52*	24	19	18	20	11	*92*	*197*
	b_2	8	35	10	*53*							

† Chain fibres 2 and 3 in spindle 6 are numbered as 3 and 2 by Banks (1981*a*), and he numbers chains 1, 2, 3 and 4 in spindle 12 as 4, 1, 2 and 3.

5 μm × 9 μm) nuclei contained within a centrally placed myotube. It is convenient to define the nuclear bag as being delimited at each end by the level at which there is a consistent presence of three myonuclei abreast in five successive transverse or longitudinal 1 μm sections; and to define a myotube region as occupying a length of fibre from this level to the end of its axial core of sarcoplasm. Thus defined, the nuclear bags measured 70 μm (b_1) and 86 μm (b_2) long and contained 52 and 53 myonuclei, respectively. The lengths of the myotubes were 100 and 172 μm (b_1) and 93 and 148 μm (b_2), the longest being adjacent to the site of the S_1 secondary ending. They contained nine and twelve (b_1) and nine and ten (b_2) myonuclei, respectively. Table 2 includes these counts together with similar counts for spindles 8, 10 and 12. In this small sample it is evident that b_1 and b_2 fibres either have a very similar number of myonuclei in the primary region or have most located in the b_2 fibre.

On the distal side of the primary region myonuclei were scattered at intervals along the length of the bag fibres, becoming fewer as these left the periaxial space for the distal pole. Each nucleus was more or less centrally aligned and enclosed within an envelope of sarcoplasm. On the proximal side, where the S_1 secondary ending was located, there were dense aggregations

FIGURE 2. Equatorial nucleation of spindle 6 as reconstructed from sections of the primary and S_1 secondary regions of the bag_1 (b_1) and bag_2 (b_2) fibres and the longest and shortest chain (c) fibres (numbered 1 and 4, as in table 2). Only myonuclei are shown; the few subsarcolemmal nuclei and satellite cells present have been omitted. The myonuclei are accurately drawn except those filling the nuclear bags, which are drawn as freehand outlines around their nucleoli. The alignment of each muscle fibre relative to the other is that which obtained in the spindle; the distal end of each fibre lies at top left. Sarcoplasm, such as that filling each myotube (mt.), is shown black. See also figures 5 and 6, and plates 1 and 2.

of myonuclei in those areas of the bag fibres that received secondary terminals (see figures 2 and 8). In the b_1 fibre there were two rows of nuclei in this area, each enclosed within a separate myotube, whereas in the b_2 fibre the nuclei were mostly contained within sarcoplasmic envelopes situated at all levels among the myofibrils. The same transverse section might include up to four. Bag-fibre myonuclei in the secondary region were usually longer and narrower than those in the primary region, typically measuring 4 μm × 14 μm.

Counts of the myonuclei present in the primary region of the *c* fibres in spindles 6, 8, 10 and 12 are given in table 2. There was a tendency for the longest fibres to have the most nuclei. In spindle 6 the myonuclei in the longest fibre were enclosed within a myotube that was more or less continuous throughout the primary and secondary regions (see figure 2). The nuclei were closer together in the primary region, and for a short length at the equator they formed a double instead of a single row so as to give the appearance of a miniature nuclear bag. By contrast, the shortest *c* fibre had a single row of myonuclei in a myotube that was confined to the primary region.

Measurements of the total cross-sectional area of the bag fibres in spindle 6, made at intervals during their course through the equatorial region, showed that there was a marked increase in the region of the S_1 secondary ending (see figure 3). The largest cross-sectional areas of each bag fibre corresponded with the sites at which they received secondary terminals. At these levels their cross-sectional areas were approximately twice those at the equivalent levels on the distal side of the primary region, where there was no secondary ending. In each bag fibre the proportion of cross-sectional area occupied by myonuclei was approximately 70–90 % in the region of the nuclear bag, 30–50 % in the myotube regions, and 10 % in the region of the secondary terminals (see figure 3).

The *c* fibres did not show a similar increase in thickness in the secondary region. Indeed they were somewhat thinner here than in the equivalent region on the distal side of the primary ending. From 40 to 60 % of the total cross-sectional area of the longest *c* fibre was occupied by myonuclei in the primary region, and about 15 % in the secondary region.

(iii) *Reconstructed primary endings*

Reconstructions of the primary endings of spindles 6 and 12 are shown in figures 4 and 5. The endings are remarkably alike in several respects. For example, in the proportional distribution of terminal contact area to the three types of intrafusal fibre, it is the b_1 fibre in each ending that is the most densely innervated. Thus, in spindle 6, 37 % of the total terminal contact area was distributed to the b_1 fibre as against 24 % to the b_2 fibre and 39 % to the four *c* fibres. This compares with proportions of 33 % (b_1), 25 % (b_2) and 42 % (*c* fibres) in spindle 12, and in each ending it was the b_1 fibre terminals that covered the largest proportion of muscle-fibre surface (see table 3).

There were also consistent differences in the form and disposition of the terminals on the two bag fibres. In the b_1 fibre, transversely orientated, regularly arranged terminals wrapped closely together around the nuclear bag were flanked on either side by an irregular array of terminals, many of which lay parallel with the fibre's axis. By contrast the terminals on the b_2 fibre were more widely spaced apart and nearly all were transversely orientated, with minimal irregularity at each end. In figure 7 these differences between the primary terminals on the bag fibres of spindle 6 have been emphasized by changing the alignment of the nuclear bags so that, instead

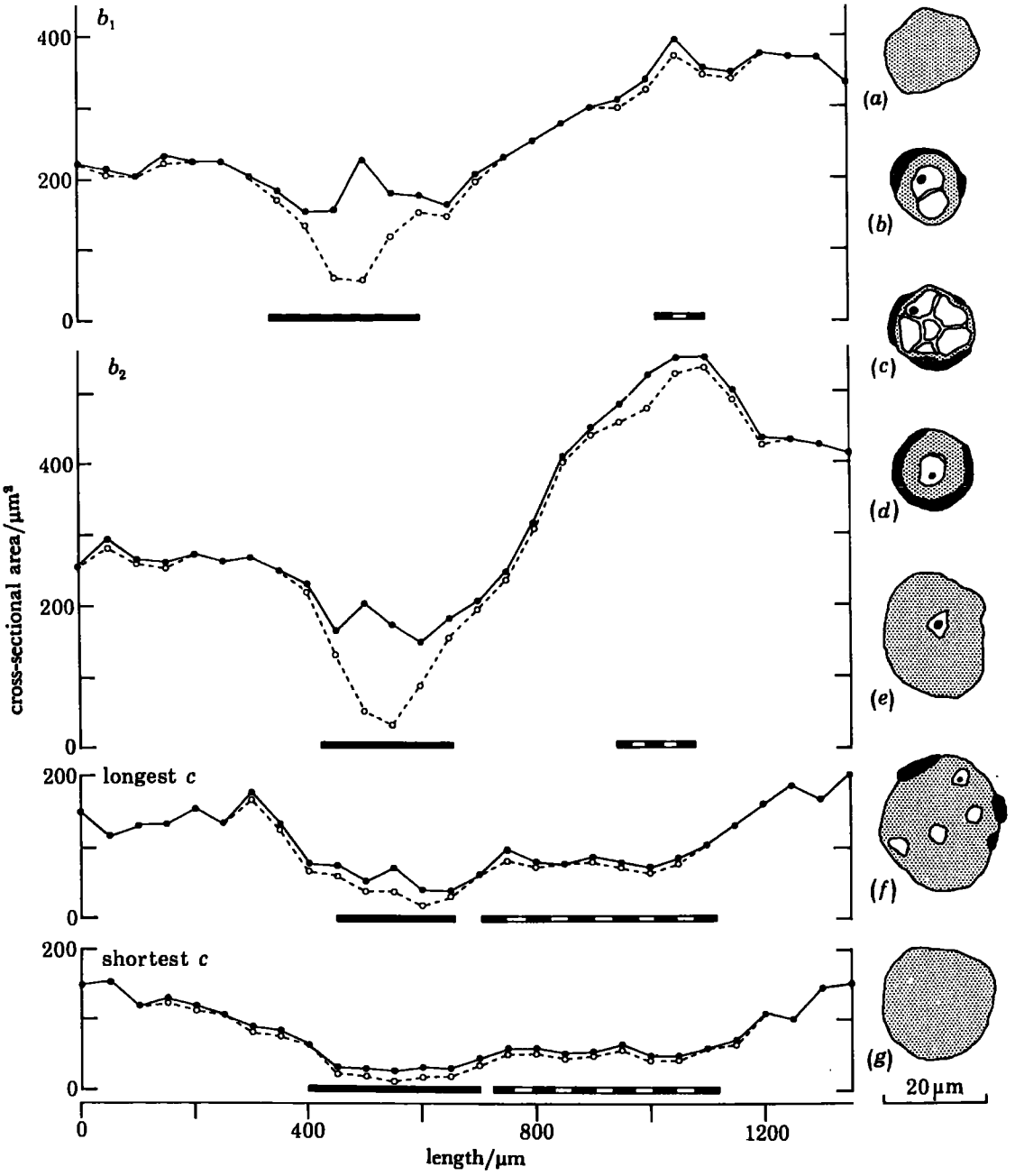


FIGURE 3. Graphs illustrating the cross-sectional areas of the bag fibres (b_1, b_2) and longest and shortest chain (c) fibres of spindle 6 during their course through the equatorial region. Areas plotted at 50 μm intervals; filled circles indicate total area of muscle fibre, unfilled circles the area occupied by myofibrils. Solid bar underneath each graph indicates length of fibre occupied by primary-ending terminals, broken bar indicates the length occupied by secondary-ending terminals.

(a)–(g) Cross-sectional profiles of b_2 fibre based on sections selected to represent condition at various levels during the course of the fibre through the equatorial region, as follows: (a) distal equatorial limit of periaxial space; (b) transition between distal myotube region and nuclear bag; (c) middle of nuclear bag; (d) proximal myotube region; (e) region between primary and secondary terminals; (f) region of secondary innervation; (g) proximal equatorial limit of periaxial space. Myofibrils indicated by dots; myonuclei as hollow profiles, some with nucleoli; sensory terminals as peripheral black shapes.

of their staggered arrangement in the ending, each is centred on the equator. In addition each bag fibre is repeated alongside to show its myonucleation. There was a close association between nucleation and innervation, but no constant relation between number of myonuclei and terminal contact area.

The terminals on each muscle fibre may be regarded as being arranged in an essentially helical fashion, with the pitch progressively increasing towards each end. Various modifications are superimposed upon this basic pattern, such as branches to form a double helix, fusion of adjacent turns, and longitudinal anastomoses, which may themselves bear lateral branches. The irregularities produced by these factors were minimal among the terminals on *c* fibres and maximal among those on the b_1 fibre. Thus regular spirals of up to four complete turns occurred among the *c* fibre terminals in both primary endings, whereas the longest stretch of regular spiral among the b_1 terminals in spindle 6 was one and a half turns around one end of the nuclear bag.

Description of figures 4–6

These figures illustrate the reconstructions of the primary and secondary endings described in the text. They should be examined with reference to figures 1–3, 7 and 8, which illustrate information abstracted from them, e.g. the branching of the I a axon supplying spindle 12, shown as reconstructed in figure 4, is shown schematically in figure 1; the primary terminals supplied to the bag figures of spindle 6, shown as reconstructed in figure 5, are schematically represented in figure 7; and so on.

The main conventions of line thickness and shading used are as follows. The *standard line* is that used to indicate the sarcolemma of the intrafusal muscle fibres. A *thick line* delineates internodal myelin and sensory terminals located on the near side of muscle fibres. A *thin line* indicates the outline of structures seen through other structures, e.g. most myonuclei. Preterminal axons and terminals have *graded shading* when located on the near side of muscle fibres, *even shading* when located on their far side.

Nuclear-bag nuclei are represented by their nucleoli only.

Figure 4. Isometric reconstruction of the primary ending of spindle 12.

(*a*) The terminals on the chain (*c*) fibres are seen to be interconnected by sensory cross-terminals (s.c.t.) and to form two separate systems each supplied by one or two short preterminal axons. One *c* fibre in the lower group is almost completely obscured by two others. Some terminals belonging to the adjacent S_1 secondary ending are shown in outline at the end on the right.

(*b*) The bag_1 (b_1) and bag_2 (b_2) terminal systems are each supplied by two preterminal axons. Note dense innervation of b_1 fibre. The gap between the bag fibres was occupied by the *c* fibres.

(*c*), (*d*) First-order branches of I a axon supplying (*c*) the b_2 and *c* fibres, and (*d*) the b_1 fibre.

Symbols: n.R., node of Ranvier; pt.a., preterminal axon; sat.c., satellite cell; sbs.n., sub-sarcolemmal nucleus; Sch.c.n., Schwann cell nucleus.

Figure 5. Isometric reconstruction of the primary ending of spindle 6. Compare with figure 4.

(*a*) Terminals on the *c* fibres; they are interconnected to form a single system supplied by two preterminal axons.

(*b*) Terminals on the bag fibres. The b_1 terminal system is supplied by three preterminal axons, two of which subdivide close to their heminodes, whereas the b_2 terminal system is supplied by two preterminal axons derived from a single heminode.

(*c*) First-order branches of the Ia axons and their subdivisions that lie in front of the muscle fibres shown in relation to the outline of the intrafusal bundle.

Symbols: b_1 br., b_2c br., first-order branches of I a axon supplying, respectively, the b_1 fibre and the b_2 and *c* fibres.

Figure 6. Isometric reconstruction of the S_1 secondary ending of spindle 6. The left end of the intrafusal bundle is continuous with the right end of that shown in figure 5.

(*a*) Terminals on the *c* fibres.

(*b*) Terminals on the bag figures.

(*c*) Part of the II axon and branches that lie in front of the muscle fibres shown in relation to the outline of the intrafusal bundle.

Note that the preterminal axons (pt.a.), as compared with those in primary endings, arise from nodes as well as heminodes and are relatively longer and more abundant.

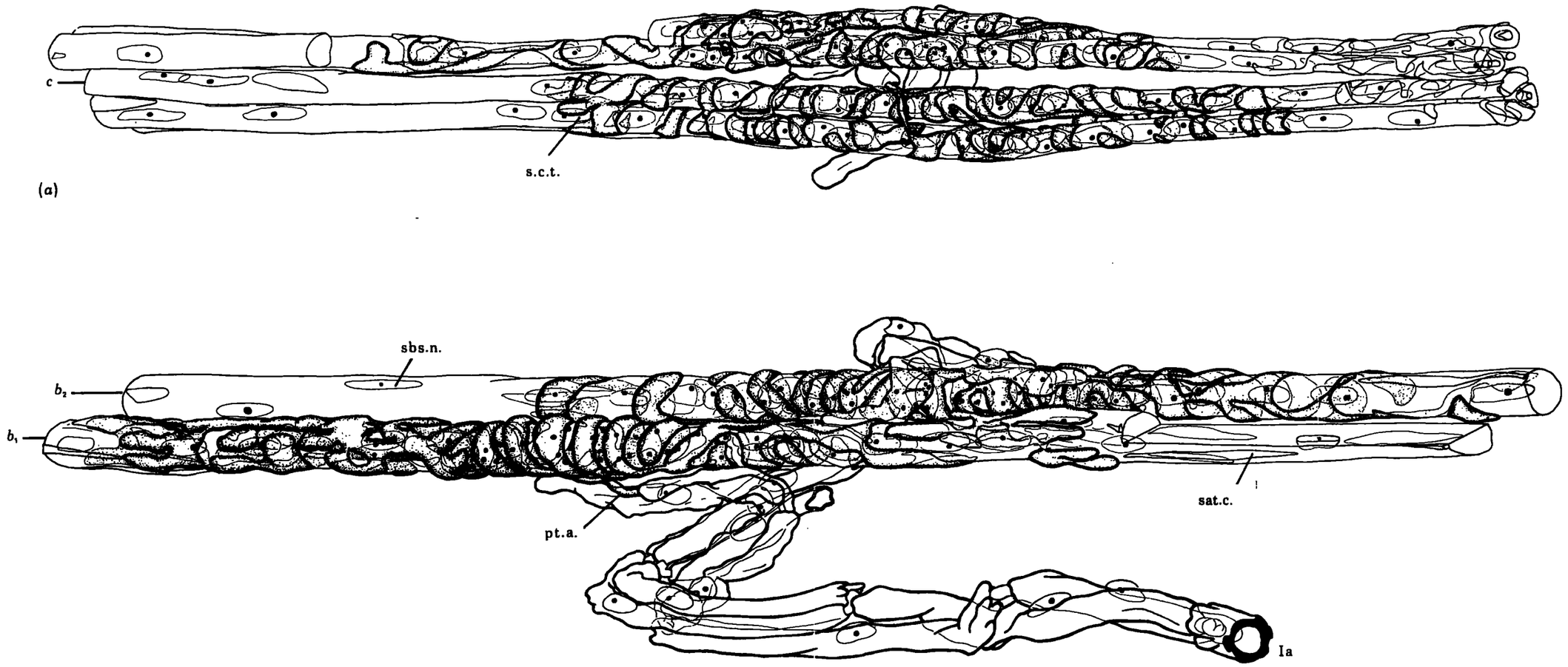


FIGURE 4. For description see opposite.

(*Facing p.* 342)

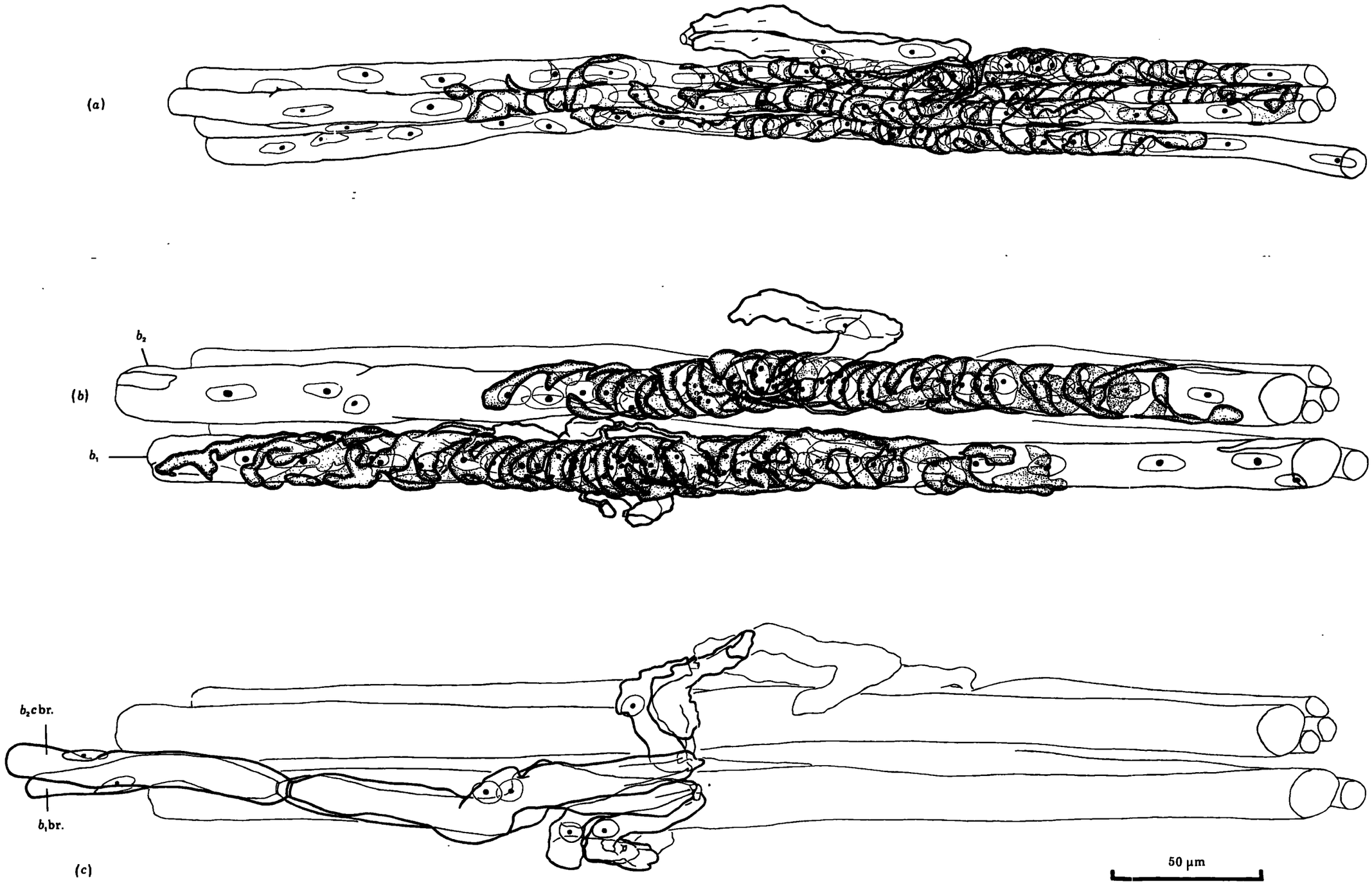


Figure 5. For description see page 342.

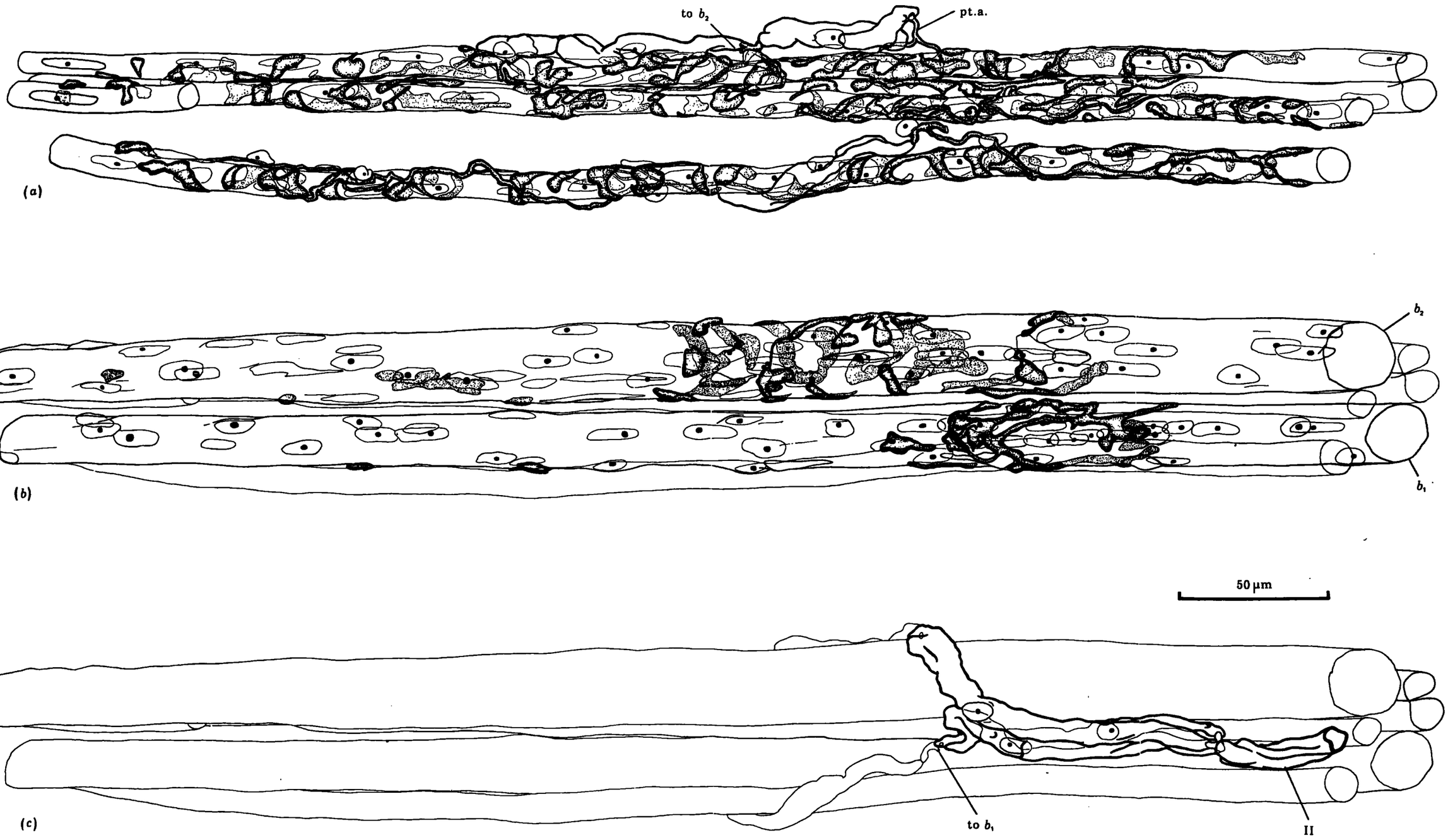


FIGURE 6. For description see page 342.



FIGURE 7. Schematic representation of parts of the reconstructed primary ending of spindle 6 shown in figure 5. The terminals shown are those supplied to the bag_1 (b_1) and bag_2 (b_2) fibres and the longest and shortest chain (c) fibres (numbered 1 and 4, as in table 2). In addition each fibre is repeated alongside to show its myonucleation and thus demonstrate the relation between nucleation and innervation. Adjustments have been made to the original alignment of each muscle fibre relative to the others, mainly in order to position the centre of each nuclear bag on a common midline and thus facilitate comparison between b_1 and b_2 terminal systems. Terminals shown in outline at bottom end of $c4$ belong to adjacent S_1 secondary ending. Asterisks alongside c terminals indicate positions of sensory cross-terminals with other c fibres.

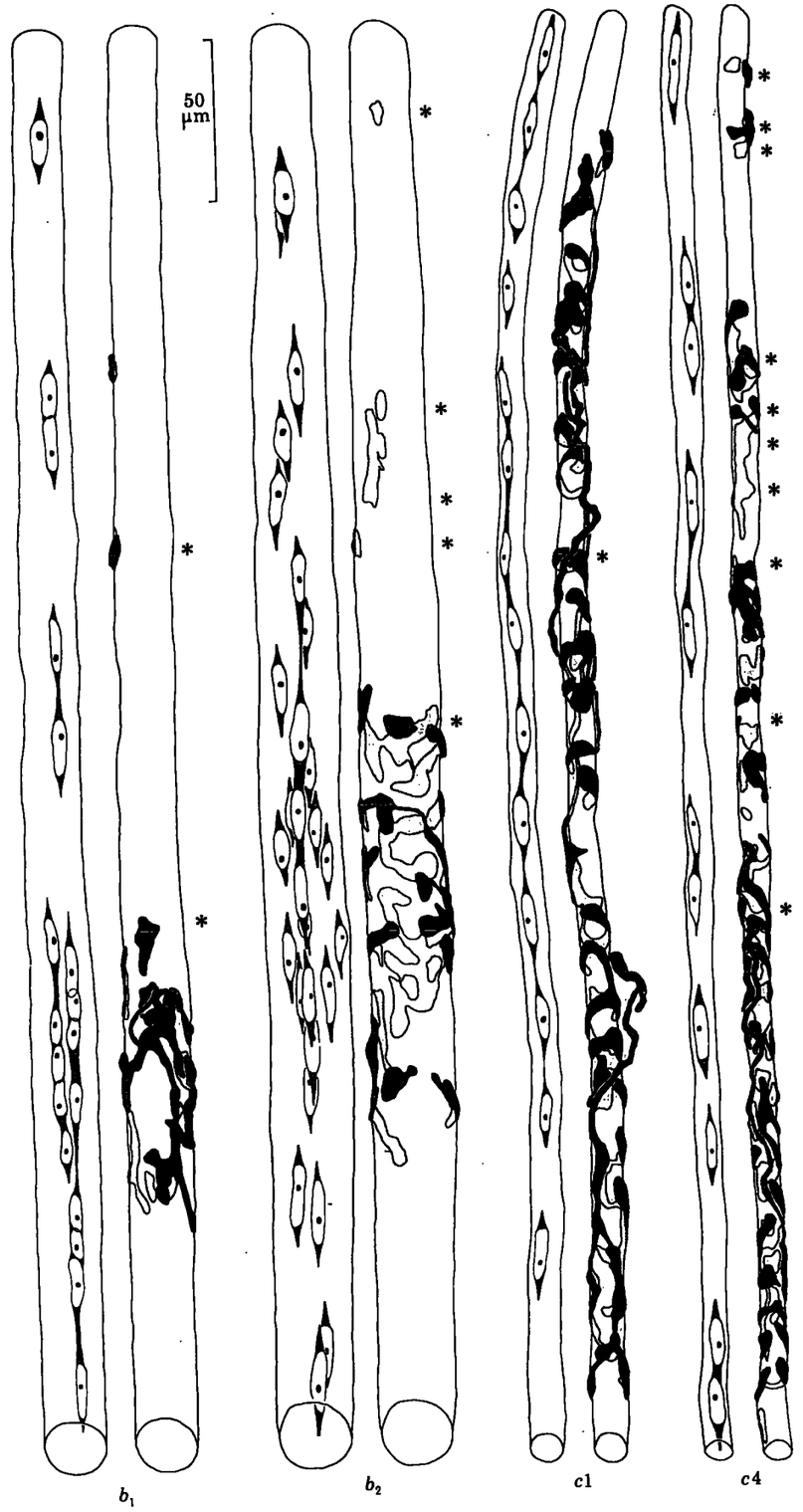


FIGURE 8. For description see opposite.

Cross-terminals occurred frequently in both primary endings and were formed exclusively among *c* fibres. There was a tendency for the shortest *c* fibres to be involved with the greatest number of cross-terminals. The interconnections were usually made between two fibres, occasionally three. They resulted in the terminals on all four *c* fibres being interconnected in spindle 6, and those in spindle 12 being interconnected among one group of two fibres and another of three. This made it possible for the chain-fibre terminals to be distributed by fewer preterminal axons than were bag-fibre terminals. Only two such axons distributed terminals to the *c* fibres in spindle 6, as compared with seven supplying the bag fibres (five b_1, two b_2). The preterminal axons measured 1–2 μm in diameter and were of much smaller cross-sectional area than either the terminals they supplied or the myelinated axons from which they were derived. They connected with the terminals somewhere within the middle third of the ending on each bag fibre and on each group of *c* fibres.

Examination of the 1 μm thick serial transverse sections used in reconstruction revealed the presence of darkly stained granules located mostly towards the ends of the terminal systems supplied to each muscle fibre. To identify the nature of these granules we cut serial longitudinal sections of the equatorial and juxtaequatorial regions of spindle 5, including some ultrathin sections for study with electron microscopy (see plate 2). These confirmed the presence and distribution of the granules, which, under high magnification, proved to be osmiophilic bodies with a membranous organization and a range of electron densities. We concluded that they represented mitochondria in various stages of degeneration (cf. Hudson & Hartmann 1961), since the least dense, most highly organized bodies closely resembled nearby mitochondria (figure 17, plate 2).

(iv) *Reconstructed secondary ending*

The reconstruction of the S_1 secondary ending supplied to spindle 6 is shown in figure 6. As in the adjacent primary ending, the terminals were distributed to all three types of intrafusal muscle fibre, but they covered the fibres more sparsely and were spread over a greater length (445 μm as against 363 μm). The total area of contact made by the terminals was less (12639 μm^2 as against 18492 μm^2), but proportionately much more was distributed to the *c* fibres (75 % as against 39 %) (see table 3). Of the remainder, the b_2 fibre received about twice (17 %) that received by the b_1 fibre (8 %). The close association between myonuclei and innervation was especially marked in the region of the bag-fibre terminals (see figure 8).

The terminals clasped and partially encircled the fibres in an irregular array that lacked spirals. Many terminals appeared to be completely isolated; presumably the strands connecting them to other parts of the ending were so thin as to escape detection with the light microscope. Cross-terminals occurred between *c* fibres, and also between bag (b_1 and b_2) fibres and *c* fibres. The shortest *c* fibre was involved with the greatest number of cross-terminals (see figure 8). The preterminal axons supplying the terminals arose from three heminodes and three penultimate

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FIGURE 8. Schematic representation of parts of the reconstructed S_1 secondary ending of spindle 6, shown in figure 6. As in figure 7, the terminals shown are those supplied to the b_1, b_2, *c*1 and *c*4 fibres, and each fibre has been repeated alongside to show its myonucleation and demonstrate the relation between nucleation and innervation. Asterisks indicate positions of 11 sensory cross-terminals; 10 of these linked terminals on the *c*4 fibre to those on the b_1 fibre (1), b_2 fibre (5), and other *c* fibres (4), and there was one cross-terminal between the b_1 and *c*1 fibres.

nodes. As in the primary endings, these axons measured 1–2 μm in diameter, but they differed in occasionally extending for considerable distances (up to 200 μm).

(*b*) *Analysis of silver preparations*

(i) *Composition of the spindle sample*

The nature of the sensory innervation of the 310 spindles studied is shown in table 4. Most of the spindles were composed of three types of muscle fibre, but a small proportion (12.9%) lacked a b_1 fibre. It is convenient to refer to these as 'b_2c spindle units', as distinct from 'b_1b_2c spindle units'. The b_2c spindle units were all portions of tandem spindles in which the b_2 fibre was continuous from one capsule, where it was accompanied by b_1 and c fibres, to another, in which it was accompanied by c fibres only (usually two or three). In these units the equatorial nucleation of the b_2 fibre usually resembled that of a c fibre. After passing through an encapsulated sensory region, each unit formed a very short pole that usually inserted into tendon, leaving the excluded b_1 fibre to insert separately. In extensively sampled muscles the proportion of b_2c spindle units was 23.8% in extensor digitorum longus, 23% in peroneus brevis, and between 6 and 11% in the rest. In muscles less extensively sampled the highest proportions of b_2c units were 28.6% in extensor digitorum brevis and 27.3% in flexor hallucis longus (see table 4).

Two-hundred-and-seventy b_1b_2c spindle units received a total of 282 Ia and 440 II axons, a Ia:II ratio of 1:1.6. Twelve of these spindles (4.4%) had primary endings formed by two Ia axons (double primaries). Secondary endings (total 444) were usually located on both sides of the primary ending when two or more were present. The majority (70.7%) terminated in the S_1 position; the rest terminated in the S_2 (23.4%), S_3 (5.0%), S_4 (0.7%) and S_5 (0.2%) positions. When several secondaries were distributed to a pole their positional arrangement was

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Description of plate 1

Photographs of some of the transverse sections used in reconstructing the sensory region of spindle 6. The distances between the sections selected for illustration in figures 9–14 are, respectively, 123, 6, 0, 39 and 466 μm. The two large muscle fibres in each section are nuclear-bag fibres (bag$_1$ (b_1) left; bag$_2$ (b_2) right), the four smaller ones nuclear-chain fibres (c). Epon sections, 1 μm thick, stained with toluidine blue. Abbreviations: ax.sh.n., axial sheath nucleus; cap., capsule; p.s., periaxial space; pt.a., preterminal axon; s.c.t., sensory cross-terminal; s.t., sensory terminal.

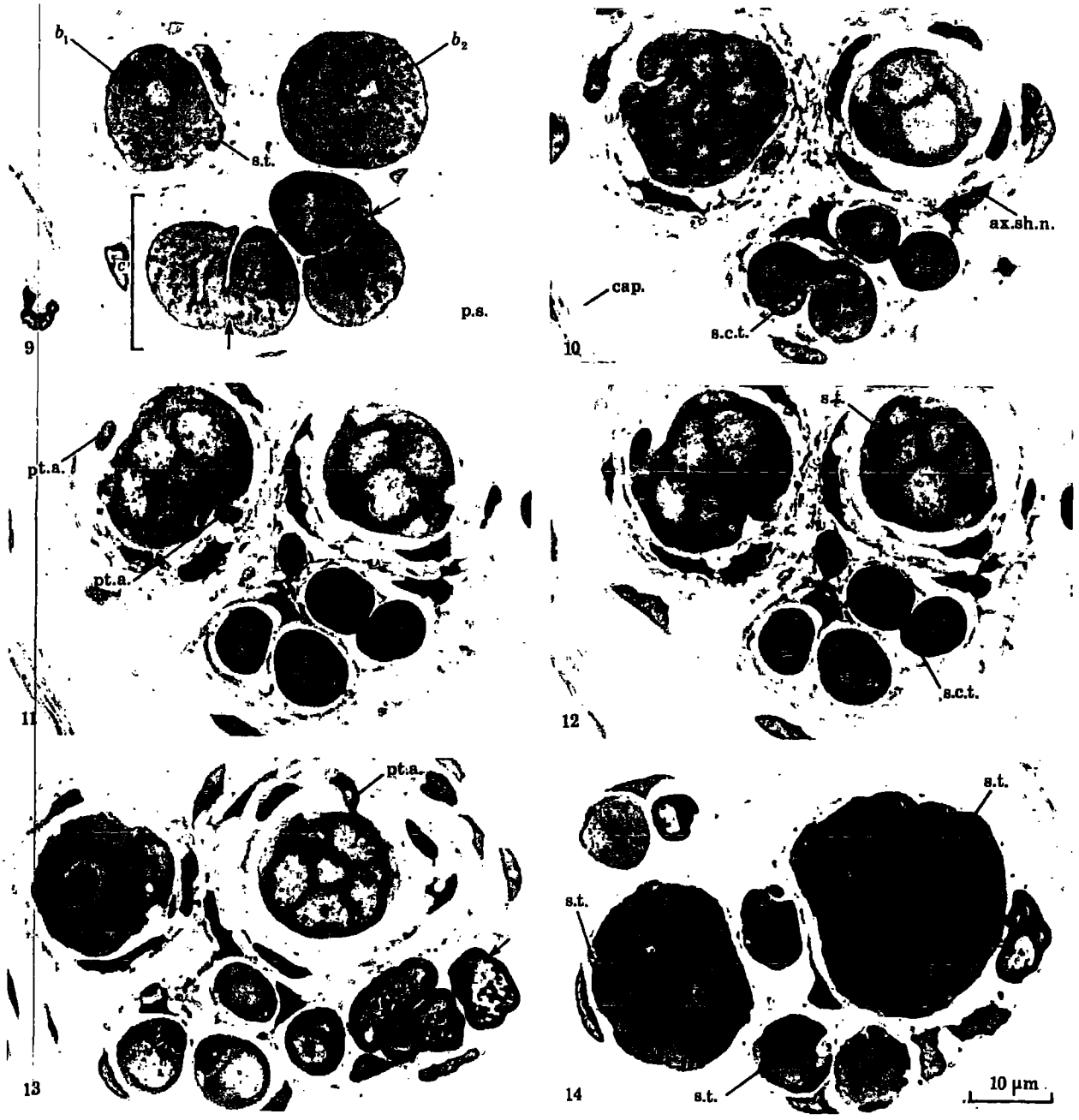
Figure 9. Section through distal myotube of the b_1 fibre, here dissociated from the b_2 and c fibres. The c fibres are joined together as two pairs by regions of close apposition (arrows) such as described by Corvaja *et al.* (1967). Primary sensory terminals present at this level only on b_1 fibre.

Figure 10. Section through nuclear bag of the b_1 fibre, distal myotube of the b_2 fibre, and nuclear chains of the c fibres. The characteristic euchromatic nature of the bag and chain myonuclei is evident in this and the next three figures. Note prominent sensory cross-terminal between two of the c fibres.

Figures 11 and 12. Adjacent sections through the nuclear bags of both bag fibres. Two preterminal axons close to the b_1 fibre in figure 11 can be seen contributing primary sensory terminals to it in figure 12. The two sections also show the start of a sensory cross-terminal between a pair of c fibres.

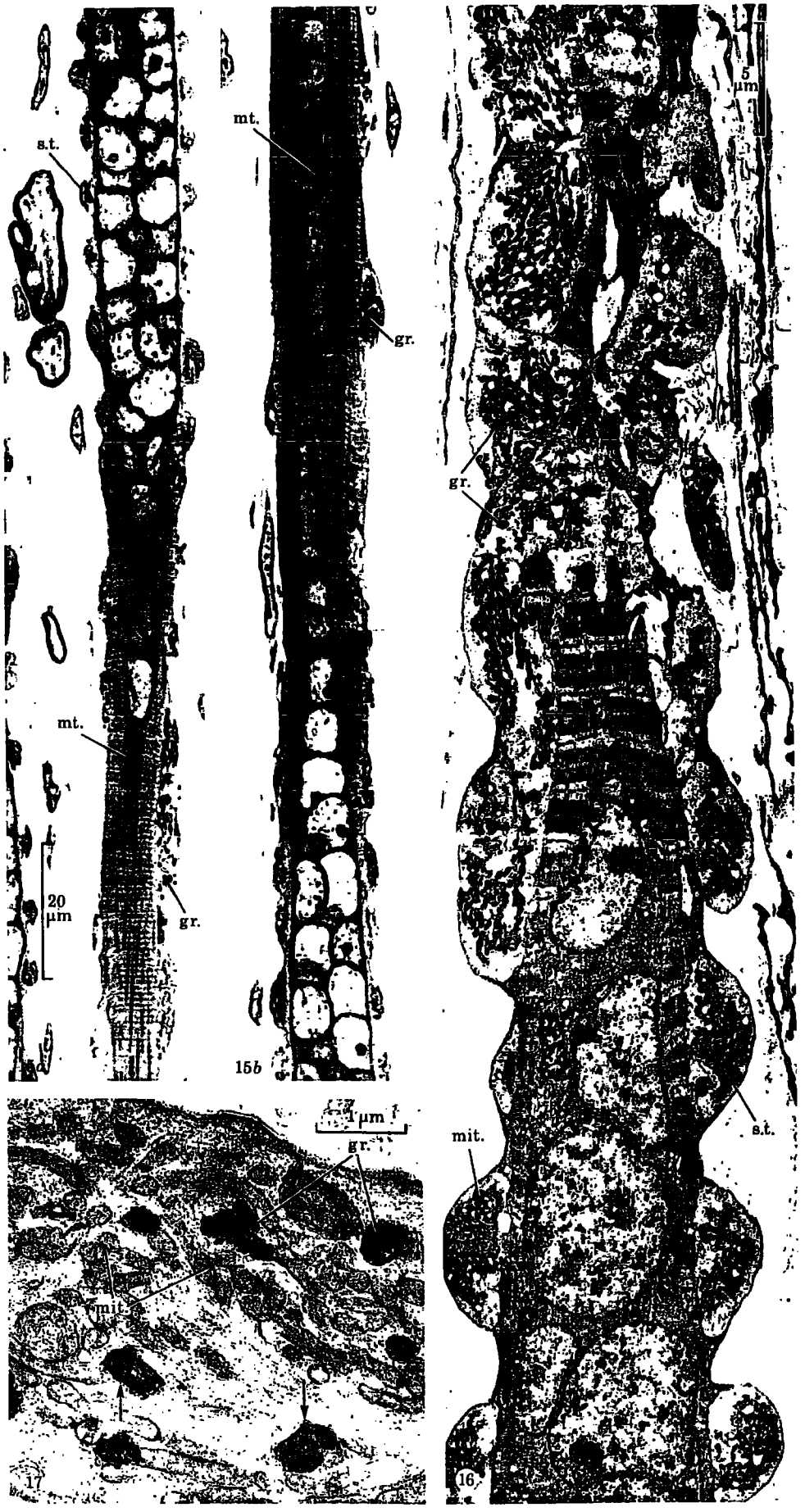
Figure 13. Section through proximal myotube of the b_1 fibre and nuclear bag of the b_2 fibre. The preterminal axon seen contributing a primary sensory terminal to the b_2 fibre was derived from the heminode of the myelinated Ia axon branch arrowed at bottom right. The other two myelinated branches supplied terminals to the c fibres.

Figure 14. Section through S_1 secondary ending in a region where terminals are present on b_1, b_2 and c fibres. Note nucleation and relatively large diameters of the bag fibres.



FIGURES 9–14. For description see opposite.

(*Facing p.* 314)



FIGURES 15–17. For description see opposite.

TABLE 3. AREAS AND DISTRIBUTION OF SENSORY ENDINGS IN TWO CAT TENUISSIMUS SPINDLES

(Symbols: b_1, bag$_1$ fibre; b_2, bag$_2$ fibre; c, chain fibre. Chain fibres numbered in order of decreasing length.)

		terminal area µm²		area of fibre surface covered (%)	proportion of ending on fibre (%)	
primary ending (spindle 12)	b_1	6154		59	33	
	b_2	4738		49	25	
	$c1$	1364	7869	40	7	42
	$c2$	2097		45	11	
	$c3$	1772		45	9	
	$c4$	1617		41	9	
	$c5$	1019		43	5	
primary ending (spindle 6)	b_1	6842		51	37	
	b_2	4391		41	24	
	$c1$	1617	7259	33	9	39
	$c2$	2267		41	12	
	$c3$	1417		26	8	
	$c4$	1959		38	11	
S_1 secondary ending (spindle 6)	b_1	974		16	8	
	b_2	2205		16	17	
	$c1$	2775	9460	28	22	75
	$c2$	2554		30	20	
	$c3$	2082		26	16	
	$c4$	2049		27	16	

normally sequential starting with S_1, but one spindle received a single secondary located in the S_2 position. Spindles were most frequently supplied with one primary and one secondary ending (PS_1, 30.0%) or a primary ending only (P, 25.2%; 42 among 270 b_1b_2c units, 36 among 40 b_2c units).

(ii) *Branching and distribution of primary (Ia) axons*

Examination of the branching undergone by 182 Ia axons before terminating showed that they either remained unbranched (3.3%) or divided to produce two (84.0%), three (11.0%) or four (1.7%) first-order branches. The axons examined included 39 supplied to b_2c spindle

DESCRIPTION OF PLATE 2

Longitudinal sections through the primary sensory region of the b_1 fibre of spindle 5 illustrating nucleation, innervation, and nature of granules located in parts of the terminal system. Abbreviations: gr., granules; mit., mitochondria; mt., myotube; s.t., sensory terminal.

FIGURE 15. Section through the entire length of the primary terminal system on the b_1 fibre. The micrograph has been divided into two parts close to the middle of the nuclear bag. Terminals forming the regular, middle portion of the system are seen as profiles on each side of the bag, whereas those forming the irregular portions at each end are seen as profiles associated with the myotube regions. These terminals contain densely stained granules that do not occur in those forming the middle portion of the system. Epon section 1 µm thick, stained with toluidine blue.

FIGURE 16. Electron micrograph of the b_1 fibre including one end of the nuclear bag and part of one myotube region; the myotube itself lies largely outside the plane of section. Note abundance of mitochondria that occur in the sensory terminals. Osmiophilic granules, presumed to correspond with those shown in figure 15, occur in the irregular portion of the primary terminal system associated with the myotube region.

FIGURE 17. Electron micrograph of part of the irregular portion of the primary terminal system, showing detail of granules, which appear to represent stages (arrows) in the degeneration of mitochondria.

units and eight involved in contributing terminals to two fibre types in double primary endings (see table 5).

The distribution of first-order branches to b_1, b_2, and c fibres was ascertained for 132 Ia axons and gave the results shown in table 6. Among tenuissimus axons, the first-order branches of 73% (24 of 33) had a segregated distribution, i.e. were exclusively supplied either to b_1 or to b_2 and/or c fibres, thereby resulting in separation of dynamic and static inputs. By contrast, the

TABLE 4. SILVER ANALYSIS: SENSORY INNERVATION OF SPINDLE SAMPLE

(Symbols and abbreviations: b_1, b_2, c, bag_1, bag_2, chain fibres; P, primary ending; S, secondary ending; ten., tenuissimus; p.b., p.l., p.t., peroneus brevis, p. longus, p. tertius; s. and d.lum., superficial and deep lumbricals; sol., soleus; e.d.l., e.d.b., extensor digitorum longus and brevis; f.h.l., flexor hallucis longus; t.a., t.p., tibialis anterior and posterior; p. int., pes interosseus; r.f., rectus femoris.)

sensory innervation	muscles sampled														
b_1b_2c spindle units	ten.	p.b.	p.l.	p.t.	s.lum.	d.lum.	sol.	e.d.l.	e.d.b.	f.h.l.	t.a.	t.p.	p.int.	r.f.	total
P	10	4	—	6	10	1	6	1	1	2	1	—	—	—	42
PS_1	15	21	8	5	11	4	9	5	1	2	3	1	4	—	89
PS_1S_2	12	6	2	3	4	—	3	3	1	—	1	—	1	—	36
P–S_2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
$PS_1S_2S_3$	6	—	—	1	1	—	1	1	—	—	1	—	—	—	11
$PS_1S_2S_3S_4$	—	—	1	—	—	—	—	—	—	—	1	—	—	—	2
$PS_1S_2S_3S_4S_5$	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
S_1PS_1	9	6	3	6	3	2	7	2	1	1	—	—	—	—	40
$S_1PS_1S_2$	5	6	2	2	3	—	2	1	1	3	2	—	—	—	27
$S_1PS_1S_2S_3$	1	—	1	—	—	—	—	—	—	—	—	—	—	—	2
$S_2S_1PS_1S_2$	2	—	—	—	—	—	1	1	—	—	1	—	—	—	5
$S_2S_1PS_1S_2S_3$	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
$S_3S_2S_1PS_1S_2S_3$	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1
PPS_1	1	—	—	—	—	—	—	1	—	—	—	—	—	—	2
PPS_1S_2	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
$PPS_1S_2S_3$	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
S_1PPS_1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
$S_1PPS_1S_2$	1	2	1	—	—	—	—	1	—	—	—	—	—	—	5
$S_2S_1PPS_1S_2S_3$	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
b_2c spindle units															
P	4	13	2	3	2	1	2	4	1	3	1	—	—	—	36
PS_1	1	1	—	—	—	—	—	1	1	—	—	—	—	—	4
total . . .	70	61	20	27	34	8	31	21	7	11	13	1	5	1	310

first-order branches of 83% (19 of 23) of Ia axons supplying superficial lumbrical spindles had a mixed distribution, and such distributions were only slightly less frequent than segregated ones among the samples of Ia axons examined from peroneus brevis, p. tertius and soleus muscles. The most common type of mixed distribution was that in which the b_1 fibre derived its terminals from the same first-order branch as one or two c fibres. Mixing was usually restricted to the dynamic input. This was so in 74.6% (44 of 59) of Ia axons in a sample where all had two first-order branches; mixing was restricted to the static input in 11.9%, and affected both inputs in 13.5%.

The distribution of Ia first-order branches was ascertained in eight double primary endings. Six of these were formed by one Ia axon supplying terminals to b_1, b_2 and c fibres in combination with another whose distribution was restricted to two fibre types, i.e. to b_1 and b_2 fibres

(2), b_2 and c fibres (2), or b_1 and c fibres (2). In one double primary each Ia axon supplied all three fibre types, and in another b_1 and c fibres were supplied by one Ia axon, b_2 and c fibres by the other.

(iii) *Branching and distribution of secondary (II) axons*

Branching was examined among 272 secondary axons, i.e. 201 S_1, 58 S_2, 11 S_3, one S_4, and one S_5. The proportion remaining unbranched was 17.7%; the rest divided to produce two (74.6%), three (7.0%) or four (0.7%) first-order branches. Unbranched axons occurred less

TABLE 5. BRANCHING AND DISTRIBUTION OF PRIMARY AFFERENTS SUPPLYING CAT HINDLIMB MUSCLES

(Abbreviations as in table 4.)

distribution to fibre types	number of first-order branches	afferents innervating							
		ten.	s.lum.	p.b.	p.l.	p.t.	sol.	others†	total
					b_1b_2c spindle units: single primaries				
b_1b_2c	0	—	—	1	—	1	1	—	3
	2	24	16	24	4	10	16	10	104
	3	6	4	2	1	1	1	2	17
	4	—	3	—	—	—	—	—	3
					b_1b_2c spindle units: double primaries				
b_2c	2	3	—	—	—	—	—	—	3
b_1c	2	2	—	—	—	—	—	1	3
b_1b_2	2	—	—	—	1	—	—	1	2
b_1b_2c	2	2	—	—	1	—	—	3	6
	3	1	—	—	—	—	—	1	2
					b_2c spindle units				
b_2c	0	—	—	2	—	—	—	1	3
	2	5	2	12	2	3	1	10	35
	3	—	—	—	—	—	1	—	1
	total ...	43	25	41	9	15	20	29	182

† Ia afferents innervating d.lum. (4), e.d.l. (9), e.d.b. (2), f.h.l. (4), t.a. (6), p.int. (2), r.f. (2).

frequently among S_1 secondary axons (12.2% unbranched) than among those terminating in more polar positions (S_2, 29.3% unbranched; S_3, 36.4%; S_4 and S_5 axons both unbranched). One secondary axon in soleus branched at spindle entry and supplied two secondary endings, one on each side of the primary in the S_1 position.

The preterminal axons that supplied secondary terminals were derived either exclusively from heminodes (as in primary endings) or from both heminodes and penultimate nodes. The latter were usually those distal to the first branching node, but this node itself, as well as that immediately proximal to it, were also occasionally involved. Secondary endings supplied exclusively from heminodes were more frequent in the S_1 position than in more polar positions. For example, among 65 S_1 endings, 42 (64.6%) were supplied exclusively from heminodes, whereas this was so in only six of 15 S_2 endings (40.0%). Generally speaking, the more polar the position of a secondary the more likely it was for the terminals to be partly derived from penultimate nodes, and for the parent axon to be unbranched.

Table 7 correlates the position of 351 secondary endings with their distribution to the three

types of intrafusal muscle fibre. The most common secondary (59.0%) was the S_1 ending distributed to b_1, b_2 and c fibres, and this kind of distribution was the most common (67.8%) in the sample as a whole. Restriction of terminals to one or two fibre types was more prevalent among secondaries terminating in the more polar positions. Thus the terminals of 68.4% of S_2 and 60.0% of S_3 endings were restricted to one or two fibre types as against only 18.2% S_1 endings.

TABLE 6. DISTRIBUTION OF FIRST-ORDER BRANCHES OF PRIMARY AFFERENTS SUPPLYING CAT HINDLIMB MUSCLES

(Symbols: b_1, bag$_1$ fibre; b_2, bag$_2$ fibre; c, chain fibre(s). Abbreviations of muscles as in table 4.)

type of distribution	number of first-order branches	distribution to muscle fibres mediating dynamic (D) and static (S) responses		ten.	s.lum.	p.b.	p.t.	sol.	others†	total
segregated	2	D:S	b_1:b_2c	19	—	14	5	8	5	51
	3	D:S:S	b_1:b_2 :c	3	—	—	—	1	—	4
			b_1:b_2c:b_2	1	—	—	—	—	—	1
			b_1:b_2c:c	—	—	—	1	—	1	2
			b_1:b_2c:b_2c	—	1	—	—	—	—	1
		D:D:S	b_1:b_1 :b_2c	1	—	1	—	—	—	2
	4	D:D:S:S	b_1:b_1 :b_2c:c	—	1	—	—	—	—	1
			b_1:b_1 :b_2 :c	—	1	—	—	—	—	1
			subtotal . . .	24	4	15	6	9	6	64
mixed	2	DS:S	b_1b_2 :c	—	—	1	—	1	—	2
			b_1b_2 :b_2c	—	1	1	—	—	—	2
			b_1c :b_2	1	—	1	—	—	—	2
			b_1c :b_2c	4	6	1	1	2	3	17
			b_1b_2c :b_2	—	—	1	1	—	—	2
			b_1b_2c :c	1	3	1	—	2	6	13
			b_1b_2c :b_2c	—	1	2	—	2	1	6
		D:DS	b_1 :b_1b_2c	1	2	2	—	—	2	7
		DS:DS	b_1b_2 :b_1b_2c	—	1	—	—	—	1	2
			b_1c :b_1b_2	—	—	—	—	1	—	1
			b_1c :b_1b_2c	—	1	—	3	—	—	4
			b_1b_2c :b_1b_2c	—	1	—	—	—	—	1
	3	D:DS:S	b_1:b_1c:b_2	1	—	—	—	—	—	1
			b_1:b_1c:b_2c	—	1	1	—	—	—	2
		DS:S:S	b_1c :b_2c:c	1	2	—	—	—	—	3
			b_1c :b_2c:b_2c	—	—	—	—	—	1	1
			b_1c :b_2c:b_2	—	—	—	—	—	1	1
			b_1b_2 :c :c	—	—	—	—	—	1	1
			subtotal . . .	9	19	11	5	8	16	68
		total . . .		33	23	26	11	17	22	132
		percentage segregated . . .		73	17	58	55	53	27	48.5
		percentage mixed . . .		27	83	42	45	47	73	51.5

† Ia afferents innervating p.l. (6), d.lum. (4), e.d.l. (3), f.h.l. (1), t.a. (4), p.int. (2), r.f. (2).

A sample of 44 S_1 secondary axons innervating b_1, b_2 and c fibres was examined to ascertain the distribution of first-order branches to the three fibre types. The sample comprised axons from tenuissimus (19), soleus (11), peroneus brevis (6), p. tertius (2) and lumbrical (6) muscles, and all had two first-order branches. A segregated distribution, in which one branch supplied the b_1 fibre while the other supplied b_2 and c fibres, was infrequent in all muscles and occurred

in only 22.7% of the sample. In mixed distributions the usual condition (61.8%) was for one first-order branch to supply the b_1 fibre together with c fibres, or b_2 and c fibres, and for the other to supply b_2 and c fibres.

(iv) *Diameters of spindle afferents*

Measurements of the intramuscular diameters of 259 Ia and 357 II axons showed that on average Ia axons were about twice as thick as II axons (mean diameters: Ia, 7.1 μm; II, 3.6 μm) (see table 8). The diameters of Ia axons supplying b_2c spindle units were generally

TABLE 7. SECONDARY ENDINGS: CORRELATION OF POSITION WITH DISTRIBUTION TO FIBRE TYPES

(Abbreviations as in table 4.)

position of ending	distribution to fibre types				
	b_1b_2c	b_1c	b_2c	c	total
S_1	207	7	38	1	253
S_2	25	9	31	14	79
S_3	6	1	3	5	15
S_4	—	1	1	1	3
S_5	—	—	—	1	1
total...	238	18	73	22	351
percentage...	67.8	5.1	20.8	6.3	

thinner than those supplying b_1b_2c spindle units. Thus the mean diameter of 37 b_2c Ia axons was 5.1 μm in a range of 2.2–9.0 μm (peak 4.0 μm), whereas 213 b_1b_2c Ia axons had a mean diameter of 7.5 μm in a range of 3.4–12.8 μm (peaks at 6.0 and 8.0 μm). There was no correlation between the diameter of b_1b_2c Ia axons and the presence, absence or number of secondary endings.

The diameter of II axons terminating as S_2–S_5 endings was generally less (mean 2.9 μm) than that of those terminating as S_1 endings (mean 3.9 μm). This decrease appeared to be related to polar position rather than to number of muscle-fibre types innervated. Thus among II axons distributed to all three fibre types the mean diameter of 196 terminating in the S_1 position was 3.8 μm as compared with a mean of 3.0 μm for 28 terminating in the S_2–S_5 positions. Similarly, among those II axons distributed to only one or two fibre types the mean diameter of 40 S_1 axons was 3.8 μm as against a mean of 2.8 μm for 65 S_2–S_5 axons.

The histograms in figure 18 compare the diameters of b_1b_2c Ia axons, firstly, with those of other Ia axons in the sample that supplied only two fibre types (figure 18*a*), and, secondly, with those of II axons that also supplied b_1, b_2 and c fibres (figure 18*b*). There is overlap in each case: the lower part of the b_1b_2c Ia diameter range includes all but one of the Ia axons supplied to two fibre types, and 75% of the II axons supplied to three fibre types. However, in all three of the possible comparisons the differences between the mean values are significant (b_1b_2c Ia > b_2c Ia) or very highly significant (b_1b_2c Ia > b_1b_2c II; b_2c Ia > b_1b_2c II).

(v) *Primary endings*

The form of primary endings supplied to b_1b_2c spindle units was remarkably constant, the only major variation being that due to the presence of bag fibres additional to the normal complement of one of each type. In the total sample of 270 such spindles (see table 4) this

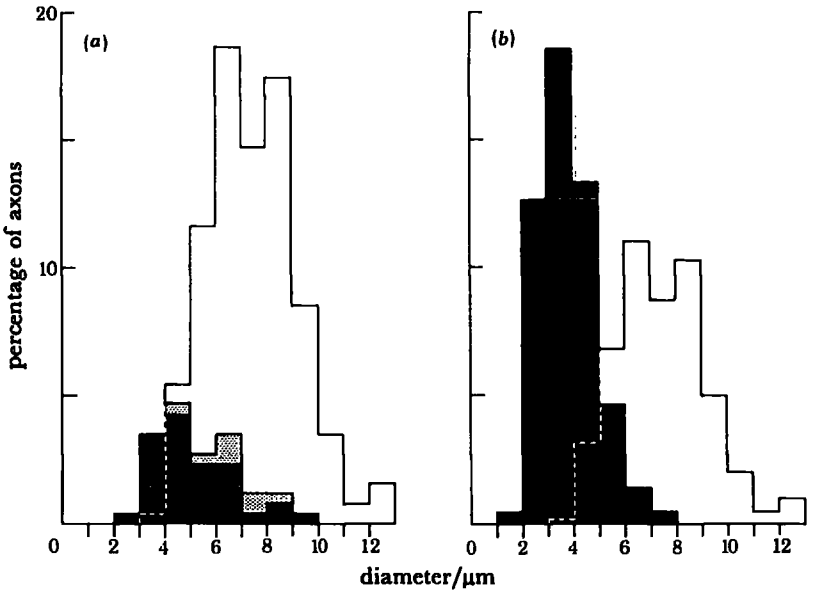


FIGURE 18. Histograms of fibre diameter for cat hindlimb spindle afferents as measured in teased, silver preparations.

(*a*) Comparison between the diameters of Ia axons supplying three types of intrafusal muscle fibre (unfilled columns; $n = 213$) and those supplying two. The latter group consisted of 37 Ia axons supplying b_2c spindle units (filled columns), and eight supplying two fibre types (stippled columns) as follows: seven to double primary endings (three b_2c, two b_1c, two b_1b_2) and one (b_1c) to a compound spindle.

(*b*) Comparison between the diameters of Ia axons supplying three types of intrafusal muscle fibre (unfilled columns; same sample as in (*a*)), and II axons that also supplied three types of intrafusal muscle fibre (filled columns; $n = 224$).

TABLE 8. INTRAMUSCULAR DIAMETERS OF SPINDLE AFFERENTS

(Abbreviations as in table 4.)

type of axon measured	number of axons measured													diameter/μm	
	ten.	p.b.	p.l.	p.t.	s.lum	d.lum	sol.	e.d.l.	e.d.b.	f.h.l.	t.a.	p.int.	total	range	mean
primary (Ia) supplying:															
(*a*) 3 fibre types															
b_1b_2c spindle units	51	37	11	17	28	7	23	12	5	7	10	5	213	3.4–12.8	7.5
(*b*) 2 fibre types															
b_2c spindle units	5	12	1	3	2	1	2	5	2	3	1	—	37	2.2–9.0	5.1
others†	5	—	1	—	—	—	—	1	—	—	2	—	9	4.6–8.1	6.5
total . . .	61	49	13	20	30	8	25	18	7	10	13	5	259	2.2–12.8	7.1
secondary (II) supplying:															
b_1b_2c spindle units															
S_1	69	40	21	23	24	8	27	14	4	4	15	4	253	1.7–7.7	3.9
S_2	28	15	5	4	6	—	7	5	—	2	9	—	81	1.4–4.6	2.9
S_3	6	1	2	1	—	—	1	1	—	—	3	—	15		
S_4	1	—	1	—	—	—	—	—	—	—	1	—	3	1.5–4.5	2.7
S_5	1	—	—	—	—	—	—	—	—	—	—	—	1		
b_2c spindle units															
S_1	1	1	—	—	—	—	—	1	1	—	—	—	4	2.6–4.3	3.1
total . . .	106	57	29	28	30	8	35	21	5	6	28	4	357	1.4–7.7	3.6

† Eight Ia axons contributing to double primary endings; one innervating b_1c fibres in an aberrant primary supplied to a compound spindle.

occurred in 32 (11.9%) and most frequently involved the duplication of the b_1 fibre: 28 spindles had two b_1 fibres, two had three, and two had two bag fibres of each type. In some spindles duplication of the b_1 fibre was accompanied by a reduction in the number of c fibres. In most of the muscles sampled the frequency of spindles with more than one b_1 fibre was below 10%, but in tibialis anterior it was 16.7% (two of 12), extensor digitorum longus 18.8% (three of 16), superficial lumbrical 28.1% (nine of 32) and pes interosseus 80.0% (four of five).

The mean length of 151 single primary endings was 359 μm in a range of 242–608 μm; the lengths of seven double primaries fell mostly in the upper part of this range (mean 466 μm). There was no consistent difference between the two types of bag fibre in the length occupied by primary terminals; the mean length innervated in b_1 fibres was 294 μm, in b_2 fibres 282 μm. There was also no significant difference between the maximum diameters of the b_1 and b_2 terminal systems; the mean of 134 b_1 measurements was 24.6 μm (s.e. 0.56 μm) as compared with a mean of 25.0 μm (s.e. 0.49 μm) for 120 b_2 measurements. According to Homma & Seki (1964) spindles in the fast tibialis anterior muscle have wider nuclear bags than those in the slow soleus, but our measurements did not reveal any significant difference.

The terminal systems supplied to bag fibres in the sample of 151 single primary endings consisted of a middle portion, in which the terminals were arranged mainly as regular transverse bands, and portions at each end, in which the terminals were disposed as irregular forms in mainly diagonal and longitudinal arrangements. The terminal systems supplied to b_1 and b_2 fibres were distinguished from each other by the following features.

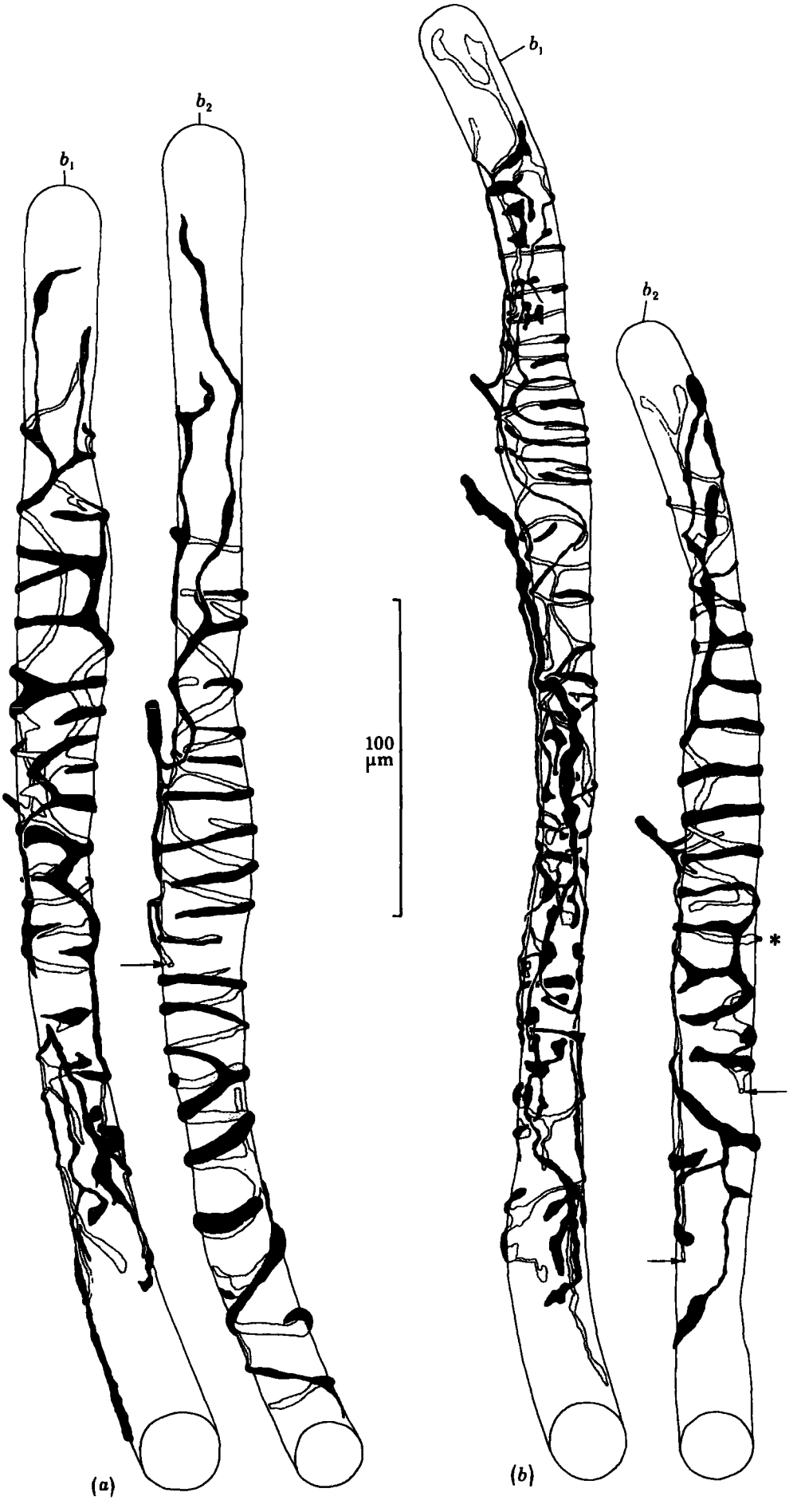
(i) Lack of an irregular portion at one end of the terminal system. This occurred frequently (43.4%) among b_2 systems, seldom (4.4%) among b_1 systems. In four endings the b_2 systems were entirely regular.

(ii) Relative proportions of regularity and irregularity in the two systems. Partly as a consequence of (i), the terminal systems supplied to b_2 fibres were mainly regular; on average their regular portions occupied 67% of their total length. By contrast, the irregular portions predominated in b_1 systems, occupying, on average, 57% of their total length.

(iii) Difference in number of bands encircling each fibre in the regular portion of its terminal system. In most endings the number of bands per unit length was greatest in the b_1 system; this was so in 78 % of the sample, there being no difference among the rest. The excess number per 50 μm length ranged from one to four, and was usually one (48%) or two (22%).

Drawings of b_1 and b_2 terminal systems traced from photographs are shown in figure 19. The most notable feature of the terminals supplied to c fibres was the fairly common occurrence of continuous sequences of spirals, sometimes with as many as six or seven complete turns, about twice the maximum number that occurred among spirals supplied to bag fibres. This, and other features of b_1b_2c primary endings, are illustrated in plates 3, 4 and 6.

The primary endings supplied to b_2c spindle units were mostly irregular in appearance and formed a straggling array that generally extended over a greater length than b_1b_2c primaries. The mean length of 32 was 402 μm in a range of 243–833 μm, with most measuring between 400 and 500 μm. Continuous sequences of spirals were sometimes present among the c fibre terminals, and regular transverse bands occurred around the most heavily nucleated part of the b_2 fibre when this constituted a bag rather than the more usual chain of nuclei. Photographs of these endings, and of the equatorial nucleation of the b_2c unit, are illustrated in plate 5.


b_1
b_2
100 µm
(a)
b_1
b_2
(b)

(vi) *Secondary endings*

A constant feature of all secondary endings was the distribution of terminals to *c* fibres, largely in the form of widely spaced spirals and incomplete loops, and in most endings this was the dominant feature. The terminals were more dispersed and generally thinner, more delicate and irregular than those supplied to *c* fibres in primary endings, but viewed collectively they presented a similar annulospiral appearance. When bag fibres were included in the innervation, their terminals were usually much more irregular, and those supplied to the b_1 fibre were often in the form of sprays. Occasionally these sprays were a dominant feature so that the appearance of the ending could then more appropriately be described as 'flower-spray'. In such endings the b_1 innervation was extensive and the *c* innervation was reduced, sometimes because the *c* fibres were fewer than usual. There was frequently a strong resemblance between the spray terminals of an S_1 secondary ending supplied to a b_1 fibre and the adjacent irregular portion of the primary terminal system supplied to the same fibre.

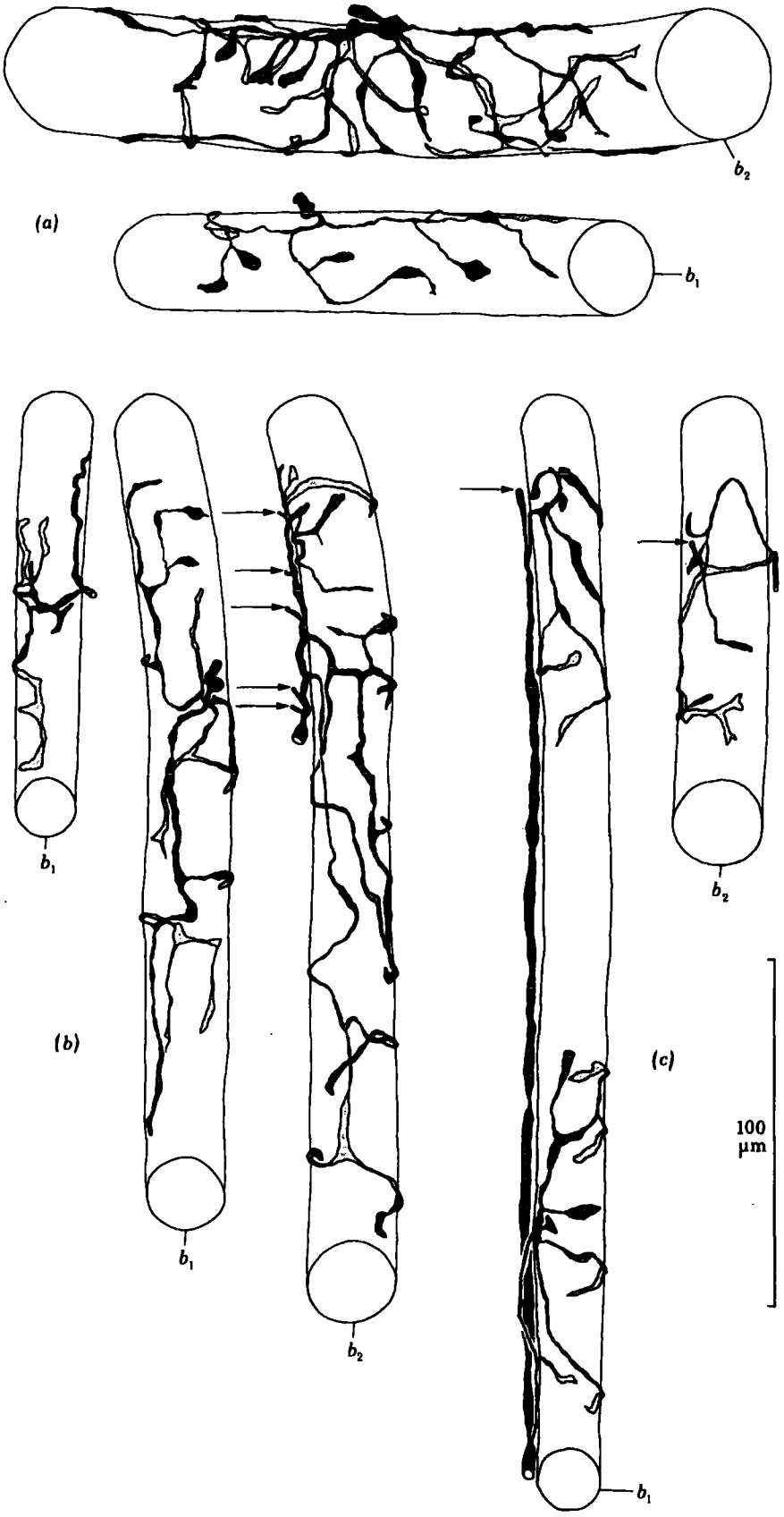
The lengths of the secondary endings studied were about the same as those of the primary endings. The mean length of 313 secondaries (221 S_1, 71 S_2, 17 S_3, 4 S_4) was 348 μm in a range of 138–716 μm; S_2 endings tended to be the longest (mean 385 μm, range 202–716 μm). Instances of overlap between sensory and motor innervation were occasionally observed in the S_1–S_3 positions involving secondary terminals on *c* fibres and trail endings on bag fibres.

A study of the supply of terminals to bag fibres in 83 b_1b_2c S_1 secondary endings was made in order to quantify the amount of innervation received and its proportional distribution between the two types. The sample comprised endings from tenuissimus (26), peroneus brevis (16), p. tertius (15), soleus (13) and superficial lumbrical (13) muscles supplied to a total of 67 spindles. The mean lengths of the b_1 and b_2 fibres innervated in these endings were, respectively, 143 μm (range 18–360 μm) and 173 μm (range 16–434 μm). On average the innervated portions represented 42% (b_1) and 51% (b_2) of the total length of the ending. In the endings sampled from tenuissimus and soleus the longest innervated portion belonged to either type of bag fibre with about equal frequency, but in 63% of all endings sampled it belonged to the b_2 fibre. The b_1 fibres received an average of ten terminals (range 2–31), the b_2 fibres an average of 13 (range 2–46). The presence of an additional b_1 fibre in the spindle did not always result in an excess of b_1 innervation, though this was often the case. The b_2 fibre received the most terminals in 64% of the endings. A supply of eight terminals provided an innervation that was arbitrarily judged to be visually significant. The proportion of endings on b_1 fibres with eight or more terminals was 65%, that on b_2 fibres 76%.

In four of the 40 b_2c spindle units the primary ending was accompanied by an S_1 secondary ending (see table 4). The predominance of terminals supplied to the b_2 fibre gave the endings a markedly irregular appearance.

In some muscles secondary endings were noticeably fewer than in others. In muscles exten-

FIGURE 19. Primary terminals supplied to bag$_1$ (b_1) and bag$_2$ (b_2) fibres in spindles from (*a*) tenuissimus and (*b*) superficial lumbrical muscles. Drawings traced from photographs of teased, silver preparations; a photograph of the tenuissimus preparation used is shown in figure 25, plate 4. Arrows indicate preterminal axons that have been cut, but which in the ending ran on to supply terminals to chain (*c*) fibres. Asterisk indicates position of a sensory cross-terminal with a *c* fibre. The b_1 terminal system in (*a*) and the b_2 system in (*b*) are each derived from a single heminode. The difference in spacing of the bands encircling the regular portions of the b_1 and b_2 systems is especially marked in (*b*). Note the characteristically irregular portions of the b_1 systems.


(a)
b_2
b_1
(b)
b_1
b_2
(c)
b_2
b_1
100 µm

sively sampled the lowest Ia:II ratio was 1:1.2 in superficial lumbrical; amongst the highest were tenuissimus (1:1.7) and peroneus longus (1:1.8). Drawings, traced from photographs, of secondary terminals supplied to b_1 and b_2 fibres are shown in figure 20, and the main features of secondary endings that have been described are illustrated in plates 3, 5 and 6.

4. Discussion

This study has shown that there are a number of constant features in the form and distribution of sensory terminals in cat hindlimb spindles. Among these are: (i) the close association between sensory terminals and equatorial myonuclei; (ii) the fact that among individual fibres the b_1 fibre always receives the most innervation in primary endings and usually the least in secondary endings; (iii) the almost exclusive restriction of sensory cross-terminals (Adal 1969) in primary endings to interconnections between the terminals supplied to c fibres; and (iv) the occurrence of S_1 secondary endings distributed to b_1, b_2 and c fibres as the most common type of secondary in all muscles sampled. We shall explore the functional significance of these features having first considered them in the context of spindle development.

The association between innervation and nucleation begins when spindle development is initiated by the contact of a Ia axon with a developing myotube that would otherwise have matured into an extrafusal muscle fibre. There follows a period of morphogenesis induced by the Ia axon during which the three types of intrafusal muscle fibre and their equatorial nucleation are differentiated in the sequence b_2, b_1, c (Zelená 1957; Landon 1972; Milburn 1973; Barker & Milburn 1982). Thereafter the Ia axon appears to play a major role in the maintenance of the adult spindle, so that, for example, after deafferentation, the equatorial nuclei disappear and are ultimately replaced by myofibrils, and the periaxial space is reduced (Tower 1932; Boyd 1962; Kucera 1980). An abundant and varied population of vesicles in the terminals of the primary ending, as well as beneath them in the muscle fibres, strongly supports Zelená's (1957, 1964) assumption that the influence of the Ia axon is mediated neurohumorally.

Secondary axons seem to have much less influence. Spindles that lack them, though slightly smaller, appear to be entirely normal. Our data indicate that secondary axons are supplied to developing spindles in a random fashion, since the frequency distribution of spindles that received different numbers of secondary endings is binomial (see figure 41). We suggest that the axons are led to the developing spindles by contact guidance, growing down paths already established by the Ia axons. They reach developing cat spindles after Ia and β axons, at about the same time as γ axons (A. Milburn 1981, personal communication). At this stage the process of separation of interlocked myotubes has started to spread from the poles to the equator and the b_1 myotube is becoming increasingly dissociated from the rest of the intrafusal bundle

Figure 20. Terminals of S_1 secondary endings supplied to bag$_1$ (b_1) and bag$_2$ (b_2) fibres. Drawings traced from photographs of teased, silver preparations.

(*a*) Tenuissimus spindle; the b_1 fibre receives nine terminals in the form of a spray, whereas the b_2 fibre is surrounded by a complex configuration of 46 terminals.

(*b*) Superficial lumbrical spindle with two b_1 fibres (a photograph of the preparation is shown in figure 38, plate 6). The b_1 fibres together receive 19 terminals, the b_2 fibre 16.

(*c*) Tenuissimus spindle; in this ending the b_1 fibre, with 19 terminals, is more densely innervated than the b_2 fibre, with six terminals. Note that each configuration of terminals supplied to the b_1 fibre is derived from a penultimate node. Arrows indicate preterminal axons that have been cut, but which in the ending ran on to supply terminals to c fibres.

Description of plate 3

Photographs of teased, silver preparations illustrating features of primary (P) and secondary (S_1) endings innervating spindles in cat hindlimb muscles. Abbreviations: Ia, primary axon; II, secondary axon; b_1, bag_1 fibre; b_1 br., b_2 br., b_1b_2 br., b_2c br., first-order branch of Ia axon supplying b_1 fibre, b_2 fibre, b_1b_2 fibres or b_2c fibres; b_1 p.t.s., b_1 primary terminal system; b_2, bag_2 fibre; b_2 p.t.s., b_2 primary terminal system; c, chain fibre(s); c sp., chain-fibre spiral terminals.

Figure 21. The primary ending of a soleus spindle is supplied by a Ia axon that has a segregated distribution; S_1 secondary endings lie adjacent to it in the upper and lower parts of the figure. The b_1 fibre is dissociated from the others, and the position of the three types of muscle fibre in the intrafusal bundle alters, left to right, from b_1, b_2, c at the top of the figure, to b_2, c, b_1 at the bottom. Note irregular terminals (i.t.) at each end of the b_1 primary terminal system (otherwise only faintly stained) and the supply of secondary terminals to the b_1 fibre at top left and bottom right of spindle. Asterisk denotes example of preterminal axon being derived from the penultimate node of a II axon.

Figure 22. Two Ia axons supply a double primary ending in a peroneus longus spindle; that at the top of the figure (b_1b_2 Ia) innervates the b_1 and b_2 fibres, whereas that at the bottom (b_1b_2c Ia) innervates all three fibre types.

Figure 23. The primary ending of a superficial lumbrical spindle is supplied by a Ia axon that has a mixed distribution; an S_1 secondary ending lies adjacent to it in the lower part of the figure. The equatorial dissociation of the b_1 fibre provides a clear view of its primary terminal system and secondary innervation. Asterisk denotes point where II axon divides to produce two first-order branches; the branch on the right gives rise to three preterminal axons, two of which travel downwards to supply terminals to the b_1 and b_2 fibres, while the third travels upwards to supply terminals to b_2 and c fibres.

Figure 24. Photographs taken at different focal planes to illustrate a primary ending supplied to a peroneus brevis spindle with two b_1 fibres. The focal plane in (a) shows primary terminal systems supplied to the b_2 fibre (left) and one of the b_1 fibres, whereas that in (b) shows the chain fibre terminals (left) and the primary terminal system supplied to the other b_1 fibre (right). The bands encircling the b_2 fibre are seen to be spaced wider apart than those around the b_1 fibres. Three first-order branches were produced by the Ia axon supplying the ending, and their distribution was segregated.

Description of plate 4

Photographs of teased, silver preparations illustrating features of primary endings innervating spindles in cat hindlimb muscles. Abbreviations as in plate 3.

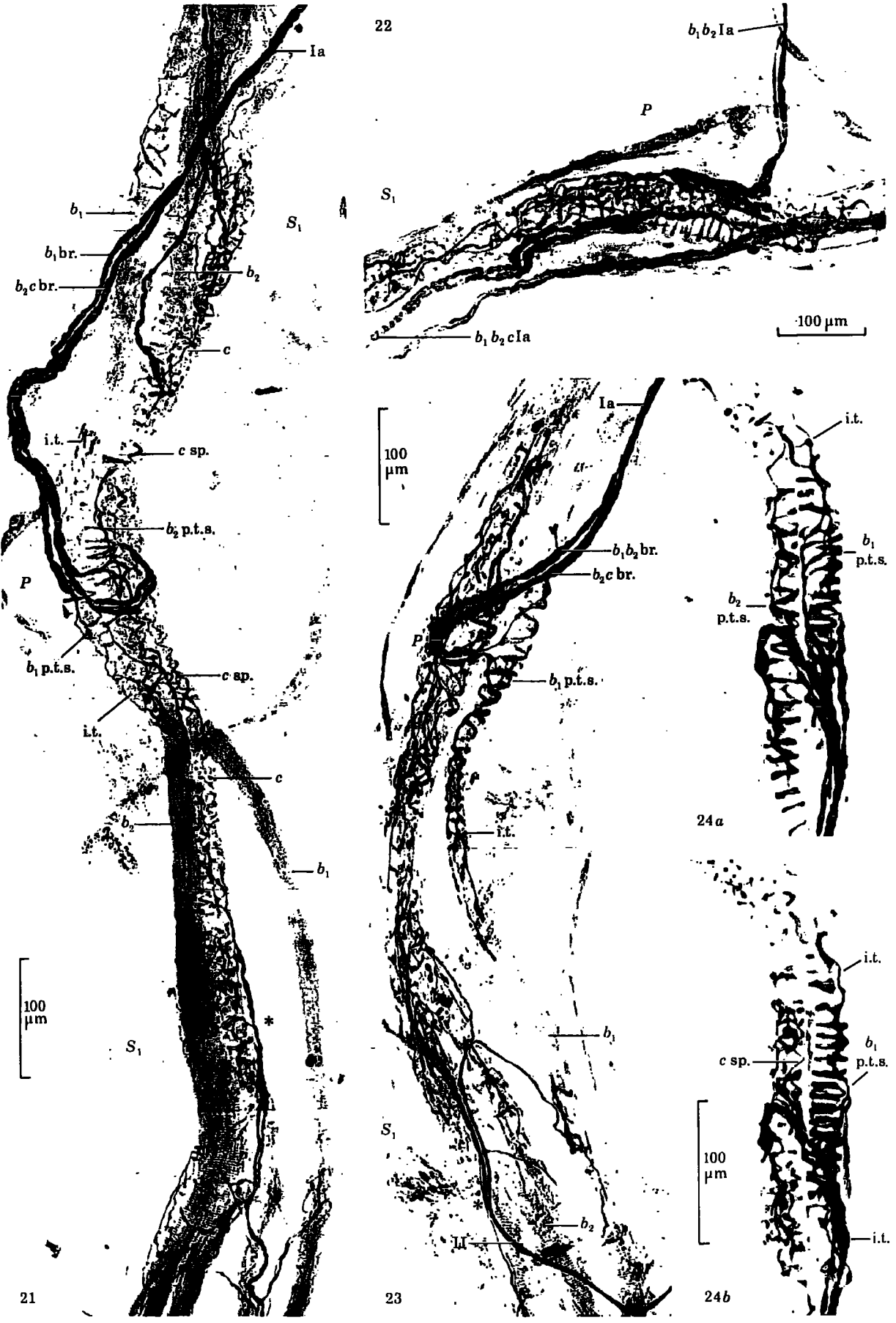
Figure 25. Primary ending of a tenuissimus spindle supplied by a Ia axon whose segregated distribution is clearly evident. The focal plane selected is that which best demonstrates the b_1 terminal system. The terminals supplied to the b_2 fibre lie on the right underneath the chain-fibre terminals. Drawings of the bag-fibre primary terminals supplied to this spindle are shown in figure 19*a*.

Figure 26. Part of a primary ending supplied to a peroneus brevis spindle provides an example of a continuous sequence of spirals around a chain fibre. Irregular terminals belonging to the b_1 terminal system lie immediately above.

Figure 27. Example of an unbranched Ia axon. The preterminal axons distributing the primary terminals are all derived from one heminode. Peroneus brevis spindle.

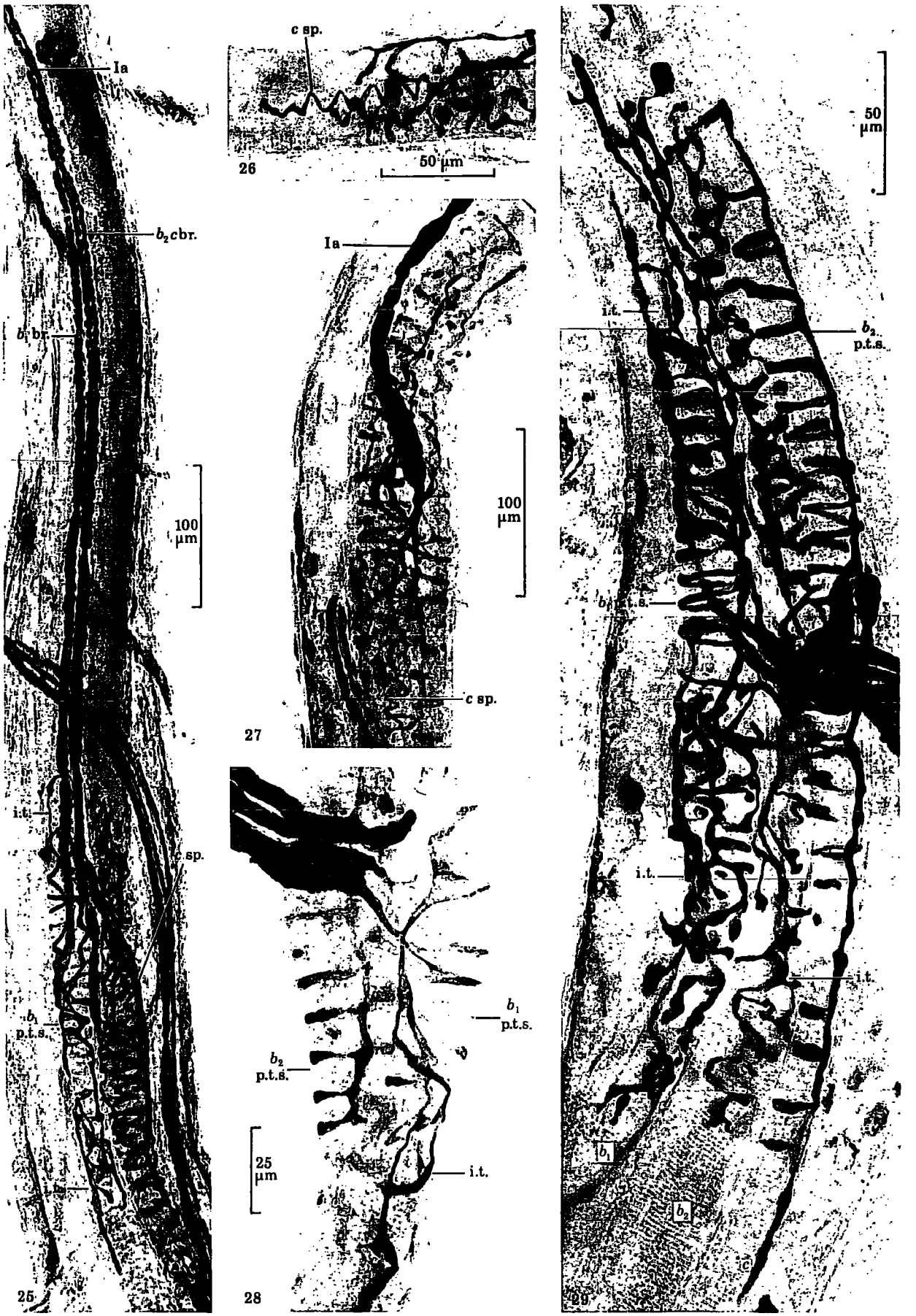
Figure 28. Part of primary ending supplied to a peroneus brevis spindle illustrating typical contrast between the end portions of the terminal systems on the b_1 and b_2 fibres.

Figure 29. Primary ending supplied to a peroneus brevis spindle. The position of the three types of muscle fibre in the intrafusal bundle alters, left to right, from b_1, c, b_2 in the top half of the ending, to b_1, b_2, c in the bottom half. The b_1 terminal system, with its irregular portions at both ends, contrasts with the terminal system supplied to the b_2 fibre, which is regular at one end (top of figure) and irregular at the other (bottom). The terminals supplied to the b_2 fibre in the top half of the ending all originate from one centrally placed preterminal axon and wrap around the fibre in ribcage fashion, a fairly common type of configuration. Terminals are similarly disposed around the chain fibre in the bottom right half of the ending.

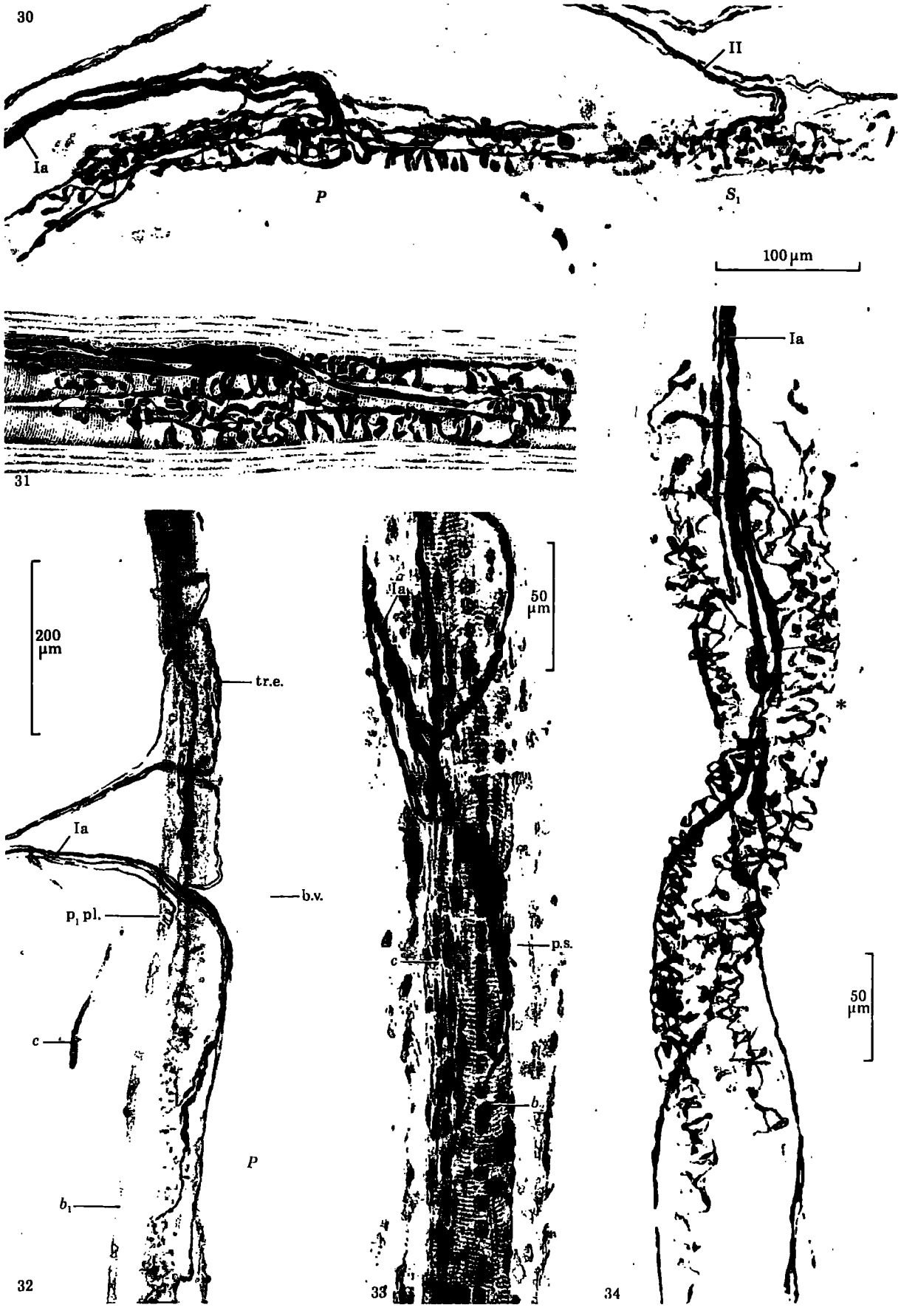


FIGURES 21–24. For description see opposite.

(*Facing p.* 356)



FIGURES 25–29. For description see page 356.



FIGURES 30–34. For description see page 357.



FIGURES 35–40. For description see opposite.

Description of plate 5

Photographs of teased, silver preparations illustrate the sensory innervation and equatorial nucleation of b_2c spindle units, and are compared with a figure from Ruffini (1898). Abbreviations: b.v., blood vessel; p.s., periaxial space; $p_1pl.$, p_1 plate; tr.e., trail ending. Other abbreviations as in plate 3.

Figure 30. A b_2c spindle unit from peroneus brevis is supplied with a primary ending and one S_1 secondary ending. The b_2 fibre lacked a nuclear bag and was accompanied by three c fibres.

Figure 31. Ruffini (1898), in his figure 3, plate 2, reproduced here, illustrates a type of primary ending in which 'the pure spiral or ring form occurs only here or there, the greater part showing S or C forms intercalated among forked, hooked or comma-shapes' (p. 199). The figure and description strongly suggest what we recognize as a primary ending supplied to a b_2c spindle unit.

Figure 32. A b_2c spindle unit from peroneus brevis illustrating the exclusion of the b_1 fibre belonging to the tandem-linked b_1b_2c spindle unit. The b_2c unit, consisting of one b_2 and three c fibres, is seen to be innervated by a Ia axon and fusimotor axons terminating as trail endings, while the excluded b_1 fibre receives a p_1 plate. A c fibre belonging to the tandem-linked b_1b_2c unit inserts alongside the b_2c unit.

Figure 33. Equatorial region of a b_2c spindle unit from tenuissimus. Three c fibres accompany the b_2 fibre, which is seen to possess a chain of myonuclei. The terminals of the primary ending are unstained.

Figure 34. Primary ending supplied to a b_2c spindle unit from peroneus brevis. The b_2 fibre was accompanied by four c fibres. The regularly spaced bands that can be seen in the ending at the level of the asterisk encircle the b_2 fibre.

Description of plate 6

Photographs of teased, silver preparations illustrating features of primary and S_1 secondary endings innervating spindles in cat hindlimb muscles. Abbreviations: tr.a., trail axon; tr.e., trail ending. Other abbreviations as in plate 3.

Figure 35. Peroneus brevis spindle illustrates overlap that may occur in the distribution of secondary and fusimotor endings. The b_1 fibre, positioned on the lower side of the intrafusal bundle, receives a typical system of terminals in the primary ending on the right, while in the adjacent S_1 position a secondary ending is distributed to the b_2 and c fibres. The trail axon on the left innervates all three muscle-fibre types; the terminals supplied to the b_1 fibre are seen to overlap in position with the S_1 secondary ending. The innervation of b_1 fibres by trail axons occurs in 17% of spindles in peroneus brevis (Barker & Stacey 1981).

Figure 36. An S_1 secondary ending supplied to a superficial lumbrical spindle clearly demonstrates the distribution of terminals to all three types of intrafusal muscle fibre.

Figure 37. Example of S_1 secondary innervation of a b_2 fibre in a peroneus brevis spindle. The b_2 fibre lies on the right of the intrafusal bundle, the b_1 fibre mostly at top centre with the c fibres beneath it. The regular end portion of the b_2 terminal system in the primary ending contrasts typically with the irregular end portion of the b_1 terminal system. The b_2 fibre received an extensive secondary innervation of 30 terminals distributed over a length of 434 μm, as compared with 11 terminals distributed to the b_1 fibre over a length of 182 μm.

Figure 38. Primary and S_1 secondary innervation of superficial lumbrical spindle that possessed two b_1 fibres. Each b_1 fibre is supplied with a primary terminal system with typically irregular portions at each end, and is also supplied with terminals in the S_1 secondary ending (cf. figure 20*b*).

Figure 39. Example of typical contrast between the regular features of primary-ending terminals (spirals on c fibres, regular end of b_2 terminal system) and the irregularity of S_1 secondary terminals. Peroneus brevis spindle.

Figure 40. The elaborate sprays of terminals supplied to the b_1 fibre in this S_1 secondary ending were its dominant feature, giving a general appearance more aptly described as 'flower-spray' than 'annulospiral'. Peroneus tertius spindle.

(Barker & Milburn 1982). Thus the first secondary axon to reach any given spindle is most likely to terminate in an S_1 position on a bundle of interlocked myotubes and supply all three fibre types, whereas any that arrive later will be obliged to terminate in more polar positions where the myotubes have already become separate and the innervation of only one or two fibre types is more probable. The proportional distribution of secondary endings to the three types

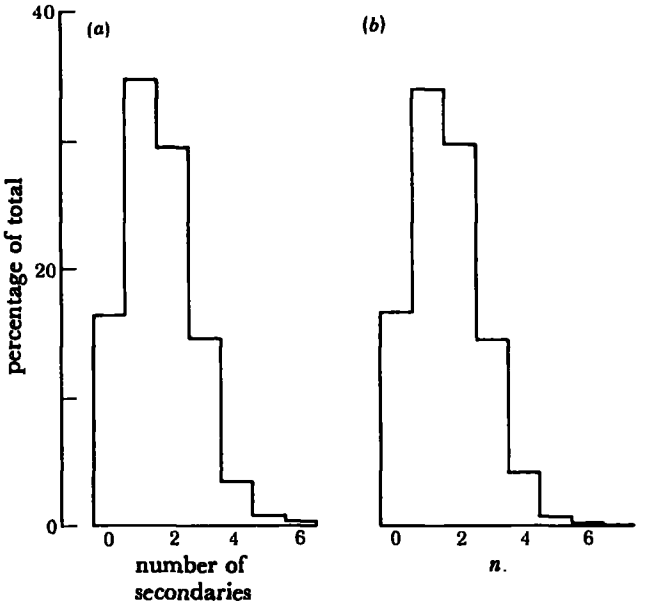


FIGURE 41. (*a*) Histogram showing the frequency distribution of b_1b_2c spindle units receiving different numbers of secondary endings. Data used are those in table 4 that relate to units supplied with a single primary ending. (*b*) Histogram showing the frequencies expected from a binomial distribution with parameters ($p = 0.22$, $n = 7$) calculated from the histogram in (*a*). (χ^2 goodness-of-fit test; $\chi^2 = 0.43$, for 5 degrees of freedom $P < 0.01$.)

of muscle fibre in adult spindles (see table 7) is thus accounted for. Equatorially the process of separation is never completed among *c* fibres, hence the regions of close apposition and sensory cross-terminals that occur among them in the adult.

Chain fibres frequently contract as a group on fusimotor stimulation (Boyd 1966, 1976), and their effect on the primary response is so powerful as to drive it at the frequency of their own contractions, up to about 60 Hz under suitable conditions (Boyd 1981). In the context of other characteristics of *c* fibres it seems likely that their cross-terminals represent a positive adaptation to such behaviour, perhaps to produce as large a generator potential as possible. Nevertheless, if the functional unity of *c* fibres is important, it is not clear why there should be so many. It may be that in view of the relatively poor vascularization of the spindle, a high surface/volume ratio is essential for their metabolism. Also more fibre surface is made available for sensory innervation in a given length of equatorial region, and, as we have shown, *c* fibres collectively receive the greatest amount of contact area with both primary and secondary terminals (see table 3).

The dense innervation of the b_1 fibre in primary endings is presumably adapted to generate a sufficiently large dynamic component in the receptor potential, since considerably more fibres (b_2 and *c*) are involved in the static input. The innervation of the b_1 fibre by secondary endings

appears to be of little functional significance. Although some secondary endings have a high dynamic sensitivity to passive stretch (Jami & Petit 1979), there appears to be no obvious correlation between this and the extent of their b_1 secondary innervation. Moreover, stimulation of dynamic fusimotor axons rarely increases the dynamic sensitivity of secondary endings (Appelberg *et al.* 1966; Boyd 1981), although it does occasionally increase their static sensitivity (L. Jami & J. Petit 1980, personal communication). The static effect may be due to the enhanced 'creep' of the b_1 fibre that occurs during dynamic fusimotor stimulation following the completion of ramp stretch (Boyd 1981). This could lead to a compensatory extension of the secondary terminals while the primary region was shortening so as to produce what Matthews (1964) envisaged as 'an inverse dynamic response, consisting of a static response developing with a lag'.

Our data on the branching of spindle afferents have important implications for the manner in which nerve impulses are generated from the endings and propagated into the axons. Quick *et al.* (1980), using a cytochemical method, have shown that within the final branches of Ia axons the heminodes and some of the penultimate nodes are all potential sites of spike initiation. If the membrane properties of these nodes are similar, the preferred sites for impulse generation would be the heminodes, resulting in a complex pattern of spikes converging on the parent axon. Since abortive spikes have been observed in isolated frog spindle preparations (Katz 1950; Ito 1976; Ito & Komatsu 1976), it may be assumed that not all spikes propagate into the parent axon. This may be attributed to the relatively low safety factor of the branch points (Krnjević & Miledi 1958) resulting in some of the nodes at these points acting as further stages in the encoding of the afferent response.

For Ia axons whose first-order branches have a segregated distribution there would presumably be separate dynamic and static pacemakers. These have been demonstrated by Hulliger & Noth (1979), whose results show that the outputs of the separate pacemakers do not summate linearly; indeed the dynamic pacemaker may be totally occluded when its output falls below that of the static one. Our reconstructions of the branching patterns of Ia axons (figure 1) suggest how this may occur. When an impulse, propagating centrally in a first-order branch, reaches the first branching node it will then propagate orthodromically into the parent axon, and, provided that it is not refractory, antidromically into the other first-order branch. During the following refractory period this branch would then be less likely to transmit another impulse, thus suppressing its pacemaker. However, the antidromic impulse might itself fail to propagate into some of the smaller branches, or be eliminated by collisions with abortive orthodromic spikes within them. Since these effects would be cumulative, a profusely branching first-order system would recover from an antidromic impulse more rapidly than one with fewer branches.

Examination of the Ia branching patterns shown in figure 1 shows that the first-order branches associated with static input (from b_2 and c fibres) tend to be more profusely branched than those associated with dynamic input (from b_1 fibres). There is thus a correlation between the morphological and physiological asymmetry. If this interpretation is correct the branching pattern of spindle 6 shown in figure 1 should produce a more symmetrical interaction between its dynamic and static pacemakers than is typical. We would suppose that the branching patterns of Ia axons exert a major influence on the pattern of the afferent response, and suspect that the dynamic pacemaker is more easily occluded in Ia axons that have a mixed distribution.

Similar considerations apply to secondary axons, though in some instances their branching allows for a terminal input from penultimate nodes as well as heminodes. Presumably these

nodes act as spike generators, or as modulators of impulse transmission. The variable dynamic sensitivity of secondary endings may partly be accounted for by a variable input from b_1 terminals, though, as already mentioned this sensitivity is rarely affected by dynamic fusimotor stimulation.

The spiking activity of the final branches of spindle afferents is presumably generated by receptor potentials produced by their terminals. An electrotonic potential has been recorded in the Ia axon about 1 mm from its terminal by Hunt & Ottoson (1975) using an isolated cat-spindle preparation in which spiking activity was blocked with tetrodotoxin. They refer to this as the receptor potential, but it is perhaps more accurately regarded as the algebraic sum of several receptor potentials, each associated with a single terminal branch system. Thus in primary endings there would be at least three receptor potentials separately produced by terminals on b_1, b_2 and c fibres. By analogy with impulse activity in a group of axons, the potential recorded by Hunt & Ottoson could be referred to as the 'compound receptor potential' (c.r.p.). However, the c.r.p. is probably an abstraction since its appearance would normally be prevented by spiking activity in the final afferent branches. Hunt *et al.* (1978) have shown that the c.r.p. is generated mainly by increased Na^+ conductance, and presumably the individual receptor potentials are generated in the same way.

The actual transduction mechanism is still a matter for speculation. In its simplest form it would involve direct coupling of the ion-permeability changes to stretch deformation of the terminal membrane adjacent to the intrafusal muscle fibre (Katz 1950), and this seems to be the mechanism favoured by Hunt *et al.* (1978) and Ito *et al.* (1980). However, consideration of the fluid mosaic model of membrane structure (Singer & Nicholson 1972) makes it difficult to see how the membrane could support the necessary longitudinal tension changes for this to occur. Moreover, since the terminals are convex structures occupying grooves in the muscle fibres (Adal 1969), it seems more likely that the membranes flatten out rather than stretch during muscle lengthening. Flattening of frog sensory terminals has been described by Karlsson *et al.* (1971). Furthermore there is no evidence that the terminals adhere directly to the muscle fibres; they appear instead to be pressed against them by the basal laminae of the muscle fibres, which pass over the free surfaces of the terminals.

We believe that the deformation leading to transduction may be due to a deformation of the whole sensory terminal brought about by increased tension in the basal lamina, the terminal being squeezed between the lamina and the underlying muscle fibre. We suggest that the Na^+ channels are distributed over the whole terminal membrane, rather than being restricted to that part adjacent to the muscle fibre, and that their permeability is affected by an intracellular messenger released from a bound state by the increase in cytoskeletal tension that may be presumed to accompany the bulk deformation of the terminal. Such a process has the advantage of introducing a possible amplification stage between the initial mechanically linked event and the permeability changes, thereby allowing for very high sensitivity. The intracellular messenger would most probably be Ca^{2+} since it is thought to play a similar role in retinal receptors (Hagins 1979) as well as acting as an intracellular messenger in a number of other subcellular systems (Kretsinger 1979). Removal of Ca^{2+} from the extracellular fluid has profound effects on crustacean muscle receptors (Wiermsa *et al.* 1953), as does the inclusion of Ca^{2+} channel blockers on frog spindles (Ito *et al.* 1980), but so far no effect has been reported for mammalian spindles (Hunt *et al.* 1978).

Soon after it was recognized that intrafusal muscle fibres were of two types, nuclear-bag and

nuclear-chain (Cooper & Daniel 1956; Boyd 1956), it became evident that pairs of spindle capsules were sometimes linked together in tandem by a single bag fibre, the smaller capsule of the two being supplied by a markedly irregular primary ending (Barker & Ip 1961; Barker & Cope 1962). We have established that the link is made by the b_2 fibre, and have recognized the smaller capsule, from which the b_1 fibre is excluded, as a b_2c spindle unit innervated by a Ia axon of relatively small diameter. Despite some features that are intermediate we regard the axon and its ending as primary rather than secondary for the following reasons: (i) the myotubal chains of nuclei, and the occasional nuclear bag that occurs in the b_2 fibre, resemble the chains and bags of myonuclei found at the equator of spindles where primary endings terminate; (ii) the relation of the ending to the surrounding capsule and periaxial space is typical of primary innervation; and (iii) the ending is occasionally accompanied by another axon and ending that have all the appearances of being S_1 secondary. Furthermore, it seems very probable that the ending is the same one that Ruffini (1898) recognized as an irregular type of primary in his original description (compare figures 30 and 31, plate 5).

These b_2c spindle units were present in most of the muscles sampled for the silver analysis. They usually inserted into tendon and were more common in some muscles than in others, e.g. they were about four times more frequent in extensor digitorum longus and peroneus brevis than in superficial lumbrical and soleus. Bakker & Richmond (1981) have found much higher frequencies in some cat neck muscles: 45% of the spindle population were b_2c units in complexus (sample of 40) and biventer cervicis (40), 33% in splenius (33). They have also demonstrated that the ATPase profile of the bag fibre is that of a b_2 fibre.

The functional properties of b_2c Ia axons and their endings remain to be elucidated. The usual criteria for the identification of primaries (axon conduction velocity, dynamic response of the ending to passive stretch) are of little use since the relatively small diameters of the axons will place their conduction velocities somewhere between 60 m/s (obviously secondary) and 80 m/s (obviously primary), and in the absence of a b_1 fibre their endings will have little dynamic sensitivity. Richmond & Abrahams (1979) found that the stretch responses of a considerable number of neck-spindle sensory endings were intermediate between primary and secondary. Many of these were probably primaries innervating b_2c spindle units, and it seems likely that these also account for most of the 'truly intermediate sensory endings' identified by Dutia (1980) in soleus spindles.

Banks *et al.* (1980) have examined the responses of peroneal spindle afferents to ramp-and-hold stretches in an attempt to identify b_2c Ia axons. These did not emerge in plots of dynamic index against static response, or of the dynamic index/static response ratio against conduction velocity. Further progress in this matter must await experiments in which the responses of b_2c spindle units are monitored during motor stimulation. Boyd (1981) has described the effects that separately activating each type of intrafusal muscle fibre has on the dynamic and position (or length) sensitivities of primary and secondary endings. Some response patterns of b_2c units could be predicted from these data, but it is perhaps unwise to ascribe particular functions to subsystems of the spindle and expect them to combine linearly under varying conditions.

The high proportion of b_2c units in muscles that raise the head, such as complexus, is due to the fact that their spindles are mostly arranged in elaborate tandem systems in which there are many capsules that exclude the b_1 fibre (Richmond & Abrahams 1975; Bakker & Richmond 1981). Spindle systems of this kind presumably have a high primary static sensitivity to cater for the exclusive antigravity function of such muscles. By contrast, occipitoscapularis, which

rotates the scapula, has a spindle population that lacks tandem systems and resembles that of a hindlimb muscle (Richmond & Abrahams 1975). In our own sampling of tenuissimus, soleus, extensor digitorum longus, peroneal and lumbrical muscles, the spindle population of the superficial lumbrical muscles stands apart as having the lowest proportion of b_2c units, the lowest supply of secondary endings, and the highest proportion of b_1b_2c units with more than one b_1 fibre. A relatively high dynamic sensitivity may thus be achieved, which presumably relates to the function that these muscles serve in carrying out finely adjusted movements of digits. With the amount of information about spindles now available, attempts such as these to correlate the function of a muscle with the structural and functional properties of its spindle population are becoming more feasible, and perhaps the time has come to pay more attention to these considerations.

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Paper 21.

R.W. Banks (1983)
A morphometric study of intrafusal motor endings in the cat.
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Journal of Physiology, **341**, 15–16*P*]

A morphometric study of intrafusal motor endings in the cat

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Barker, Banks, Harker, Milburn & Stacey (1976) and Banks (1981) have suggested that some features of intrafusal motor endings may be related to the distance of the endings from the primary sensory terminals. For at least one feature, the extent to which the endings are indented into the muscle fibres, this suggestion seems to be borne out.

Twenty-seven endings were analysed from three spindle poles, each pole from a different tenuissimus muscle. Measurements, to the nearest 0·5 mm, were made of the maximum width (x), thickness (z) and indentation into the muscle fibre (y) of every terminal on photomicrographs enlarged 1800× from serial, 1 μm thick, transverse sections. Three ratios, termed for convenience ellipsity ($E = x/z$), indentation ($I = y/x$) and superficiality ($S = y/z$), were obtained from the data. The ratios varied widely within and between individual terminals of each ending, but due to the large amount of data the standard errors of the mean values for complete endings were small.

Mean values of E ranged from 1·42 to 2·55 (exceptionally 3·97), clustering around 2·0. They showed no systematic variation with respect to location of ending. Both I and S, therefore, effectively estimated the amount by which the terminals were embedded in the muscle fibres and their mean values were found generally to increase with greater distance between the motor and primary sensory endings on all three types of muscle fibres. Moreover, endings from the same axon and on the same muscle fibre, or fibre type, were almost always more deeply embedded when located further from the primary endings (nineteen of twenty endings from eight axons). Where endings on different muscle-fibre types occurred close together, those on the chain fibres were more deeply embedded than those on the bag fibres. Thus the mean values of I for 5 bag_1, 6 bag_2 and 5 chain motor endings, located between 1·0 and 1·5 mm from the primary ending, were 0·15, 0·14 and 0·20 respectively. However, the most deeply embedded endings were found on bag_1 fibres, due mainly to their being furthest from the primary ending.

Arbuthnott, Ballard, Boyd, Gladden & Sutherland (1982) claim that there are two types of ending on chain fibres derived from static axons, one predominantly innervating bag_2 fibres and lying superficially on the muscle fibres, the other surrounded by sarcoplasmic protrusions and derived from axons predominantly supplied only to chain fibres. My results do not support this; there was no tendency for the endings on chain fibres to fall into two groups, and the most deeply embedded endings were supplied by axons that also innervated bag_2 fibres.

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Paper 22.

R.W. Banks & D. Barker (1983)
Recovery of cat hindlimb muscle afferents following nerve section.
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Recovery of cat hindlimb muscle afferents following nerve section

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The common peroneal nerve was cut in nine adult cats and repaired with 10/0 polyamide epineurial sutures under aseptic conditions. Cats were prepared at various times during a 6–50 week postoperative period for single-unit, dorsal-root recording of peroneus brevis afferent responses to ramp-and-hold stretches of 1·8 mm amplitude up to maximum physiological length applied at 10, 5, or 2·5 mm s^{-1}. Dorsal and ventral roots L7 and S1 were cut. Sodium pentobarbitone anaesthesia was used for all procedures.

Overall conduction velocities increased up to 40 weeks without segregation of groups I and II. Functionally identifiable spindle and tendon-organ afferents were present from 7 weeks. The number of spindle afferents, and the proportion of those tonically firing, increased up to 16 weeks, but even at 50 weeks three of twenty-three responded only to phasic stretch. Neither dynamic nor static responses showed any tendency to increase with longer recovery periods. The mean peak dynamic response of tonically firing units to medium-rate stretches was 67% of the control value, whereas the mean static firing level during the hold phase reached 78% of the control. The mean tonic firing level increased up to 40 weeks reaching 86% of the control. At 40 and 50 weeks the responses of units conducting within the control group II velocity range were indistinguishable from those of normal secondary endings, whereas spindle units conducting within the control group I velocity range showed initial bursts and dynamic indices that were reduced as compared with those of normal primary endings. This suggests that the bag_1 fibres may have been less completely reinnervated than bag_2 and chain fibres.

As judged by the numbers of afferent units recovered, many spindles and tendon organs remained functionally deafferented, and the loss of secondary endings appeared to be disproportionately high. This was confirmed histologically, but in addition to terminals showing some similarity to normal primary and secondary endings, spindle sensory regions often contained wholly abnormal, fine axon terminals. These were widely distributed in the periaxial space and remained mostly apart from intrafusal muscle fibres. After 7 weeks recovery sensory axons had returned to all spindles to supply either one or both types of innervation. It seems likely that the abnormal terminals did not respond to stretch and were not originally distributed to spindles.

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Paper 23.

R.W. Banks & D. Barker (1983)
The current wiring diagram of the muscle spindle.
Proceedings of the International Union of Physiological Sciences 15: 111.01.

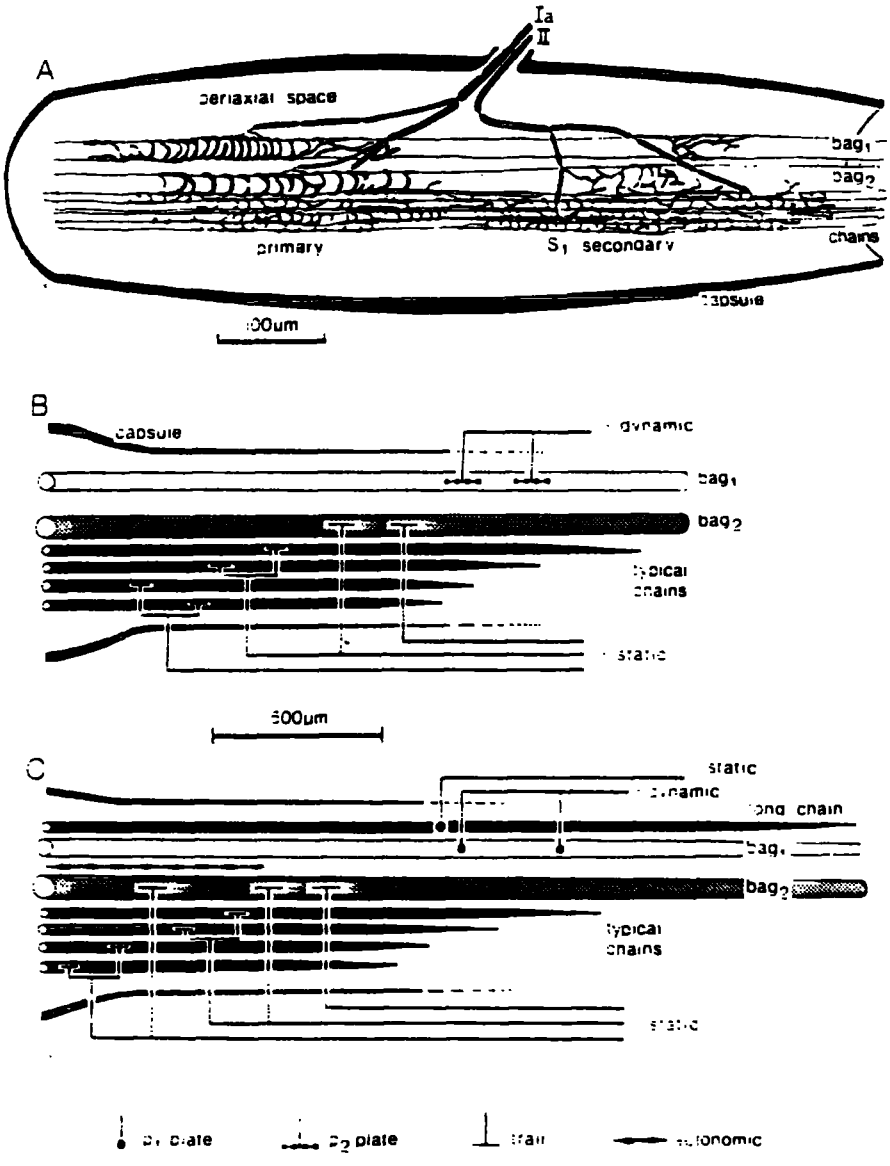
Proc. IUPS XXIXth Congress 15, 111.01 (1983)

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R.W. Banks and

THE CURRENT WIRING DIAGRAM OF THE MUSCLE SPINDLE

D. Barker
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Cat tenuissimus spindles. A. Sensory innervation. Primary terminals mainly innervate bag fibres, secondary terminals chain fibres. The distribution of the total terminal contact area of a primary ending is about 35% bag1 ($\dot{b}_1$), 25% bag2 ($\dot{b}_2$), 40% chain (c); of an S_1 secondary 10% $\dot{b}_1$, 20% $\dot{b}_2$, 70% c. B, C. The motor innervation of each pole typically consists (B) of a γ dynamic axon supplying 2 p_2 plates to the $\dot{b}_1$ fibre; and 2-3 γ static axons supplying trail endings to the $\dot{b}_2$ and c fibres. About twice as many γ static axon branches enter spindles to supply both $\dot{b}_2$ and c fibres as to supply $\dot{b}_2$ or c fibres. About 1 in 4 fusimotor axons innervate both poles. The most common variation (C) is for static and dynamic β axons to participate in the motor innervation (though seldom of the same spindle), terminating as p_1 plates on long c and $\dot{b}_1$ fibres, respectively. Some spindles receive a non-vascular autonomic innervation.

Paper 24.

R.W. Banks (1984)
On the attachment of elastic fibres in cat tenuissimus muscle spindles.
J. Physiol. 348; 16P.

MODELLING OF CHAOTIC AND REGULAR Ia AFFERENT DISCHARGE DURING FUSIMOTOR STIMULATION

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Introduction

Muscle spindle Ia afferents often show 'driving', the firing of action potentials time-locked to the stimulus pulse, during static fusimotor stimulation. Driving is thought to be caused by rapid unfused contractions of nuclear chain fibres.

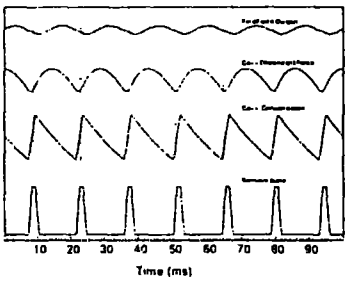
In order to give a more quantitive account of the mechanisms involved in driving, a model study was conducted based on detailed mathematical discriptions of both mechanical events and ionic events .

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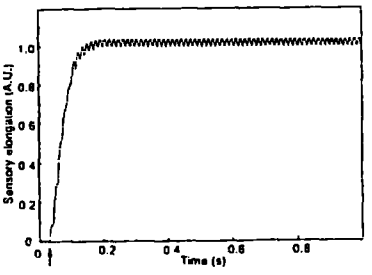
Bessou et al, 1968, J. Physiol. Lond. 196:47-6
Boyd et al, 1985. The muscle spindle. pp. 195-199.
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Methods

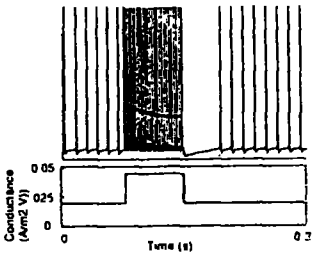
The mechanical model simulated the process of force production, using equations describing this process for type II extrafusal muscle fibre (Otten, 1987).



Stimulation pulses at 70 Hz. cause an unfused tetanus. The final force fluctuates at the stimulation frequency with an amplitude of 5% of the total force level.

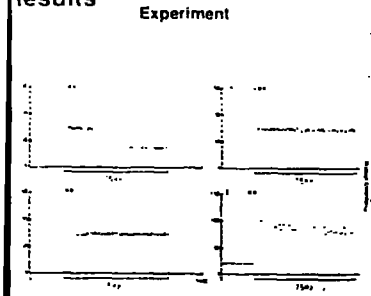


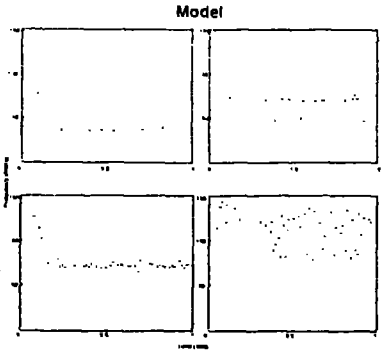
The sensory ending is stretched by the contracting fibre. The sensory elongation is tranduced into a depolarising conductance, proportional to the amount of sensory elongation. Note the remaining 5% oscillation.



The conductance is used as an input for the ionic model, which generates action potentials. The ionic model is based on t Frankenhaeuser-Huxley equations. A slo K-channel (τ = 70 ms) was added to enal low frequency repititive firing. It also causes spike frequency adaptation after step change.

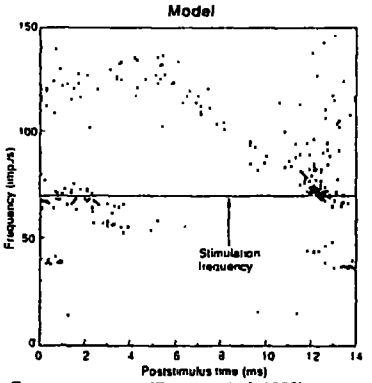
Results



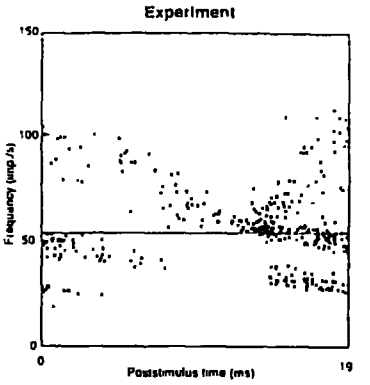


Experimental data (cat, peroneus tertius) from Boyd et al. (1985) shows that the initial muscle length can alter the driving pattern. 1:2 (top left), 1:1 (top right, bottom left) and irregular firing occur at resp. minimal, intermediate and maximal physiological muscle lengths.

All driving patterns could be reproduced with the model by adjusting the level of input conductance. The irregular firing occurs when the average afferent firing rate exceeds the stimulation frequency.



Frequencygrams (Bessou et al.,1968) were constructed from the model responses. Irregular firing (above stimulation frequency) forms a pattern in the frequencygram which resembles the intrafusal contraction. Irregular firing due to random noise will show no pattern.

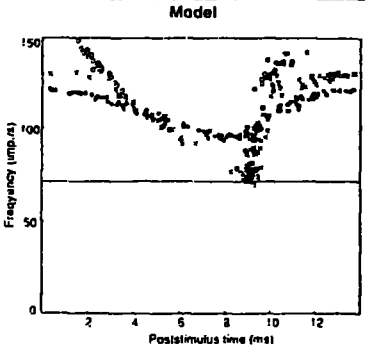


The frequencygram of experimental data (cat, soleus) recorded during an 8 mm ramp and hold stretch with static fusimotor stimulation is comparable to that of the model.

The hypothesis that the frequencygram reflects the contraction dynamics of the chain fibre was tested with the model (crosses).

When the amplitude of the mechanical oscillation is doubled, the modulation in the frequencygram increases (open circles).

Removing the slow K-channels reduces the modulation (closed circles). Therefore the frequencygram also reflects ionic mechanisms.



The irregular firing pattern caused by the mechanical oscillation is enhanced by the slow potassium conductance. In other words, the process of spike initiation itself is sensitive to dynamic changes in receptor potential.

Discussion

Small oscillations of the sensory elongation (5%) can cause different patterns of driving.

The type of driving is determined by the average afferent firing rate, which in turn is determined by the average amount of sensory elongation.

The observation (Boyd et al, 1985) that stable 1:1 driving can occur at all muscle lengths, can only be explained by the present model by assuming that the change in sensory elongation is small.

Possibly a transducer process sensitive to dynamic changes is needed to account for this phenomenon.

Conclusions

The irregular firing pattern observed during chain fibre stimulation is a chaotic form of driving which is expressed when the average afferent firing rate is higher than the stimulus frequency.

Driving is enhanced by the presence of a slow K-channel at the encoder site.

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Journal of Physiology, **348**, 16*P*]

On the attachment of elastic fibres in cat tenuissimus muscle spindles

By R. W. Banks. *Department of Zoology, University of Durham, Durham DH1 3LE*

Elastic fibres are a prominent passive mechanical component of mammalian muscle spindles. They form a mainly longitudinally orientated network condensed round the bag_2 fibre in the polar regions and dispersed amongst the inner and outer capsules in the equatorial regions (Cooper & Gladden, 1974). I have examined their attachment to intrafusal muscle fibres using longitudinal sections of cat tenuissimus muscle spindles.

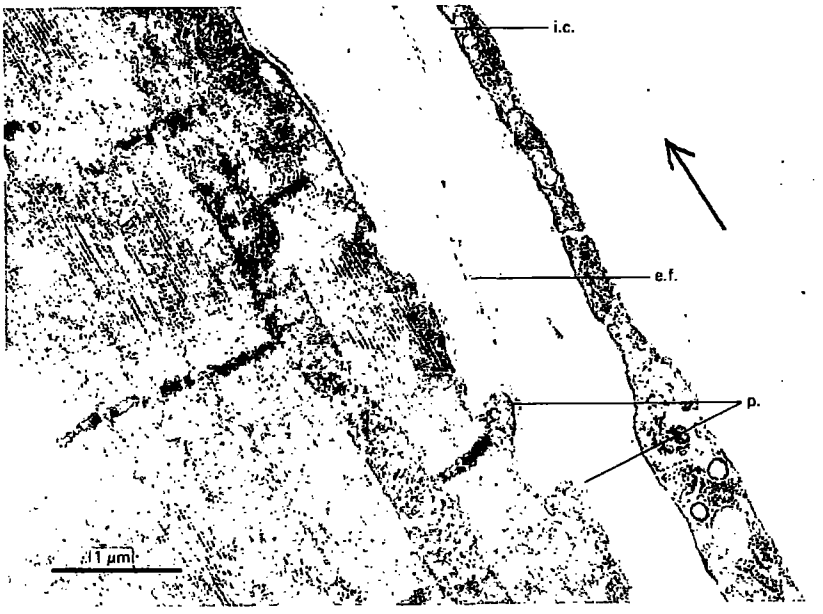


Fig. 1. Longitudinal section through a bag_1 fibre, showing surface projections (p.) with an attached elastic fibre (e.f.). The arrow indicates the direction of the primary ending: i.c., inner-capsule cell.

The surfaces of the bag_1 and bag_2 fibres have minute projections for distances of about 300 μm on either side of the primary ending. The projections were not consistently associated with a particular sarcomeric component, but the plasmalemmal covering appeared to be thickened. Elastic fibres were often seen attached, terminally rather than *en passant*, to the basal lamina overlying the projections (Fig. 1). Many of the projections were asymmetric, pointing in the direction of the primary ending, and suggesting the transmission of tension from the elastic fibres to the sarcolemma.

The elastic fibres thus appeared to arise from the opposite pole and were effectively in parallel with the primary sensory region, but in series with the polar and juxta-equatorial regions to which they were attached. This arrangement might be expected to reduce the compliance of the primary sensory region.

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Reinnervation of cat muscle spindles by foreign afferents after nerve section

By R. W. Banks, D. Barker and M. J. Stacey. *Department of Zoology, University of Durham, Durham DH1 3LE*

The fact that foreign afferents are capable of reinnervating muscle spindles after nerve section (Banks & Barker 1983) led us to carry out experiments designed to ascertain the degree of specificity required for the reinnervation to be successful. Cross-unions were effected unilaterally between hindlimb sensory and muscle nerves in adult cats such that the interosseous (INT) nerve reinnervated flexor digitorum longus (FDL) in four cats, and the superficial peroneal (SP) nerve reinnervated peroneus brevis (PB) in three. The INT nerve contains 150–200 myelinated afferents that supply free endings and an average of 60 pacinian corpuscles, 18 tendon organs and 3 spindles (Barker, 1962); the SP nerve supplies cutaneous and joint receptors in the foot. The appropriate stumps of the severed nerves were united with 10/0 polyamide epineurial sutures and the proximal stumps of the muscle nerves were ligated, all procedures being carried out under aseptic conditions using sodium pentobarbitone anaesthesia. Two of the reinnervated FDL muscles were examined in teased, silver preparations after 10 weeks; the other two were left for a further week after cutting the FDL proximal stump so as to eliminate any reinnervation from this source.

In the INT/FDL cross-unions myelinated afferents reinnervated spindles with either an extensively branched network of free endings; annulospiral endings in primary and secondary regions; or tapers and bulbs resembling pacinian terminals. Of 205 afferents that reinnervated 83 spindles, 84 formed free endings, 60 annulospirals and 43 tapers and bulbs; a further 18 entered and left without terminating.

Cats in the SP/PB series were prepared for single-unit, dorsal-root recording under sodium pentobarbitone anaesthesia after 16–22 post-operative weeks. Overall conduction velocities of the reinnervating afferents ranged from 13·7 to 62·4 m s^{-1}; in each experiment they showed similar unimodal distributions that peaked at about 40 m s^{-1}. Ramp-and-hold 1·8 mm stretches applied to the PB tendon at 5 mm s^{-1} elicited one or more spikes in 40 of 120 afferents. Additionally, four units responded to localized probing of the muscle belly, but not to stretch. Motor endings were absent from the muscles and spindles were reinnervated by freely ending afferents of various diameters.

These experiments suggest that (i) tendon–organ afferents are capable of reinnervating spindles by terminating in sites originally occupied by primary or secondary endings; and that (ii) some afferents normally innervating cutaneous or joint receptors are capable of reinnervating spindles and responding to stretch.

Financial support from the M.R.C. is gratefully acknowledged.

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Paper 26.

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On the classification of intrafusal motor endings.
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9

On the classification of motor endings in mammalian muscle spindles

R. W. Banks, D. Barker and M. J. Stacey

In mammalian muscle spindles, motor neuromuscular junctions (i.e. the composite structures of axonal terminals and the muscle fibres below them) are much more varied in form than those on the surrounding extrafusal muscle fibres. Figure 1 shows transverse sections of six intrafusal neuromuscular junctions, each extensively sampled by electron microscopy. In each case the surface of the muscle fibre is both indented by the motoneuronal terminals and secondarily folded by various amounts. Sole-plate nuclei are usually present. The neuromuscular junctions on the bag_2 and the three chain fibres were all associated with the same axon, yet their form varies considerably. Thus, secondary folding appears to be most developed on the bag_2 fibre and least on the two chain fibres (4 & 5), whose motor endings were located closest to the spindle's primary sensory innervation. In addition, the post-junctional membrane of chain 1 is thrown into finger-like processes, but similar processes did not occur in the neuromuscular junctions on the other two chain fibres. In contrast, the two neuromuscular junctions on the bag_1 fibres, though superficially similar, were associated with different types of axon, one purely fusimotor (γ) the other mixed skeletofusimotor (β). Thus attempts to classify intrafusal neuromuscular junctions into distinct types are complicated by the lack of a simple correlation linking axonal type with post-junctional features.

The amounts of post-junctional indentation and secondary folding vary considerably within single neuromuscular junctions and are difficult to quantify. However, Banks (1983) analysed serial 1μm-thick sections by light microscopy and found that the mean indentation increased significantly with greater distance between the motor and primary sensory endings. Generally, our experience is that the type of muscle fibre and the location of the motor endings with respect to the primary sensory endings are important factors associated with post-junctional form, but that the type of axon has little or no influence.

In seeking a classification of intrafusal motor endings (i.e. axonal structures), we are primarily concerned with the morpho-

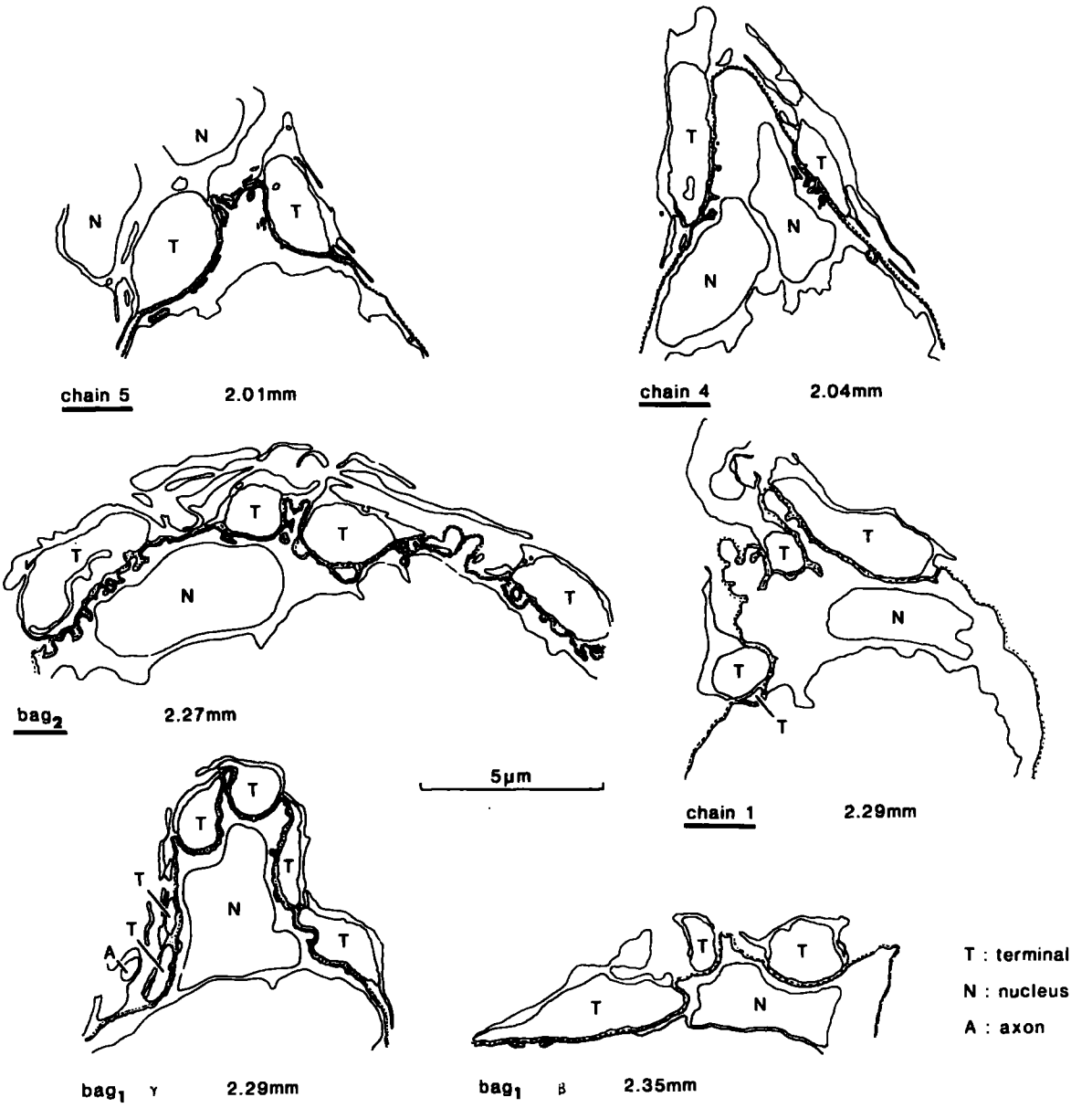


Figure 1 Transverse sections of six intrafusal neuromuscular junctions traced from electron micrographs. The location of the junction with respect to the primary sensory ending is given together with the muscle-fibre type below each profile. Terminals on the bag_2 and the three chain fibres were all derived from the same axon.

logical characteristics of the various functional groups of fusimotor and skeletofusimotor innervation. It seems reasonable, therefore, to restrict our attention to the pre-junctional features.

Figure 2 shows a sample of intrafusal motor endings as seen in silver-impregnated material, to illustrate some of the variety of forms. The endings have been grouped according to the known or presumed parent-fibre type. Their location on the different types of intrafusal muscle fibre would have determined whether they would have had a dynamic (bag_1) or static (bag_2 and various types

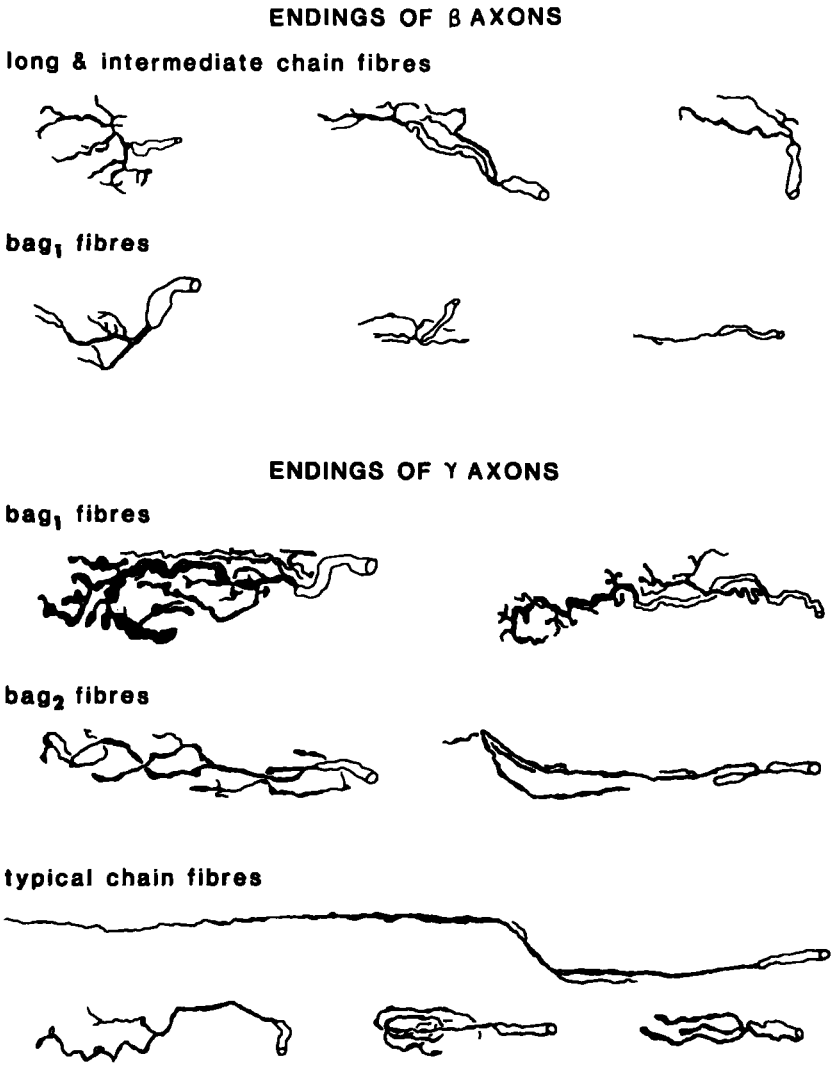


Figure 2 Camera-lucida drawings of intrafusal motor endings from teased, silver-impregnated muscle spindles, illustrating the variety of forms encountered. The endings have been grouped according to the known or presumed parent-fibre type. Axons innervating bag_1 fibres would have had a dynamic effect on the spindle's primary ending, whereas those innervating bag_2 and chain fibres would have had a static effect.

of chain) effect on the responses of the primary sensory endings.

Barker and Stacey (1984) have analysed 219 intrafusal motor endings in terms of their overall lengths (L) and the total lengths of their terminal and unmyelinated preterminal branches (PTL). It was necessary to include the preterminal branches since the precise transitions to terminal branches can not be determined in silver-impregnated material. Unmyelinated preterminal axons were regarded as belonging to the ending supplied by them as far proximal as the nearest branch point from which another ending was supplied or a recurrent axon arose. The endings were derived from normal spindles, as well as from bag_1 fibres deprived of their β innerva-

tion by differential degeneration, and from spindles in which single β or static γ axons remained following otherwise complete de-efferentation (Barker et al, 1973; 1980). Thus, several comparisons were possible, the most important being summarised in table 1. From these we may conclude that the endings of skeleto-

Table 1 Comparisons of values of PTL for populations of endings on different types of muscle fibre in normal (N) and experimental (E) muscles.

Null Hypothesis endings A ≡ endings B		Value of P (Wilcoxon's rank sum test for two samples)
A	B	
β on bag_1 (E)	diff. degen. on bag_1 (E)	<0.0005
N on typ.chain	N on long & i chain	<0.01
N on long & i chain	β on bag_1 (E)	N.S.
N on typ.chain	N on bag_2	N.S.
all static γ (E)	β on bag_1 (E)	<0.001
all static γ (E)	diff. degen. on bag_1 (E)	<0.001

abbreviations: diff. degen., remaining after differential degeneration; i., intermediate; typ., typical.

fusimotor (β) axons on bag_1 fibres are indistinguishable from the endings, presumed to be of β axons, on long and intermediate chain fibres; that this type is distinctly different from the innervation, presumed to be dynamic γ, remaining on the bag_1 fibre a short time after axotomy; and that both these types are different from the bag_2 and typical chain innervation, which is a single group supplied by static γ axons.

The recent suggestion (Boyd, et al. 1977) that there are two sorts of static γ axon, one supplying predominantly chain fibres, the other predominantly bag_2 fibres, has been supported by histological (Arbuthnott et al., 1982; Sutherland et al., this publication) and physiological (Boyd et al., 1983) evidence. The histological evidence relies on a distinction being made between two types of post-junctional structure, which we have argued above is related to muscle-fibre type and location of endings. The physiological evidence is based on the inferred distribution of single static γ axons to bag_2 and chain fibres in three spindles. Of 11 examples tabulated by Boyd et al. (1983) the recorded distributions were chain only, 2; predominantly chain, 4; bag_2 only, 2;

and predominantly bag_2, 3.

These observations must be assessed in the light of our most complete knowledge of static γ axonal distribution, which at present is provided by the single-axon studies of Barker et al. (1973), the identity of the bag fibres having been confirmed by Barker and Stacey (1981). The relevant results are summarized in table 2, which also shows the effect of hypothetically applying the method of Boyd et al. to the axon with the most mixed distribution, CT8. The chances of correctly identifying this axon as mixed would have been almost 2:1 against.

We conclude, then, that three types of motor ending are recognizable histologically, at least as populations, and that they correspond to the β and dynamic and static γ motoneurons. The dynamic and static divisions of the β axons are associated with

Table 2 The distribution of single static γ axons. (based on Barker et al. 1973).

Distribution	Numbers of spindles					
	CT2	CT3	CT4	CT8	CT10	CT11
mixed	3	2	2	4	2	2
bag_2	-	-	2	2	1	2
chain	1	1	-	1	4	1

CT8 overall 6 bag_2 and 10 or 11 chain fibres in 7 spindles. Selecting 3 spindles at a time $^7C_3 = 35$.

	Possible observations	chance of identification
predominantly chain	6	5:1 against
predominantly bag_2	17	evens
mixed	12	2:1 against

distinct post-junctional structures (Banks, 1981; Kucera and Hughes, 1983) but these must be properly regarded as properties of the bag_1, long and intermediate chain fibres, which alone allow the endings to be identified histologically. We propose to use the established nomenclature p_1 (β), p_2 (dynamic γ) and trail (static γ), since it corresponds closely to the present classification.

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Proc. R. Soc. Lond. B 225; 195-212.

Proc. R. Soc. Lond. B **225**, 195–212 (1985)
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Form and classification of motor endings in mammalian muscle spindles

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(*Communicated by J. Z. Young, F.R.S. – Received 2 January* 1985)

[Plates 1 and 2]

The presynaptic features of 234 motor endings supplied to cat hindlimb muscle spindles have been studied in teased, silver preparations, and the postsynaptic features of a further 27 endings have been studied in serial, 1 μm thick, transverse sections. In the presynaptic study motor endings received by the three types of intrafusal muscle fibre were compared with the endings supplied to spindles by the various functional categories of motor axon. Three forms of motor ending were found that had significantly different presynaptic features. These forms correspond closely to those previously identified in the literature as p_1 (β), p_2 (dynamic γ) and trail (static γ). The results of the postsynaptic study showed that the degree of indentation of the intrafusal muscle fibres by motor axon terminals increases with greater distance from the primary ending, irrespective of muscle-fibre type. We conclude that the postsynaptic form of intrafusal motor endings is determined by distance from primary ending and muscle-fibre type. It is not determined by type of motor axon, and cannot be correlated with presynaptic form so as to produce a unified classification of intrafusal motor endings.

INTRODUCTION

The classification of mammalian intrafusal motor endings into two types of plate (p_1, p_2) and trail endings, described in detail by Barker *et al.* (1970), was based on an analysis of the presynaptic features of the endings as seen in teased, silver preparations of cat muscle spindles. It was shown that the three categories could further be distinguished by their different rates of degeneration after nerve section. The same types of motor ending were recognized in silver-stained spindles of other mammals, namely, rat (Gladden 1969), rabbit (Barker & Stacey 1970; Barker *et al.* 1972), and man (Kennedy 1970; Swash & Fox 1972). At the time this work was carried out mammalian spindles were generally thought to be composed of two types of muscle fibre (nuclear-bag and nuclear-chain fibres), and discussion centred on how these two types were innervated by the functional categories of dynamic and static γ axons (Matthews 1972).

Since that time a number of important advances have occurred that make it necessary to re-examine the p_1, p_2, trail classification. It is now accepted that there are three kinds of mammalian intrafusal muscle fibre (bag_1, bag_2, and chain fibres),

and that dynamic actions are carried out by the bag_1 fibre (the dynamic bag fibre) and static actions by the bag_2 (static bag fibre) and chain fibres. The manner in which β and γ motor axons are distributed to these fibre types in cat spindles is now generally agreed (see reviews by Boyd 1981; Barker & Banks 1985). Chain fibres are now referred to as 'long', 'intermediate', or 'typical' in terms of their length relative to the capsule (Barker *et al.* 1976*a*; Kucera 1980*a*, 1982*a*). These subtypes are of functional significance since in cat spindles there is a selective innervation of long and most intermediate fibres by static β axons (Harker *et al.* 1977; Jami *et al.* 1979) and of typical chains by static γ axons (Barker *et al.* 1976*b*). Finally, detailed information has become available about the sensory innervation of cat spindles (Banks *et al.* 1982) that can be used in identifying intrafusal muscle-fibre types in teased, silver preparations and so assist in the analysis of their motor innervation.

In re-examining the presynaptic features of cat intrafusal motor innervation our approach was to study the motor endings received by the various types of intrafusal muscle fibre, and to compare these with the endings supplied to spindles by the various functional categories of motor axon. We found three forms of motor ending that had significantly different presynaptic features. These forms correspond closely to those described in the p_1, p_2, trail classification.

When the postsynaptic form of cat intrafusal motor endings is considered, the question arises: is it mainly determined by the type of motor axon that supplies the endings, or by the type of muscle fibre that receives it? Barker's group initially held the former view (Barker *et al.* 1970), but later work convinced them that muscle-fibre type and distance of the ending from the equator were the determining factors (Barker *et al.* 1976*a*; Barker *et al.* 1978). Recent studies by Banks (1981, 1983) and Kucera (1980*a*, *b*, 1981, 1982*b*, *c*) have provided evidence in support of this. Meanwhile ultrastructural studies by Arbuthnott *et al.* (1982) have led them to propose a new classification of cat intrafusal motor endings in which all are regarded as plates. Five types of plate are recognized mainly by the degree and manner of the indentation of their axon terminals into the muscle-fibre surface. Type of plate is seen as being determined either by type of muscle fibre (for example, as in the case of 'm_b plates' supplied to bag_1 fibres by dynamic β and dynamic γ axons), or by type of motor axon (for example, as in the case of 'm_d plates' supplied to long chain fibres by static β axons).

In this paper we examine both presynaptic and postsynaptic features of cat intrafusal motor endings, and discuss whether it is possible to correlate these so as to produce a unified classification. Preliminary accounts of some of the results have been published (Banks 1983; Barker & Stacey 1984; Banks *et al.* 1985).

Material and methods

Silver-stained spindles

Presynaptic features of the motor endings received by the various types of intrafusal muscle fibre were studied in spindles teased from the peroneus brevis and tenuissimus muscles of normal cats. For the study of endings belonging to the different functional types of motor axon we were able to use muscles from previous

work in which chronic degeneration experiments had been carried out to isolate single surviving static γ axons (Barker *et al.* 1973) or β axons (Barker *et al.* 1980). We also used peroneal muscles from which the β innervation had been eliminated by degeneration for 54 h after nerve section. Some of these muscles had been prepared by Barker *et al.* (1970), others were similarly prepared for the present study. The silver technique used was either that described by Barker & Ip (1963), or the modification of this reported by Barker *et al.* (1984). Full details regarding the material used are given in table 1.

TABLE 1. SILVER-STAINED MATERIAL STUDIED

source	muscle	number of muscles	number of spindles	number of motor endings
normal muscles	peroneus brevis	4	11	76
	tenuissimus	4	5	32
differentially degenerated muscles: surviving spindle motor axons:				
static γ	tenuissimus	5	15	76
β	tenuissimus	3	5	7
	superficial lumbrical	2	5	13
	abductor digiti quinti medius	1	2	3
γ	peroneus brevis	3	12	17
	peroneus longus	2	7	8
	peroneus tertius	2	2	2
	totals	**26**	**64**	**234**

For the purposes of this study we have regarded a motor ending as a discrete group of axon terminals supplied by one or more preterminal axons to the polar region of a single intrafusal muscle fibre. We have interpreted 'discrete' to mean that the terminals are supplied to part of the muscle-fibre surface within which there is no portion free of terminals for more than a length of 10 μm. This definition allows for an ending to be supplied by more than one parent axon, but this rarely occurred in our sampling.

In silver-stained material it is not possible to decide precisely where the transition from preterminal axon to axon terminal occurs. Hence as part of the attempt to quantify motor-ending form we measured the total length of unmyelinated axon present in each ending, and designated this measurement 'PTL' (that is, preterminal plus axon terminal length). The measurement was made from the origin of the preterminal axon at the heminode of the supplying axon, to the end of each axon terminal. In those instances where the preterminal axon branched to provide a further ending, the origin of its preterminal axon was regarded as the branch point from which the further ending was supplied (see figure 1*c*). Measurements of PTL were made with a map-measuring wheel on tracings prepared by using a Nikon Optiphot with a × 100 objective coupled to a television projection system. Since the tracings formed a projection plan of the ending the transverse component of the PTL was reduced. This would have been

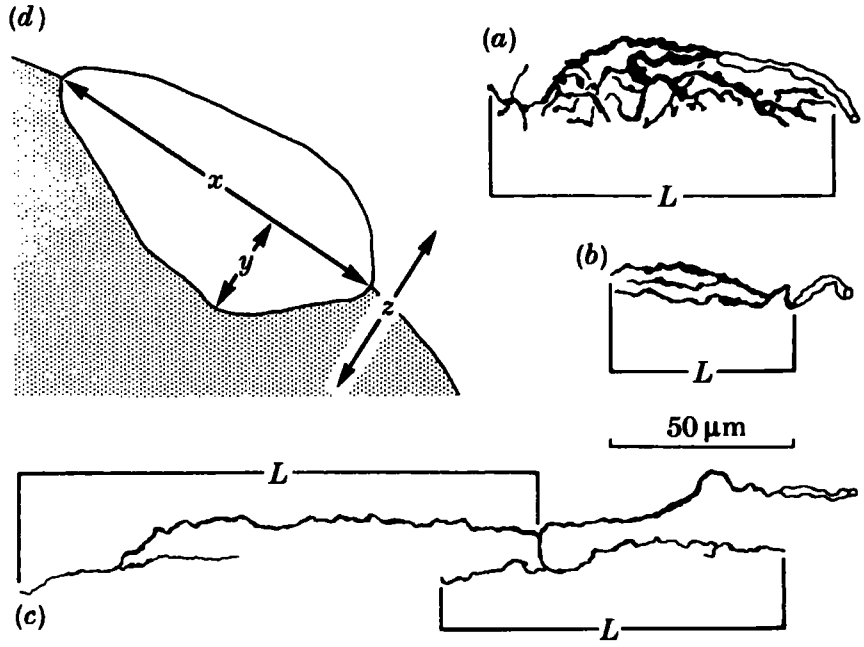


FIGURE 1. (*a*)–(*c*) Tracings of silver-stained preparations of motor endings from cat peroneus brevis spindles to illustrate examples of measuring ending length (*L*), the ending being regarded as including one or more preterminal axons and their axon terminals. In each tracing the ending is shown black, and the myelinated axon that supplies it is unshaded. The endings were located on a bag_1 fibre (*a*), a chain fibre (*b*), and two chain fibres (*c*). The disposition of the endings in (*c*) is unusual. (*d*) Diagram to illustrate the terminal profile presented by a motor axon terminal (hollow outline) embedded in the surface of an intrafusal muscle fibre (stippled) as seen in a 1 μm thick transverse section of a cat spindle stained with toluidine blue. Arrowed lines indicate measurements made of the maximum width (*x*) and depth (*z*) of the terminal and its indentation (*y*) into the muscle-fibre surface.

relatively more important in richly branched endings such as the most complex of those supplied to the bag_1 fibre. Consequently the observed difference between these and less complex endings will have been artificially reduced.

We also measured the length (*L*) of each ending parallel to the longitudinal axis of the muscle fibre (figure 1*a*–*c*); since *L* included preterminal axon it would usually have exceeded the length of the actual neuromuscular junction. Other data recorded for each ending were number of axon terminals, and location with respect to the spindle regions A, B, and C defined by Barker *et al.* (1976*a*). Finally, the internodal diameter of the parent axon was measured (excluding myelin), and the number of times it branched within the spindle was counted.

The three types of intrafusal muscle fibre, and the subtypes of chain fibre, were identified on the basis of their length and diameter, and details of their sensory innervation. When sensory endings were absent owing to their degeneration together with the β innervation after nerve section, bag-fibre types were distinguished mainly by the scarcity (bag_1) or abundance (bag_2) of elastic fibres associated with them (Gladden 1976).

Serially sectioned spindles

Serial, 1 μm thick, transverse sections of 27 tenuissimus intrafusal motor endings containing 442 axon terminals were examined to study the relationships made between their terminals and the underlying muscle-fibre surface. The endings

formed part of the sample studied by Banks (1981) and comprised all those supplied to the proximal poles of spindles 6 and 9 in that study, as well as those supplied to the distal pole of spindle 12. Enlarged photographs (× 1800) of 3189 terminal profiles were used to measure, to the nearest 0.5 mm, the maximum width (x) and depth (z) of each axon terminal and its indentation (y) into the muscle-fibre surface (see figure 1*d*). Sampling of several endings for electron microscopy confirmed that individual terminals were readily identifiable in the light-

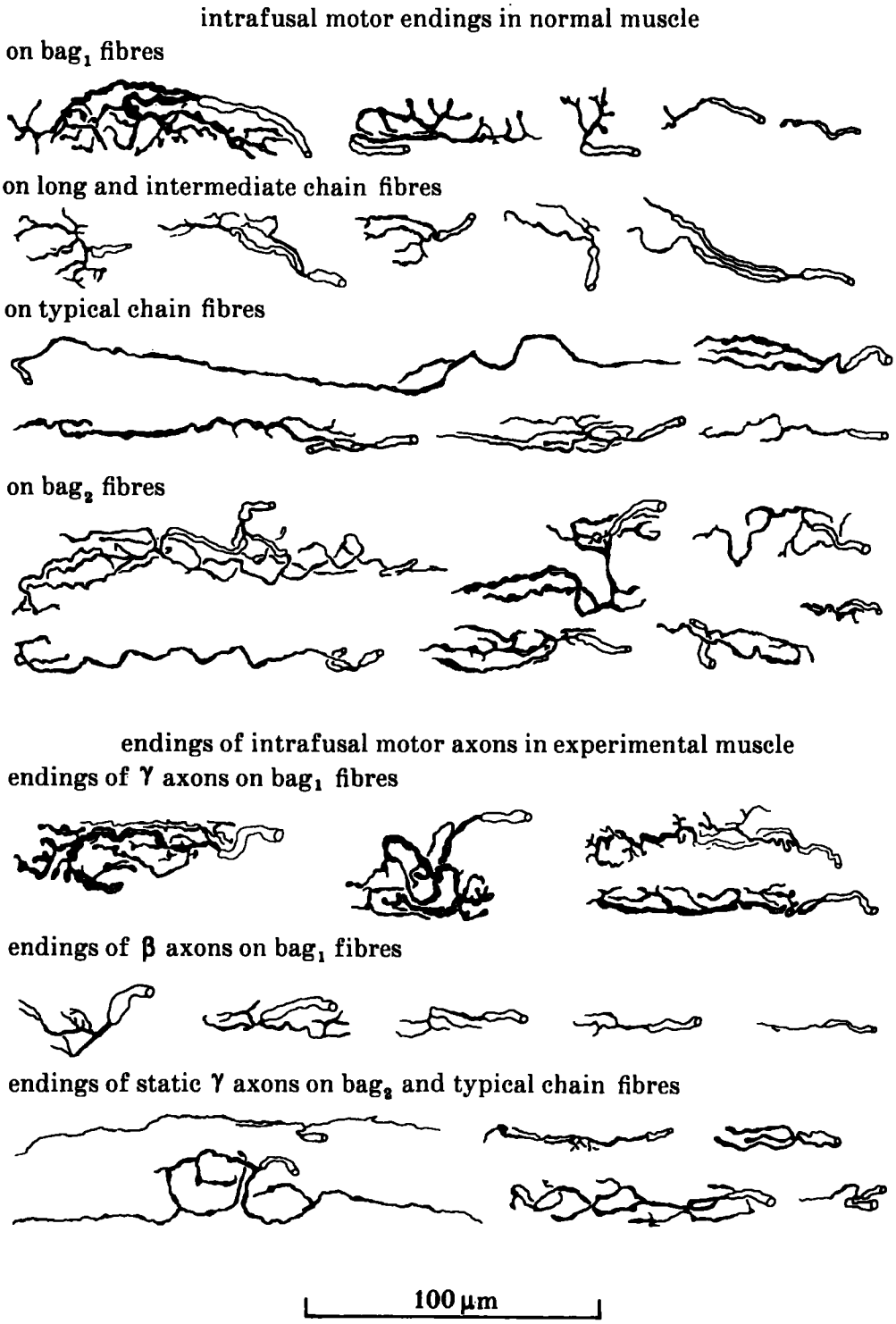


FIGURE 2. A sample of cat intrafusal motor endings traced from silver preparations teased from normal and experimental muscles (mainly tenuissimus and peroneus brevis). In each tracing the ending (that is, the preterminal axon and axon terminals) is shown black, and the myelinated axon that supplied it is unshaded.

microscopical preparations (plate 2). Thin Schwann-cell processes covering the terminals could not be separately resolved in the photographs, but their thickness is considerably less than the error inherent in each measurement.

Three ratios were obtained from the data, namely, eccentricity ($E = x/z$), indentation ($I = y/x$), and superficiality ($S = y/z$). The ratios varied widely within and between individual terminals of each ending, but standard errors of the mean values for complete endings were small due to the large amount of data (see figure 7). The number and length of terminals in each ending were also recorded as well as the number of nuclei in its sole plate. Finally, the location of a motor ending on a given muscle fibre with respect to the primary ending was recorded as the distance between the midpoint of the ending's neuromuscular junction and the midpoint of the primary terminals supplied to the fibre.

Most of the data from both the silver and serial-section analyses were clearly not normally distributed. We therefore used a non-parametric method of comparison, namely, Wilcoxon's rank sum test for a two-sample comparison (Bailey 1981).

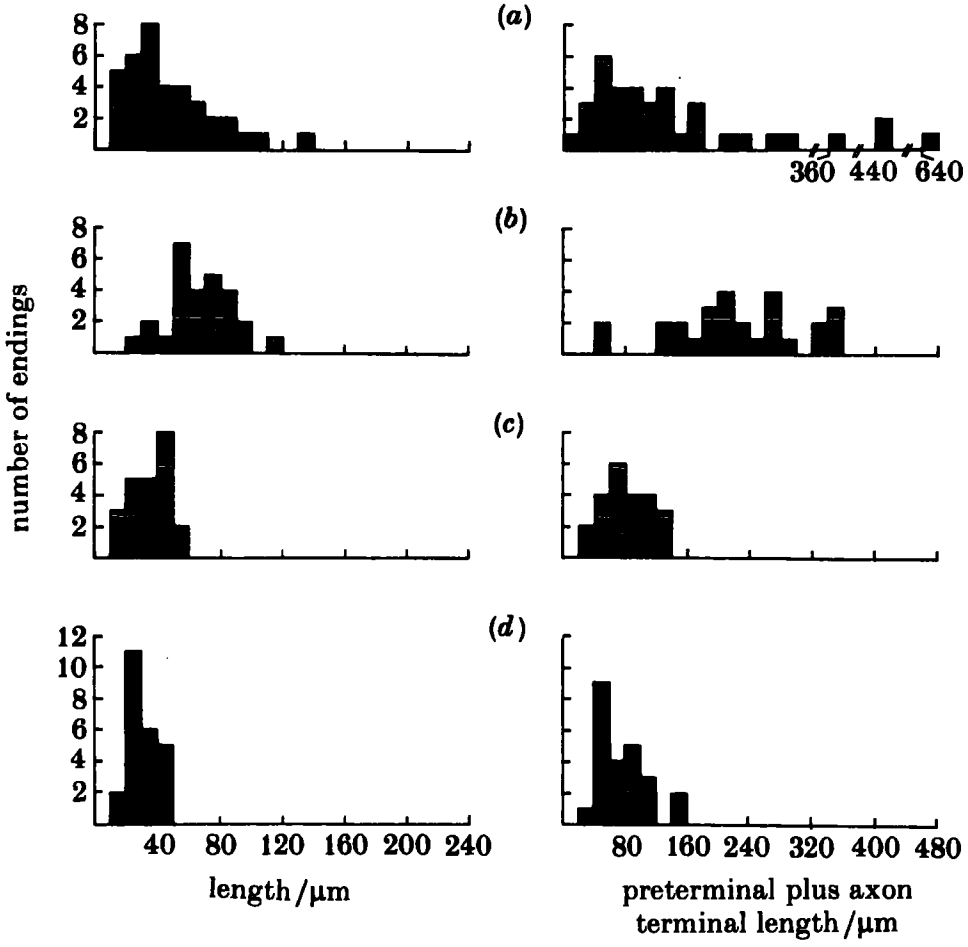


FIGURE 3. Histograms of L and PTL measurements of cat intrafusal motor endings. These show that the endings supplied to bag_1 fibres in normal spindles (*a*) consist of different populations belonging to γ (*b*) and β (*c*) axons; and that the β endings are very similar to those supplied to long and intermediate chain fibres (*d*). The endings measured in (*a*) and (*d*) belonged to spindles in normal peroneus brevis and tenuissimus muscles; those measured in (*b*) were supplied to bag_1 fibres by γ axons that remained after the β endings had degenerated in peroneal muscles following nerve section; and those measured in (*c*) belonged to β axons that had survived in the chronic degeneration experiments of Barker *et al.* (1980) on tenuissimus, superficial lumbrical, and abductor digiti quinti medius muscles.

Results

Silver-stained spindles

The complexity of intrafusal motor innervation is such that some endings, especially in normal spindles, had to be excluded from the analysis. Nevertheless comparison of L and PTL measurements of intrafusal motor endings from the normal and experimental muscles (see figures 3 and 4) strongly suggests that the total sample of endings studied (234, 46.2 % from normal spindles) is representative of the whole population. A sample of tracings of endings from normal and experimental muscles, selected to illustrate the range of form encountered, is shown in figure 2 and photographs of some silver-stained preparations are shown in figures 8–14, plate 1.

The results of the L and PTL measurements of the endings are illustrated by the histograms in figures 3 and 4 and are summarized in table 2. Comparisons of the populations of intrafusal motor endings on different types of muscle fibre in normal and experimental muscles are shown in table 3.

The following conclusions may be drawn.

(i) *Endings on bag_1 fibres*

The motor endings supplied to the bag_1 fibres of normal spindles are indistinguishable from those supplied by known β and γ axons to bag_1 fibres. However,

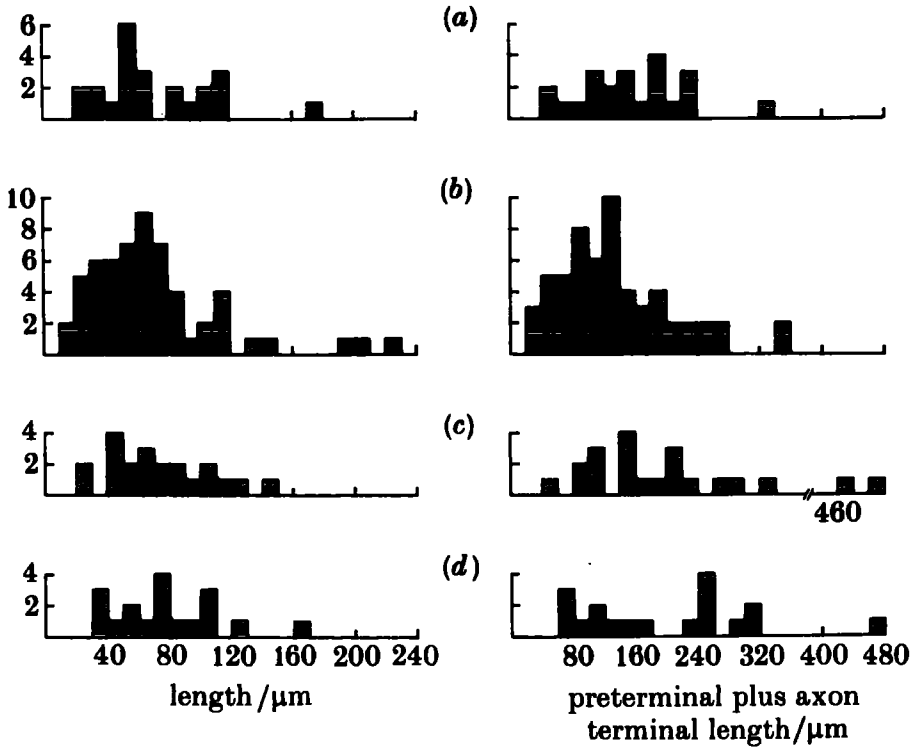


Figure 4. Histograms of L and PTL measurements of cat intrafusal motor endings. These show that the measurements of endings supplied to typical chain fibres (*a*) and bag_2 fibres (*c*) in normal spindles are similar, and compare closely with those of endings supplied to these fibre types by static γ axons (*b* and *d*). Comparison of the histograms in (*a*) with those in figure 3*d* shows that endings supplied to typical chain fibres are markedly different from those supplied to long and intermediate chain fibres. The endings measured in (*a*) and (*c*) belonged to spindles in normal peroneus brevis and tenuissimus muscles; those in (*b*) and (*d*) belonged to static γ axons that had survived in the chronic degeneration experiments of Barker *et al.* (1973) on tenuissimus muscles.

TABLE 2. MEANS, RANGES, AND SAMPLE SIZES FOR L AND PTL MEASUREMENTS OF INTRAFUSAL MOTOR ENDINGS IN NORMAL AND EXPERIMENTAL MUSCLES

intrafusal muscle-fibre type	endings in normal muscle			endings in experimental muscle: static γ			endings in experimental muscle: β			endings in experimental muscle: γ		
	mean	range	number	mean	range	number	mean	range	number	mean	range	number
					L/μm							
bag_1	45.6	13–107	37	—	—	2	36.1	15–55	23	65.5	21–115	27
bag_2	71.3	23–142	21	77.4	35–165	18						
chains												
typical	92.2	25–466	26	70.2	18–220	58						
long and intermediate	30.3	16–47	24									
					PTL/μm							
bag_1	147.2	15–653	37	—	—	2	79.7	20–150	23	222.2	48–355	27
bag_2	215.1	43–575	21	198.5	60–474	18						
chains												
typical	187.7	43–576	26	149.8	23–343	58						
long and intermediate	74.9	29–147	24									

TABLE 3. COMPARISON OF POPULATIONS OF MOTOR ENDINGS ON DIFFERENT TYPES OF INTRAFUSAL MUSCLE FIBRE IN NORMAL AND EXPERIMENTAL MUSCLES

(Abbreviations: ex.m., experimental muscle; int., intermediate; n.m., normal muscle.)

null hypothesis endings A ≡ endings B		value of p (Wilcoxon's rank sum test for 2 samples)	
A	B	L	PTL
	endings on bag$_1$ fibres		
all n.m. endings on bag$_1$	all ex.m. endings on bag$_1$	n.s.	n.s.
β endings on bag$_1$ (ex.m.)	γ endings on bag$_1$ (ex.m.)	< 0.001	< 0.0005
	endings on chain fibres		
n.m. endings on typical chains	n.m. endings on long and int. chains	< 0.0005	< 0.0005
n.m. endings on typical chains	n.m. endings on bag$_2$	n.s.	n.s.
n.m. endings on long and int. chains	β endings on bag$_1$ (ex.m.)	n.s.	n.s.
n.m. endings on long and int. chains	γ endings on bag$_1$ (ex.m.)	< 0.0001	< 0.0001
	static γ endings		
all static γ endings (ex.m.)	n.m. endings on bag$_2$ and typical chains	n.s.	n.s.
all static γ endings (ex.m.)	β endings on bag$_1$ (ex.m.)	< 0.0005	< 0.001
all static γ endings (ex.m.)	γ endings on bag$_1$ (ex.m.)	n.s.	< 0.001

the endings of known β axons on bag$_1$ fibres differ from those that remain on this fibre after the β innervation has degenerated.

(ii) *Endings on chain fibres*

The motor endings supplied to typical chain fibres in normal spindles differ from those supplied to long and intermediate chain fibres, but are indistinguishable from those supplied to bag$_2$ fibres.

The motor endings supplied to long and intermediate chain fibres in normal spindles are indistinguishable from the endings supplied by known β axons to bag$_1$ fibres.

(iii) *Static γ endings*

The motor endings supplied to bag$_2$ and typical chain fibres in normal spindles are indistinguishable from the endings supplied by known static γ axons. These endings have greater L and PTL values than those of known β axons, but differ from the endings of presumed dynamic γ axons only with respect to their PTL values. This implies that the endings of dynamic γ axons are more highly branched than those of static γ axons, and axon-terminal counts confirm this (see figure 5*b*).

The parent axons of static γ endings branched more frequently within the spindle than those supplying dynamic β and γ endings. In our samples the mean branching frequencies were 3.9 and 0.28, respectively (see figure 5*a*). In this respect

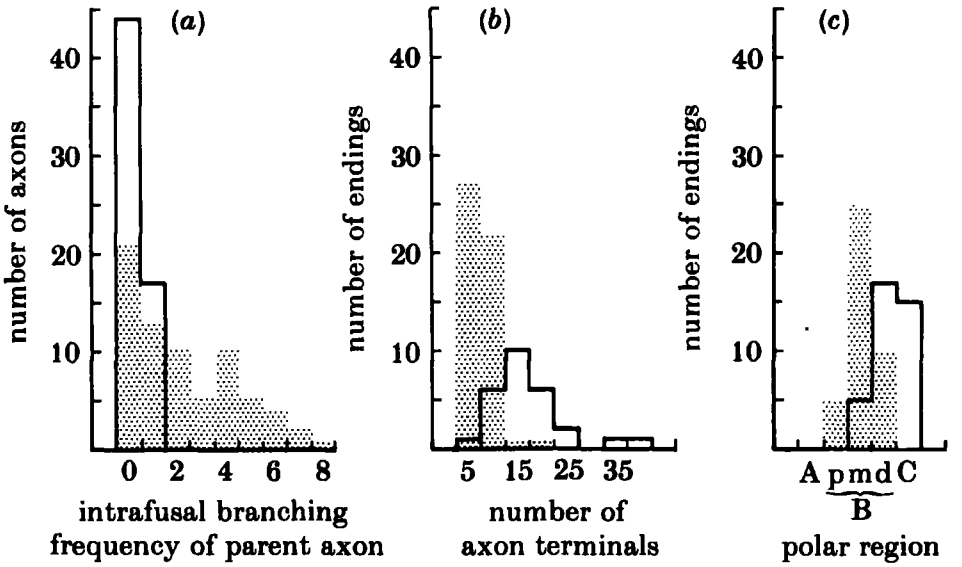


FIGURE 5. Histograms contrasting differences between static (stippled) and dynamic (unshaded) γ axons and endings. They show that, as compared with dynamic γ axons, static γ axons branch more frequently within spindles (*a*) and tend to supply endings that have fewer axon terminals (*b*) and are located nearer to the equator (*c*). Data in (*a*) and (*c*) from spindles in normal peroneus brevis and tenuissimus muscles; data in (*b*) from spindles in the experimental muscles referred to in figures 3*b* and 4*b*, *d*. p, m, d, Proximal, middle and distal thirds of polar region B.

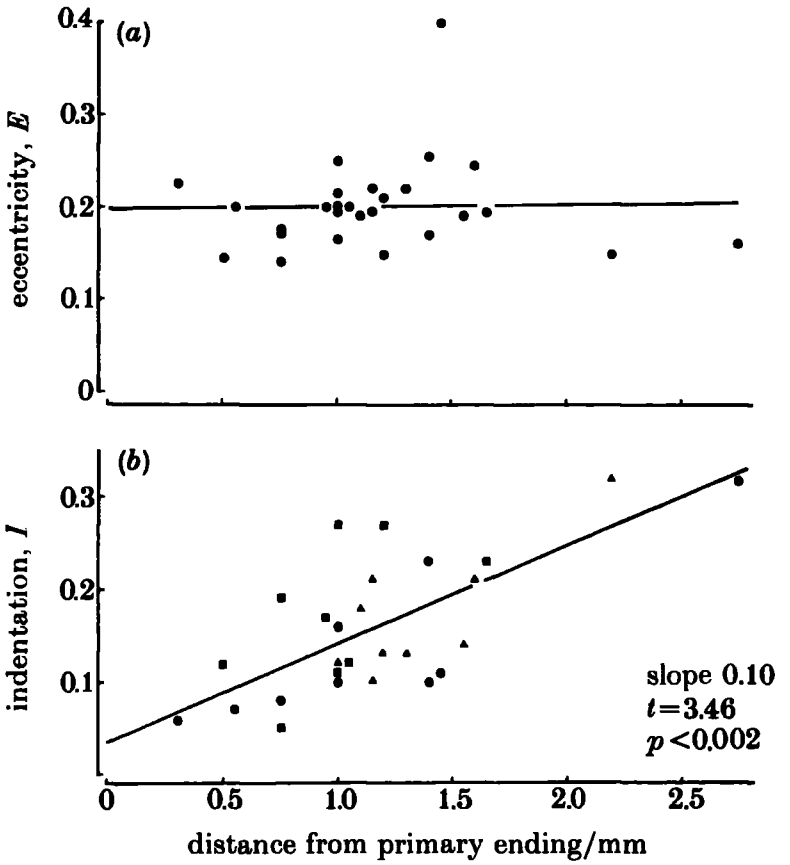


FIGURE 6. Mean values of the eccentricity (*a*) and indentation (*b*) of axon terminals in 27 motor endings from cat tenuissimus spindles plotted against the distance of the endings from the primary ending. Lines drawn through the symbols are least-squares fits for regression of the ordinates on the abscissae. Note that eccentricity shows no systematic variation related to ending position, whereas indentation does. In (*b*) the separate origins of the data from three spindles in three different cats are indicated by the use of three different symbols.

it may be significant that the mean diameter of static γ axons in normal spindles (2.14 μm) was greater than that of dynamic axons in the same spindle sample (1.87 μm, $p < 0.01$), functional categories being inferred from the type of muscle-fibre innervated. The endings of static γ axons were located in region B, mostly in the middle third, whereas the endings of dynamic axons occurred almost exclusively in the distal third of B and in C (see figure 5*c*).

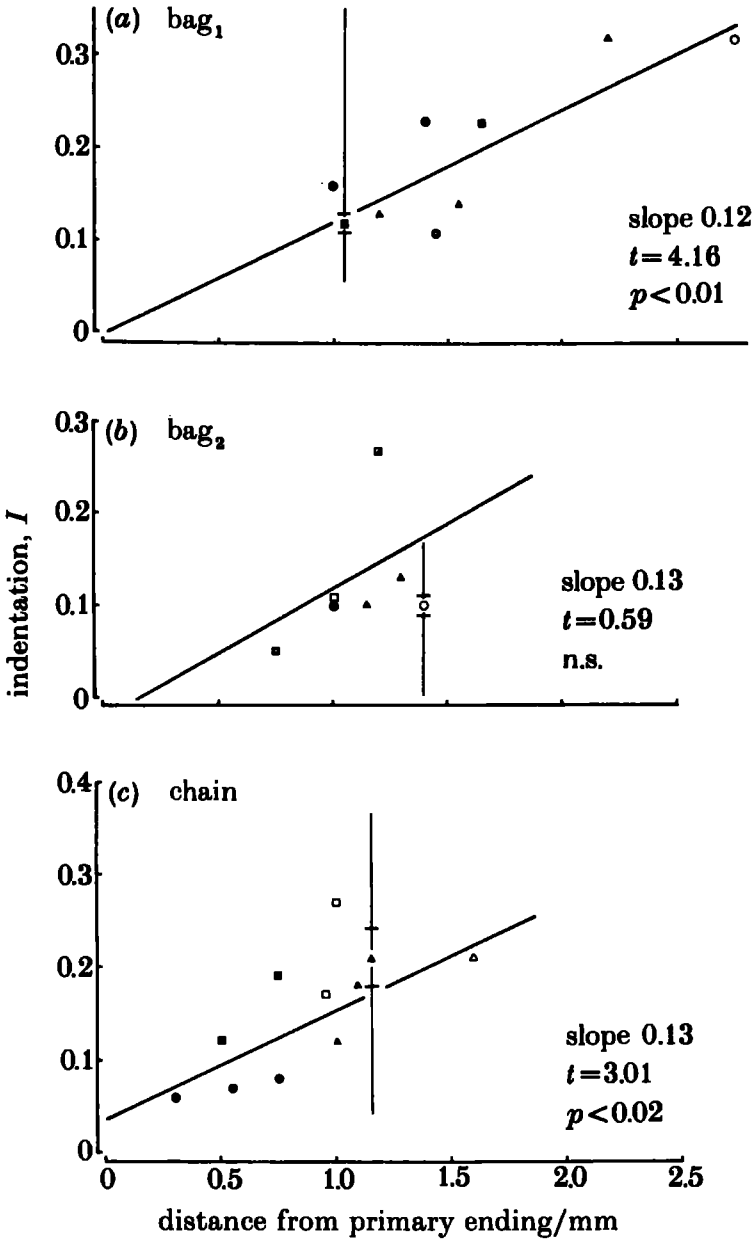


FIGURE 7. Mean values of the indentation (I) of axon terminals in 27 motor endings from cat tenuissimus spindles plotted against the distance of the endings from the primary ending and segregated according to their location on different muscle-fibre types. Lines drawn through the symbols are least-squares fits for regression of the ordinates on the abscissae. Circles, squares, and triangles represent the separate origins of the data from three spindles in three different cats. Endings supplied by different axons in the same spindle are indicated by different versions (filled, unfilled, half-filled) of the same symbol, so that the occurrence of two or more identical symbols indicates endings supplied by the same axon. The vertical line in each graph illustrates the range of mean values of I for individual axon terminals in a single ending. Horizontal bars on this line indicate the standard error of the mean value for the whole ending.

TABLE 4. MORPHOMETRIC DATA RELATING TO NEUROMUSCULAR JUNCTIONS OF 27 INTRAFUSAL MOTOR ENDINGS

fibre type innervated	number	length/μm range	length/μm mean	sole-plate nuclei: number range	sole-plate nuclei: number mean	sole-plate nuclei: number per 10 μm length range	sole-plate nuclei: number per 10 μm length mean
bag_1	9	40–106	64.7	2–7	4.9	0.43–1.13	0.79
bag_2	7	24–105	62.7	2–7	4.1	0.36–1.25	0.73
chains	11	35–59	45.9	1–5	3.0	0.27–1.28	0.67

		axon terminals: number range	axon terminals: number mean	axon terminals: mean length/μm range
bag_1	9	6–35	21.3	5.3–15.8
bag_2	7	7–34	15.3	4.4–15.3
chains	11	5–22	12.1	4.7–12.6

Serially sectioned spindles

Mean values for the eccentricity (ratio E) of the axon terminals in whole endings ranged from 1.42 to 2.55 (exceptionally 3.97) and clustered around 2.0. They showed no systematic variation with respect to location of the endings (see figure 6*a*). Since E, I and S are interdependent, both the ratios for indentation (I) and superficiality (S) measure the degree to which terminals were embedded in the muscle-fibre surface. Hence only the results for I will be considered further.

Figure 6*b* shows the mean values of I for all 27 endings plotted against their distances from the primary ending. The graph clearly illustrates that the further away an ending is from the primary, the more embedded in the muscle fibre its terminals become. The results for the endings in each spindle are indicated by

DESCRIPTION OF PLATE 1

Photographs of teased, silver preparations of motor endings supplied to normal cat peroneal muscles. Typical examples of the three types of intrafusal motor ending (figures 8–11, 13, 14) are illustrated together with an extrafusal motor ending (figure 12) for comparison. The ending in figure 11 is from peroneus tertius; the rest are from peroneus brevis. Scale in figure 8 applies throughout.

FIGURE 8. Two typical chain fibres each receive a trail plate.

FIGURE 9. A bag_2 fibre receives a trail plate.

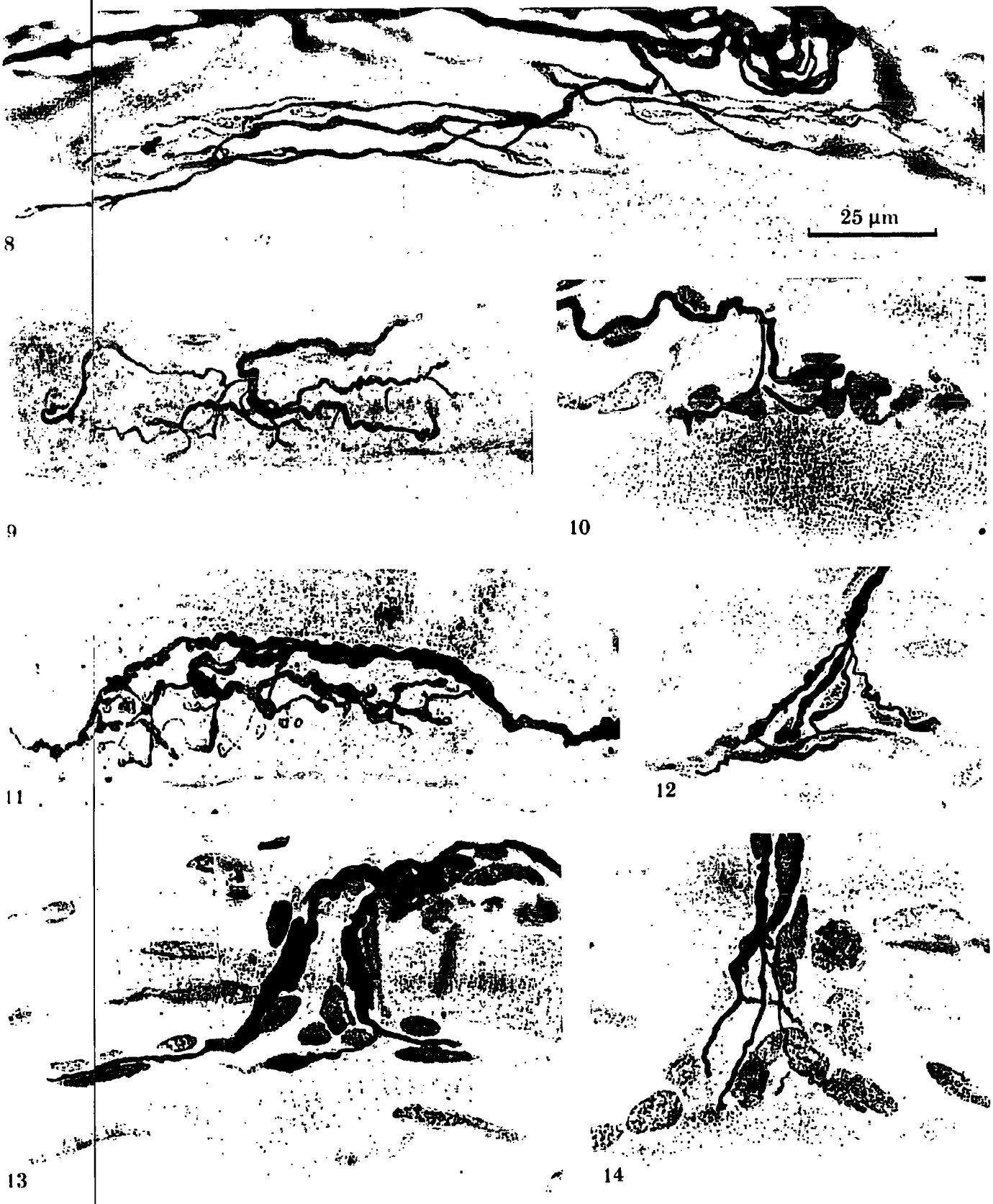
FIGURE 10. A bag_1 fibre receives a p_1 plate.

FIGURE 11. A bag_1 fibre receives a p_2 plate.

FIGURE 12. An extrafusal motor endplate.

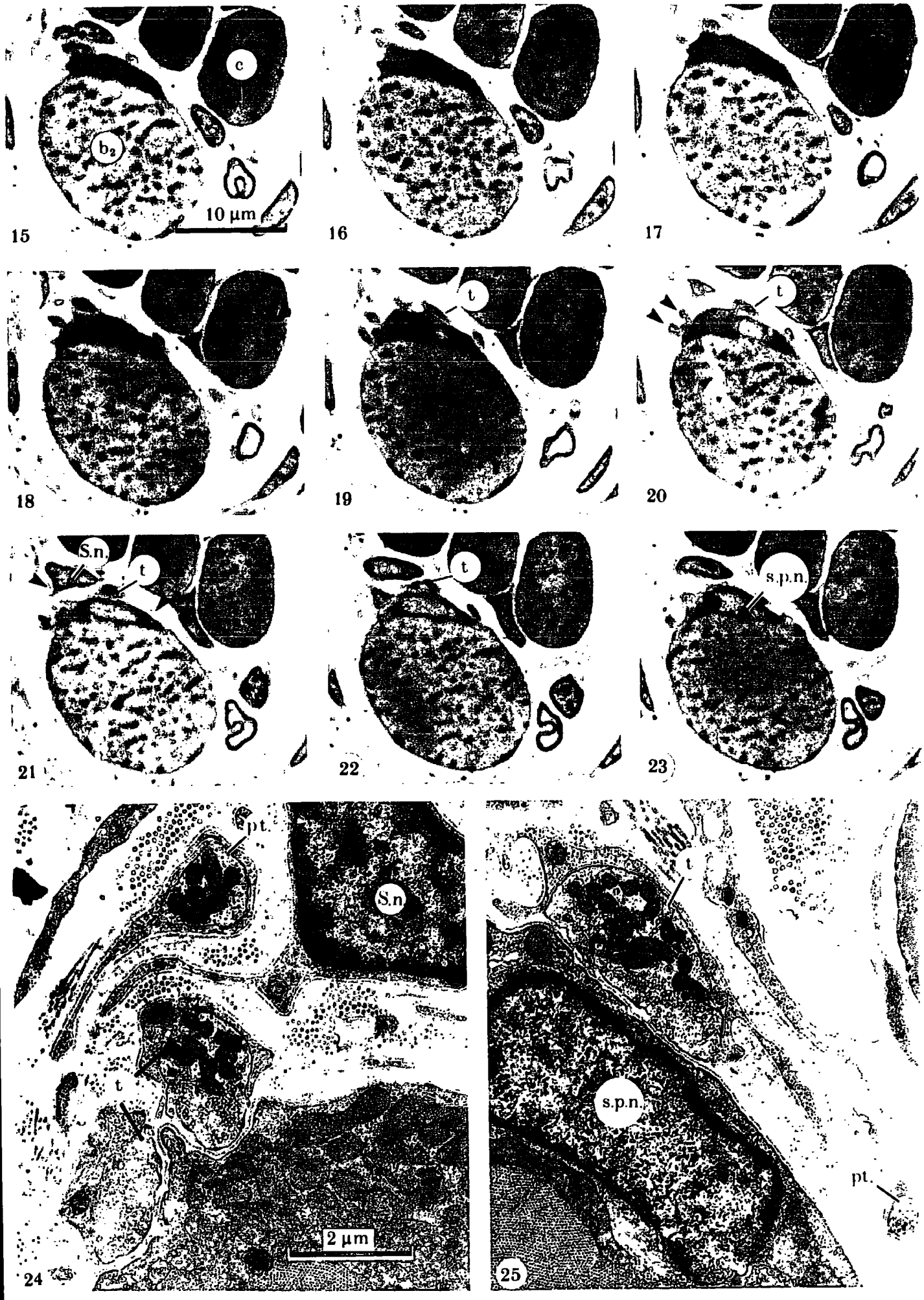
FIGURE 13. The p_1 innervation supplied to a bag_1 fibre consists of two plates 20 μm apart.

FIGURE 14. A long chain fibre receives a p_1 plate. Note nucleated sole plate and Doyère eminence. These features are usually obvious in p_1 and extrafusal plates, but not in p_2 or trail plates.



FIGURES 8–14. For description see opposite.

(Facing p. 206)



FIGURES 15–25. For description see opposite.

different symbols; these also represent different cats since each spindle was originally located in a tenuissimus muscle of a different cat. This shows that some of the total variability arises from differences between each source of material, such as differences between individual cats, and uncontrollable variations in details of preparation.

When the results are plotted separately for the endings on each type of intrafusal muscle fibre, the mean values of I increase with greater distances from the primary ending in each case. Moreover when an axon supplied two or three endings to the same muscle fibre, or the same type of muscle fibre, the fibre surface in the neuromuscular junctions of the more distal endings almost always had a greater mean indentation than that found in the junctions of the more proximal endings (see figure 7). The small number of endings sampled on bag_2 fibres (7), and their relatively restricted location, may account for the fact that the slope of the regression line is not significantly different from 0 in this case, though it is nevertheless similar to that in the graphs for the bag_1 and chain fibres. The vertical bar in each graph illustrates the variability of I within a single ending (see also figures 15–25, plate 2). For example, in figure 7*c* the ending selected was located on a chain fibre 1.15 mm from the primary ending in region B. There were nine axon terminals in a neuromuscular junction 39 μm long; their mean length was 5.1 μm in a range of 2.0–8.0 μm. The mean value of I for all terminals was 0.21 ± 0.03 s.e., and the mean values for individual terminals varied over the range 0.04–0.37. Finally, values of I for individual terminal profiles ranged from 0 to 0.75.

The remaining data relating to the serially sectioned endings are summarized in table 4. The neuromuscular junctions on chain fibres appear to be shorter overall than those on bag fibres, but the only significant difference is that between the mean lengths of the junctions on bag_1 and chain fibres ($p < 0.05$, Wilcoxon's rank sum test). The junctions on these fibre types also differ significantly ($p < 0.05$) in their number of axon terminals. This may be a consequence of the difference in junction length, but the two differences are not proportional, and the larger number of terminals on the bag_1 fibres may reflect a greater terminal density in their neuromuscular junctions.

Description of plate 2

Photographs of transverse sections through a cat tenuissimus muscle spindle showing part of a trail plate on a bag_2 fibre. Abbreviations: b_2, bag_2 fibre; c, chain fibre; pt., preterminal axon; s.p.n., sole-plate nucleus; S.n., Schwann-cell nucleus; t, axon terminal.

Figures 15–23. Serial, 1 μm-thick, toluidine-blue-stained sections show axon terminals of various sizes, profile, and degrees of indentation into the sole plate. The axon terminals marked by arrowheads in figure 20 are shown in detail in figure 24. The preterminal axons similarly marked in figure 21 are shown in detail in figures 24 and 25. The axon terminal labelled t in figures 19–22 is slightly more than 4 μm long. It is shown in detail in figure 25.

Figures 24 and 25. Electron micrographs of an ultrathin section taken between the sections shown in figures 20 and 21. The axon terminals are thinly covered by Schwann-cell cytoplasm which cannot be separately resolved in the light micrographs (figures 15–23). Nevertheless, the light micrographs do show fine detail; for example, the preterminal axons visible in the electron micrographs can be clearly identified in the light micrograph figure 21.

Discussion

Presynaptic features

We conclude from our results that β axons are associated with a single type of intrafusal motor innervation irrespective of whether it is supplied to bag_1, long chain, or intermediate chain fibres. This type of innervation is distinctly different from the presumed dynamic γ innervation that remains on the bag_1 fibre after the β endings have degenerated following nerve section; and both these types are different from the bag_2 and typical-chain innervation, which is a single group supplied by static γ axons. In our sample of normal bag_1 innervation it is interesting to note the presence of a large peak corresponding to the β supply (see figure 3), confirming the increasing recognition of the importance of this dynamic input.

Since these three groups of intrafusal motor endings are similar to those previously identified in the literature as p_1 (β), p_2 (dynamic γ), and trail (static γ), we shall continue to use this nomenclature. We do so on the understanding that the classification is based solely on the presynaptic features of the endings, and that there is often overlap between the categories with respect to any single feature.

Postsynaptic features

Our results show that the degree of indentation of the intrafusal muscle fibres by motor axon terminals increases with greater distance from the primary ending, irrespective of fibre type. Arbuthnott *et al.* (1982) use degree of indentation as a major defining characteristic to classify intrafusal motor endings into various types of plate, the most deeply indented ones (their 'm_b plates') occurring on the bag_1 fibre. Our findings indicate that this is because the bag_1 fibre usually receives endings most distant from the primary ending (see figure 6). At equal distances from the primary there is little difference between the indentation of motor terminals on the bag_1 fibre and the other fibre types. For example, the mean value of I for the five endings in our sample that were located on bag_1 fibres 1.0–1.5 mm from the primary endings was 0.15. This compares with six bag_2 and four chain endings located within this distance whose mean values of I were 0.14 and 0.20, respectively.

Apart from the degree of indentation (primary folding) of the postsynaptic membranes, there is the amount of junctional (secondary) folding to consider. There is now good evidence that this is related to muscle-fibre type and tends to increase in motor endings on bag_1, bag_2, and chain fibres, respectively (Barker *et al.* 1978; Kucera 1980*a*, 1981, 1982*b*, *c*; Banks 1981). This gradation is matched by the cholinesterase (ChE) content of the endings, there being a parallel increase in the density and thickness of the ChE reaction product (Kucera 1982*b*). It may be that this reflects differences in the electrical and contractile activity of the muscle-fibre types (Pachter & Eberstein 1983). The factor of distance from the primary ending appears to operate regardless of fibre type such that the greater the distance, the higher the ChE content of an ending (Kucera 1982*c*), and the greater the amount of junctional folding. It follows from this, and from our own data on indentation, that maximal primary and secondary folding should be found

in motor endings supplied to long chain fibres in region C, and the observations of Arbuthnott *et al.* (1982, their 'm_d plate') and Kucera & Hughes (1983*a*) confirm this.

Into this scheme of things Arbuthnott *et al.* (1982) and Sutherland *et al.* (1985) have introduced the idea that there are two types of static γ axon that preferentially innervate bag_2 and typical chain fibres, respectively. Both types are said to innervate chain fibres; one, mainly distributed to bag_2 fibres, supplies terminals that lie superficially on the muscle-fibre surface as 'm_a plates', whereas the other, mainly distributed to chain fibres, supplies terminals deeply embedded in the muscle-fibre surface as 'm_c plates'. The m_a plates are correlated with trail endings, but the ending identity of the m_c plates is left open. The evidence put forward by Arbuthnott *et al.* (1982) for the existence of the m_c plate rests mainly on the distribution of five such plates to typical chain fibres by a static γ axon in one pole of a single tenuissimus spindle. One other such plate in another spindle is acknowledged by them to have been supplied by a dynamic γ axon, and two others in a further spindle are located on long chain fibres and were presumably supplied by a static β axon. Their study was confined to the motor innervation of six spindle poles. A study of eleven spindle poles is reported by Sutherland *et al.* (1985), that claims to confirm the existence of m_a and m_c plates as distinct entities, but this is simply an extension of the earlier work, and, indeed, apparently includes the data from it.

We doubt whether this adds up to a valid case. In particular we doubt the histological basis for the conclusions drawn. This consisted of sampling each plate by cutting ultrathin sections at two or more levels at variable distances apart (Arbuthnott *et al.* 1982). In view of the variability of postsynaptic form both within and between individual intrafusal motor neuromuscular junctions, it is desirable that observations be made on serial sections.

Boyd *et al.* (1983) have advanced physiological evidence in support of two types of static γ axon that predominantly or exclusively innervate either bag_2 or typical chain fibres. This consists of identifying the type of muscle fibre activated by a single static γ axon in three of the spindles that it supplies. As we have shown elsewhere (Banks *et al.* 1985), the selection of a small number of spindles from those actually innervated by an axon inevitably tends to exaggerate any preponderant innervation of one muscle-fibre type, particularly when only two categories are recognized. This occurs even though the initial selection is unbiased. Although most static γ axons that innervate cat spindles undoubtedly show some degree of predominant distribution to bag_2 or typical chain fibres, analysis of the available histological evidence (Barker *et al.* 1973; Barker & Stacey 1981; Banks 1981; Kucera 1982*d*, 1983; Kucera & Hughes 1983*b*; Arbuthnott *et al.* 1982) indicates that this is subject to random variation.

Classification

When the cholinesterase technique was first applied to mammalian muscle spindles (Coërs & Durand 1956; Hess 1961) it became evident that, in addition to the presence of discrete plates in the poles, there were multiple diffuse endings near the equatorial region. In gold chloride preparations Boyd (1962) distinguished

these fusimotor components as 'γ_1 plates' and 'γ_2 endings', and although he illustrated several discrete γ_2 endings he chose not to describe them as plates 'to avoid confusion with the typical end-plates of γ_1 nerve fibres'. The distinction made between 'plates' and 'endings' is thus of long standing in descriptions of mammalian intrafusal motor innervation and is expressed in the plate (p_1, p_2) and trail-ending classification of Barker *et al.* (1970). Indeed, the multiterminal nature of the trail ending led Barker (1967) to see it as closely resembling the grape endings that innervate the slow extrafusal muscle fibres present in some vertebrate muscles. However, the serial-section studies of Kucera (1980*a*, *b*), Banks (1981), and Arbuthnott *et al.* (1982) have revealed that the trail innervation is in fact distributed in the form of plates, and that in any given spindle pole the typical chain fibres, which receive most of the innervation, usually do so in the form of a single plate. We therefore propose that in future the term 'trail ending' should be replaced by the term 'trail plate'.

It is clear that the postsynaptic form of intrafusal motor endings (determined by muscle-fibre type and distance from primary ending) cannot be correlated with their presynaptic form so as to produce a unified classification. The overlap in location in spindle poles is such that components of different function, or from different systems, may have the same postsynaptic form, for example, as in the case of dynamic γ and static γ endings on typical chain fibres (Arbuthnott *et al.* 1982), or dynamic β and γ endings on bag_1 fibres (Banks 1981; Arbuthnott *et al.* 1982). There is the additional variable of fibre type, and the fact that any unified classification would have to distinguish between the static and dynamic components of both β and γ systems. We doubt whether such a classification would serve any useful purpose, though attempts to devise one have not yet been abandoned by others (Boyd & Gladden 1985; Kucera & Walro 1985).

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Paper 28.

R.W. Banks, D. Barker & H.G. Brown (1985)
Sensory reinnervation of muscles following nerve section and suture in cats.
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Sensory Reinnervation of Muscles Following Nerve Section and Suture in Cats

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The common peroneal nerve was transected and repaired by epineurial suture in nine cats. In a further nine the nerve was transected twice and similarly repaired so as to produce a short autograft. Recovery of stretch receptors in peroneus brevis was monitored histologically and physiologically from six to fifty weeks. In recovery after single neurotomy functionally identifiable muscle-spindle and tendon-organ afferents were reduced to 25% and 45% of normal, respectively; after double neurotomy (autograft) both were reduced to about 10% of normal. Muscle spindles were reinnervated with annulospiral terminals, or wholly abnormal fine axon terminals, or both. Recovery evidently entails not only a reduction in number of stretch afferents, but also the making of some incorrect reconnections that presumably result in abnormal proprioceptive feedback and reflex action. When a graft is used the sensory impairment is compounded.

Nerve repair by epineurial suture is now widely used in the restoration of motor function following injury or pathological change. The ability of the reinnervated muscles to generate tension on electrical stimulation of their nerves may be similar to normal (Luff, 1984), but the degree to which their central nervous control is restored is often disappointing (Omer, 1980). In view of the preponderance of sensory axons in muscle nerves it is more likely that this deficiency is due to the disturbance of proprioceptive feedback than to the altered pattern of motor units in the reinnervated muscles. Sensory axons account for about two thirds of the total somatic component in nerves supplied to cat hindlimb muscles, whereas skeletomotor (α) axons account for only about one fifth (Boyd, 1968; Barker, 1974). Despite the obvious importance of muscle proprioceptors, relatively little attention has been paid to their recovery after nerve injury, perhaps partly because the prime need has been to find out more about the normal structure and function of the most complex of these, the muscle spindle.

Nevertheless, it has been established that muscle spindles and tendon organs can be successfully reinnervated under suitable conditions. For example, after a nerve-crush injury, which severs the axons but leaves the connective-tissue sheaths largely intact, the sense organs are restored to almost normal function and histological appearance, provided that reinnervation is not too long delayed (Brown, 1976; Ip, 1977; Barker, 1980; Hyde, 1983; Scott, 1983; Barker, 1985). Such is not the case in the reinnervation that follows nerve section, for this not only leads to a reduction in the number of normally responsive spindle and tendon-organ afferents, but also results in the occurrence of various kinds of abnormally responsive afferents (Brown, 1976; Gregory, 1982; Banks, 1983). Brown (1976) regards the regeneration of afferents with responses similar to normal as evidence of neuronal specificity. If this view is held then functional deficiencies that remain after recovery from nerve section are presumably attributable to the failure of an axonal guidance mechanism that remains more or less intact after a less serious lesion.

In the experiments of Brown (1976) and Gregory (1982) nerves supplying single muscles in the cat hindlimb were cut and their stumps either left to unite without suture or tied together in close opposition with fine silk thread. Gregory found abnormally responsive afferents in both self- and cross-reinnervated muscles. The use of long survival times allowed them to rule out Brown's suggestion that the abnormal responses were temporary, but they did not explicitly abandon the idea of neuronal specificity.

In the experiments reported here we have made histological and physiological observations on the proprioceptive recovery of the cat peroneus brevis muscle that results from its reinnervation after section and epineurial repair of the common peroneal nerve. We have also examined the effect of sectioning the nerve at two levels so as to isolate a segment that could then be regarded as an autograft. It is hoped that the results of this work will help to assess the quality of proprioceptive recovery that might be expected when similar repairs are carried out clinically.

Materials and Methods

The common peroneal nerve of the left hindlimb was exposed where it crosses the lateral head of gastrocnemius in eighteen adult cats anaesthetized with sodium pentobarbital (Sagatal 45mg/kg i.p.). The fascia covering the nerve was removed and the nerve sectioned with fine scissors about 4mm proximal to its point of entry into the gastrocnemius lateralis muscle. At this level the nerve consists of seven or eight fascicles of which two are cutaneous and form the superficial peroneal nerve, and the rest innervate the peroneal and

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other crural muscles. The nerve was repaired using about ten 10/0 polyamide epineurial sutures, fascicular alignment being preserved as far as possible. In nine cats the nerve was sectioned a second time 2mm distal to the first lesion, and similarly repaired. This procedure inevitably isolated the intervening segment from its blood supply and produced a short autograft with optimal fascicular alignment.

After a recovery period of from six to fifty weeks, each cat was prepared for single-unit, dorsal-root recording under sodium pentobarbital anaesthesia. The left hindlimb was extensively denervated leaving the nerve to peroneus brevis intact. Ipsilateral L7 and S1 dorsal and ventral roots were cut, and latencies of functionally single afferent axons were recorded in dorsal-root filaments during stimulation of the muscle nerve. Ramp-and-hold stretches of 1.8mm amplitude up to maximum physiological length were applied to the tendon of peroneus brevis at 2.5, 5.0, and 10mms^{-1}. Whenever possible, afferents were identified as originating from tendon organs or muscle spindles according to whether they tended to fire during or after a muscle twitch. Afferent responses were recorded on magnetic tape, unless only a dynamic response of one to three spikes was present.

At the end of the experiment the length of nerve between the stimulating and recording electrodes was measured, and the segment that included the repair was excised for subsequent histology. Peroneus brevis was stripped out so as to retain its periosteal origin and processed according to the silver technique of Barker (1963) with the modifications reported by Barker (1985). Muscle spindles and tendon organs were then teased out from the muscle and examined in whole mounts.

Four unoperated adult cats were used to provide the physiological and histological data required for control purposes.

Results

Normal peroneus brevis receptors

The population of encapsulated sense organs in the normal peroneus brevis muscle of the cat is about thirty muscle-spindle units (mean of 25 muscles = 29.6 ± 1.1 S.E.) and about twenty-five to thirty tendon organs. Paciniform corpuscles are rarely present; only one was found in twenty-five muscles. Each spindle unit is supplied with a primary sensory ending and usually also receives one or two secondary endings (see Figure 1A). Histological analysis of the four control muscles revealed an average provision of thirty primary endings and thirty-eight secondary endings; about a quarter (23.5%) of the spindle units lacked secondary endings. Since the afferent axons that innervate spindles and tendon organs rarely branch to supply more than one receptor, it follows that the total number of these axons in the nerve to peroneus brevis is close to one hundred.

Conduction velocities of afferent axons obtained in control experiments and classified into functional types are given in Table 1 (see also Figure 2A).

TABLE 1
Conduction velocities (ms^{-1}) of three functional types of afferent axon in the normal cat peroneus brevis nerve.

Afferent	
Group I	c.v. (mean ± S.E.)
Ia muscle-spindle primary	83.6 ± 6.6
Ib tendon organ	74.2 ± 8.6
Group II	36.5 ± 15.1

Sensory reinnervation following single neurotomy

Regenerating afferent axons were present in the peroneus brevis nerve after six weeks, and the establishment of functional mechanosensory contacts were most rapid between six and seven weeks. By this time spindle and tendon-organ afferents could be identified, but there were always some afferents of high threshold that could not be assigned to a particular receptor (at fifty postoperative weeks four of thirty-eight were of this type) as well as many afferents that did not respond to stretch. The average overall conduction velocity of mechanosensory afferents increased up to forty weeks, most rapidly before twelve weeks, but without any tendency to segregate into groups I and II.

Reinnervation of spindles and tendon-organs appeared to have been completed by the twelfth week, but the number of functionally identifiable afferents innervating the receptors was considerably reduced, tendon-organ afferents to an average of twelve per muscle (about 45% of normal) and spindle afferents to about seventeen per muscle (25% of normal). Conduction velocities were also reduced: at fifty weeks spindle afferents conducted at an average velocity of 62ms^{-1} (intermediate between the normal means for groups I and II), and tendon-organ afferents at an average velocity of 46ms^{-1} (62% of normal).

Throughout the recovery period of six to fifty weeks the responses of spindle afferents showed various abnormalities. The most obvious of these were the absence of a resting (tonic) discharge and the ability to respond only to the ramp phase of stretch. Though proportionately more common in the early stages of recovery, as following nerve-crush injury (Hyde, 1983), eighteen of forty afferents still lacked a tonic discharge at forty to fifty weeks, and five of these responded only during dynamic stretch (see Figure 2C). At this late

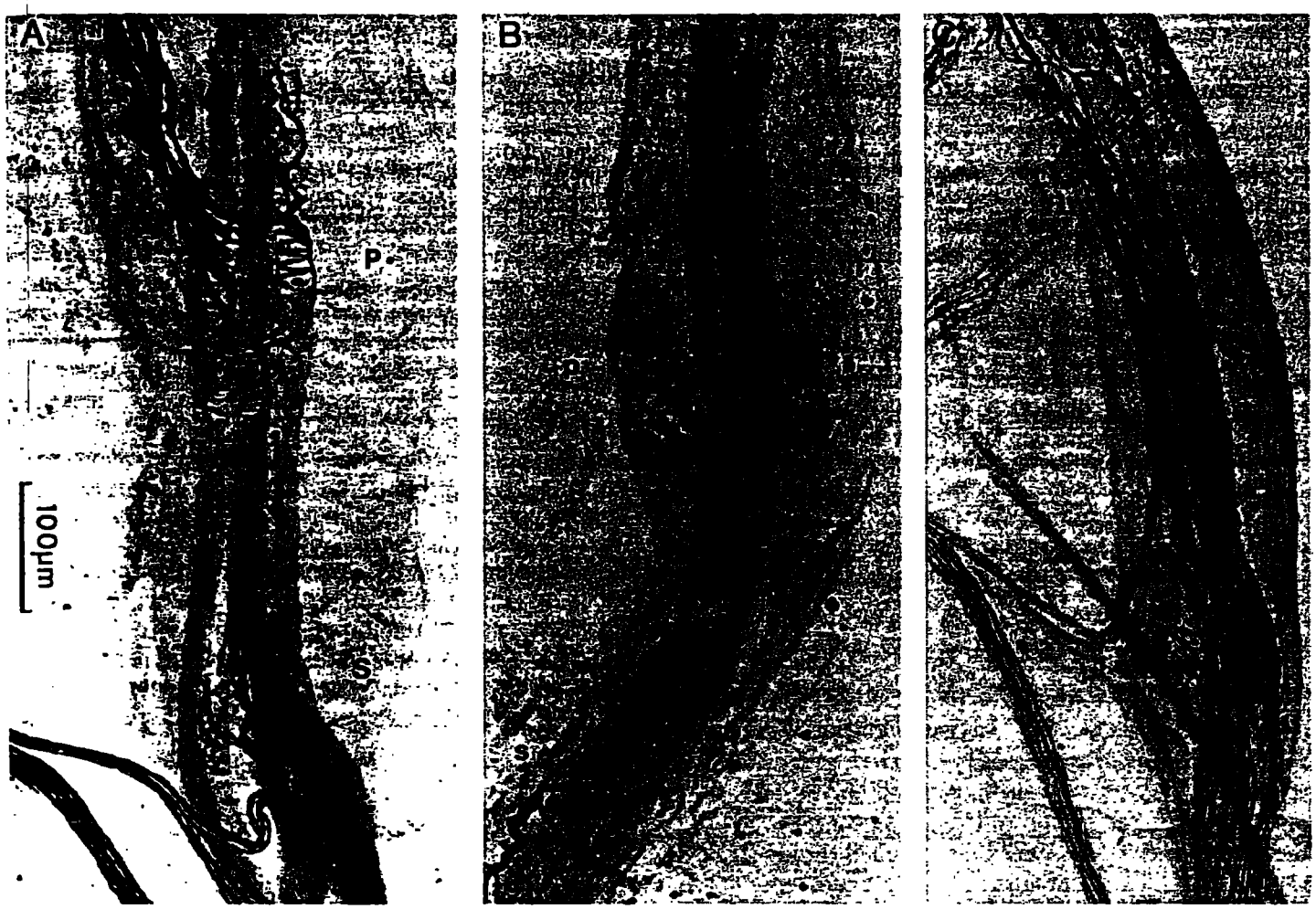


Fig. 1 Photographs of the sensory region of silver-stained muscle spindles teased from peroneus brevis. A. Sensory region of a normal spindle with primary (P) and secondary (S) endings. B. Double neurotomy (autograft) experiment after thirty weeks' recovery. One of few spindles to receive regenerated annulospiral endings, in this case reinnervating both primary (p) and secondary (s) regions. C. Single neurotomy experiment after sixteen weeks' recovery. Sensory region has been reinnervated by fine axon terminals that most probably belong to cutaneous afferents.

of recovery tonically firing afferents that conducted within the control group II velocity range were indistinguishable from normal secondary afferents, but were particularly rare. As a group, afferents conducting within the control group I velocity range still responded abnormally to dynamic stretch (Banks, 1983), though individual endings responding to a single rate of stretch could appear normal (see Figure 2B).

The reduction in number of spindle and tendon-organ afferents was confirmed histologically. Thus in a sample of eighty-four tendon organs teased from muscles that had recovered for eight to fifty weeks, thirty-nine (46.4%) had been reinnervated by variously abnormal terminals, whereas the rest remained deafferented or contained a few very fine axons. In the spindles teased from the twelve to fifty week muscles there were, on average, twelve endings per muscle that had annulospiral terminals more or less resembling those of normal primary and secondary endings (see Figure 1B). These were located on the intrafusal muscle fibres as in normal spindles, but the sensory regions of the reinnervated spindles more frequently contained wholly abnormal fine axon terminals that remained mostly apart from the intrafusal fibres and were widely distributed in the periaxial space (see Figure 1C). After seven weeks' recovery sensory axons had returned to all spindles to supply either one or both types of innervation.

Between the twelve- and fifty-week recovery stages most of the axons in the spindle nerves measured less than 3μm in diameter in the teased, silver preparations. Of the regenerated afferents that had annulospiral terminals, 62%, on average, had diameters of 3μm or more and their maximum diameter was 5.0μm. This compares with a sample of 113 normal peroneus brevis spindle afferents in which 74.3% had diameters of 3μm or more and the maximum diameter was 10.1μm (data from measurements used for Table 8 (Banks, 1982)). On average the thicker annulospiral afferents outnumbered the thicker afferents with fine terminals by about two to one.

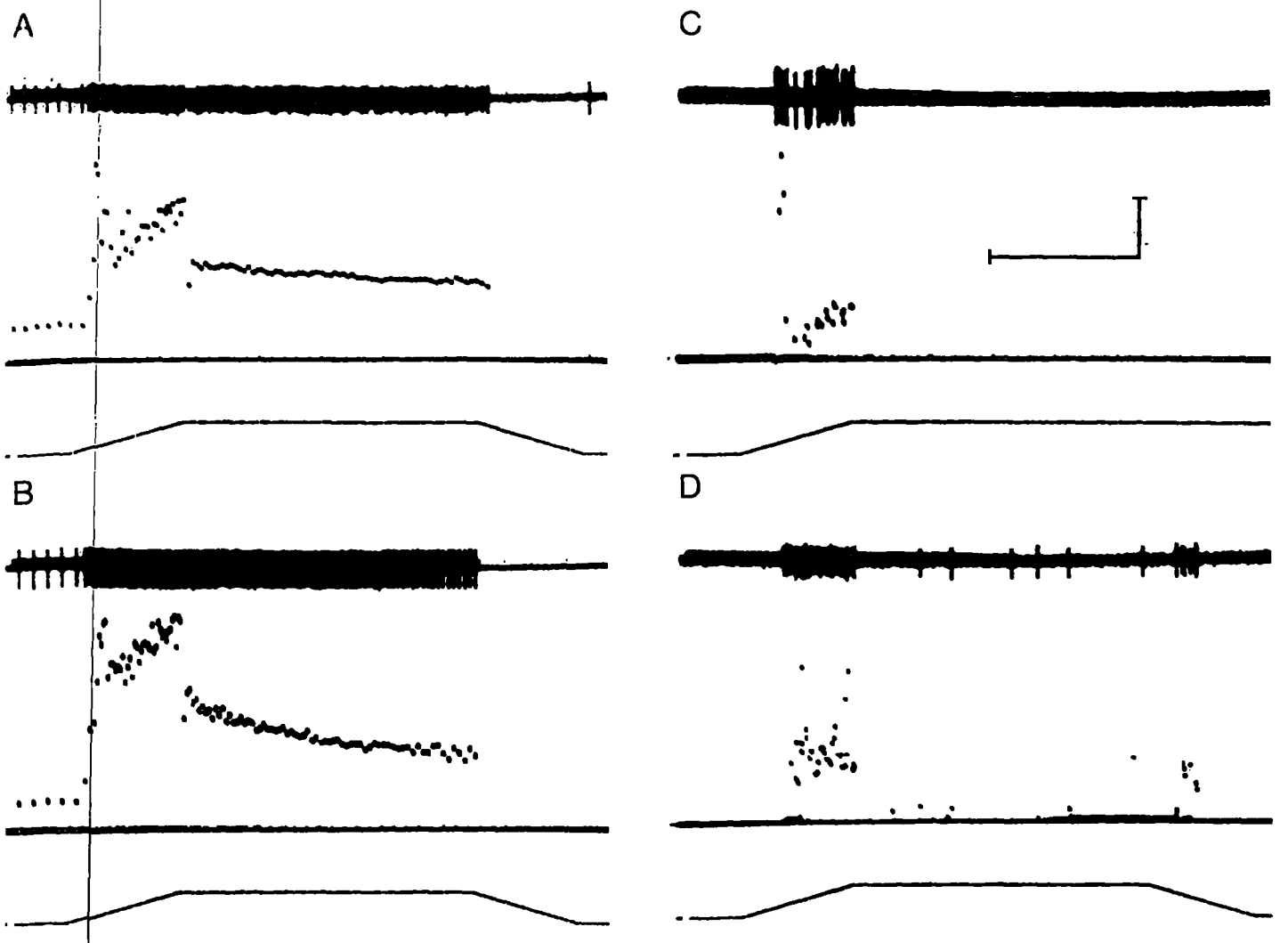


Fig. 2 Responses of afferent axons, identified as originating in peroneus brevis muscle spindles, to ramp-and-hold stretch of the muscle. Stretches were of 1.8mm amplitude up to maximum physiological length and were applied at 5mm s^{-1}. Each record shows, from top to bottom: spikes in a dorsal-root filament; a plot of instantaneous firing frequency calculated as the reciprocal of interspike interval; a baseline of zero frequency; and the phases of the ramp-and-hold stretch. Calibration (in C) 50 impulses s^{-1} (vertical) and 0.5s (horizontal).
A. Response of a primary ending supplied to a normal spindle by a Ia axon whose conduction velocity was 78.8 ms^{-1}.
B. Response from a reinnervated spindle after fifty weeks recovery in a single neurotomy experiment. The axon had a high conduction velocity (72.5ms^{-1}) and the response of its ending closely corresponds to that of a normal primary ending.
C. Highly abnormal response of a purely phasic type from a reinnervated spindle ending in the same experiment as in B; axon conduction velocity 34.4ms^{-1}. Three traces superimposed.
D. Abnormal response from a reinnervated spindle after forty weeks recovery in a double neurotomy (autograft) experiment. There are phasic responses to both the ramp and release phases of stretch; axon conduction velocity 18.2ms^{-1}.
Three traces superimposed. This type of response occurred less frequently than the abnormal response shown in C.

Sensory reinnervation following double neurotomy (autograft)

The inclusion of a short autograft in the nerve lesion was followed by reinnervation that was qualitatively similar to that following single neurotomy, but quantitatively worse in almost every respect. Regenerating afferents were present in the peroneus brevis nerve from six weeks onwards, and the most rapid increase in their conduction velocity occurred before twelve weeks, as after single neurotomy, but functional mechanosensory contacts were fewer and were established more slowly. Identifiable spindle and tendon-organ afferents were each reduced to about 10% of normal, averaging respectively seven and three per muscle at sixteen to fifty weeks. The average overall conduction velocities of mechanosensory afferents remained very low (44ms^{-1} at fifty weeks), and the proportion of unidentified afferents remained very high (eighteen of thirty-seven at forty to fifty weeks). However, the proportion of tonically firing spindle afferents, and the nature of their responses to dynamic and static stretch, were similar to those present following single neurotomy. An example of an abnormally responding ending is shown in Figure 2D.

Silver-stained spindles teased from the twenty- to fifty-

week muscles had, on average, seven annulospiral endings per muscle. Of their afferents only 37% had diameters of 3μm or more, and only 45% of all the afferents of that calibre in spindle nerves ended in annulospirals.

Discussion

Our results show that proprioceptive function is seriously impaired in the reinnervation and recovery that follow the severance and suture of a cat's musculocutaneous nerve, despite the single-cut injury being immediately repaired by epineurial suture that achieved optimal fascicular alignment. Not only is there an absolute reduction in the number of stretch- and tension-sensitive afferents, but those that do successfully reinnervate muscle spindles and tendon organs show various degrees of abnormality in both structure and function. Moreover, these deficiencies remain unchanged after fifty weeks and appear to be permanent. Although there are many outstanding problems concerning the proprioceptive role of muscle spindles (Matthews, 1981) and other muscle receptors, this sensory impairment can only be detrimental to the degree of central nervous control of muscle activity.

Clinical assessments of recovery following the suture of human peripheral nerves (Omer, 1980) suggest that in most circumstances there may be considerable loss of proprioceptive function. It seems probable that the impairment would be greatest in those muscles with a high density of spindles such as the lumbricals of the hand and the intrinsic muscles of the thumb (see Table 1 in Cooper, 1960).

Evaluation of the reinnervation and restoration of function that follows the repair of severed muscle nerves raises the question as to whether the muscle receptors have to be reconnected with the same types of afferent they received before the injury in order for functional success to be achieved. We have carried out cross-union experiments (Banks, Barker and Stacey, 1984) which indicate that the regenerating afferents are, in fact, not specific as to the receptor site they innervate. The experiments suggest (i) that cutaneous afferents are able to reinnervate muscle spindles with fine axon terminals, such as have been observed in the present study, and that some of these are capable of responding to stretch; and (ii) that tendon-organ (Ib) afferents are capable of reinnervating spindles by terminating in sites originally occupied by endings supplied by primary (Ia) or secondary (II) afferents.

Thus the sensory impairment that follows the repair of severed muscle nerves not only involves afferent loss, but also some incorrect reconnections that presumably result in abnormal proprioceptive feedback and reflex action. It seems that nerve severance results in an irreduceable level of axonal mismatching in the regeneration and reinnervation that follows, and this is naturally compounded when a graft is used, since this effectively involves two lesions. Clinically, grafting is the only feasible way to bridge a wide gap between nerve stumps and so avoid the excessive tension that end-to-end suture would produce. Opinions vary as to what the critical distance between stumps should be beyond which a graft should routinely be used (Miyamoto, 1981 and subsequent discussion). Our results clearly encourage the choice of longer rather than shorter gap distances (e.g. 2cm rather than 1.5cm) if additional sensory loss is to be avoided.

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Paper 29.

R.W. Banks & D. Barker (1986)
Responses of cat muscle spindles reinnervated by afferents formerly terminating in tendon organs.
J. Physiol. 372; 24P.

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Journal of Physiology, **372**, 24*P*, 1986]

Responses of cat muscle spindles reinnervated by afferents formerly terminating in tendon organs

By R. W. Banks and D. Barker. *Department of Zoology, University of Durham, Durham DH1 3LE*

Tendon–organ (group Ib), Pacinian-corpuscle (group II) and free-ending afferents almost exclusively comprise the myelinated component of the cat hind-limb interosseous (INT) nerve. All three types are able to reinnervate muscle spindles with their own characteristic terminals (Banks, Barker & Stacey, 1984). Here we describe the responses of such reinnervated spindles to passive stretch.

Cross-unions were made unilaterally between the INT nerve (proximal) and the flexor digitorum longus (FDL) nerve (distal) in two adult cats anaesthetized with sodium pentobarbitone. After 17 or 18 weeks the cats were deeply anaesthetized with sodium pentobarbitone and prepared for single-unit, dorsal-root (L6–S1) recording. The hind limb was extensively denervated, including the proximal stump of the FDL nerve so as to eliminate any afferent reinnervation from that source. Efferent reinnervation was advantageous since it enabled the muscle to twitch in response to electrical stimuli applied to the FDL nerve distal to the cross-union. This allowed afferents exhibiting rebound excitation following muscle twitch to be identified as being located in spindles. Mechanical stimulation by ramp-and-hold stretch of 1·8 mm amplitude up to maximum physiological length was applied at 10, 5 or 2·5 mm s^{-1} to the FDL tendon.

Of 79 group I or II afferents present in the reinnervated FDL nerves, 31 showed maintained responses to stretch and were located in spindles. Their conduction velocities (mean 73·7, S.D. 8·18 m s^{-1}) were similar to those of normal INT tendon–organ afferents (mean 77·2, S.D. 6·98 m s^{-1}). In terms of dynamic index, initial burst and static discharge, their responses were all within the range of normal primary and secondary endings of FDL and flexor hallucis longus spindles. The occurrence of such responses correlates well with the histology of three similar cross-unions in which large-diameter afferents, presumed to be Ib, were observed in FDL spindles terminating in sites previously occupied by the endings of Ia and II afferents.

Only eight of the remaining afferents showed responses to mechanical stimulation or muscle twitch. One was a group I axon (81·8 m s^{-1}) located in a tendon organ; two were group II axons (47·4 and 51·4 m s^{-1}) in Pacinian corpuscles; and the rest were group II axons with weak responses to ramp stretch or muscle twitch.

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Sensory reinnervation of cat muscle spindles after nerve section.
Proceedings of the International Union of Physiological Sciences. 16; P462.08.

Proc. IUPS XXXth Congress **16**, 462.08 (1986)

P462.08

SENSORY REINNERVATION OF CAT MUSCLE SPINDLES AFTER NERVE SECTION.

M.J. Stacey, R.W. Banks and D. Barker. Department of Zoology, University of Durham, Durham, U.K.

After section and suture of the common peroneal nerve, spindles were reinnervated with annulospiral terminals, or abnormal fine axon terminals, or both, and their responses to ramp-and-hold stretch showed various abnormalities throughout a recovery period of 6-50 weeks. Cross-union experiments indicated that the fine axon terminals belonged mainly to free-ending afferents, and that some responded to stretch. Other cross-union experiments, designed to elucidate the identity of the annulospiral terminals, provided the opportunity for Ib tendon-organ afferents to reinnervate spindles. We found that such afferents could form endings in sites previously occupied by the endings of Ia and II spindle afferents and respond to stretch in an entirely comparable manner. This has enabled the afferent recovery of peroneus brevis after nerve section to be quantified histologically in terms of the number of regenerated Ia and II afferents restored to spindles expressed as a percentage of the estimated normal supply, as follows: single transection of common peroneal nerve, 15%; double transection (short autograft), 9%; single transection of peroneus brevis nerve only, 33%.

Paper 31.

R.W. Banks, D. Barker & M.J. Stacey (1986)
Regenerated sensory innervation of muscle receptors following nerve section.
Neurosci. Letters Suppl. 26 S565.

Neurosci. Letters Suppl. 26: S565 (1986)

REGENERATED SENSORY INNERVATION OF MUSCLE RECEPTORS FOLLOWING NERVE SECTION

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Recovery of stretch receptors in the peroneus brevis muscle of the cat was monitored 6-50 weeks after section and suture of the common peroneal nerve. Spindles were reinnervated with annulospiral terminals, or wholly abnormal fine axon terminals, or both, and their responses to ramp-and-hold stretch showed various abnormalities throughout the recovery period (Banks and Barker, J. Physiol. 345, 97P, 1983; Banks, Barker and Brown, J. Hand Surg. 10-B, 340, 1985). The reinnervation of peroneus brevis spindles by cutaneous afferents in cross-union experiments indicated that the fine axon terminals belonged mainly to free-ending afferents, and that some were capable of responding to stretch (Banks, Barker and Stacey, J. Physiol. 357, 21P, 1984). Other experiments were designed to investigate the nature of the annulospiral terminals. Cross-union of the interosseous and flexor digitorum longus nerves provided the opportunity for tendon-organ (Ib) afferents to reinnervate spindles in the absence of spindle (Ia and II) afferents. We found that the Ib afferents were capable of forming annulospiral endings in sites previously occupied by the endings of Ia and II afferents, and of responding comparably to stretch (Banks and Barker, J. Physiol. 372, 24P, 1986). Self-reinnervation of the tenuissimus allowed Ia and II afferents to reinnervate spindles in the virtual absence of Ib afferents.

Paper 32.

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In Engel, A.and Banker, B.Q. (eds.) Myology, pp. 309-341 McGraw-Hill: New York.

CHAPTER

10
The Muscle Spindle

DAVID BARKER & ROBERT W. BANKS

Morphology
- INTRAFUSAL MUSCLE FIBERS
- CAPSULE AND VASCULAR SUPPLY
- TYPES OF SPINDLE UNIT
- NUMBER AND DISTRIBUTION
- SENSORY INNERVATION
- MOTOR INNERVATION
- AUTONOMIC INNERVATION

Physiology
- THE SPINDLE AS A RECEPTOR
- EFFECTS OF SYMPATHETIC STIMULATION

Muscle spindles are mechanoreceptors sensitive to muscle length and changes in muscle length. They are composed of small (*intrafusal*) muscle fibers that lie as bundles in parallel with ordinary (*extrafusal*) muscle fibers, their ends attached to connective tissue, tendon, or extrafusal endomysium. They receive both a motor and a sensory innervation. The sensory innervation, which responds to active and passive changes in muscle length, is protected by a fusiform, fluid-filled capsule and occupies the *equatorial region* of the intrafusal bundle, whereas the motor innervation is distributed to the *polar regions* that extend on each side. Activation of the motor innervation elicits contractions in the polar regions that modify the sensory discharge.

Muscle spindles occur in the somatic muscles of vertebrates. They were first noticed and described in frog muscle by Weismann[1] in 1861. For many years it was believed that the receptor first appeared in early tetrapods in antigravity muscles associated with posture and locomotion, but it now seems likely that there was an earlier debut in the jaw muscles of fish, since Maeda, Miyoshi, and Toh[1a] have recently reported finding monofibral spindles in the jaw-closing muscle of the Japanese salmon.

The nonmammalian spindle is supplied with one sensory ending and receives its motor innervation from branches of axons that also innervate extrafusal muscle fibers. Mammalian spindles are supplied with a *primary* sensory ending (the homologue of the sensory ending in nonmammalian spindles) and may, in addition, be supplied with one or more *secondary* sensory endings. Two kinds of motor system are involved in their motor innervation: a *fusimotor* (γ) system, which is exclusively intrafusal and unique to mammals, and a *skeletofusimotor* (β) system, in which intrafusal and extrafusal muscle fibers share a common innervation, as in nonmammalian spindles. Each system contains two functionally different types of motor axon whose stimulation produces different effects on the dynamic response of the primary ending. (The *dynamic response* is defined as the alteration in the rate of impulses discharged by the ending that is related to the rate of change of muscle length. It contrasts with the *static response*, which is the alteration in discharge that arises as a result of the muscle's changing from one steady state length to another). Stimulation of dynamic γ motor axons increases the dynamic sensitivity of the primary ending, whereas stimulation of static γ axons decreases it[2]; there is the same functional difference between dynamic and static β axons.[3–5] The static response is increased by stimulating either static or dynamic axons, γ or β.

This chapter is about mammalian spindles. It is mostly about those of the cat because far more is known about them than any others. Such information as there is about human spindles indicates that they do not differ in any radical respect from those in the cat.

A typical spindle in a cat's hindlimb muscle (see Fig. 10-1) consists of a 7- to 10-mm-long bundle of six to nine muscle fibers that is richly vascularized, partly encapsulated (generally the middle third), and innervated by a spindle nerve that leaves a nearby intramuscular nerve trunk to enter the equatorial region. Two kinds of muscle fiber can be recognized on the basis of differences in length, diameter, and equatorial nucleation. The longest and thickest are called *nuclear-bag fibers* because for a short length in the equatorial region they contain few myofibrils and are full of round vesicular nuclei, thus forming what Barker described as a *nuclear bag*.[6] Each bag tapers off on either side into a single row of elongated nuclei within a central core of sarcoplasm to form a *myotube region*.[6] The shortest and thinnest fibers contain a single central row of nuclei in the equatorial region and are called *nuclear-chain fibers*[7] (see Fig. 10-1*D*). There are usually two bag fibers and four to seven chain fibers.

The site of the nuclear bags, myotube regions, and chains is innervated by a group Ia axon that terminates as an annulospiral primary ending. This is usually accompanied by one secondary ending supplied by a group II axon that distributes less regular rings and spirals predominantly to the chain fibers. The motor innervation consists of a diffuse multiterminal trail ending[8] and two types of plate,

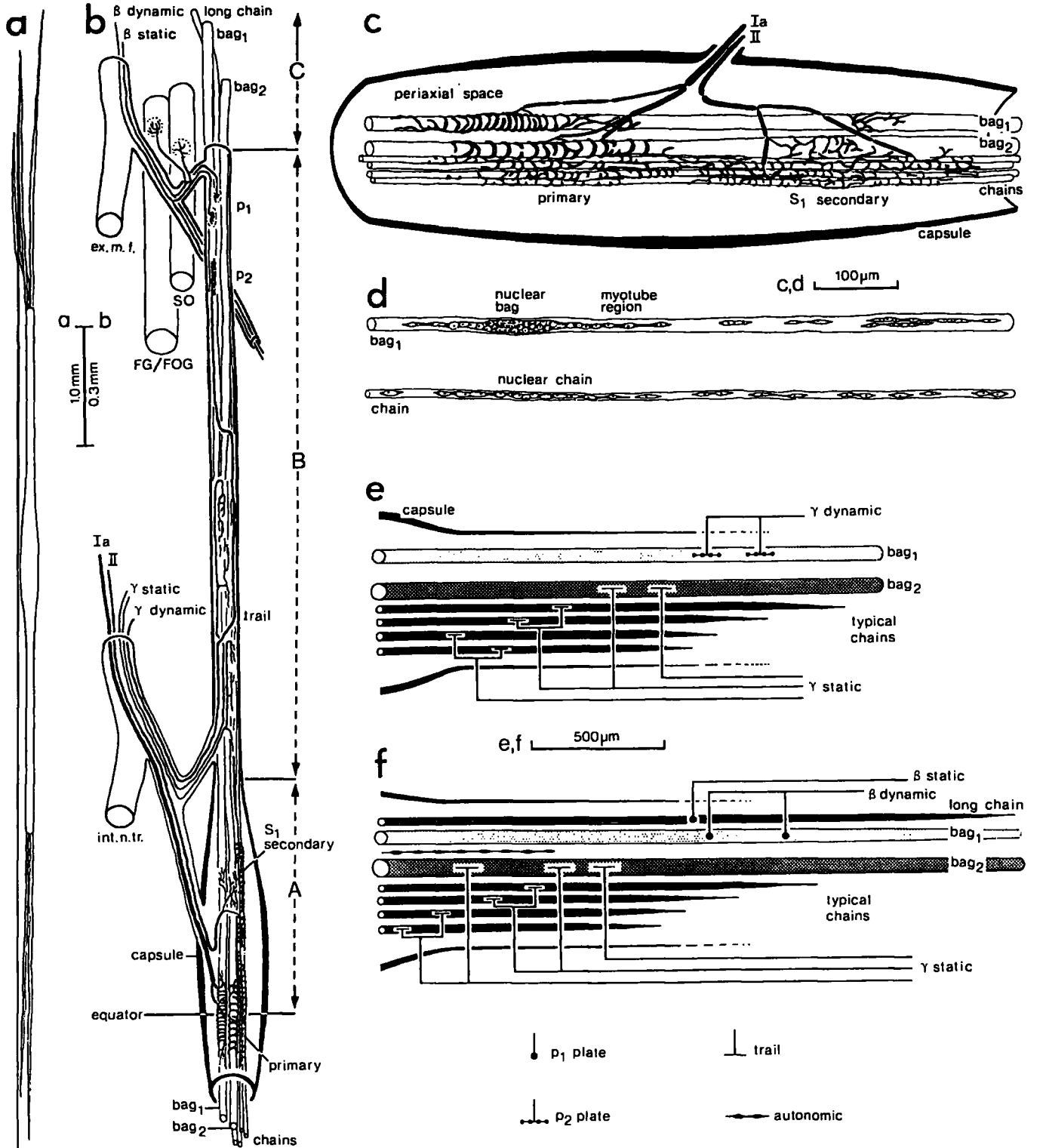


Figure 10-1. Schema illustrating the structure and innervation of cat tenuissimus muscle spindles. *A*. The encapsulated bundle of intrafusal muscle fibers that constitutes a spindle. *B*. The equatorial region and part of one pole illustrating regions A, B, C and innervation by Ia and II sensory and β and γ motor axons. ex.m.f. = extrafusal muscle fibers; FG/FOG = fast glycolytic or fast oxidative-glycolytic muscle fiber; SO = slow oxidative muscle fiber. *C*. Sensory innervation comprising a primary ending and an S_1 secondary ending. The distribution of the total terminal contact area of a primary ending is about 35% bag_1, 25% bag_2, 40% chain; of an S_1 secondary 10% bag_1, 20% bag_2, 70% chain. *D*. Nuclear-bag and nuclear-chain intrafusal muscle fibers showing nucleation in primary and S_1 secondary regions. *E,F*. Motor innervation of a typical pole (*E*). The most common variation (*F*) is for static and dynamic β axons to participate in the motor innervation (though seldom of the same spindle). Some spindles receive a nonvascular autonomic innervation. *A–D* depict features drawn to the scale of average dimensions; *C*, *D* are based on reconstructions[29]; *E*, *F* are schematic diagrams.

designated p_1 and p_2.[9,10] The p_1 plates are supplied by β axons, the p_2 plates and trail endings by γ axons. Apart from this somatic innervation, some spindles also receive autonomic axons that are in neuroeffective association mainly with the intrafusal muscle fibers.[11]

The manner in which the motor innervation is distributed to the bag and chain muscle fibers has been the subject of considerable controversy. The original idea was that dynamic motor axons innervated the bag fibers terminating as plates, whereas the static motor axons innervated the chain fibers terminating as trail endings. Such selective innervation had the attraction of providing a basis whereby the static and dynamic responses of the spindle could be under separate motor control and operate through two intrafusal systems. This dual model of the mammalian spindle was generally accepted during the sixties and early seventies, despite an increasing amount of histological and histophysiological evidence that failed to support it. This acknowledged that dynamic motor axons were almost exclusively distributed to bag fibers but maintained that static axons were most frequently distributed to both bag and chain fibers. The conflict was resolved when it gradually came to be realized that there are two types of bag fiber that differ with respect to ultrastructure, histochemical profile, contractile properties, and development. They were designated as *bag*$_1$ and *bag*$_2$ fibers. It is now accepted that there are three kinds of mammalian intrafusal muscle fiber and that dynamic actions are carried out by the bag_1 fiber (the dynamic bag fiber) and static actions by the bag_2 (static bag fiber) and chain fibers.

The original work involved in this switch from a dual to a triple spindle model is referred to in the following sections; these discussions summarize details of the structure, innervation, and development of the cat spindle and give some account of how it functions. The most recent reviews of work on mammalian spindles are those of Laporte,[12] Kennedy, Poppele, and Quick,[13] Boyd,[14,15] and Matthews[16]; for reviews that deal with both mammalian and nonmammalian spindles see those of Barker[17] and Hunt.[18]

Morphology

INTRAFUSAL MUSCLE FIBERS

The dual model of the mammalian spindle could not satisfactorily account for histochemical observations made in the sixties that distinguished three types of intrafusal muscle fiber. When the histochemical evidence was correlated with observations made on ultrastructure and teased silver preparations, it became clear that there were two kinds of bag fiber (see review by Barker[17]). These were designated as *nuclear bag* and *intermediate* by Barker and Stacey[19] and as *bag*$_1$ and *bag*$_2$ by Ovalle and Smith.[20] The correlation of the histochemistry with the electron microscopy was somewhat conjectural as the observations reported by various workers had been made on separate preparations of different spindles, and for a time there was some confusion about how the two kinds of bag fiber should be categorized.

The matter was resolved when Banks, Barker, Harker, and Stacey[21] devised a technique that allowed adjacent sections of the same spindle to be prepared for either histochemical or ultrastructural study. Descriptions by Banks, Harker, and Stacey[22] of the ultrastructural and histochemical characteristics of the two types of bag fiber then followed, and Ovalle and Smith's[20] terms *bag*$_1$ and *bag*$_2$ were adopted to designate them.[23] Besides their different ultrastructure and histochemistry, it transpired that the two types could also be histologically distinguished by the abundance (bag_2) or scarcity (bag_1) of elastic fibers associated with them in the extracapsular polar regions[24]; and by the fact that the bag_1 fiber often lay apart from the bag_2 and chain fibers during its course through the equatorial region.[23]

Barker, Banks, Harker, Milburn, and Stacey[23] found it convenient to distinguish three regions, A, B, and C, between the equator and the origin or insertion of a spindle pole, a practice that has since been generally adopted. The regions are defined as: A, that part of the equatorial region lying between the equator and the equatorial end of the *periaxial space* (i.e., the space between the intrafusal, or axial, bundle and the capsule wall); B, that part of the pole extending from the equatorial end of the periaxial space to the end of the capsule; and C, the extracapsular part of the pole (see Fig. 10-1*B*).

In some spindles one of the chain fibers extends for a considerable distance beyond the capsule and has been called a *long* chain fiber.[23] Kucera[25,26] distinguishes two further subtypes, namely, *typical* chain fibers, which mostly attach within the capsule, and *intermediate* chains, which extend beyond it but not so far as long chain fibers. These subtypes are of functional significance, since there is evidence of selective innervation of long and intermediate chains by static β axons and of typical chains by static γ axons (see Motor Innervation, below).

Length, Diameter, and Nucleation

In most spindles the bag fibers are the longest, the bag_2 generally being longer than the bag_1. Kucera[26] reports mean polar lengths of 2947 μm for bag_2 fibers, 2760 μm for bag_1, and 1231 μm for typical chains (tenuissimus, frozen sections). In 77 percent of 313 spindle poles the bag_2 was the longest fiber, in 14 percent the bag_1; in 3 percent the bag fibers were of equal length, and in 6 percent the longest fiber was a long chain. The long chain fibers (i.e., those extending 1.0 mm or more beyond the capsule[23]), when considered as a group, proved to be the longest fibers in Kucera's study,[26] their mean polar length being 2990 μm. This compares with one of 1929 μm for intermediate chain fibers (i.e., those extending for less than 1.0 mm beyond the capsule). Long and intermediate chain fibers occurred in a minority of spindles and usually lay close to the bag_1 fiber for much of their course.[27]

Mean juxtaequatorial (inner region B) diameters for bag and chain fibers given by Boyd[28] are 16.86 ± 2.35 μm for bag fibers and 8.37 ± 1.85 μm for chains (tenuissimus, Susa fixation, paraffin sections). No systematic study of intrafusal muscle fiber diameters has been made since it was established that there are two types of bag fiber and three subtypes of chain. All types of fiber become thinner as they pass through the equatorial region, and those that extend well into region C tend to become thickest in this region. The presence of a secondary ending adjacent to the primary results in the bag fibers' undergoing a marked increase in diameter at the sites where they receive secondary terminals.[29]

The nuclei of intrafusal muscle fibers are located either peripherally underneath the sarcolemma (subsarcolemmal nuclei), as in the polar regions, or internally among the myofibrils (myonuclei), as in the equatorial region. In light microscope preparations it is sometimes difficult to distinguish between subsarcolemmal nuclei and those of satellite cells and endomysial fibrocytes. Satellite cells occur mostly in association with bag_2 fibers in region C; they are less frequently associated with the bag_1 fiber and rarely occur on chain fibers.[30]

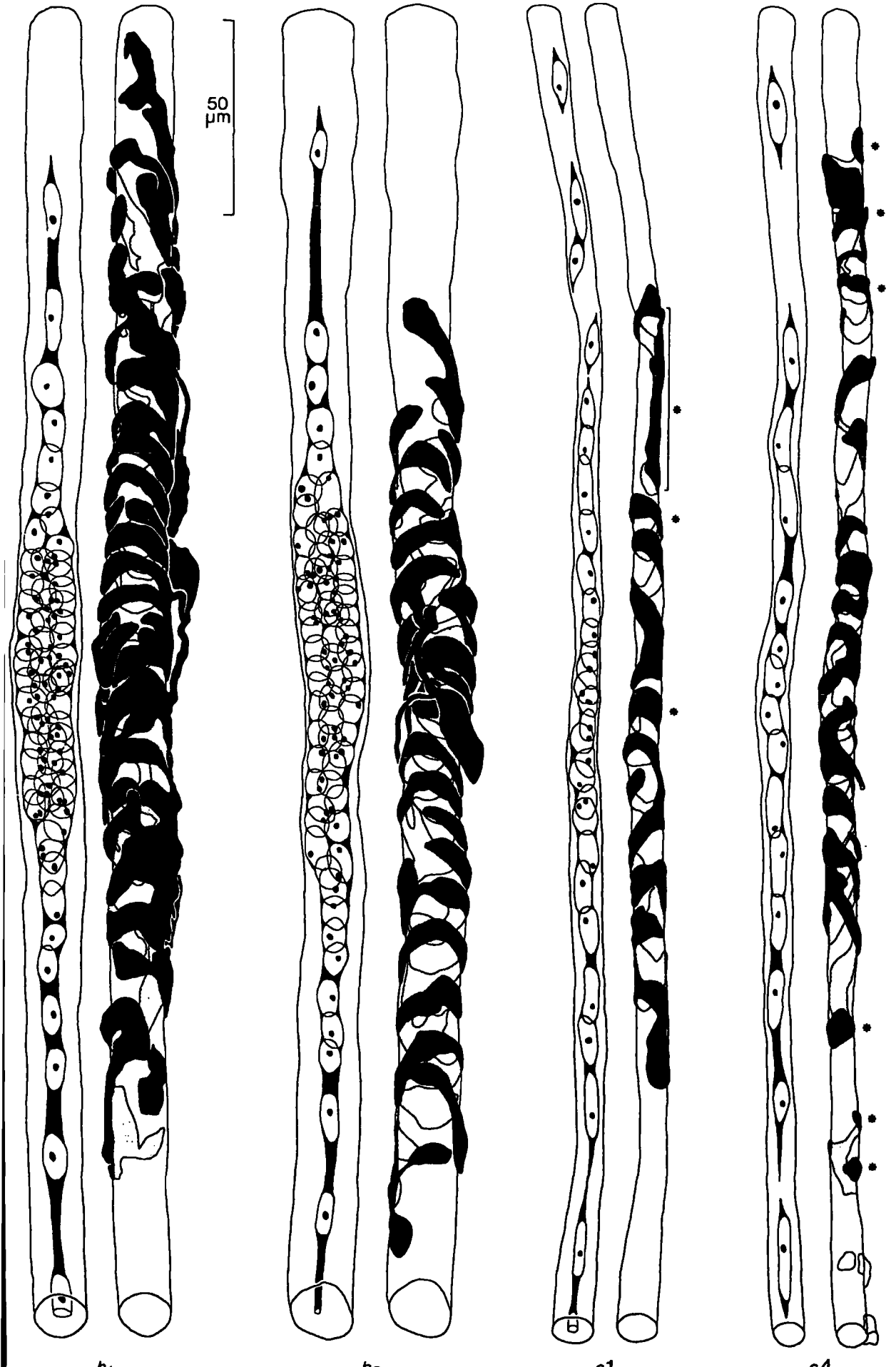
The most detailed information about equatorial nucleation is that recently obtained by Banks, Barker, and Stacey[29] from reconstructions of four tenuissimus spindles (see Fig. 10-2). They found that nuclear bags contained 52 to 106 myonuclei, those of bag_1 and bag_2 fibers averaging 68 and 80, respectively. In the myotube regions there were 6 to 12 myonuclei, average 9; in the nuclear chains there were 11 to 38, average 24. There was a tendency for the longest chain fibers to be the most densely nucleated and for their myonuclei to aggregate equatorially to form miniature nuclear bags (see also Kucera[27]). Myonuclei occupied 70 to 90 percent of the cross-sectional area of each nuclear bag, 30 to 50 percent of each myotube region, and 40 to 60 percent of the longest chain fibers in the primary region. By contrast, myonuclei in the region of secondary terminals on bag fibers occupied only 10 percent of the cross-sectional area.

Ultrastructure

Observations on the ultrastructure of intrafusal muscle fibers made before the present classification of fiber types was established are reviewed by Barker.[17] It is now apparent that the fibers display two types of myofibrillar ultrastructure, which for convenience have been designated M or dM according to the appearance of the M line.[21,23] In the M condition the M line crosses the middle of each sarcomere as a single prominent line (low power) composed of five parallel faint lines (high power), whereas in the dM condition the M line either cannot be seen or appears as two parallel faint lines, according to the orientation of the myofibrils.[22]

In the M condition the myofibrils are packed as discrete units in sarcoplasm that is rich in glycogen and contains many thick, long mitochondria and a sarcoplasmic reticulum (SR) that is well developed at the level of the I and Z bands. Transverse sections at this level show the myofibrils almost completely encircled by SR elements. By contrast, in the dM condition there is very little interfibrillar sarcoplasm, little glycogen, poorly developed SR, and the mitochondria are thin, short, and scarce. Transverse

Figure 10-2. Schematic representation of parts of a primary ending reconstructed from a cat tenuissimus spindle. The terminals shown are those supplied to the bag_1 (b_1) and bag_2 (b_2) fibers and the longest and shortest chain (c) fibers (numbered 1 and 4). Each fiber is repeated alongside to show its myonucleation and thus demonstrate the relation between nucleation and innervation. Adjustments have been made to the original alignment of each muscle fiber relative to the others, mainly in order to position the center of each nuclear bag on a common midline and thus facilitate comparison between b_1 and b_2 terminal systems. Terminals shown in outline at bottom end of c4 belong to adjacent S_1 secondary ending. Asterisks alongside c terminals indicate positions of sensory cross-terminals with other c fibers. *(Banks RW et al, Philos Trans R Soc Lond 299:329, 1982.)*


50
µm
b1
b2
c1
c4

sections show the myofibrils to be poorly defined and tightly packed together so as to form a more or less continuous bundle, with the SR only occasionally encountered at the level of the I and Z bands. Chain fibers have the M type of ultrastructure, whereas, remarkably, the bag fibers are a mixture of both M and dM. In the bag_1 fiber the ultrastructure is dM in region A and most of region B, then changes to the M condition toward the outer end of region B. The sarcomere length is consistently longer than in the bag_2 or chain fibers.[22] In the bag_2 fiber the condition is dM in region A, changing to M at level A/B.[22,23] Recent observations by us suggest that the transition from M to dM is from a five- to a four-line substructure, the middle line being lost.

In the equatorial region the spaces in between the myonuclei in the myotube regions and nuclear chains are full of sarcoplasm that contains many small mitochondria, ribosomes, Golgi complexes, and occasional lipoid droplets. Corvaja, Marinozzi, and Pompeiano[31] describe how two or three chain fibers (presumably typical chains) may share the same endomysial envelope in the equatorial and juxtaequatorial regions. Here and there they become enclosed within a common basal lamina and *zonulae adhaerentes* form between their closely apposed surfaces. Microladders occasionally occur in both bag and chain fibers, usually situated near the surface in the sarcoplasm underneath axon terminals.

According to the descriptions of Cooper and Gladden[32] and Gladden,[24] elastic fibers are most numerous around the bag_2 fiber and anchor the spindle at each end to the elastic-fiber network among extrafusal muscle fibers. In passing through the spindle, they travel alongside muscle fibers, or within intercellular spaces in the axial sheath, or between layers of the capsule. Recent observations by Banks[32a] have revealed that the bag fibers have peglike projections on their surface over a length of 300 to 400 μm on either side of the primary region (see Fig. 10-3). Each projection slants toward the equator and appears to serve as an anchoring point for an elastic fiber originating from the opposite pole. Such attachments must greatly enhance the elastic properties of the primary region.

Histochemistry

The histochemical profiles of intrafusal muscle fibers are similar to those of extrafusal ones in that they vary according to fiber type but dissimilar in that they are subject to regional variation. The three fiber types—bag_1, bag_2, and chain—differ in their glycogen content and in their profiles of the enzymes myofibrillar adenosine triphosphatase (ATPase*), phosphorylase, and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR),[20,22,33] among others.

The technique most favored for demonstrating fiber type is that which stains ATPase, as it enables the types to be checked against the different staining reactions that follow alkaline or acid preincubation of the sections. As Kucera[33] has demonstrated, the profiles of the bag fibers show regional variation, but not those of the chains, and the optimum level for distinguishing between fiber types is mid-region B (see Fig. 10-4). In transverse sections cut at this level the staining intensities for alkali-stable ATPase are low for bag_1, medium or high for bag_2, and high for chains, whereas the staining for acid-stable ATPase is low for bag_1, high for bag_2, and low for chains. The three subtypes of chain fiber can be distinguished histochemically by staining for NADH-TR activity, which either increases (intermediate and typical chains) or decreases (long chain) from equator to pole.[33]

The enzyme profiles of intrafusal muscle fiber types are similar, but not identical, to those of extrafusal ones. In terms of the extrafusal muscle fiber types defined by Ariano, Armstrong, and Edgerton[34] the nearest approximation would regard the bag_1 fibers as slow oxidative (SO) and the bag_2 and chain fibers as falling within the fast glycolytic (FG) and fast oxidative-glycolytic (FOG) types. In this context it is relevant to note that dynamic β axons, which preferentially innervate bag_1 fibers, are branches of axons that innervate SO extrafusal muscle fibers[5,35] (Fig. 10-7*D*) and that static β axons, which preferentially innervate long chain fibers, are branches of axons that innervate FG or FOG extrafusal muscle fibers.[36–38]

Development

Electron microscopic investigations of spindle development in rat,[39,40] mouse,[41] and cat[42,43] have revealed close parallels between intrafusal and extrafusal myogenesis. Both involve the sequential production of myotubes following a stage before innervation when myoblasts fuse to form primary myotubes in the muscle primordium.

The generation of secondary extrafusal myotubes is dependent on innervation and the electrical or

*A list of the abbreviations used in this chapter is given at the end of the chapter.

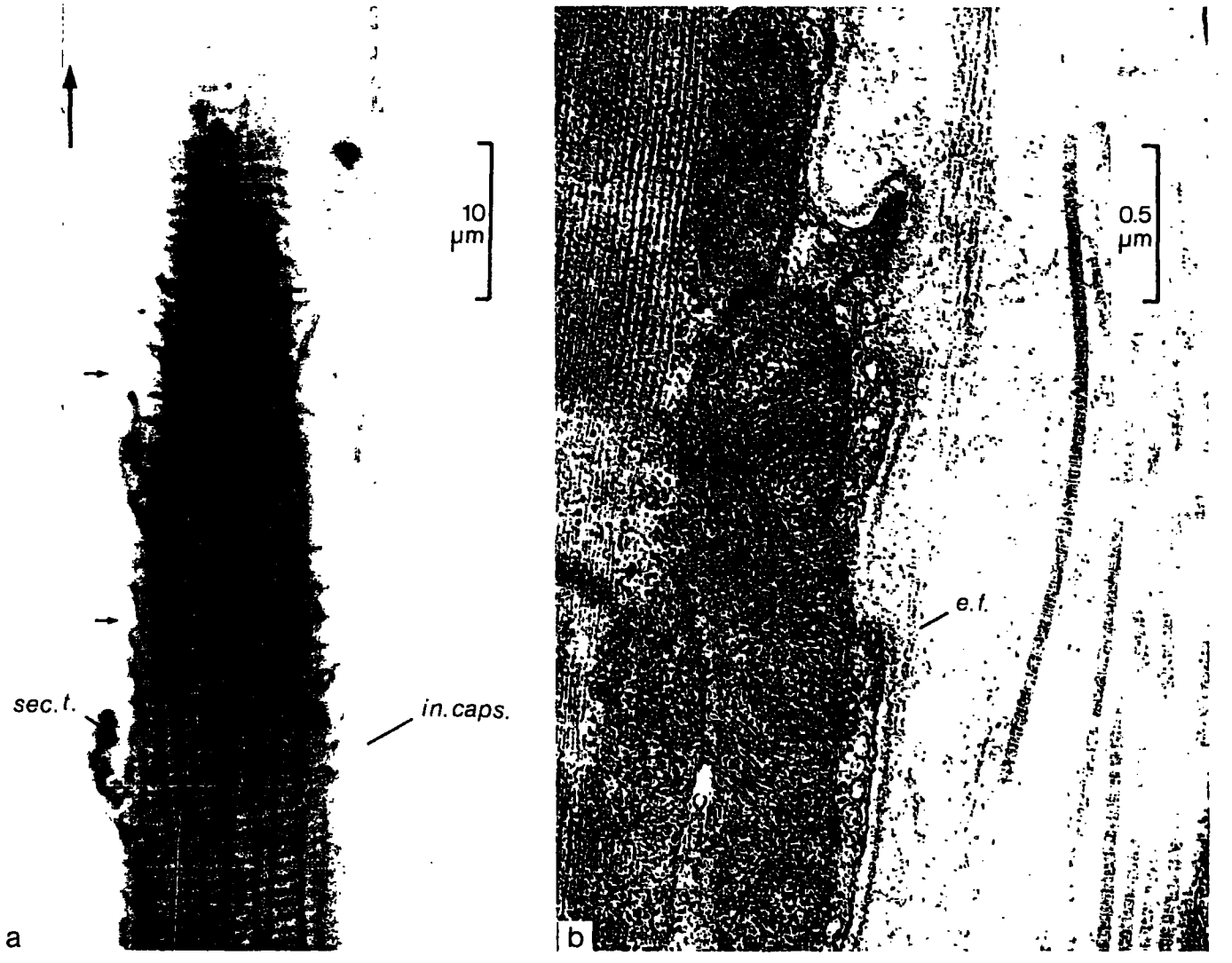


Figure 10-3. The attachment of elastic fibers in the juxtaequatorial region of nuclear-bag fibers is illustrated by longitudinal sections through the S_1 region of the bag_1 fiber of a cat tenuissimus spindle. *A*. Toluidine blue-stained, 1-μm-thick plastic section shows small projections on the muscle fiber surface, many pointing in the direction (large arrow) of the primary ending. The straight faintly stained fibers (small arrows) are elastic. sec.t. = terminal of secondary ending; in.caps. = inner capsule. *B*. Electron micrograph showing attachment of an elastic fiber (e.f.) to the basal lamina of the bag_1 fiber. The surface of the muscle fiber seems to be drawn out into small projections (such as those seen in (*A*) by the tension in the elastic fiber. At higher magnification a slight thickening of the plasmalemma is noticeable at these attachment sites.

contractile activity of the muscle.[44] They assemble and develop in close apposition to primary myotubes so as to form multicellular muscle clusters from which they subsequently separate, each within its own basal lamina, to form independent muscle fibers.[45–48] According to Milburn,[43] in cat peroneal muscle the sequential process of assembly, maturation, and separation occurs so as to produce successive series of secondary myotubes beginning with the separation of the first series at the 38 to 41 days fetal (df) stage. Meanwhile, subsequent series have begun to assemble in association with primary myotubes, and eventually these also separate, so that by birth (60 to 63 df) primary and secondary muscle fibers are distinguishable only where their separation from fetal muscle clusters is incomplete. Milburn regards the pattern of spindle development as fundamentally similar to that of extrafusal fascicles and correlates the two processes as shown in Fig. 10-5. Her account of the development of cat spindles in peroneal muscles may be summarized as follows (see also Reference 48a).

By the 34 to 38 df stage, motor and sensory axons have grown into the peroneal muscle primordium; primary myotubes have begun to receive the terminals of α motor axons; and secondary extrafusal myotubes have begun to assemble. Those primary myotubes that receive terminals from Ia afferents

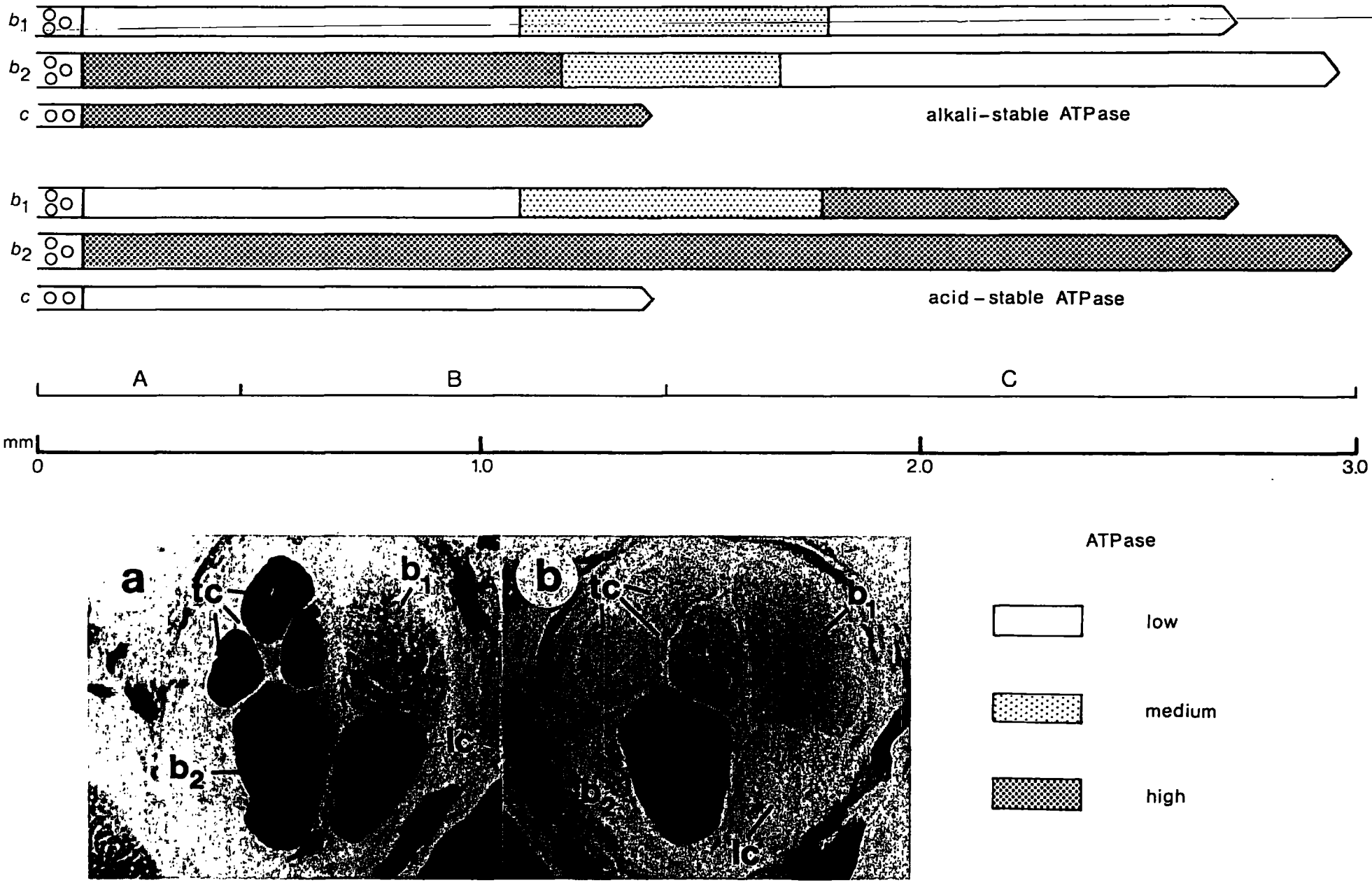


Figure 10-4. Schematic representation of the usual staining profiles of bag_1 (b_1), bag_2 (b_2), and chain (c) fibers in cat tenuissimus spindles reacted for alkali-stable and acid-stable ATPase. Scale indicates distance from the equator for a spindle pole of average length subdivided into regions A, B, and C. Photos illustrate 8-μm-thick transverse sections through region B of the same spindle stained for alkali-stable ATPase (*A*) and acid-stable ATPase (*B*). The intrafusal bundle comprises one bag_1 (b_1) fiber, one bag_2 (b_2) fiber, three typical chain (tc) fibers and one long chain (lc) fiber. *(Modified from Kucera J, Histochemistry 73:397, 1981.)*

will ultimately become intrafusal bag_2 muscle fibers. The effects of this initial contact are to produce an accumulation of myonuclei beneath the sensory terminals and to stimulate the formation of a thin capsule that isolates the sensory region from neighboring extrafusal myotubes. Simple motor terminals are also present at this early stage, indicating that both Ia and motor axons arrive at the site of spindle formation together.

These pioneering axons initiate a sequential generation of secondary intrafusal myotubes, which develop in association with the primary myotube within a common basal lamina. Their assembly begins beneath the Ia terminals which are in contact with the outer surface of the developing intrafusal bundle, and spreads toward the poles. As development proceeds, the successive myotubes are shorter and thinner and contain fewer equatorial myonuclei, so that those formed last become the shortest and thinnest typical chain fibers in the mature spindle. At the 41 to 43 df stage the presumptive bag_1 myotube (often accompanied by the long chain myotube when present) separates from the primary bag_2 myotube and acquires individual Ia terminals. At the same time most of the nuclear-chain myotubes assemble exclusively in association with the primary bag_2 myotube from which they subsequently separate, often as a group. Sensory cross-terminals between bag_2 and chain myotubes are thereby lost but are retained between chain fibers in the mature spindle.[49]

Separation of the developing myotubes begins in the poles and spreads toward the equator, where the influence of the Ia afferent renders it incomplete. During separation, the Ia innervation is remodeled and the mature form of the primary ending is established. This probably gives rise to the postnatal increase in size of the nuclear bags noted by Maier and Eldred.[50] As the intrafusal bundle develops, the capsule increases in girth and length, and the motor innervation increases in complexity. By birth, both plate and trail terminals are present. The periaxial space develops during the first two postnatal weeks.

The intrafusal muscle fibers thus develop in the order bag_2, bag_1, long chain, intermediate chain, typical chains; and their equatorial position in the mature spindle[23,27] reflects the pattern of their assembly and separation. According to Milburn[43] spindle units that are linked in tandem (see Types of Spindle Unit, below) develop as a consequence of the synchronized multiple innervation of a single primary myotube by two or more Ia afferents. Each encapsulated sensory region then recruits its own population of secondary intrafusal myotubes, which either remains exclusive to its own capsule or becomes confluent with the intrafusal bundle of an adjacent spindle unit in a shared capsule. The absence of the bag_1 fiber from certain tandem spindle units (see Types of Spindle Unit) is seen as due to asynchrony in the time of arrival of separate Ia afferents. That which arrives last innervates a primary bag_2 myotube already engaged in spindle development initiated by the first. The bag_1 fiber is excluded from the second unit because its polar separation from the primary bag_2 myotube has already occurred (see Fig. 10-5).

CAPSULE AND VASCULAR SUPPLY

The *capsule* is a lamellated structure that encloses the sensory innervation within a fusiform dilation and extends as a sleeve on each side to enclose part of each pole. The width of tenuissimus spindle capsules at the equator is 100 to 150 μm where their walls are 10 to 15 μm thick; their length usually falls within 2.0 to 4.0 mm and varies according to the number of sensory endings present.[17]

The capsule lamellae are composed of layers of thin flat cells arranged in concentric tubular fashion alternating with layers filled with collagenous fibrils. Each *capsular sheet cell*[51] is surrounded by a basal lamina and closely interdigitates with its neighbor to form a continuous layer one cell thick; regions of intimate contact between the cells have been described as *zonulae adhaerentes*.[52] The outermost capsule layer is composed of thick collagenous fibrils and scattered fibrocytes. The innermost layer is composed of a lining of fibrocytes, some of which cross the periaxial space to join other cells of the same type that form the axial sheath and the endomysial enclosures of the intrafusal muscle fibers.

The capsular sheet cells are continuous with the cells that form the perineurium of the spindle nerve and, according to Low,[53] may confidently be equated with them. He regards the capsule as a modified extension of the perineurium and connective tissue sheaths (epineurium, endoneurium) that enclose the spindle nerve, and we agree with this interpretation. The endoneurial connective tissue space in the spindle nerve is thus continuous with the periaxial space within the capsule, which is in turn continuous with the connective tissue space outside the spindle via the open end of each capsule sleeve.

Tight junctions between capsular sheet cells[54] act as a barrier to the diffusion of substances into the

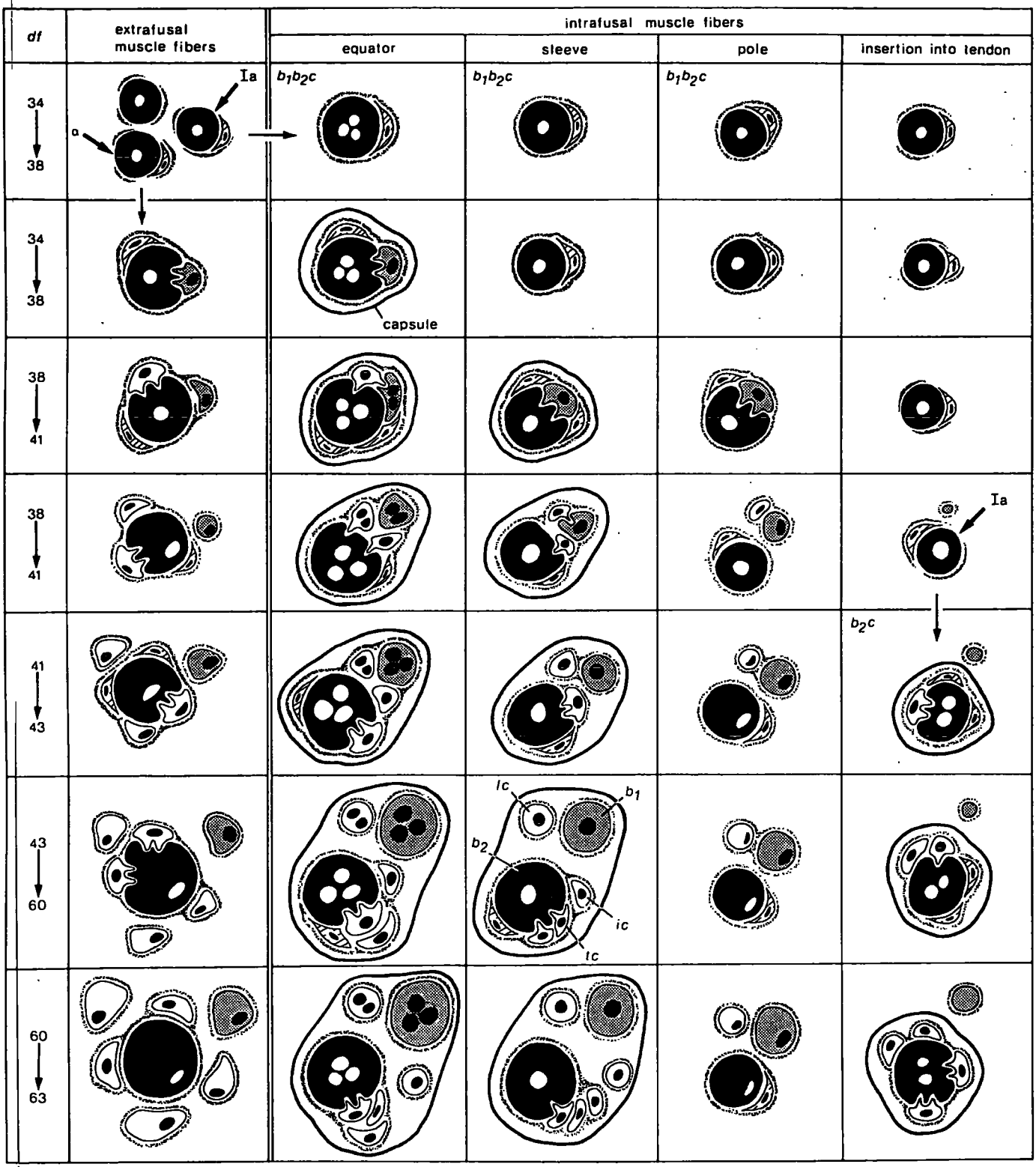


Figure 10-5. Schematic diagrams of transverse sections of developing extrafusal and intrafusal muscle fibers in cat peroneal muscles. In the extrafusal fascicle note how the first-series secondary myotube (stippled) separates from the primary myotube (black), acquiring its own basal lamina (stippled halo) before the assembly of subsequent series of secondary myotubes (white). The thin fusiform cells (hatched) are myoblasts. The diagrams of intrafusal muscle fibers show how myotube assembly begins at the equator and spreads to the poles, in contrast to their maturation and separation, which begins at the poles and spreads to the equator. The column headed Insertion into Tendon shows the later arrival of a b_2c Ia axon as compared with a b_1b_2c Ia axon; it is shown innervating the primary bag_2 myotube of a developing spindle and initiating the subsequent development of a b_2c spindle unit (see types of spindle unit under Intrafusal Muscle Fibers, above) of a tandem spindle. α = alpha motoneuron; b_1 = bag_1 fiber; b_2 = bag_2 fiber; b_1b_2c = developing b_1b_2c spindle unit; b_2c = developing b_2c spindle unit; c = chain fiber; df = days fetal; ic = intermediate chain fiber; lc = long chain fiber; tc = typical chain fiber; Ia = Ia axon. *(Milburn A, J Embryol Exp Morph 82:177, 1984.)*

perixial space, in the same way that the perineurium acts as a diffusion barrier in peripheral nerves. Such junctions are presumably located in the inner layers of the capsule, since horseradish peroxidase (HRP) flooded directly onto the living spindle penetrates the outer layers but fails to enter the periaxial space.[55] After systemic injection of HRP, Dow, Shinn, and Ovalle[56] showed that, whereas passive flow of the tracer into the periaxial space was prevented by the capsular cell tight junctions, there was some leakage into the poles through the open end of each capsule sleeve. The small amount of HRP actively transported across the capsular cells via cytoplasmic vesicles was phagocytosed by fibrocytes in the axial sheath.

The periaxial space is full of a highly viscous fluid, probably a thin mucopolysaccharide gel,[57] which may contain macrophages. Following long-term deafferentation the space disappears or is greatly diminished.[58] The origin of the periaxial fluid and its functions are uncertain. It may be that it is secreted by the sensory terminals as a contribution to their own metabolic insulation and mechanical protection.

Capillaries course for long distances between capsule layers; they are invariably present in the periaxial space of rabbit spindles[59] but only occasionally so in those of cat. In rabbit tenuissimus spindles Miyoshi and Kennedy[60] have shown that there is a short direct pathway from the main muscle artery to the spindle capillaries, which are separate from those supplying extrafusal muscle and different from them in being larger and having intercellular tight junctions. The capillaries supplying intramuscular nerve are similar, and HRP injected intraaortically does not leak from either nerve or spindle capillaries, whereas it leaks rapidly from those supplying extrafusal muscle.[55] A blood/nervous-system barrier, therefore, obtains in both endoneurial and periaxial spaces.

TYPES OF SPINDLE UNIT

Spindles occur singly or may be variously combined in groups or intimately associated with tendon organs. The functional significance of the single encapsulated receptor with its sensory and motor innervation is sometimes stressed by using the term *spindle unit*. Spindle units may be linked in series as *tandem spindles*[61] or combined together in pairs in which the intrafusal bundles either remain separately encapsulated or equatorially share a common capsule. Richmond and her colleagues[62–64] have shown that in cat neck and intervertebral muscles many spindle units are linked together in tandem and compound fashion to form *spindle complexes*, a type of organization so far previously observed only in the frog's extensor digitorum longus IV muscle.[65,66]

The standard spindle unit is provided with one bag_1 fiber, one bag_2 fiber, and about half a dozen typical chain fibers. Variations of intrafusal complement occur with respect to the bag fibers and subtypes of chain fiber. Some spindle units have three, rarely four, bag fibers. In a sample of spindles from various hindlimb muscles we identified the extra bag fibers from details of their sensory innervation as usually being bag_1.[29] However, in a histochemical study of tenuissimus spindles Kucera[66a] found that extra bag_1 or bag_2 fibers were about equally common and that, more rarely, some were of mixed type.

Absence of the bag_1 fiber occurs in certain tandem spindle units. In the early sixties Barker and Ip[67] observed that the most common tandem spindle in hindlimb muscles consists of a large and a small capsule linked together by a single bag fiber, the small capsule being supplied with a markedly irregular primary ending. Later studies of the sensory innervation of hindlimb spindles,[29,68] and the ATPase profiles of spindles in neck muscles,[63] established that the continuous bag fiber in such linkages is a bag_2 fiber and that it is the only bag fiber present in the small capsule. Here it is accompanied by a few typical chain fibers and usually has a single row of myonuclei instead of a nuclear bag. These *one-bag spindles*,[63] or b_2c spindle units,[29] typically insert into tendon. Their frequency among spindle units sampled by Banks et al.[29] from various hindlimb muscles was 23.8 percent in extensor digitorum longus, 23 percent in peroneus brevis, and 6 to 11 percent in the rest. In neck muscles Bakker and Richmond[63] have found much higher frequencies of 45 percent in complexus and biventer cervicis and 33 percent in splenius.

In one of Kucera's studies[27] of tenuissimus spindles, long chain fibers occurred in 13.3 percent of spindle poles ($n = 430$), intermediate chain fibers in 21.6 percent ($n = 333$). The frequencies of occurrence of these subtypes of chain fiber will presumably prove to be highest in muscles whose spindles are known to have a high degree of β static innervation, e.g., peroneus tertius.[5]

NUMBER AND DISTRIBUTION

The spindle density of a muscle is expressed in terms of the number of spindles per gram of the mean weight of the adult muscle. Densities based

on counts of spindle capsules are the most accurate and may also be regarded as estimates of Ia connections per gram. Generally speaking, high spindle densities characterize muscles initiating fine movement (e.g., interossei) or maintaining posture (e.g., soleus); low densities characterize those initiating gross movements (e.g., gastrocnemius). Thus among cat muscles, the forelimb fifth interosseus has a spindle capsule density of 119, soleus 23, and lateral gastrocnemius 5.[17] Slow muscles generally have higher spindle densities than fast, and spindle density is usually high in muscles that have many of their spindle units linked together in tandem or compound fashion. Thus among cat neck muscles occipitoscapularis, with mostly single spindle units, has a spindle density of 16, whereas complexus, with a high proportion of spindle complexes, has a spindle density of 88.[62] In the cat intervertebral centrotransverse muscle, Bakker and Richmond[64] found that low weight, combined with frequencies of spindle complexes as high as 79 percent, gave a spindle density of 328, by far the highest at present recorded in mammalian muscles.

In many muscles the spindles are distributed without restriction in close association with the nerve supply, but in others they are confined to certain regions because of various features of muscle design, as illustrated by the following examples. (1) In an extraocular muscle such as sheep superior rectus the spindles are necessarily confined to the orbital layer of nontwitch muscle fibers because these contribute to the composition of the receptors as bag fibers.[69] (2) Some fast hindlimb rat muscles, e.g., medial gastrocnemius and plantaris, consist of a core of mainly SO and FOG fibers surrounded by superficial fascicles of FG fibers. Spindle distribution is restricted to the core.[70] Similar core-restricted spindle distributions occur in certain cat neck muscles, e.g., splenius (fast) and occipitoscapularis (slow).[62,71] (3) Spindles in cat intervertebral and large neck muscles are distributed mostly in association with tendinous inscriptions and within compartments created by them.[62,64] In the centrotransverse muscle most spindles are distributed as spindle complexes in the ventrolateral region, where they are contained within a series of subsections, each with a different tendinous origin and insertion.[64,72] Some complexes span the whole intramuscular length as tightly associated chains of up to 12 spindle units that appear to be aligned so as to monitor specific lines of pull on the muscle.[64]

It is clear from the manner in which spindles develop (see Development, under Intrafusal Muscle Fibers, above) that when a primary myotube is a target for contact by either motor or Ia axons, it is a matter of chance as to whether it develops into an extrafusal muscle fiber or a bag_2 intrafusal muscle fiber. The pattern of spindle distribution within a muscle must therefore depend on developmental control of the distribution of Ia axons among intramuscular nerve trunks. Example 1 above is, therefore, presumably achieved by restricting the distribution of Ia axons to intramuscular nerve trunks that carry motor axons innervating nontwitch muscle fibers.

The presence of many spindle complexes in the cat complexus muscle, which raises the head, contrasts with the complete lack of them in occiptoscapularis, which rotates the scapula,[62] and is strikingly paralled in the frog by the presence of similar spindle complexes in the fourth toe extensor and their absence from the sartorius and pectoral cutaneous muscles.[66] What is the functional significance of such variations in composition of spindle populations in different muscles? Banks et al.[29] suggest that the high frequency of b_2c units in the spindle population of complexus provides the high primary static sensitivity required by the muscle's antigravity function. In superficial lumbrical muscles they found that a low frequency of b_2c units and secondary endings was associated with a high frequency of spindle units possessing more than one bag_1 fiber. They suggest that a high dynamic sensitivity will thus be achieved in the spindle population, and they relate this to the function that these muscles serve in carrying out finely adjusted movements of digits.

SENSORY INNERVATION

A study of the sensory innervation of cat hindlimb spindles published by Banks et al.[29] describes the form of the terminals and their distribution to bag_1, bag_2, and chain fibers as studied by reconstruction, electron microscopy, and examination of teased silver preparations. This is the first detailed account of spindle sensory innervation in terms of its distribution to the three types of intrafusal muscle fiber and, unless otherwise indicated, what follows is based upon it.

Primary Endings and Axons

The primary ending terminates on the densely nucleated equatorial parts of the intrafusal muscle fibers (the nuclear bags, myotubes, and nuclear chains) and occupies a length of about 350 μm. The ending is annulospiral in form,[73] and the terminals

consist of spirals, half rings, and a few complete rings. Spirals are more common and more extensive around chain fibers than bag fibers. The terminal systems supplied to bag fibers consist of a middle portion, in which the terminals are arranged mainly as regular transverse bands, and portions at each end, in which they are disposed as irregular forms in mainly diagonal and longitudinal configurations. Bag_1 terminal systems can be distinguished from those supplied to bag_2 fibers because they have more bands per unit length in the middle and more extensive irregular portions at each end (see Figs. 10-2 and 10-6*C*). Primary endings supplied to b_2c spindle units are mostly irregular in appearance (see Fig. 10-6*B*).

Reconstructions of two primary endings supplied to tenuissimus spindles showed that bag_1 terminal systems covered the largest amount of muscle fiber surface, the proportional distribution of the total terminal contact areas being: bag_1 fibers, 33 and 37 percent; bag_2 fibers, 25 and 24 percent; chain fibers, collectively 42 (five chains) and 39 (four chains) percent, individually 5 to 12 percent. The dense innervation of the bag_1 fiber is presumably adapted to generate a sufficiently large dynamic component in the receptor potential, as considerably more fibers (bag_2 and chain) are involved in the static input.

The reconstructions showed a close association between nucleation and innervation (Fig. 10-2), but no constant relation between number of myonuclei and terminal contact area. Sensory cross-terminals[49] occurred frequently in both endings. They were formed exclusively among chain fibers, a feature which, taken together with regions of close apposition between fibers of this type (see Ultrastructure, under Intrafusal Muscle Fibers, above), may be attributed to their incomplete separation during development (see Development, under Intrafusal Muscle Fibers). These features correlate with the fact that chain fibers frequently contract as a group on fusimotor stimulation[74] and thus produce an effect on the primary response that is sufficiently powerful to drive it at the frequency of their own contractions up to about 60 Hz under suitable conditions.[75]

Ultrastructural studies of primary axon terminals (reviewed by Barker[17]) have shown that they lie in shallow grooves on the surface of the muscle fibers forming smooth myoneural junctions. They are not covered by Schwann cells. In transverse sections they are cut into various shapes and may sometimes almost completely encircle a muscle fiber in a horseshoe configuration. The terminals contain neurofilaments, microtubules, glycogen granules, mitochondria, and a mixed population of vesicles. Degenerating mitochondria tend to accumulate toward the ends of the terminal systems. Dense-core, coated, and complex vesicles occur both in the terminals and in the sarcoplasm beneath them; the terminals also contain populations of large and small clear vesicles.[75a] It is probable that these pre- and postjunctional vesicles are engaged in neurosecretory processes that maintain the intrafusal muscle fibers and their equatorial nucleation.

The primary axons that supply b_1b_2c spindle units are generally thicker than those supplying b_2c spindle units (see Fig. 10-7*A*). The manner in which their first-order branches distribute terminals to the three types of intrafusal muscle fiber is either segregated (bag_1 fiber supplied separately from bag_2 and chain fibers, thereby resulting in separation of dynamic and static inputs) or mixed. Segregated distributions are usual in tenuissimus, mixed in the superficial lumbrical muscles, but in most hindlimb muscles sampled by us neither type of distribution predominated. Mixing was usually restricted to the dynamic input and resulted from the bag_1 fiber sharing a supply of terminals with a few chain fibers.

Quick, Kennedy, and Poppele[76] have shown that the sites for impulse generation in Ia axons are the heminodes, and some of the penultimate nodes, of the final branches. For Ia axons whose first-order branches have a segregated distribution there would presumably be separate dynamic and static pacemakers, and these have been demonstrated by Hulliger and Noth.[77] The spiking activity of the final branches of spindle afferents is presumably generated by receptor potentials produced by their terminals. The transduction mechanism is a matter for speculation (see Input-Output Conversion, under Physiology, below).

Secondary Endings and Axons

Secondary endings terminate on one or both sides of the primary. The most we have seen on one side is five, and on both sides six. Each occupies a length of about 350 μm and is designated S_1,S_2,S_3, and so on, according to its position relative to the primary.[29] Most secondaries terminate next to the primary in the S_1 position, and the most common type of sensory innervation among cat hindlimb spindles is for the primary to be accompanied by one S_1 secondary (Fig. 10-6*A*). Chain fibers are innervated in all secondary endings, but few secondaries are restricted to chain fibers only; most are distributed to all three fiber types. Restriction of terminals to one or two fiber types is more prevalent among secondaries terminating in the more polar positions.



Figure 10-6. Photographs and drawings traced from photographs of teased silver preparations illustrating features of the sensory innervation of spindles from cat hindlimb muscles. *A*. Sensory innervation of a b_1b_2c spindle unit from a superficial lumbrical muscle. The primary ending (P) is supplied by a Ia axon that has a mixed distribution, one first-order branch supplying the bag_1 and bag_2 fibers (b_1b_2 br.), the other supplying the bag_2 and chain fibers (b_2c br.). An S_1 secondary ending lies adjacent to it in the lower part of the figure. The equatorial dissociation of the b_1 fiber provides a clear view of its secondary innervation and primary terminal system (b_1 p.t.s.) with characteristic irregular terminals (i.t.). Asterisk denotes point where II axon divides to produce two first-order branches; the branch on the right gives rise to three preterminal axons, two of which travel downward to supply terminals to the b_1 and b_2 fibers, while the third

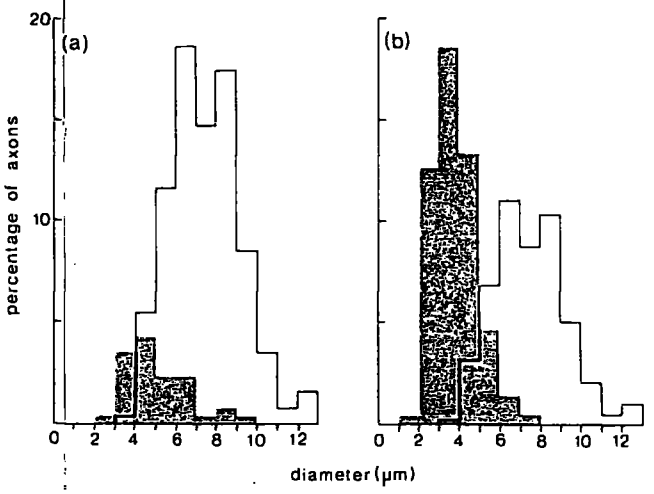


Figure 10-7. Histograms of fiber diameter for cat hind limb spindle afferents as measured in teased silver preparations. *A.* Comparison between the diameters of Ia axons supplying b_1b_2c spindle units (unfilled columns; $n = 213$) and b_2c spindle units (shaded columns; $n = 37$). *B.* Comparison between the diameters of Ia axons supplying b_1b_2, and c fibers (unfilled columns; $n = 213$) and II axons supplying b_1, b_2, and c fibers (shaded columns; $n = 224$). *(Modified from Banks RW et al, Philos Trans R Soc Lond 299:329, 1982.)*

These features are explained in terms of development as follows. Our data indicate that secondary axons are supplied to developing spindles in a random fashion, as the frequency distribution of 258 spindles that received different numbers of secondary endings was binomial. We suggest that the axons are led to the developing spindles by contact guidance, growing down paths already established by the Ia axons. Studies of development[42,43] show that at the time they arrive the separation of myotubes has started to spread from the poles to the equator. Thus the earliest secondary axon to reach any given spindle is most likely to terminate in the S_1 position on a bundle of interlocked myotubes and supply all three fiber types, whereas any that arrive later will be obliged to terminate in more polar positions where the myotubes have already become separate and the innervation of only one or two fiber types is more probable.

Secondary terminals supplied to chain fibers are annulospiral but generally thinner and more dispersed and irregular than those supplied to chain fibers in primary endings. When bag fibers are included in the innervation, their terminals are generally much more irregular and the bag_1 fiber usually receives fewer than the bag_2, often in the form of sprays. The ultrastructure of the terminals is very similar to that of primary terminals. Cross-terminals occur between chain fibers and also between chain fibers and both types of bag fiber.

The total terminal contact area of a reconstructed S_1 secondary ending was 32 percent less than that of the adjacent primary. The chain fibers received 75 percent (individually 16 to 22 percent), the bag_2 fiber 17 percent and the bag_1 8 percent. Using NADH-TR staining, Kucera[78] reports that long-chain fibers receive less secondary innervation than other types of chain. The secondary innervation of bag_1 fibers appears to be of little functional significance. Stimulation of dynamic fusimotor axons rarely increases the dynamic sensitivity of secondary endings[75,79]; the particularly high dynamic sensitivity of most secondary endings in peroneus tertius reported by Jami and Petit[80] is not matched by an unusually extensive secondary innervation of bag_1 fibers in this muscle.

Some hindlimb muscles have less secondary innervation than others. In superficial lumbrical muscles, for example, we found a Ia/II axon ratio of 1:1.2, as compared with 1:1.8 in peroneus longus. Secondary axons terminating as S_1 endings are generally thicker than those terminating in more polar positions. There is considerable overlap between the intramuscular diameters of Ia and II axons that innervate all three types of muscle fiber (see Fig. 10-7*B*).

MOTOR INNERVATION

Intrafusal Distribution of Motor Axons

Analysis of the intrafusal distribution of motor axons has proved to be one of the most difficult problems in studies of the mammalian spindle. Some of the difficulties have arisen because the methods used either were incapable of resolving

Figure 10-6. *(Continued)* travels upward to supply terminals to b_2 and c fibers. *B.* Sensory innervation comprising one primary and one S_1 secondary ending supplied to a b_2c spindle unit from peroneus brevis. The b_2 fiber lacked a nuclear bag and was accompanied by three c fibers. *C.* Primary terminals supplied to b_1 and b_2 fibers in a superficial lumbrical spindle. Note difference in spacing of the bands encircling the regular portions of the b_1 and b_2 systems and also the characteristically irregular portions of the b_1 system. Arrows indicate preterminal axons that have been cut but which in the ending ran on to supply terminals to c fibers. Asterisk indicates position of a sensory cross-terminal with a c fiber. *(Banks RW et al, Philos Trans R Soc Lond 299:329, 1982.)*

important details or led through artifact to false conclusions. Another difficulty has been that overlapping variability occurs between any categories so far described, and this allows for a theory to be supported by an unwittingly biased selection of results.

Retrospect (1962–1982). The problems began with the recognition that intrafusal muscle fibers were not a homogeneous group but instead consisted of two types, the bag and chain fibers. In 1962 Boyd[28] maintained that these were selectively innervated by γ axons that differed both in diameter and mode of termination, the bag fibers being supplied by thick (γ_1) axons with end plates, the chain fibers by thin (γ_2) axons terminating as *networks*. Boyd's observations were made on teased gold chloride preparations, and the axon diameters were measured close to the spindles. Although he illustrated several discrete γ_2 endings, Boyd chose not to describe them as end plates "to avoid confusion with the typical end-plates of γ_1 nerve fibers."

Meanwhile Barker and Cope,[81] on the basis of reconstructing a number of spindles from silver-stained, serial, transverse sections, confirmed that there were thick and thin γ axons at spindle entry, but they could find no correlation between axon diameter and type of muscle fiber innervated and concluded that all fusimotor axons terminated as plates. However, Boyd's dual model of the spindle catered admirably for the operation of the two functionally distinct types of γ axon that had just been established by Matthews,[2] and this model was generally accepted until well into the seventies.[82]

Histological evidence of separate groups of stem axons for the γ_1 and γ_2 innervation would have provided powerful support for the dual model, and Boyd and Davey[83] showed that there were candidates for these in some muscle nerves. But Adal and Barker[84] were able to show, in teased osmium preparations, that there was no correlation between the diameters of γ-stem axons and their terminal branches, "no more than exists between the thickness of the twigs of a tree and the girth of its trunk."[85] Nevertheless, the concept of two types of fusimotor innervation derived from separate γ axons persisted and continued to be supported by physiological evidence. An important consequence of the method used by Adal and Barker[84] was the histological demonstration of a "mixed" skeletofusimotor (now called β) innervation for which convincing physiological evidence had recently been provided by Bessou, Emonet-Dénand, and Laporte.[3] As osmium stains only myelin, no information was obtained on the form and location of the intrafusal β terminals.

Barker and his colleagues were now using teased silver preparations, and one of the first results obtained with this technique[86] was a description, in 1965, of the γ *trail* innervation,[8] so called because of the characteristically long distances traveled by the preterminal axons. This clearly corresponded with Boyd's[28] γ_2 network, but trail endings differed from γ_2 endings in not being connected together in a network and not being restricted to chain fibers but distributed to bag fibers, chain fibers, or both. In the following year Barker[9] reported that, in addition to the trail innervation, two types of end plate could be identified, which he designated as p_1 and p_2. The p_1 plate was sometimes seen to be supplied by a branch of a β axon and was described as similar in all respects to an extrafusal end plate; the p_2 plate was a much longer and more elaborate ending that lacked a nucleated sole plate and Doyère eminence. After nerve section, p_1 plates and extrafusal plates degenerated simultaneously, whereas p_2 plates and trail endings persisted for a further 24 to 36 h. The p_1 plates were seen more frequently on bag than on chain fibers, but the p_2 plates were described[10] as more or less equally distributed to both bag and chain fibers.

In a detailed account of these findings published in 1970, Barker, Stacey, and Adal[87] gave the bag/chain ratio of the plate innervation as p_1 75/25, p_2 90/10. Though bag fibers were occasionally seen to lack trail endings, it was concluded that the trail innervation was completely nonselective in its distribution and that most chain fibers received trail endings only. The axons that supplied p_1 plates were shown mostly to measure below 2.0 μm in diameter and to have a diameter range comparable to that of the terminal branches of skeletomotor axons, whereas those axons that supplied p_2 plates mostly measured above 2.0 μm in diameter and were among the largest motor axons to enter the spindle. The p_1 innervation proved to be widespread in the hindlimb muscles studies, rendering it improbable that the presence of a skeletofusimotor innervation in mammals was a vestigial feature as had been suggested.[3,88] Indeed, it was argued that the high frequency of the p_1 innervation found in flexor hallucis longus made it unlikely that it could all be supplied by the collaterals of slow-conducting skeletofusimotor axons and indicated that fast axons of this kind also contributed.

At this time the identification of γ static and γ dynamic axons with specific fusimotor axons innervating bag, chain, or both types of fiber was still

largely a matter for speculation. It seemed safe to identify β axons, which had been shown to have a dynamic action,[3] as those supplying the p_1 innervation; and if the case, based on circumstantial evidence, for identifying γ static axons with trail axons was accepted, then by inference γ dynamic axons were those that supplied the p_2 plates (see Barker[89] and Barker et al.[87]). According to Barker et al.,[87] the fact that the intrafusal distribution of motor axons was nonselective implied that an intrafusal muscle fiber, like an arthropod muscle fiber, could contract in different ways according to the type of motor ending initiating the contraction. This has proved not to be so, but at the time it seemed the most plausible way of reconciling nonselective innervation with the known physiology.

Boyd[88,90] and Matthews[82] accepted that there was some nonselectivity in the distribution of the trail innervation but did not consider it to be of any functional importance. Such a view became increasingly untenable in the light of convincing evidence obtained from various histophysiological experiments conducted in the early seventies. The first of these, carried out by Barker, Emonet-Dénand, Laporte, Proske, and Stacey,[91] entailed the isolation of single γ static axons innervating tenuissimus by cutting all other motor axons supplying the muscle and allowing a few days for them to degenerate. Subsequent examination of the terminals of the surviving static axon in teased silver preparations revealed that they were trail endings and that they were most frequently distributed to both bag and chain fibers. In another series of experiments on tenuissimus, carried out by Barker, Bessou, Jankowska, Pagès, and Stacey,[92] procion yellow dye was injected electrophoretically into intrafusal muscle fibers impaled by microelectrodes and activated by single γ static axons. The types of muscle fiber impaled were then determined by fluorescence and electron microscopy; they proved to be either bag or chain fibers. In one experiment, two muscle fibers activated by the same γ static axon were impaled and injected in one pole of a spindle; one proved to be a bag fiber, and the other a chain fiber (see Fig. 10-8C). Finally, Brown and Butler,[93] using the glycogen-depletion technique, showed that prolonged stimulation of single γ static axons depleted glycogen in both bag and chain fibers. This result led Brown to withdraw his previous suggestion[94] that the terminals on the bag fiber might not be functional.

Meanwhile the nonselectivity of γ static innervation was also being demonstrated in experiments that involved direct observation of the effects of fusimotor stimulation on intrafusal muscle fibers. Bessou and Pagès[95] found that of 38 γ static axons stimulated, 15 elicited visible contractions in bag fibers, 11 in both bag and chain fibers, and 12 in chain fibers only. In similar experiments Boyd, Gladden, McWilliam, and Ward[96] found that of eight γ static axons, four activated chain fibers only, whereas the rest produced "some movement in one of the nuclear bag fibers . . . in addition."

A crucial observation made by Bessou and Pagès[95] was that a γ static axon never activated the same bag fiber that could be activated by a γ dynamic axon. The significance of this important finding seems to have been largely unheeded at the time, perhaps partly because of the confusion there was then about identifying two types of bag fiber. There were also some reservations about the obvious difficulties of interpretation in the cinematographic analysis of intrafusal contractions in living spindles. For example, as late as 1975, Boyd and Ward[97] maintained that both bag fibers were activated together in 40 percent of the tenuissimus spindles observed by them, though a year later Boyd[74] withdrew this claim.

As the recognition of two types of bag fiber became firmly established in the mid-seventies on histological grounds,[21,22] controversy about their selective motor innervation replaced that which had concerned bag and chain fibers. It was now generally agreed that bag_1 fibers were responsible for dynamic actions and that bag_2 and chain fibers were responsible for static actions. This had become evident from more cinematographic analyses of intrafusal contractions[98,99,100] and further glycogen-depletion studies[101,102]

It was clear that in most spindles dynamic axons, γ or β, exclusively innervated the bag_1 fiber and that such axons seldom supplied other types of fiber. In tenuissimus the combined results of glycogen-depletion[102] and procion-yellow[103] experiments indicated that γ dynamic axons activated bag_2 or long chain fibers, in addition to the bag_1 fiber, in about one in every four or five spindles. Glycogen-depletion experiments on β dynamic axons[35] showed that these had a very similar distribution (see Fig. 10-8*D*).

What was not clear was whether the bag_1 fiber could also be activated by γ static axons. Glycogen-depletion studies indicated that there was such activation, the frequency of bag_1 depletions produced by stimulating single γ static axons being about 50 percent in tenuissimus.[93,102] Moreover, experiments by Emonet-Dénand, Laporte, Matthews, and Petit,[104] in which they surveyed the effects on the primary-ending response of stimulating single γ

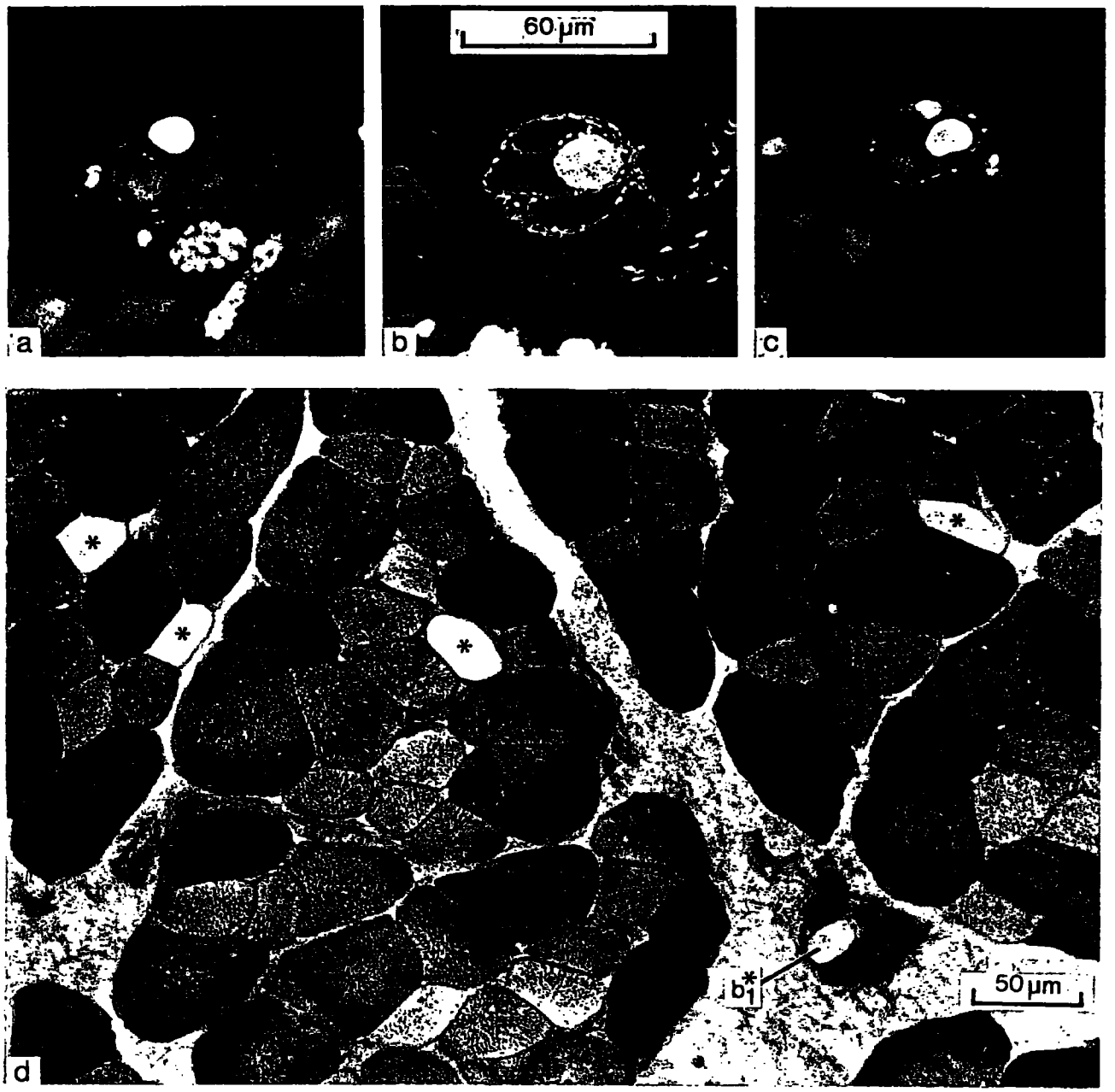


Figure 10-8. *A–C.* Transverse sections (10–15 μm thick) of cat tenuissimus spindles showing fluorescence of muscle fibers injected with procion yellow following recording of their membrane potentials on activation by single static γ axons. In (*A*) the muscle fiber is a chain fiber from which an action potential was recorded; in (*B*) it is a bag_2 fiber from which a junctional potential was recorded. In (C) two fibers that were activated by the same static γ axon were impaled and injected; the large fiber is a bag_2 fiber from which a junctional potential was recorded, whereas the small fiber is a chain fiber from which an action potential was recorded. *(Barker D et al, C R Acad Sci (Paris) 275:2527, 1972.) D.* A 10-μm frozen transverse section of a cat peroneus brevis muscle stained for glycogen (PAS method) following repetitive stimulation of a single dynamic β axon (conduction velocity 77 m/s) to produce glycogen depletion. Asterisks indicate depleted extrafusal muscle fibers and a depleted bag_1 fiber in the muscle spindle in the lower right quadrant. Histochemical tests applied to adjacent sections showed that the extrafusal muscle fibers were of the slow oxidative type. *(Barker D et al, J Physiol 266:713, 1977.)*

axons during stretch, lent support to this conclusion. They recognized categories of response intermediate between "pure" static and "pure" dynamic and showed that these could be mimicked by mixing the effects produced when stimulating two γ axons supplying the same spindle, one purely static and the other purely dynamic in action. They concluded that 33 percent of the responses from peroneus brevis spindles to single γ axon stimulation were produced by an admixture, in various proportions, of static and dynamic actions. Though less than might be expected from the frequency of the

glycogen depletion of bag_1 fibers by γ static stimulation in tenuissimus, this compared well with the 38 percent of such depletions subsequently found among peroneus brevis spindles by Emonet-Dénand, Jami, Laporte, and Tankov.[105] Nevertheless the matter remained in doubt because dynamic bag fibers could not be seen to contract in response to γ static stimulation.[98,100]

A direct histological approach was once more required. Since γ static axons terminate as trail endings,[91] were trail endings to be found on bag_1 fibers? The glycogen-depletion results[102] for tenuissimus suggested that they should be and that virtually every bag_1 fiber should receive them. However, reconstructions of 3½ tenuissimus spindles made by Banks[106,107] from serial 1-μm-thick transverse sections did not reveal a single example of an ending or supplying axon that was both typical of trail innervation and distributed to a bag_1 fiber (Fig. 10-9). Such reconstructions could feasibly be made of only a few spindles, but fortunately they revealed consistent features of sensory innervation that enabled bag_1 and bag_2 fibers to be identified with confidence in larger samples of teased silver preparations. The frequency of bag_1 trail innervation in such preparations was found by Barker and Stacey[108] to be 8 percent in tenuissimus and 17 percent in peroneus brevis, much less than the glycogen-depletion results had indicated. These results indicate that innervation by γ static axons, as by γ dynamic axons, is only occasionally nonselective. This implies that the variability of the static response is largely the result of the activation of bag_2 and chain fibers, either on their own or in various combinations in one or both poles of a spindle, with the additional activation of the bag_1 fiber making only an occasional contribution.

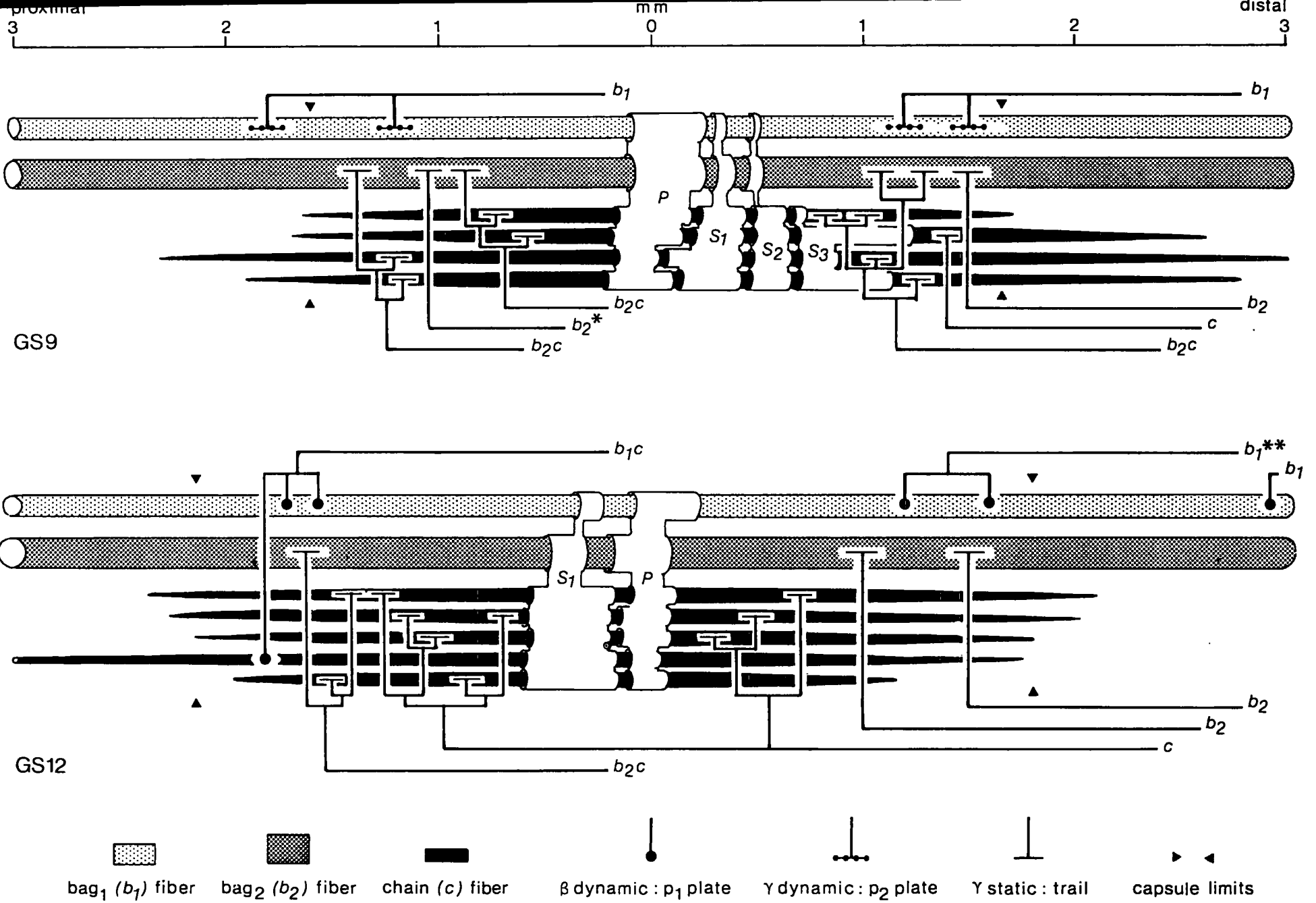
It is clear that the glycogen-depletion technique produces some activation of the bag_1 fiber that is nonneural; this could either be a stretch-induced contraction,[109,109a] or some physicochemical factor, e.g., accumulation of potassium ions liberated from contracting bag_2 and chain fibers.[108,108a] With this exception, it has proved an excellent method for mapping the intrafusal distribution of motor axons. This was illustrated, for example, when it became evident that fast β axons (above 85 m/s) were involved as well as slow (40 to 85 m/s),[36] as had been predicted from studying teased silver preparations[87] (discussed earlier in this section). Glycogen-depletion studies showed that fast β axons selectively deplete long chain fibers,[36] are static in action,[5,38] and are seldom supplied to the same spindle as slow β axons.[5,37] The existence of β static axons had earlier been revealed in rabbit[4] and rat[110] spindles. In rabbit spindles the disposition of the p_1 innervation[19] suggests that they selectively activate the bag_2 fiber.

The present picture. It is useful to summarize the present view of how motor axons are "wired up" in a typical cat hind limb spindle (see Fig. 10-1*E* and *F*). Inevitably the view is based on tenuissimus because most is known about spindles in this muscle. The following is derived from data obtained by serial transverse-section analysis[106,107,111] and chronic degeneration experiments.[91]

A tenuissimus spindle usually contains one bag_1 fiber, one bag_2 fiber, and four typical chain fibers. Each bag-fiber pole receives two to three motor endings, whereas each chain-fiber pole receives one. The endings are located mostly on the polar half of each fiber nearest to the primary ending, except within 0.3 mm from the equator (Fig. 10-10). Endings on the bag fibers may occur anywhere within this region, whereas those on the chains tend to be midpolar. Each pole of the bag_1 is innervated by a single axon, β or γ (only one in four axons innervating bag_1 fibers supply both poles); and each pole of the bag_2 fiber is innervated by two to three axons, at least one of which also innervates chain fibers. The chain fibers in each pole are supplied by two axons, each chain-fiber ending being supplied by an axon that also innervates other intrafusal muscle fibers. About one in four γ static axons operates both poles.

Of the branches of γ static axons entering spindle poles in our sample, half supplied bag_2 and chain fibers, a quarter chain fibers only, and a quarter bag_2 fibers only. As the motor unit of each γ static axon includes both bag_2 and chain fibers, the activation of a bag_2 fiber alone in one spindle is accompanied by chain-fiber activity in others. Motor axons entering spindle poles to supply bag_1 and either or both the other fiber types are rare. In our total sample of 65 axons only 3 (5 percent) did so.

Based on the above data, the fusimotor innervation ratio for tenuissimus may be calculated as follows. We may assume a spindle content of 15[112] supplied by 21 γ axons[83] of which, on the basis of a γ static/γ dynamic ratio of 6:1,[100] we may regard 18 as static and 3 dynamic. Assuming that the distribution of 75 endings to 30 bag_1-fiber poles is shared equally between γ dynamic and β dynamic axons, the 3 γ dynamic axons will innervate 15 bag_1-fiber poles, each supplying 12 to 13 endings to 5 bag_1-fiber poles. Similarly, as the 18 γ static axons will distribute 75 motor endings to 30 bag_2-fiber poles, and 1 ending to each of 120 chain-fiber poles, a single γ static axon will distribute 11 to 12 endings to 1.7 bag_2-fiber poles and 7 chain-fiber poles. Com-


proximal
distal
mm
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2
1
0
1
2
3
b_1
P
S_1
S_2
S_3
b_2c
b_2*
b_2
c
GS9
b_1c
b_1**
b_1
S_1
P
b_2
b_2
c
b_2c
GS12
bag$_1$ (b_1) fiber
bag$_2$ (b_2) fiber
chain (c) fiber
β dynamic : p$_1$ plate
γ dynamic : p$_2$ plate
γ static : trail
capsule limits

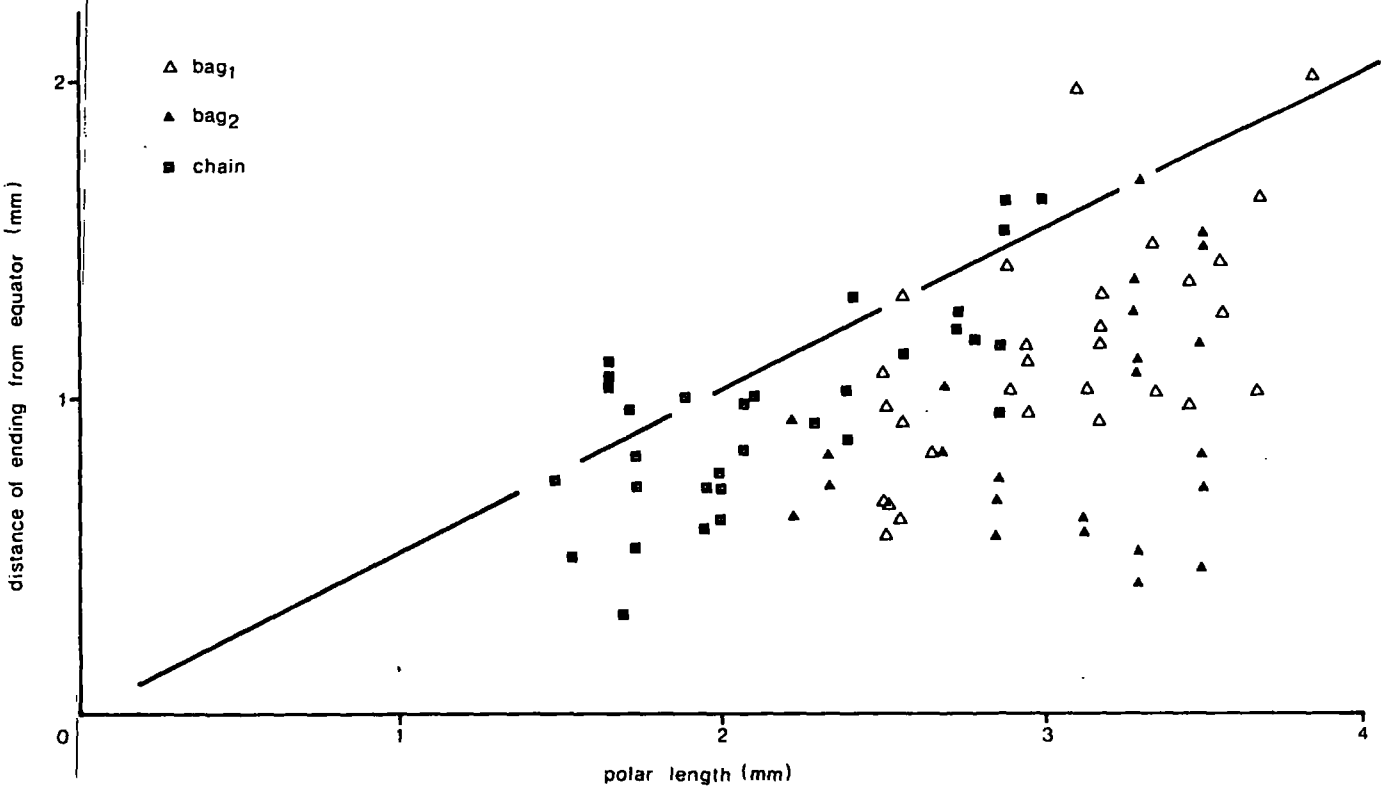


Figure 10-10. Scatter diagram showing locations of motor endings on bag_1, bag_2, and chain fibers. Each ending is represented by a symbol plotted as the distance of the ending from the equator against the length of the muscle fiber pole on which the ending occurred. Almost all the endings were located at sites less than half the polar length (shown by the sloping line) from the equator. Overlap between the endings on bag and chain fibers is probably exaggerated owing to different preparation techniques used. *(Data on bag fibers from Kucera J, Histochemistry 67:291, 1980; on chain fibers from Banks RW, J Anat 133:571, 1981.)*

pared with most skeletomotor innervation ratios these are remarkably low but are nevertheless in line with Adal and Barker's[84] results for the cat's 1st deep lumbrical muscle. They arrived at a ratio of 1 γ or β axon to 9 intrafusal-fiber poles and calculated the skeletomotor innervation ratio to be 1:300.

Since the numbers of spindles[113] and γ axons[88] are known for several hindlimb muscles, their fusimotor innervation ratios can be calculated on a similar basis, providing that probable figures are used for the average number of intrafusal fibers per spindle. Assuming this figure to be 6, as in tenuissimus, the number of intrafusal-fiber poles supplied by each γ axon in flexor digitorum longus, flexor hallucis longus, gastrocnemius medialis, semitendinosus, soleus, tibialis anterior, and tibialis posterior would range from 4 to 6 (average 5); the number for tenuissimus is 8.4. Assuming the figure to be 8, the number would range from 5.3 to 8.0 (average 6.7).

The main factor to vary in the motor innervation of spindles in different muscles is the amount of β innervation. As judged by the proportion of spindle poles innervated by axons terminating as p_1 plates, the frequency of β innervation is high in flexor hallucis longus (73 percent), peroneus tertius (59 percent), and soleus (56 percent), but low in peroneus longus (21 percent).[87] Estimates based on the physiological identification of β axons indicate that 50 percent of the spindles in peroneus tertius receive a β innervation and that one-third of the β axons supplied to this muscle are dynamic and two-thirds static.[5] In tenuissimus, a glycogen-depletion study[37] showed that β axons (about half dynamic, half static) supply at least 40 percent of the spindles. Some distal muscles of the rat's tail contain spindles that lack any γ innervation and are activated entirely by β axons, dynamic and static.[110,114]

Figure 10-9. Schematic representations of the innervation of two cat tenuissimus spindles reconstructed from serial 1-μm-thick transverse sections. Single asterisk in GS9 indicates uncertain identification of this motor axon and ending; double asterisk in GS12 indicates possibility that this axon may be dynamic γ rather than dynamic β as shown. *(Banks RW et al, in Taylor A, Prochazka A (eds): Muscle Receptors and Movement. London, Macmillan, 1981, pp 5–16.)*

The most recent work on the motor innervation of rat spindles[114a] has shown that the amount of nonselective innervation is much higher than in cat spindles. For example, a considerable number of axons innervate both types of bag fiber. This is a salutary warning against thinking of spindles entirely in terms of those in the cat's hindlimb.

The Form of Intrafusal Motor Endings

The classification of intrafusal motor endings into p_1 and p_2 plates and trail endings, described in detail by Barker et al. in 1970,[87] was based on an analysis of silver preparations that was mainly concerned with distinguishing differences in presynaptic features such as the location and form of the axon terminals and the diameter and mode of branching of the supplying axon. It was shown that the three categories could further be distinguished by their different rates of degeneration after nerve section; and chronic degeneration experiments, designed to isolate single surviving motor axons, later demonstrated that γ static axons terminated as trail endings[91] and β axons as p_1 plates[115] (see Fig. 10-11). This classification has proved to be valid and useful and has recently been revised and restated by ourselves in terms of endings distributed to three types of intrafusal muscle fiber and subtypes of chain fiber.[115a,115b]

When postsynaptic features are considered, intrafusal motor endings do not fall into categories that correspond with plate (p_1 and p_2) and trail endings. Barker et al.[87] initially maintained, on the contrary, that each type of ending could be distinguished ultrastructurally by its degree of postsynaptic folding, but further observations by Barker's group led them to discard this view and to suggest that postsynaptic folding might instead be related to muscle fiber type and distance from the equator.[23]

Recent studies by Barker et al.,[103] Kucera,[25,33,116,117] and Banks[107,118] provide evidence in support of this. Ultrastructural complexity, in terms of prominence of sole plate and degree of postsynaptic folding, tends to increase in endings on bag_1, bag_2, and chain fibers, respectively.[107] This gradation is matched by the cholinesterase (ChE) content of the endings, there being a parallel increase in the density and thickness of the ChE reaction product.[116] The factor of distance from the equator, or primary ending, appears to operate regardless of fiber type such that the greater the distance, the higher the ChE content of an ending[117] and the more deeply embedded it is into the surface of the muscle fiber.[118] It follows that the terminals of an ending supplied to a long chain fiber in region C should mostly be deeply embedded in a prominent sole plate that has considerable postsynaptic folding. Kucera[119] recently demonstrated this to be so, thus incidentally confirming the original description by Barker et al.[87] of the ultrastructure of a p_1 plate on chain fibers.

Into this scheme of things Boyd, Gladden, McWilliam, and Ward[100] and Arbuthnott, Ballard, Boyd, Gladden, and Sutherland[120] recently introduced the idea that there are, in fact, two types of γ static axon that preferentially innervate bag_2 fibers and chain fibers, respectively. The ultrastructure of the myoneural junctions formed by these axons is described,[120] and they are all called *plates*. Both types of axon are said to innervate chain fibers. One, mainly distributed to bag_2 fibers, supplies terminals that lie superficially on the muscle fiber surface as *m_a plates*, whereas the other, mainly distributed to chain fibers, supplies terminals deeply embedded in the muscle fiber surface as *m_c plates*. Our own studies[118,118a] have clearly demonstrated that the extent to which motor axon terminals are embedded in intrafusal muscle fibers increases with greater distance from the primary ending, irrespective of fiber type. The evidence put forward by Arbuthnott et al.[120] for the existence of the m_c plate rests mainly on the distribution of five such plates to typical chain fibers by a γ static axon in one pole of a tenuissimus spindle. One other such plate in another spindle is acknowledged by them to have been supplied by a γ dynamic axon, and two others in a further spindle are located on long chain fibers and were presumably supplied by a β static axon. We doubt whether this adds up to a valid case.

AUTONOMIC INNERVATION

Until recently it was widely held that skeletal muscle fibers are not innervated by sympathetic axons. It was acknowledged that such axons enter muscle spindles, but opinion was divided as to whether they supplied a vascular or nonvascular innervation. In 1981 Barker and Saito[11] demonstrated unequivocally that an autonomic innervation is distributed to some extrafusal muscle fibers and that it also has a nonvascular presence in some spindles. Earlier claims made in favor of an intrafusal autonomic innervation by Banker and Girvin[121] and Santini and Ibata[122] were thus substantiated and an observation by Ballard[123] confirmed.

Barker and Saito[11] made most of their observations on cat hindlimb muscles deprived of their somatic innervation by degenerative spinal-root section and then prepared for fluorescence microscopy, silver staining, or electron microscopy. They found

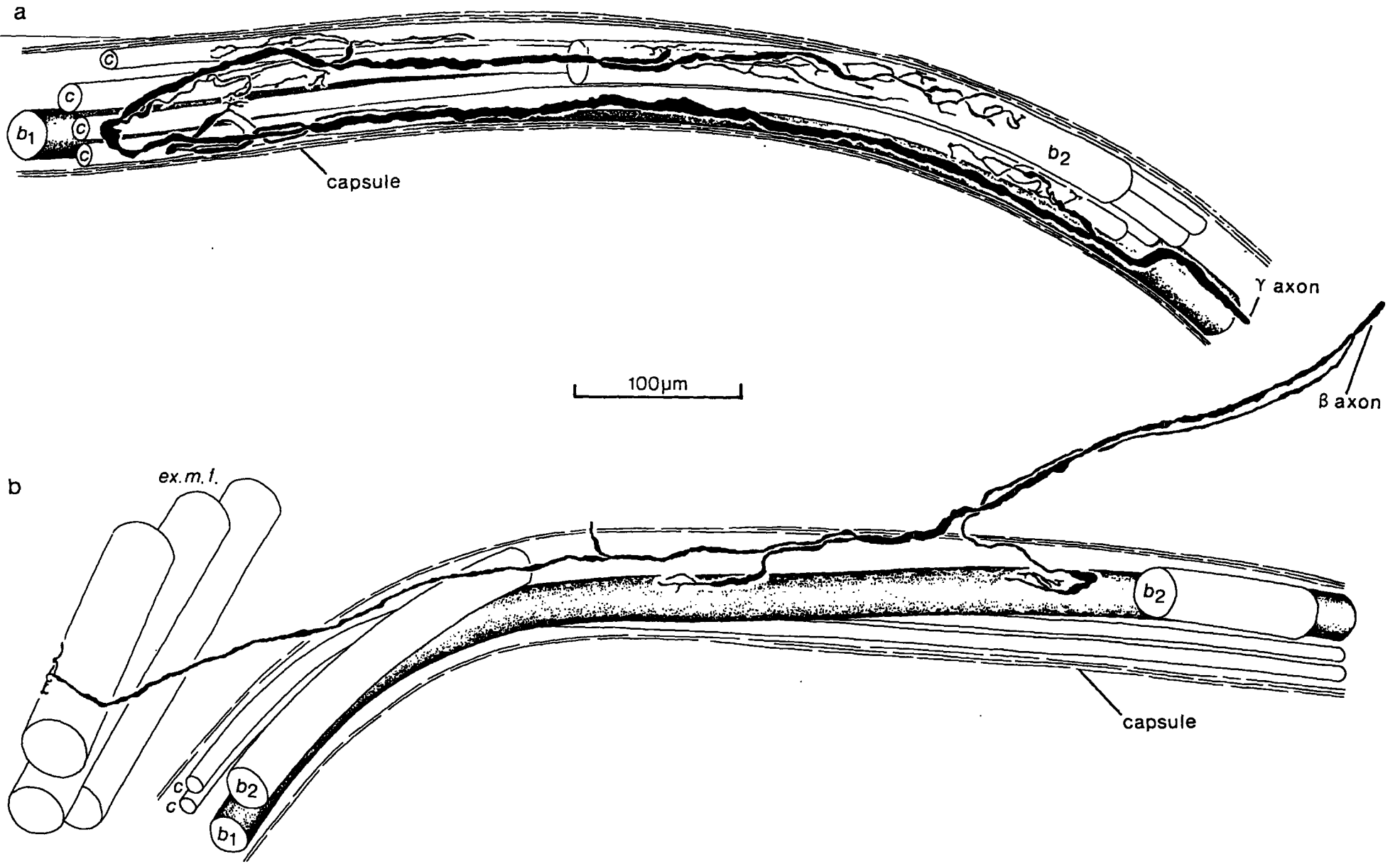


Figure 10-11. Drawings of teased silver preparations. *A.* Part of a pole of a cat tenuissimus spindle showing trail innervation supplied by a branch of the single static γ axon isolated in one of the chronic degeneration experiments performed by Barker, Emonet-Dénand, Laporte, Proske, and Stacey.[91] The axon innervates the bag_2 (b_2) fiber and four chain (c) fibers. All other motor axons supplying the muscle had previously been cut and allowed to degenerate for 168 h. *B.* Part of one pole of a cat spindle deprived of its γ innervation in one of the chronic degeneration experiments carried out by Barker, Emonet-Dénand, Laporte, and Stacey.[115] The spindle was teased from an abductor digiti quinti medius muscle innervated by five fast-conducting motor axons that had been left intact. One of these was the β axon shown supplying two p_1 plates to a bag_1 (b_1) fiber and one plate to an extrafusal muscle fiber (ex.m.f.). *(Drawing in (B) modified from Barker D et al, Brain Res 185:227, 1980.)*

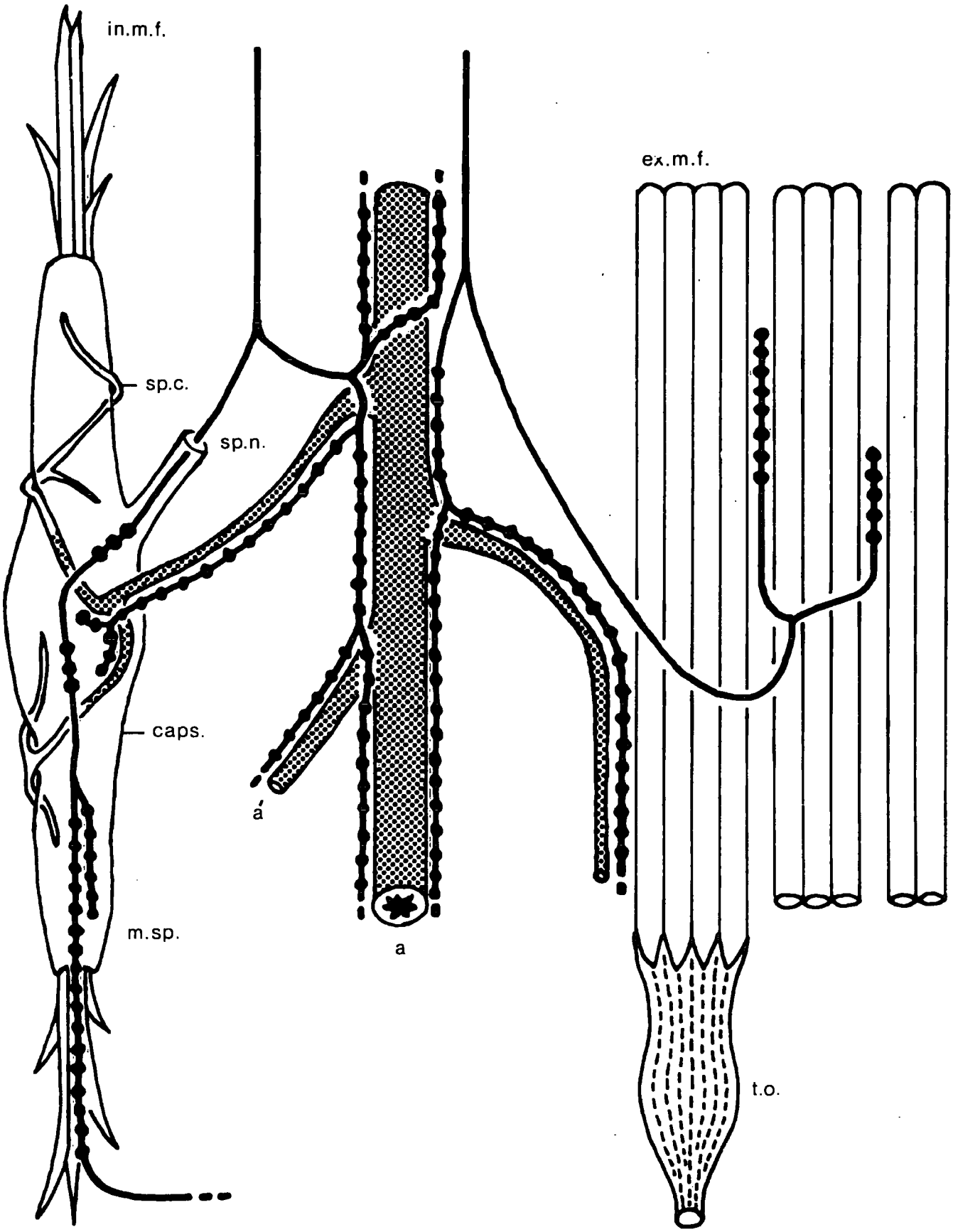


Figure 10-12. Schema of the noradrenergic autonomic innervation of cat skeletal muscle. The distribution of the varicosities of two noradrenergic axons is shown. Note varicosities among both intra- and extrafusal muscle fibers as well as those lying between arteriole and muscle fiber. a = artery; a′ = arteriole; caps. = capsule; ex.m.f. = extrafusal muscle fibers; in.m.f. = intrafusal muscle fibers; m.sp. = muscle spindle; sp.c. = spindle capillary; sp.n. = spindle nerve; t.o. = tendon organ. *(Barker D, Saito M, Proc R Soc Lond 212:317, 1981.)*

that autonomic axons were not supplied to all spindles; in the muscles sampled the proportion of spindles receiving autonomic axons was highest in the lumbricals (65 percent) and lowest in peroneus brevis (8 percent). They were absent from 18 tendon organs examined. Fluorescence microscopy revealed a noradrenergic innervation distributed to some spindles by axons supplied either through the spindle nerve or from nearby perivascular nerves, which they left to enter the equatorial and polar regions at various points. Branches of perivascular axons were also occasionally seen ending among extrafusal muscle fibers (Fig. 10-12). It was impossible to determine whether any noradrenergic axons were exclusively distributed to spindles or extrafusal muscle fibers.

On the basis of the vesicle content of varicosities examined by electron microscopy, the extrafusal innervation was identified as noradrenergic (32 axons traced) and the spindle innervation as involving noradrenergic, cholinergic, and nonadrenergic axons (14 traced). Varicosities were located within the capsule lamellae, inside the periaxial space, and in neuroeffective association with intrafusal muscle fibers (without preference for fiber type) in regions B and C.

Physiology

THE SPINDLE AS A RECEPTOR

Input-Output Properties

When a ramp-and-hold stretch is applied to an adapted, deefferented spindle, the primary ending fires a short, high-frequency burst of impulses at the start of the ramp, and this is followed by a more or less steady increase in the rate of firing until a peak is reached at the end of the ramp. During the held phase there is an initial adaptation, rapid at first, then slower, until a new maintained level of firing is reached. The relation between this static firing level and the extension of the muscle is approximately linear and has been called *length, or position, sensitivity*[82,124] (see Fig. 10-13). As the velocity of stretch increases, the frequency of the initial burst becomes higher and the slope of the response during the ramp becomes steeper, leading to higher peak rates at the end of the ramp. However, stretch velocity does not affect length sensitivity, so that the difference between the peak rate and the adapted rate at some arbitrary later time (0.5 s in the case of the widely used dynamic index) is some measure of the dynamic response of the ending. Thus for an ending with low dynamic sensitivity, or one in a muscle stretched at a low velocity, the slope of the dynamic response closely corresponds to the length sensitivity (indeed Boyd[75] uses the term length sensitivity to mean the slope of the dynamic response). All these features are recognizable in secondary endings, although the initial burst and dynamic response are usually much less well developed (see Fig. 10-14).

The effects of motor stimulation (β or γ) on the primary-ending response depend on the rate of stimulation and the type of muscle fiber activated (see Figs. 10-14 and 10-15). Stimulation of a dynamic axon activates the bag_1 fiber and produces an increase in the static firing level, though not in the length sensitivity. When a ramp-and-hold stretch is applied in addition, the initial burst is abolished and the slope of the dynamic response is increased. Stimulation of a static axon under these conditions activates the bag_2 fiber, or chain fibers (long chain only if β static), or both, and again there is an increase in the static firing level and the initial burst is abolished. In this case, however, the slope of the dynamic response is usually unaffected so that, although the actual dynamic response remains constant during static stimulation, the dynamic index is usually reduced. Moreover, stimulation of some γ static axons increases the length sensitivity, an effect that can usually be produced when several γ static axons supplying the same spindle are stimulated together.[125] This effect may be attributed to the mechanical properties of the bag_2 fiber or the chain fibers; the bag_2 fiber is strongly implicated by the finding that the effect is fatigue-resistant, whereas other effects of static stimulation are not.[126] The ef-

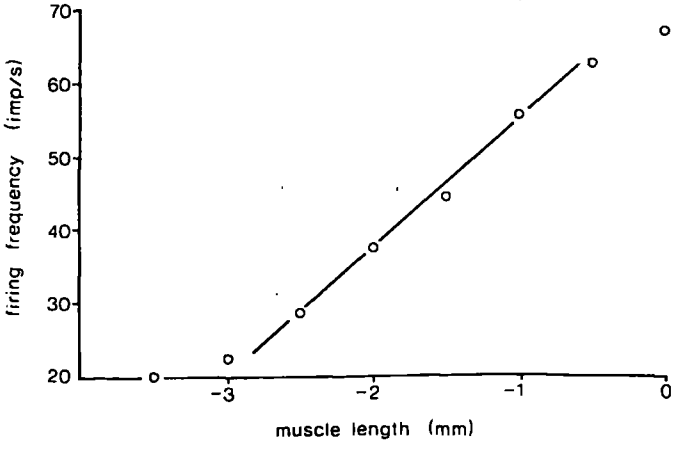


Figure 10-13. Length sensitivity of a spindle afferent unit, probably a secondary ending, shown by the slope (18 imp/s per mm) of the adapted firing frequency against muscle length. Maximum physiological length corresponds to zero on the abscissa. With the muscle completely slack, the ending had a tonic discharge of 20 imp/s. This is the same unit as in Fig. 10-14*B*; conduction velocity 63 m/s, cat peroneus brevis.

fects of fusimotor stimulation on secondary endings are qualitatively similar to those on primary endings, though the static effects are much more prominent, reflecting the relatively sparse supply of secondary terminals to bag_1 fibers.

The behavior of the primary ending described above suggests that it is measuring the length (length sensitivity), velocity (dynamic index), and, perhaps, acceleration (initial burst) of the muscle in which it occurs, and this interpretation is usually found in introductory accounts of the muscle spindle (see e.g., Kuffler and Nicholls[127]). If this were so, it would be a simple matter to describe the output of the primary ending mathematically by a linear transfer function.[128] However, the sensitivity to velocity declines rapidly with increasing velocity,[129] and for large amplitude stretches there is no particular response to acceleration.[130] Furthermore, the use of sinusoidal stretches has shown that the primary ending is about twenty times more sensitive to small amplitude stretches (up to 0.1 mm for cat soleus[131]) than to larger stretches. These complications make the fitting of a linear transfer function more difficult, though various attempts have been made, ranging from power functions[132] to a second-order relation.[133]

Nonlinear functions give better fits to the observed behavior,[124,129] but even these do not fit the complete pattern of impulse activity. To obtain a fit it seems necessary to suppose that there is more than one site of impulse initiation, such that their individual outputs may be variously summed or occluded. This phenomenon of *pacemaker switching* is to be expected in a branched axonal system.[134] Evidence that it occurs in mammalian spindles was first presented by Crowe and Matthews[135]; it has since been extended by Hulliger and Noth[77] and has been discussed on the basis of actual branching patterns by Banks et al.[29]

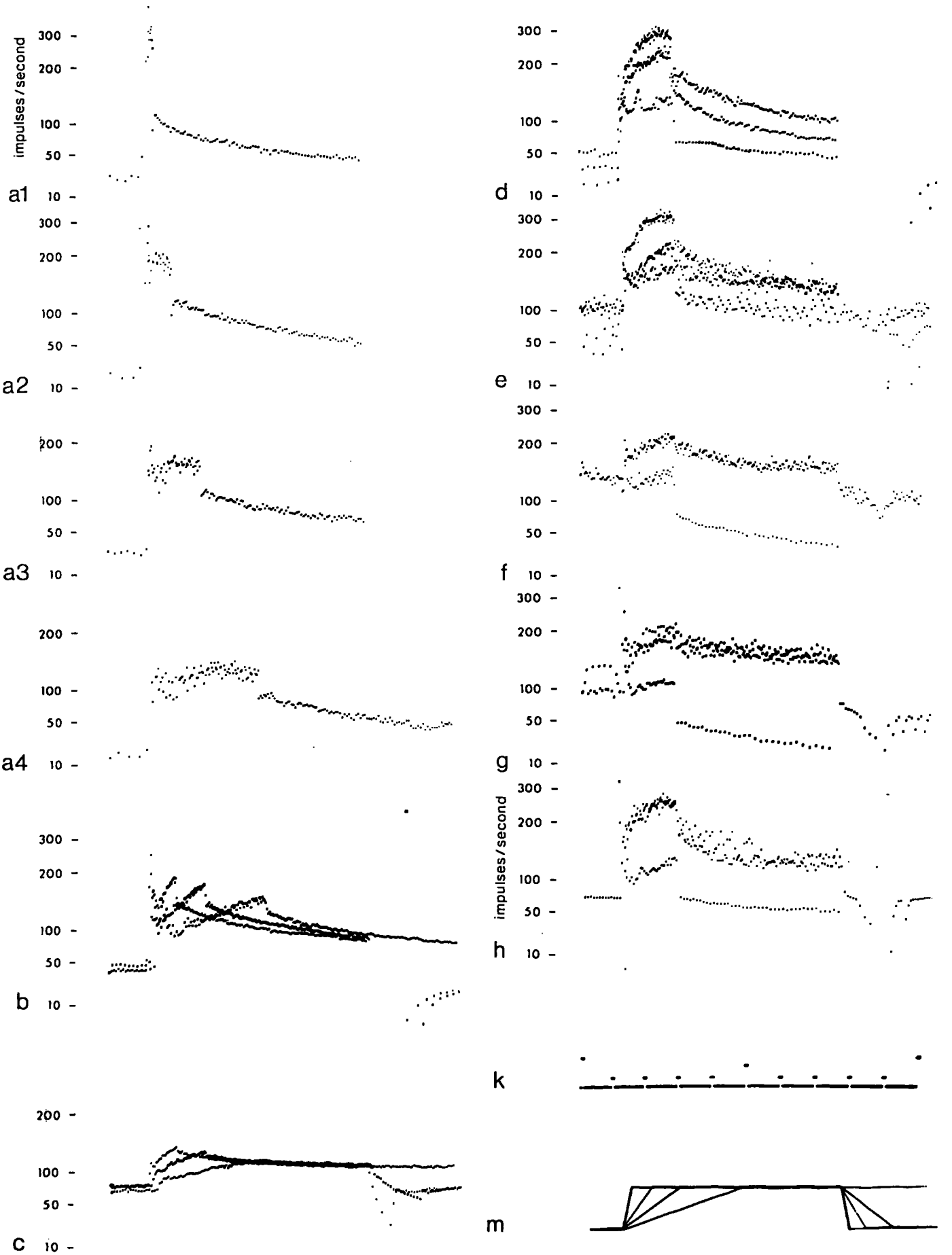
Input-Output Conversion

How does the spindle transducer work, and what may be the cause of its nonlinearities? Hunt and Wilkinson[136] analyzed receptor potentials recorded from afferent axons whose impulse activity had been blocked with tetrodotoxin. The responses of primary and secondary endings were essentially similar, the main difference being the greater sensitivity of the primary endings. Moreover, the overall tension of these isolated spindles showed similar nonlinearities in response to sinusoidal amplitude and frequency, as did the receptor potentials.

The receptor potentials are generated mainly by an influx of Na^+ into the sensory terminals,[137] and we have suggested[29] that the permeability of the Na^+ channels is affected by an intracellular messenger (probably Ca^{2+}) released from a bound state by the rise in cytoskeletal tension that accompanies bulk deformation of the terminals. As a first approximation we may further suppose that the receptor potential is linearly related to the longitudinal tension through the terminals, this being locally modified by such elements as the elastic fibers that insert into the juxtaequatorial regions of bag fibers and thus differ, particularly in phase, from the overall tension (see Ultrastructure, under Intrafusal Muscle Fibers, earlier in this chapter).

The cause of the nonlinearities in the sensory responses may then be sought in the mechanical properties of the intrafusal muscle fibers on the basis of the cross-bridge model of muscle activation. *Cross-bridges* may be regarded as elastic elements of low compliance whose breakage under tension is

Figure 10-14. Instantaneous-frequency displays of responses of spindle afferents to ramp-and-hold stretches applied to cat peroneus brevis. *A*. Primary ending (conduction velocity of supplying axon, 87 m/s) stretched at 36 (*A*1), 10 (*A*2), 5 (*A*3), and 2.5 (*A*4) mm/s. Note that both initial burst and peak of dynamic response increase with greater rates of stretch and that the ending ceases firing on release of stretch. *B*. Probably a secondary ending (conduction velocity of supplying axon, 63 m/s). Superimposed responses to 10, 5, and 2.5 mm/s stretches. Note increase in slope of dynamic response to greater rates of stretch. The ending stops firing on release. *C*. A typical secondary ending (conduction velocity of supplying axon, 35 m/s). The initial high sensitivity to dynamic stretch is clear. The ending continues to fire during release. *D–H*. Effects of separately stimulating five motor axons on the response of a primary ending (conduction velocity of supplying axon, 79 m/s) to a 5-mm/s stretch. The lowest trace in *D*, *F*, *G* and *H* is a test response in the absence of motor stimulation. In *D* a dynamic axon (conduction velocity not recorded) was stimulated at 60 and 100 Hz. Note increase in slope of dynamic response and absence of firing during release. In *E* stimulation of a motor axon with a conduction velocity of 57 m/s (probably a β axon) produces a mixed effect: stimulation at 10 and 40 Hz (lower two traces) increases the static response of the primary ending, whereas stimulation at 100 Hz further increases the dynamic, but not the static, response. Static γ axons were stimulated in *F*, *G* (conduction velocity, 28 m/s), and *H* (conduction velocity, 25 m/s) nominally at 50 Hz, but the driving in *H* before the stretch shows that the actual rate was about 60 Hz. The primary ending fires during the release of stretch in each case, and the slope of the dynamic response is unaltered from that in the test response. *K*. Time calibration 0.2, 1, 2 s. *M*. Superimosed signals generating the stretches. Release from the hold phase following the slowest ramp is not shown.


impulses / second
300
200
100
50
10
a1
a2
a3
a4
b
c
d
e
f
g
h
k
m

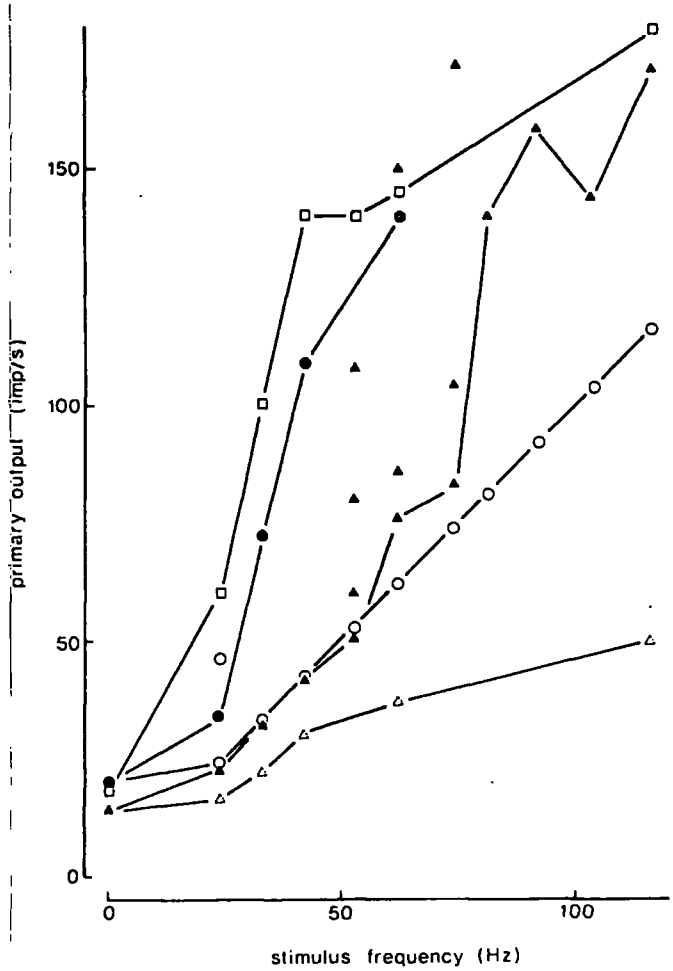


Figure 10-15. Effects on the static discharge of a primary ending (same unit as in Fig. 10-14*D–H*) of the separate stimulation of five motor axons at various frequencies recorded 1–2 s after the onset of stimulation. Open triangles: A dynamic axon (conduction velocity not recorded; see Fig. 10-14*D*); with stimulation at 200 Hz the output of the primary ending reached 56 imp/s. Open circles: A static γ axon (conduction velocity, 25 m/s; see Fig. 10-14*H*); the secure driving of the primary ending produced by stimulating this axon was used to calibrate the stimulus frequency. Filled triangles: A static γ axon (conduction velocity, 28 m/s; see Fig. 10-14*G*); stimulation at lower frequencies produced driving, with harmonics becoming irregular above 50 Hz. Filled circles: A static γ axon (conduction velocity not recorded; see Fig. 10–14*F*); stimulation did not produce driving at any frequency. Squares: An axon (conduction velocity, 57 m/s, probably β; see Fig. 10-14*E*) producing a mixed effect; the static effect below 50 Hz is very clear. The extension of the muscle (cat peroneus brevis) was close to half the physiological range.

manifested as viscosity.[138] The number of cross-bridges at any one time will be a function of the rate at which they are being formed, the rate at which they spontaneously break, and the rate at which they break under tension. Each of these factors will have an associated time constant, the actual value of which will vary according to muscle fiber type, particularly in the case of the first two. The bag_1 fiber is especially interesting on account of its peculiar properties, which include the discrepancy between the locations of its motor endings and the convergent sarcomere movements seen during the stimulation of dynamic motor axons.[111] If the bag_1-fiber pole normally has a large number of cross-bridges, activation may produce only a modest shortening restricted to the region with M-line sarcomeres, whereas the stiffness will increase. The time course of the shortening[74] may be much longer than that of the activation giving rise to it[139] because of series viscoelastic components.

The prominent dynamic response of the primary is thus seen as a consequence of the stiffness of the bag_1 fiber. Exposure of potential cross-bridge sites by breakage of cross-bridges under tension will automatically lead to an increased rate of cross-bridge formation, recognizable as stretch activation,[109,140] that tends to maintain the muscle fiber stiffness. At the peak of a ramp stretch the longitudinal tension in the primary terminals on the bag_1 fiber may fall abruptly, perhaps aided by the effectively in-parallel elastic fibers, to be followed by the compensatory length change ("creep"[74,141]) with a longer time course. As the output of the terminals on the bag_1 fiber falls below that of the terminals on the bag_2 and chain fibers, the common pacemaker becomes predominantly influenced by the latter.

The mechanical properties of chain fibers are diametrically opposed to those of the bag_1 fiber in that they show lack of stiffness, low viscosity, and rapid, overt contraction. They can be accounted for by assuming that chain fibers have a small number of cross-bridges with a rapid turnover as a result of a high rate of spontaneous breakage. Static fusimotor activation leads to shortening of the chain fiber poles with little, if any, increase in stiffness. Because of the lack of viscosity, small fluctuations in polar length are readily transmitted to the equatorial region, and the primary response can be "driven" at the static fusimotor stimulation rate over a wide range of frequencies.[75]

What is the muscle spindle signaling to the central nervous system? Because of the length-tension relation of muscle, the answer to this question is inherently ambiguous, but it is conceivable that under different conditions the length or tension aspect may be of paramount functional importance. Both primary and secondary endings are good length sensors under static or low dynamic conditions, whereas under highly dynamic conditions the primary ending may be a good sensor, not of rate of change of length but of rate of change of tension. Muscle spindles are thus admirably adapted to feedback sensing of muscle length and stiffness and may

participate in reflex control of both these aspects of muscle activation.[129]

EFFECTS OF SYMPATHETIC STIMULATION

Recent histological evidence demonstrating the presence of a nonvascular autonomic innervation in some spindles (see Autonomic Innervation) prompted Passatore and Filippi[142,143] and Hunt, Jami, and Laporte[144] to study the effects of sympathetic stimulation on the discharge of spindle afferents. The responses are variable and complex. At the shortest latency (0.6–2.5 s) 40 percent of rabbit jaw-muscle spindle afferents decreased their firing rate during repetitive stimulation of the cervical sympathetic nerve.[142] This does not seem to be due to a reflex inhibition of fusimotor tone,[143,144] but could , perhaps, be due to a direct effect on the afferent axons or endings. We have some unpublished evidence in favor of this in the form of a typical autonomic varicosity lying close to a node of Ranvier on a spindle secondary afferent.

Somewhat later (8–15 s in rabbit jaw muscles[143]; a few seconds in the cat hindlimb[144]) a weak excitation is shown by some spindle afferents. This is now recognized by all the investigators to be due to a direct effect on the intrafusal muscle fibers.[144a] It is simultaneously accompanied by a development of tension in the rabbit jaw-closing muscles, maximally 5 g.[144a] Passatore, Filippi, and Grassi[144b] attribute this as being mainly due to intrafusal contraction, but it seems unlikely, on the basis of studies on isolated spindles,[74] that the number of intrafusal muscle fibers present in rabbit jaw muscles could produce tension of that order.

On prolonged stimulation the direct intrafusal excitation is transitory and is followed after about 30 s by a second excitation of vasomotor origin.[144] Hale, Kidd, and Kucera[145] and Hale and Kidd,[146] using a preparation in which the rat's tail was maintained independently of its blood supply, found that reflex sympathetic activity, induced by carotid occlusion or nitrogen breathing, led to a long-lasting increase in the regularity and firing frequency of spindle primary afferents[146] and that superfusion with adrenaline produced a similar excitation that lasted as long as the adrenaline was present.[145] The transitoriness of the early excitation seen in cat spindles by Hunt et al.[144] could thus be a further consequence of vasoconstriction. However, all these effects are very weak and unlikely to be functionally significant. Hunt et al.[144] looked for an antifatigue effect of sympathetic stimulation on intrafusal muscle fibers comparable with the classical Orbeli effect on extrafusal muscle fibers, but one was not clearly present. The present position is thus that the significance of intrafusal autonomic innervation remains to be elucidated.

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List of Abbreviations

ATPase	adenosine triphosphatase
ChE	cholinesterase
df	days fetal
FG	fast glycolytic
FOG	fast oxidative-glycolytic
HRP	horseradish peroxidase
NADH-TR	nicotinamide adenine dinucleotide tetrazolium reductase
SO	slow oxidative
SR	sarcoplasmic reticulum

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Observations on the primary sensory ending of tenuissimus muscle spindles in the cat

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Summary. The arrangement of preterminal and terminal axon branches in the primary sensory endings of cat tenuissimus muscle spindles was studied using whole-mount and serial-section techniques. Although in every case one first-order preterminal branch was supplied exclusively to the bag_1 type of intrafusal muscle fibre, the preterminal branching patterns differed considerably in detail.

Terminals varied widely in size and location. Their precise form varied according to their position on the intrafusal muscle fibres rather than their relationship to preterminal branches. Terminals derived from separate preterminal branches remained separate and did not fuse with themselves or each other. Individually bag_1 fibres had most terminals, chain fibres least. The surface of the muscle fibres were differentially indented by the terminals, least in bag_1 fibres and most in chain fibres.

The results are discussed in relation to mechanosensory transduction and to the factors involved in determining the form of the primary ending.

Key words: Muscle spindle – Sensory innervation – Mechanosensory transduction – Morphogenesis – Cat

The primary sensory ending of the mammalian muscle spindle is usually supplied by a single, large-diameter axon which distributes terminals to a small bundle of specialized muscle fibres via a system of preterminal branches (Ruffini 1898; Barker 1948; Boyd 1962). The basic form of the ending is classically described by the term "annulospiral", because of the way in which the terminal branches wind around the intrafusal muscle fibres, often forming apparently complete rings.

The muscle fibres are of three types, known as bag_1, bag_2 and chain, whose different mechanical properties characteristically affect the response of the primary ending to stretch (Banks et al. 1977; Boyd 1981; Barker and Banks 1986). Cat hindlimb muscle spindles typically contain one bag_1, one bag_2 and four or five chain fibres, and at least in these spindles each type is constantly associated with certain features of the primary ending (Banks et al. 1982). Thus the annulospiral turns are set most closely on the bag_1 fibre, resulting in this part of the ending accounting for about a third of the total area of neuromuscular contact; they are most widely separated on the chain fibres, among which they are directly interconnected (the sensory cross-terminals of Adal 1969); and the distal terminals tend to a more irregular disposition, particularly on the bag_1 fibre. Additionally, one of the first-order preterminal branches usually innervates the bag_1 fibre exclusively, especially in the tenuissimus muscle; and the bag_1 fibre usually receives more preterminal branches than either the bag_2 fibre or individual chain fibres.

But it is now clear that the use of transverse sections and graphic reconstruction in the work of Banks et al. (1982) obscured important details of the terminals, and it is the purpose of this paper to clarify some of those details. In particular the contribution that individual terminals make to the overall form of the ending and the degree to which terminals are indented into the different types of muscle fibre are described. Their possible functional significance is discussed, and the possible morphogenetic influence of the intrafusal muscle fibres on the overall form of the primary ending is considered.

Materials and methods

Muscle spindles were obtained from eight tenuissimus muscles, each from a different adult cat. Three muscles were prepared according to the modified silver impregnation technique of Barker and Ip (1963) as described in Barker et al. (1985). The preterminal branching patterns of the Ia afferent axons were determined from teased, whole spindles using ×100 oil immersion objectives. The remaining muscles formed part of the sample described in the histophysiological study of Banks et al. (1978). Details of the processing can be found there and in Barker et al. (1978). Each of these muscles yielded one spindle, and the original reference numbers, 4, 5, 6, 10 and 12, will be retained here. In each case, the equatorial region, including the whole of the primary sensory ending, was serially sectioned at 1 μm thickness and stained with 1.7 mg/ml toluidine blue in 1.7 mg/ml borax solution.

Spindles 6 and 12 were sectioned transversely and graphic reconstructions of their primary sensory endings have been published previously (Banks et al. 1982). Spindles 4, 5 and 10 were sectioned longitudinally. The question of whether the terminals can form closed configurations such as rings could not be settled by the method of graphic reconstruction, therefore the primary sensory ending of

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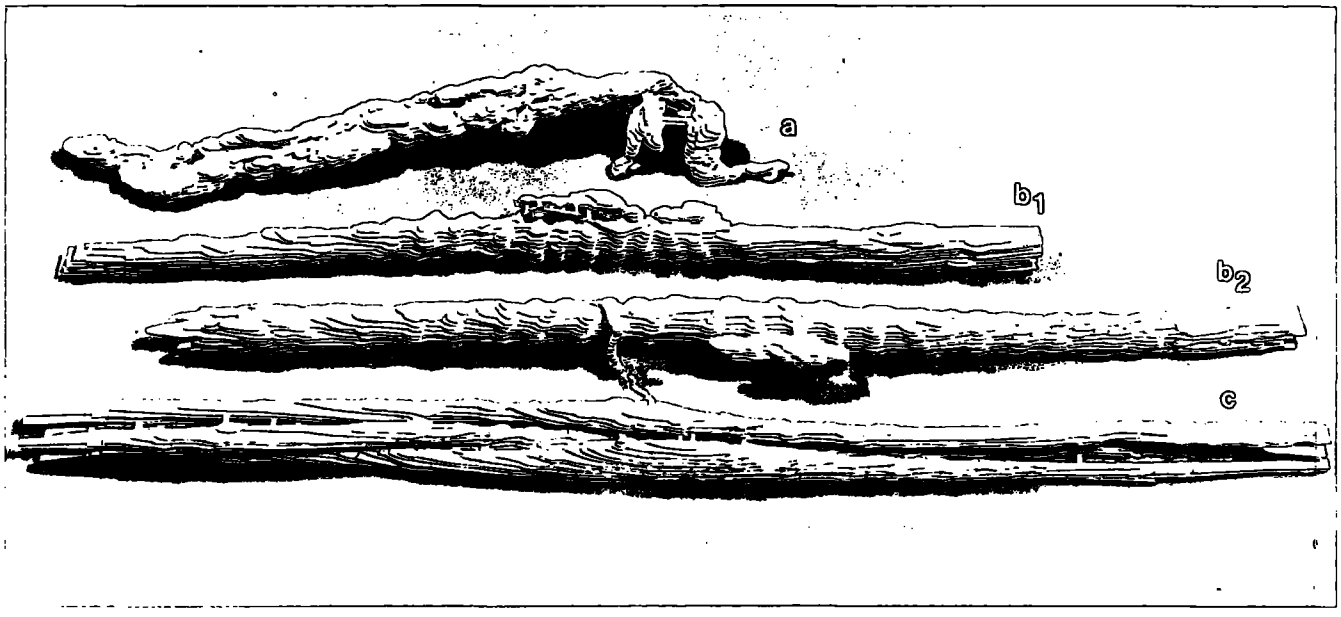


Fig. 1. The primary ending and associated muscle fibres of a cat tenuissimus muscle spindle as reconstructed from serial longitudinal sections, prior to contour filling (cf. Fig. 2). The reconstruction is in four parts: (*a*) most of the preterminal branches of the myelinated axon, (b_1) the bag_1 fibre, (b_2) the bag_2 fibre, and (*c*) the three chain fibres, all with their associated terminals. Several temporary locating bridges are visible

spindle 10 was reconstructed in three spatial dimensions. The presence in the terminals of a darkly stained central core containing many mitochondria facilitated the resolution of separate terminals even when they were closely adjacent. This is best seen in longitudinal section, since the terminals run predominantly transversely. The three-dimensional reconstruction allowed the sensory ending to be viewed from any angle, clearly revealing possible closed configurations of terminals which could then be checked in the serial sections.

The process of reconstruction involved tracing onto thin card photomontages enlarged ×4.5 from 35 mm negatives taken with a Zeiss Ultraphot using a ×100 planapo objective. The tracings were cut out and mounted with card spacers of the correct scale thickness. Since there were no external references, the successive sections were aligned using subjective judgement of minimal displacement. The whole ending was reconstructed in four parts: (i) most of the preterminal branches, (ii) the bag_1, (iii) the bag_2, and (iv) the chain fibres, the terminal branches being included with the intrafusal muscle fibres (Fig. 1). The contours were smoothed using a cellulose-based filler, and the surface was painted to differentiate the various components.

Two sets of measurements were obtained from the longitudinally sectioned parts of spindles 4, 5 and 10. Firstly, standard circles were used to estimate the radii of curvature of the outer (free) and inner (neuromuscular) boundaries of a sample of terminal profiles, together with the length of the imaginary chord cutting the profile in the line of the muscle-fibre surface. The terminal profiles on each muscle fibre were selected in the sections that contained the greatest equatorial diameter of each fibre. Those closest to the middle of the primary ending were used provided that they did not show evidence of branching nearby, such as a bilobed appearance or an unusually large size. Secondly, sarcomere lengths were estimated at various regions by counting, to the nearest half, the number of sarcomeres in a 50 μm length of fibre.

Supplementary observations were made on lumbrical muscle spindles thin-sectioned for electron microscopy and kindly provided by Dr. M.J. Stacey.

Results

Preterminal branches

The pattern and distribution of the myelinated preterminal branches of 16 primary endings were established from teased, whole spindles (13) and serially sectioned spindles (3). There were in total 79 ultimate preterminal branches in a range of 3 to 8 per ending, and of the following orders: 1st, 7; 2nd, 47; 3rd, 22; 4th, 2. With one exception, each supplied only one type of intrafusal muscle fibre, as shown in Table 1. Bag_1 fibres tended to be supplied by the lowest-order ultimate branches, chain fibres by the highest order. Individually, however, bag_1 fibres received on average more ultimate preterminal branches (1.8 ± 0.19 SE) than either bag_2 (1.3 ± 0.15 SE) or chain fibres (0.48 ± 0.06 SE).

With one exception every ending possessed two first order branches which supplied the bag_1 fibre exclusively, and the bag_2 and chain fibres together. In the remaining ending there were three first order branches, each supplying one type of muscle fibre. If the endings are considered to begin at the first branching nodes, then they included a total of 125 nodes of which 45 (36%) branched dichotomously and 9 (7%) branched trichotomously. Trichotomous nodes were most common in the distal parts of the bag_2/chain branching system.

The proportion of branched nodes in individual endings ranged from 22% to 67%. Most of the data were obtained from two cats, and the mean proportion of branched nodes from one animal (60.2%, $n=5$) was significantly greater

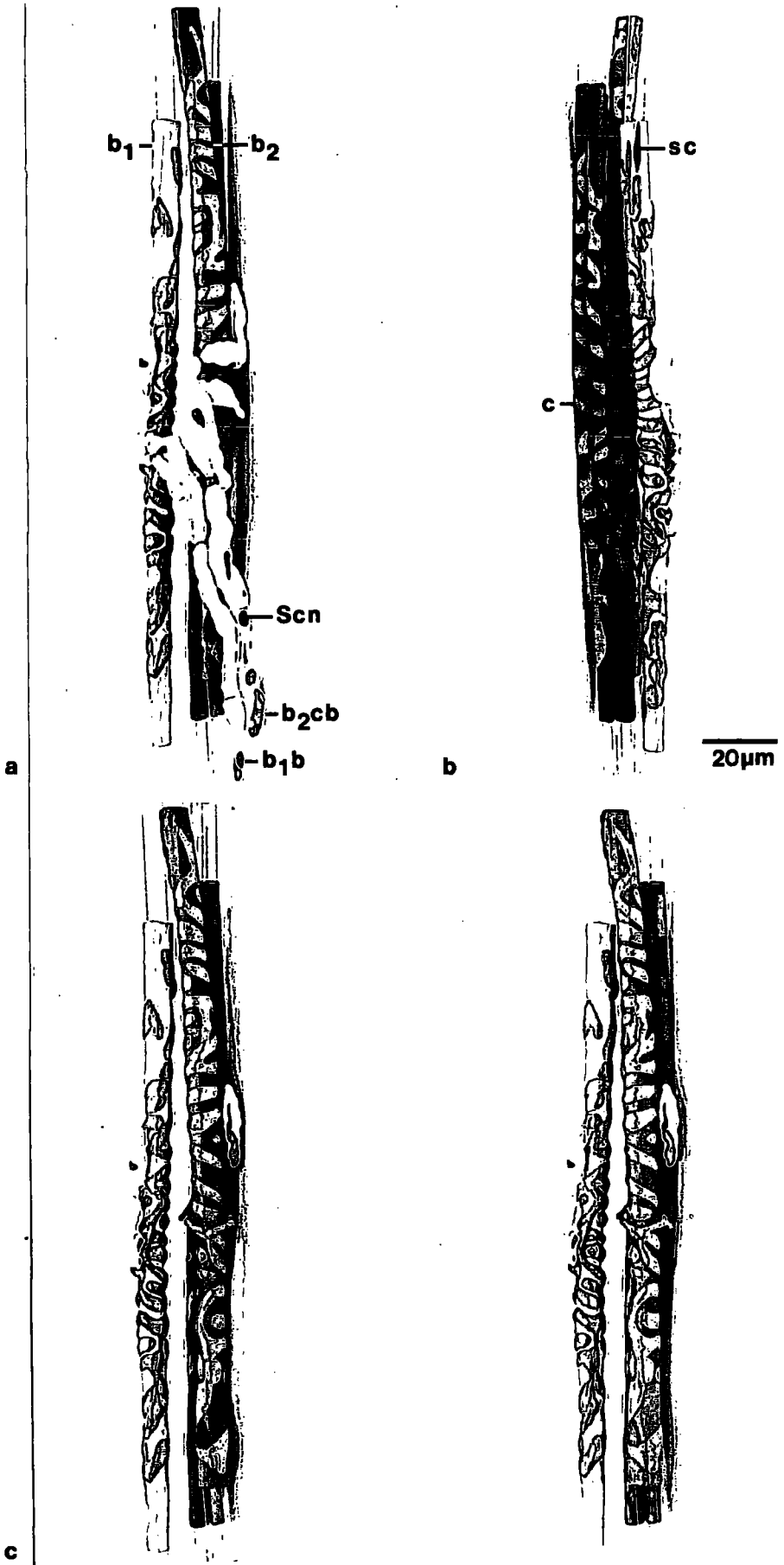


Fig. 2a–c. The completed reconstruction of the primary ending and associated muscle fibres of a cat tenuissimus muscle spindle (cf. Fig. 1), as seen from two viewpoints (**a, b**), and in a stereo-pair (**c**) from the same viewpoint as (**a**) but with the myelinated preterminal axons removed to show the terminals on the bag fibres more clearly. The bag_1 fibre has characteristically crowded terminals with very irregular parts at each end. The most regular spirals occur, as usual, on the chain fibres. b_1 bag_1 fibre; b_1b bag_1-fibre branch of Ia axon; b_2 bag_2 fibre; b_2cb bag_2-chain-fibre branch of Ia axon; *c* chain fibres; *sc* satellite cell; *Scn* Schwann-cell nucleus

than that from the other (35.5%, $n=6$; $P<0.02$, Mann-Whitney U test), perhaps reflecting variability in an inherent tendency of the sensory axons to branch. However, the 16 endings all differed in the details of their preterminal branching, indicating the importance of random effects in pattern formation.

Nonmyelinated preterminal axons arose only from the terminal heminodes of the myelinated preterminal branches. They were about 1 μm in diameter and 10–20 μm in length, and were sometimes branched.

Form and distribution of terminals

The terminals of the reconstructed primary ending of spindle 10 were generally similar in form to those of spindles 6 and 12, published previously (Banks et al. 1982). The characteristic features associated with each type of muscle fibre were all present (Fig. 2). There were several possible closed-loop or ring configurations, particularly among the crowded terminals of the bag_1 fibre. However, careful scrutiny indicated that they were all actually open, being formed by closely adjacent separate terminals or by a single terminal encircling the muscle fibre and abutting on, but not fusing with, itself.

The terminals on all the muscle fibres are shown diagrammatically in Figs. 3–5. Compared with the nonmyelinated preterminal branches they were of larger diameter, usually continuously so, though in some cases a single terminal consisted of more than one expanded portion linked by narrow connectives. Terminals of this sort were particularly common on the bag_2 fibre. At each end of the complete terminal system on the bag_1 fibre there were some small, apparently isolated portions of terminals whose connexions were perhaps too tenuous to detect.

The whole ending contained 9 terminals, 4 on the bag_1 fibre, 3 on the bag_2 fibre, and 2 distributed among the 3 chain fibres by sensory cross-terminals. However, the terminals were not symmetrically distributed, so the form of each one depended on its location in the complete system. On the bag fibres, for example, pure spirals were confined to those parts of the terminals that happened to occupy the region overlying the nuclear bag.

The domains of individual terminals are shown in greatly simplified form in Fig. 6, together with the preterminal branches supplying them. For comparison the endings of spindles 6 and 12 are shown similarly, but including only the terminal domains which may be confidently located. In spindle 6 there were 7 terminals on the bag_1 fibre, and 2 on the bag_2 fibre. In spindle 12 there were 6 terminals on the bag_1 fibre, and 3 on the bag_2 fibre. By inference from the number of nonmyelinated preterminal branches there were 2 and 5 terminals respectively on the chain fibres. For all three spindles the mean numbers of terminals per fibre were: bag_1, 5.7; bag_2, 2.7; chain, 0.7.

Terminal indentation

The longitudinal section of the bag_1 fibre of spindle 4 shown in Fig. 7 is typical of those used in the analysis. The equatorial region of the fibre was occupied by a mass of euchromatic myonuclei, which were surrounded by myofibrils in places so tenuous as to be scarcely visible. The outlines of the important features of this and similar sections of the bag_2, bag_1 and one chain fibre from spindles 4, 5 and 10 are shown in Fig. 8. It is immediately apparent that the terminals are differentially indented into the three types of muscle fibre, most deeply in the chain and least in the bag_1 fibres. There is also some indication of increasing amounts of terminal deformation associated with each spindle in the order 4, 10, 5, which may be related to the amount of static stretch during fixation as reflected in the measurements of sarcomere lengths.

Table 1. The distribution of the ultimate myelinated branches of 16 primary endings of cat tenuissimus muscle spindles

Muscle-fibre type	Branch order				Totals
	1	2	3	4	
bag_1	6	19	4		29
bag_2	1	11	7	1	20
chain		16	11	2	29
bag_2 & chain		1			1
					79

The terminal profiles are bounded on their outer (free) and inner (neuromuscular) surfaces by curves mostly approximating to segments of circles. Mean values of the radii of curvature, O and I respectively, are given in Table 2, together with the half-length, C, of the corresponding chord as defined in the Methods. Also included are the ratios that may be derived from these values. If $O=I=C$, the terminal would be circular in section and half embedded in the muscle fibre. This condition was most closely approached on the bag_2 fibre of spindle 4.

When O and I exceed C the terminal profile was lenticular, but the terminals would remain half embedded if $O=I$, as was approximated on the bag_2 fibre of spindle 10. When O exceeds I the terminals were deeply embedded in the muscle fibre, typical of those on the chain fibres; whereas when I exceeds O the terminals were relatively prominent, typical to varying degrees of those on the bag_1 fibres, but also present on the bag_2 fibre of spindle 5.

The terminals were almost always located at the surface of the muscle fibres. In this position their outer surfaces are known to be covered by basal lamina continuous with that of the underlying muscle fibres (see e.g., Merrillees 1960; Landon 1966; and Fig. 9). On rare occasions longitudinally oriented terminals were completely engulfed by the muscle fibre, and it is worth noting that their transverse-sectional profiles were approximately circular. The plasmalemma of both the outer and inner surfaces of a terminal presented a finely corrugated appearance in longitudinal or transverse section (Fig. 9). The remarkable regularity of the corrugation and its absence from the membranes of other types of cell in the same section indicate that there was a genuine rather than an artefactual basis for it.

Discussion

Impulse generation

In vertebrate muscle spindles, as in other receptors, the afferent impulse frequency is determined by the amplitude of a graded receptor potential (Katz 1950; Hunt and Ottoson 1975), which is presumably set up in the terminal branches of the sensory neuron and spreads passively to

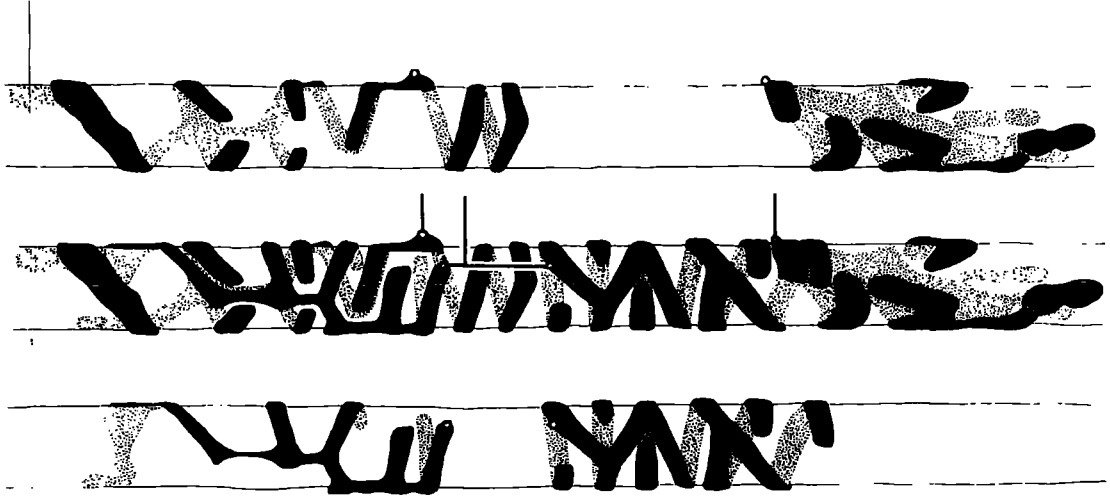


Fig. 3. Diagrammatic representation of the four terminals supplied to the bag_1 fibre in the reconstructed primary ending. The terminals are shown separately (above and below) and combined (middle), to emphasize their different domains within the overall form. Nonmyelinated preterminal branches are shown as straight lines, and their points of continuity with the terminals as small circles. Compare with Fig. 2c. Not to scale

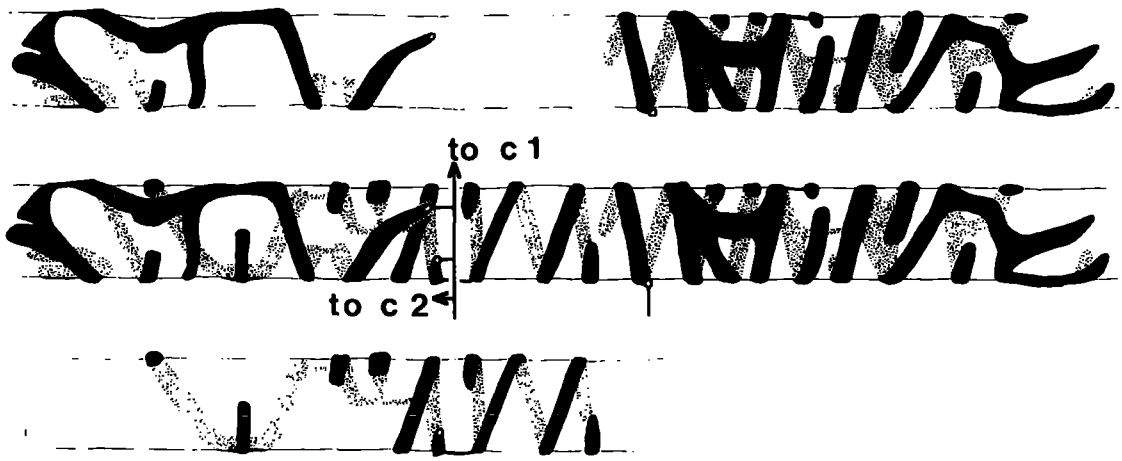


Fig. 4. Diagrammatic representation of the three terminals supplied to the bag_2 fibre in the reconstructed primary ending. The terminals are shown separately (above and below) and combined (middle), to emphasize their different domains within the overall form. Nonmyelinated preterminal branches are shown as straight lines, and their points of continuity with the terminals as small circles. Compare with Fig. 2c. Not to scale. *c1*, *c2* chain fibres 1 and 2 on Fig. 5

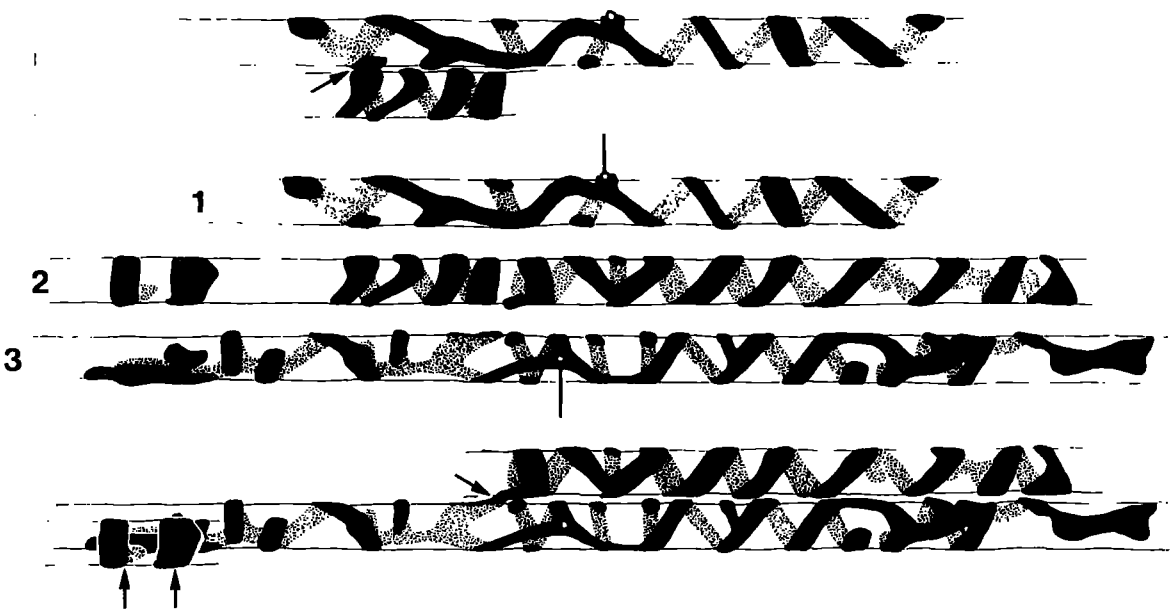


Fig. 5. Diagrammatic representation of the two terminals supplied to the three chain fibres in the reconstructed primary ending. The terminals are shown separately at the top and bottom of the figure: and the chain fibres, numbered *1–3*, are shown separately in the middle. Nonmyelinated preterminal branches are shown as straight lines, and their points of continuity with the terminals as small circles. The terminals cross directly between the muscle fibres at four points (sensory cross-terminals, *arrowed*), and fibre 2 receives all its share of the terminals in this way. Compare with Fig. 2b. Not to scale

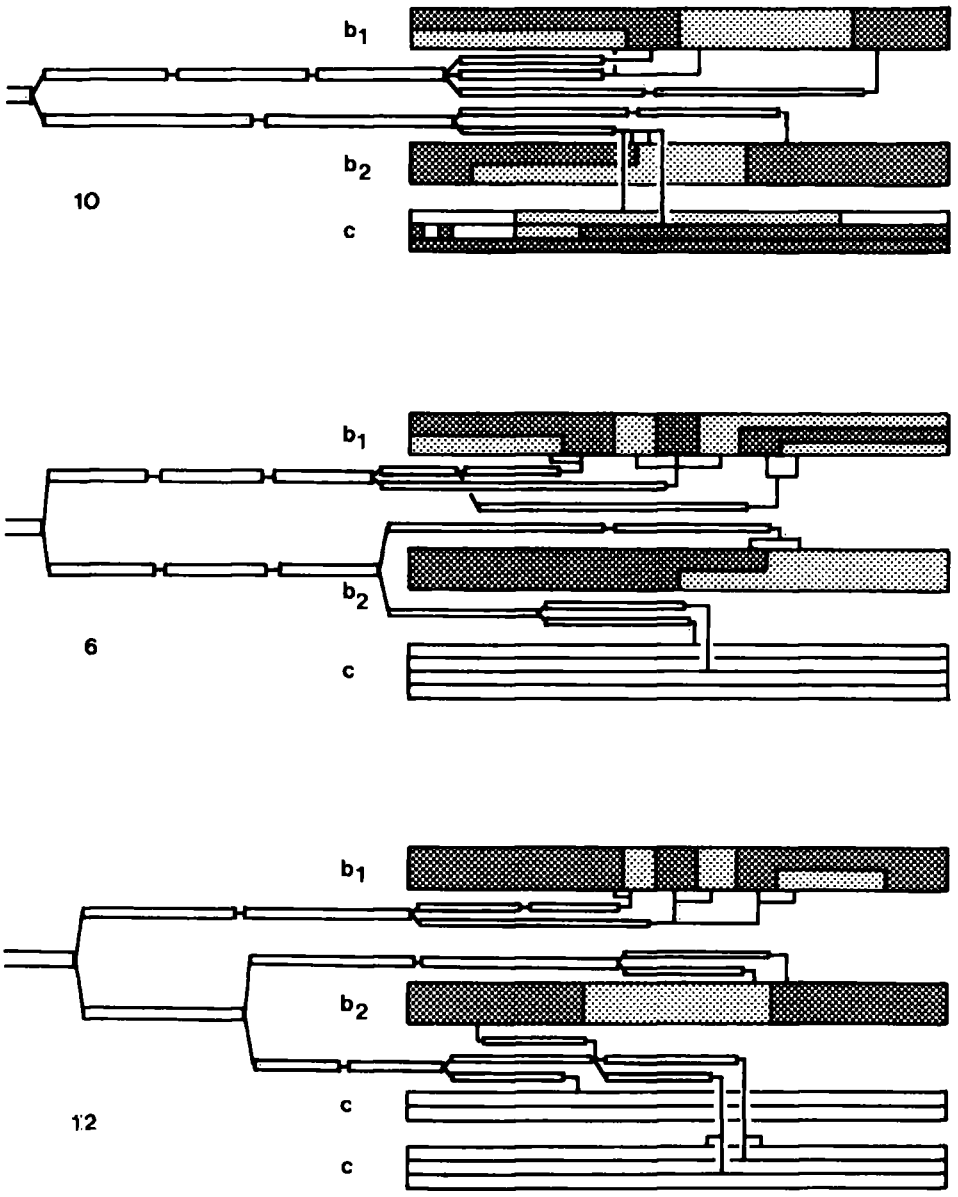


Fig. 6. Schematic representation of the preterminal branches and terminal domains of the primary ending reconstructed from serial longitudinal sections (10) compared with two endings reconstructed from serial transverse sections (6 and 12). In each case the parent axon, to the left, first divides to form myelinated branches which ultimately give rise to several nonmyelinated branches. The approximate locations and extents of individual terminal domains are shaded. Those of the chain fibres in 6 and 12 could not be determined. Graphic isometric reconstructions of 6 and 12 are given in Banks et al. (1982). Despite close similarity in the overall form of all three endings the details of the preterminal branching patterns and terminal domains vary considerably; b_1 bag_1 fibre; b_2 bag_2 fibre; c chain fibre

low threshold, electrically excitable pacemaker sites. The penultimate nodes of Ranvier and the ultimate heminodes in the neuron's preterminal branches have been plausibly identified as the pacemakers (Quick et al. 1980). The present work has demonstrated that the terminals supplied by separate preterminal branches remain separate and do not fuse. Therefore each terminal with its associated pacemaker is potentially an independent transduction unit, generating its own impulses. The large number of such units on the bag_1 fibre is particularly interesting; in one of the present examples (spindle 6) it exceeds the combined total of separate units on all the remaining intrafusal fibres.

The possible significance of the preterminal branching pattern for the integration of the separate pacemaker outputs has been discussed elsewhere (Banks et al. 1982).

Transduction

The receptor potential of the muscle spindle is generated mainly by an influx of Na^+, presumably across the terminal membranes (Hunt et al. 1978). The nature of the transducer is still a matter for speculation, but it is usually supposed that Na^+-channels themselves undergo conformational changes in response to longitudinal stress in the terminal membrane (see for example Kennedy et al. 1975; Shepherd 1983; Quick 1984). However, such a physical system cannot account for the observed temperature dependence of the response (Duncan 1967), nor is it easy to see how a fluid-mosaic membrane (Singer and Nicholson 1972) could support a one-dimensional stress.

Stimulation of the muscle spindle by passive stretch or by active intrafusal-fibre contraction is accompanied by extension of the equatorial region and, at least in the frog spindle, by deformation of the sensory nerve terminals in that region (Ottoson and Shepherd 1970; Boyd 1976; Fukami and Hunt 1977; Bendeich et al. 1978; Poppele et al. 1979; Kim et al. 1985). Since the terminals are almost all situated at the surface of the muscle fibres it might be supposed that some kind of shearing stress is transmitted directly to the terminal membrane at the sensory neuromuscu-

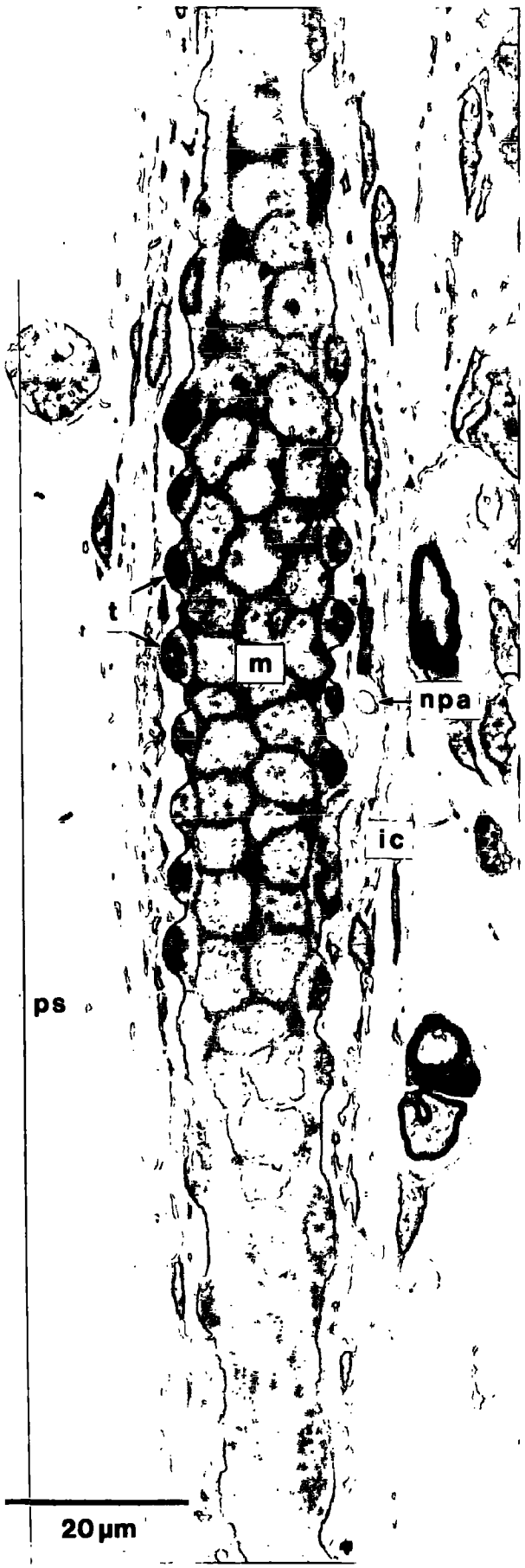


Table 2. Radii of curvature of the outer (free) and inner (neuromuscular) boundaries of terminal profiles on the three types of intrafusal muscle fibre, and of the half-length of the corresponding chord (see Methods), together with the ratios of these

	O µm	I µm	C µm	I/O	I/C	O/C
Bag_2 fibres						
4	3.8	3.5	2.9	1.0	1.2	1.3
10	4.9	5.1	3.4	1.1	1.5	1.4
5	3.8	7.7	3.2	2.3	2.4	1.2
Bag_1 fibres						
4	3.1	5.2	2.9	1.4	1.8	1.1
10	3.6	20.1	3.3	6.1	6.3	1.1
5	3.8	31.9	3.2	9.2	10.6	1.2
Chain fibres						
4	7.3	3.4	2.8	0.5	1.2	2.6
10	11.3	3.2	3.3	0.4	1.1	3.9
5	17.6	6.1	3.6	0.6	1.7	4.5

O, radius of outer boundary; I, radius of inner boundary; C, half-length of chord. All values are means of 10 observations

lar junction (Kennedy et al. 1975; Quick 1984). However, intercellular connexions capable of transmitting the stress seem to be restricted to an inadequate number of desmosomes.

The deformation of sensory terminals in mammalian muscle spindles has not been systematically studied in relation to intrafusal muscle-fibre length or contractile activity, but the results of this study are consistent with an alternative possibility that the terminals are compressed between longitudinal-tension bearing elements, usually the muscle fibres and their associated basal laminae (Banks et al. 1982). Although these cannot be entirely independent it seems likely that, at lengths close to L_0, tension due to passive stretch might be largely transmitted through the basal lamina, whereas active tension would be largely transmitted through the muscle fibre, the balance of tensions determining whether the terminals are compressed symmetrically or not as expressed by the ratio I/O. The differential indentation of the sensory terminals into the three types of intrafusal muscle fibre, expressed by the ratios I/C and O/C, may then be explained when the contractile properties of the fibres are also taken into account (reviewed by Barker and Banks 1986).

Furthermore, terminal compression can provide a mechanical basis for much of the rapid accommodation characteristic of the dynamic behaviour of the primary ending (Hulliger 1984 for review), which deformation by terminal length change cannot, since it allows the possibility of a sudden reduction in net terminal deformation if the stiffness of an intrafusal fibre is suddenly reduced (for example at

Fig. 7. A 1 µm-thick longitudinal section through the equatorial region of the bag_1 fibre of a cat tenuissimus muscle spindle (4), stained with toluidine blue. Several profiles of the sensory terminals (*t*) may be seen indenting the surface of the muscle fibre, which in this region contains a large collection of myonuclei (*m*), the nuclear bag, with the myofibrils restricted to a very thin rim; *ic* inner capsule; *ps* periaxial space; *npa* nonmyelinated preterminal axon

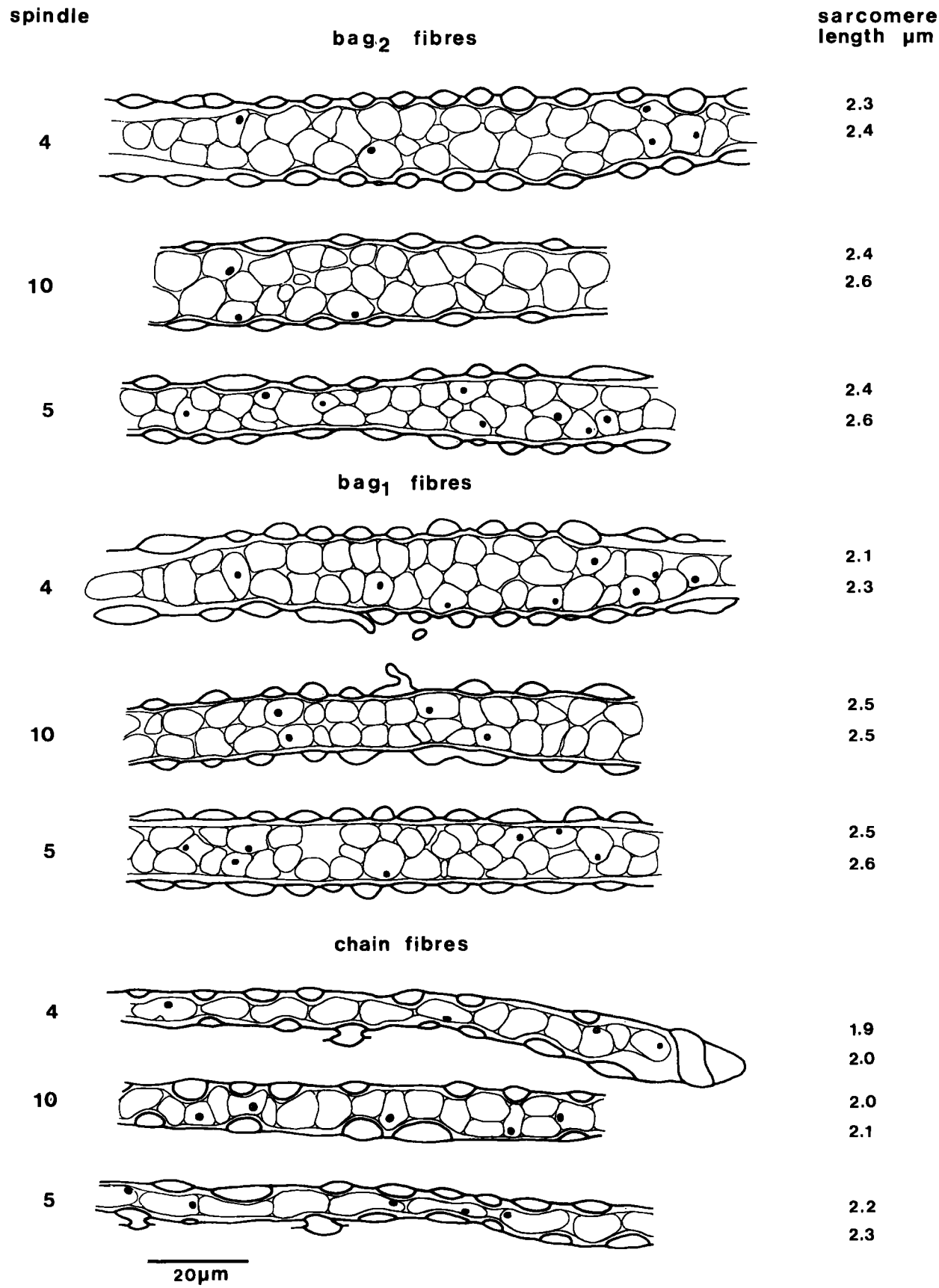


Fig. 8. Tracings of longitudinal sections through the equatorial regions of the bag_2 fibre, bag_1 fibre and one chain fibre from each of three tenuissimus muscle spindles of cat. Sections passing closest to each fibre's diameter were selected. Mean sarcomere lengths of 50 sarcomeres on each side of the primary ending are given for each fibre. They are consistently shortest in spindle 4 and longest in spindle 5 suggesting increasing amounts of static stretch during fixation in the order 4, 10, 5. The terminals are progressively less indented into the bag fibres in the same order but among the chain fibres only that of spindle 5 is clearly less indented than others (see Table 2; for example, all possible comparisons of I/C for each fibre type, except the chain fibres of 4 and 10, show significant differences, $P < 0.05$ Mann-Whitney U test)

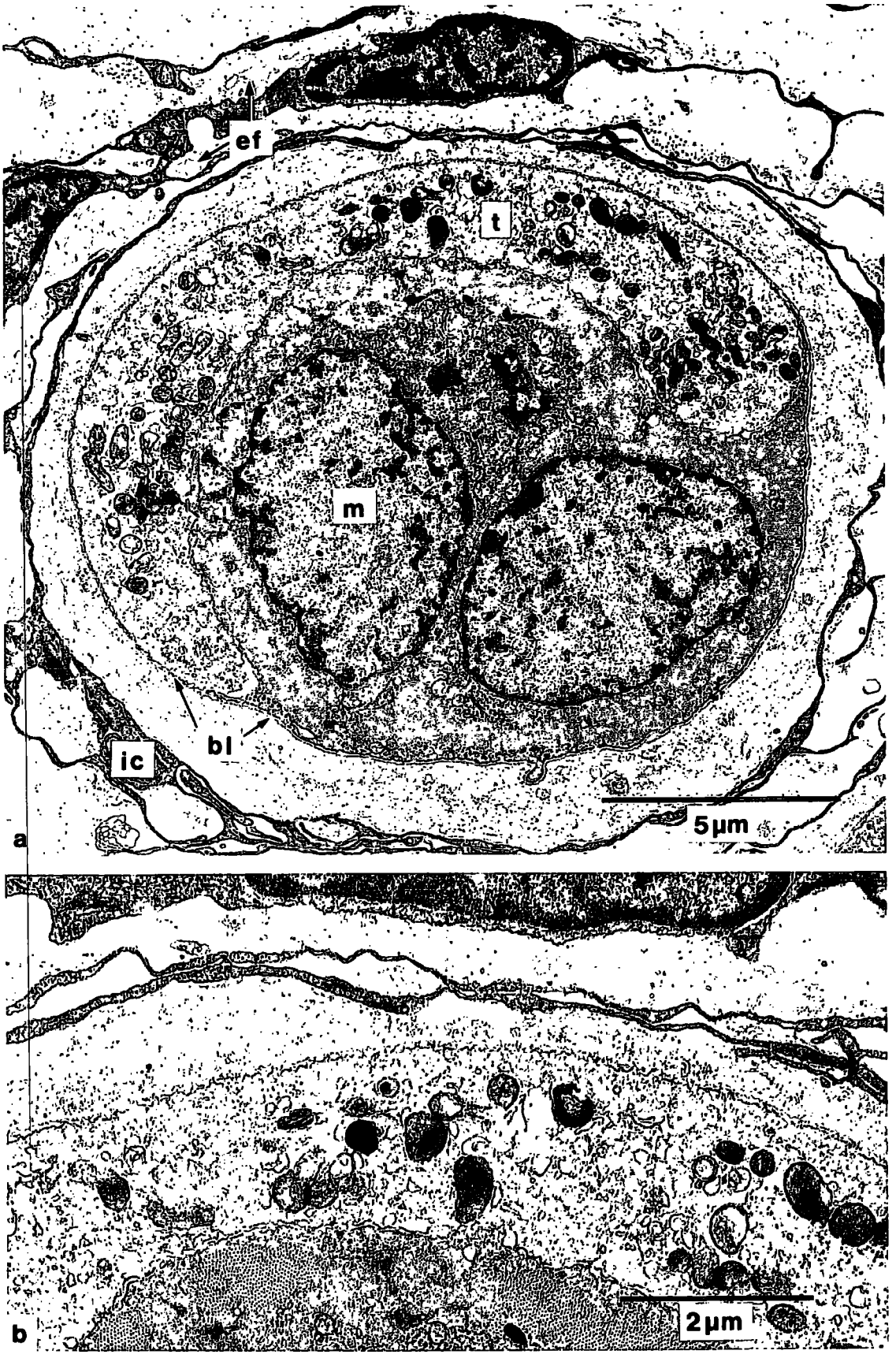


Fig. 9. **a** Electron micrograph of a transverse section from the equatorial region of a bag fibre in a hind-limb lumbrical muscle of cat; passing through a primary-ending terminal (*t*). The terminal is located between basal lamina (*bl*), continuous with that of the muscle fibre, and the plasmalemma of the muscle fibre: *ef* elastic fibres; *ic* inner capsule; *m* myonucleus. **b** Enlargement of part of (**a**) to show the corrugated appearance of the sensory-terminal membrane, which may be compared with the much smoother membranes of the fibrocytes in the adjacent inner capsule. Fibrillar material on the protoplasmic side of the terminal membrane may be part of a cytoskeleton which helps to maintain the regularity of the corrugation and which may be the primary site of mechanosensory transduction

the end of a ramp stretch). Stretch-activation, expressed as an increased stiffness of the fibre while it is being stretched, confers the required contractile property on the bag_1 fibre (Poppele and Quick 1981; Laporte et al. 1985).

The deeply indented terminals on the chain fibre require the maintenance of a passive stretch even at relatively short spindle lengths (such as spindle 4). That this passive stretch is present in the equatorial region is indicated by the occurrence and location of chain-fibre kinking described by Boyd (1976). Moreover, it is interesting that it is not opposed by the attachment of elastic fibres on each side of the primary ending, such as occurs on the bag fibres (Banks 1984), even though elastic fibres are present in the inner capsule surrounding the chain fibres.

Terminal compression would result in increased surface area of the terminals provided that their volume does not decrease. Length changes of the terminals are probably insignificant in this respect since their lengths are typically an order of magnitude larger than their radii, and under this condition the rate of change of surface area with respect to length is very low, by analogy with a cylindrical model. Moreover, the stiffness of the sensory region and the annulospiral form of the sensory terminals will tend to minimize terminal length changes, during active or passive length changes of the intrafusal fibres. The fine corrugation of the sensory-terminal membrane, seen in well-fixed material not only from spindles but from other mechanoreceptors such as the tendon organ (Fig. 18 of Schoultz and Swett 1972), can effectively allow the membrane to accommodate the increase in terminal surface area without any deleterious effects. Although the corrugation may be exaggerated by routine tissue processing (Quick 1984), its remarkable regularity leaves little doubt that it has a genuine basis and presumably is maintained by membrane-associated cytoskeletal elements. It is in these cytoskeletal elements, which must be subject to increased tension as the terminal surface area increases, that the primary event of transduction should be sought (Guharay and Sachs 1984). As in other types of sensory system this would be linked to the Na^+-channels by an intracellular messenger.

Factors affecting primary-ending form

The constant features of primary-ending form and its main divisions on the three types of intrafusal muscle fibre (Banks et al. 1982) might be taken to indicate a stereotyped morphogenesis depending only on intrinsic neuronal properties, especially as formation of the muscle spindles is initiated by the growing afferent axon (Landon 1972; Milburn 1973, 1984) and subsequent differentiation and maintenance of characteristic intrafusal muscle fibres depend on the continued presence of the axon (Zelená 1964; Zelená and Soukup 1974; Milburn 1984). However, the present work has revealed a finer level of form of the ending which varies independently of the constant features. The apparently random variations in details of preterminal branching and in the size and distribution of terminal domains strongly suggests that the overall form of the ending must be determined or limited by some extra-neuronal influence. The importance of the intrafusal muscle fibres in this respect is indicated by the way in which the precise form of a terminal varies according to the position of the terminal with respect to the myonuclei within its domain; for example the restriction of annulospiral turns on the bag_1 fibre to the region of the nuclear bag. The terminals appear to be competing among themselves for a limited amount of a suitable contact site on each muscle fibre; evidence that such site specificity occurs has been obtained in studies of reinnervation of muscle spindles (Banks and Barker 1983; Banks et al. 1984, 1985). Similar competitive behaviour on a larger scale is thought to be involved in the development of sensory neurons in leech skin (Kramer et al. 1985).

The proper functioning of nervous systems depends upon specific neuronal connexions, which are reflected in characteristic sizes and shapes of neurons. Analysis of neuronal form is therefore a valuable technique in the study of how the specificity is achieved (Cowan 1979). Neuronal form might be expected to be determined by an interaction of intrinsic, random and extrinsic factors, both in the extension of neurites and in the development of dendritic and axonal branches. In central nervous systems considerable progress has been made with respect to the main features of neuronal form (see for example Eccles 1970; Pinto Lord and Caviness 1979; Katz et al. 1980; Shankland and Goodman 1982; Robain et al. 1985), but the complexity of the finer divisions of many neurites makes their detailed analysis a formidable problem. In this context the primary ending of the mammalian muscle spindle provides a useful example, combining stereotyped form with readily analyzable arborization and geometrical simplicity of the associated muscle fibres.

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Paper 34.

R.W. Banks (1987)
Responses to small-amplitude sinusoidal stretching of cat peroneus brevis muscle spindles reinnervated after nerve section.
J. Physiol. 391; 56P.

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Journal of Physiology, **391**, 56*P*, 1987]

Responses to small-amplitude sinusoidal stretching of cat peroneus brevis muscle spindles reinnervated after nerve section

By R. W. Banks. *Department of Zoology, University of Durham, Durham DH1 3LE*

Low-threshold mechanosensory endings are formed in muscle spindles by groups Ia, Ib and spindle II afferents following nerve section. Their responses to large-amplitude ramp-and-hold stretches are mostly similar to those of normal primary and secondary endings, but their excitability is reduced (Banks, Barker & Stacey, 1986). Normal spindle endings are especially sensitive to small-amplitude stretches, one consequence of which is that the firing rate of primaries can be driven at the frequency of very small sinusoidal stretches (Brown, Engberg & Matthews, 1967). The responses to such stretches of spindles reinnervated after nerve section are described here.

The left common peroneal nerve was sectioned and repaired in 5 adult cats anaesthetized with sodium pentobarbitone (45 mg kg^{-1} I.P.). Sufficient time was allowed for reinnervation to be fully established, then 23–33 weeks after the operation each cat was deeply anaesthetized with sodium pentobarbitone and prepared for single-unit, dorsal-root recording of L7. Ramp-and-hold (10 mm s^{-1}, 1·8 mm) and sinusoidal (100 Hz, 100 μm maximum) stretches were applied to the peroneus brevis muscle using an electromagnetic puller. Of 82 low-threshold afferents located in spindles, 53 were driven by sinusoidal stretch, but only 33 of these conducted at more than 50 m s^{-1}. A further 8 afferents conducted at more than 50 m s^{-1} but were not driven by a similar stretch. In a control experiment 25 of 30 afferents were driven and 23 of these were probably Ia, conducting at more than 60 m s^{-1}. All of the 5 afferents that were not driven conducted at less than 60 m s^{-1}.

The amplitude of the initial highly sensitive phase (initial burst) at the onset of a ramp-and-hold stretch compared to that of the static response differentiates most primary (at least 35 impulses s^{-1} difference) from secondary (less than 35 impulses s^{-1} difference) endings in normal spindles. In the reinnervated spindles 11 afferents were driven and had initial burst/static response differences of at least 35 impulses s^{-1}, but conducted at less than 50 m s^{-1}. It seems likely that these were group II afferents that had regenerated to old primary sites. Conversely, 6 afferents were not driven, had initial burst/static response differences of less than 35 impulses s^{-1}, but conducted at more than 50 m s^{-1}. They are likely to have been Ib afferents that had regenerated to old secondary or more polar sites.

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Paper 35.

R.W. Banks (1988)
Studies on the motor innervation of tenuissimus muscle spindles in the anaesthetized cat.
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Journal of Physiology, **406**, 70*P*, 1988]

Studies on the motor innervation of tenuissimus muscle spindles in the anaesthetized cat

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After extensive denervation of the left hindlimb in four adult cats (1·8–2·4 kg), anaesthetized with sodium pentobarbitone (Sagatal 45 mg kg^{-1} I.P.), the tenuissimus muscle was exposed by removal of the biceps femoris, and its distal part was freed from surrounding tissues to be reflected into a bath through which an artificial interstitial fluid circulated (three cats), or was immersed in a pool of warm (37°) mineral oil formed from skin flaps.

The effects of stimulating 23 motor axons (20 static γ, 2 dynamic γ, 1 static β) on the responses to ramp-and-hold stretches of 15 primary and 1 secondary endings were studied in spindles whose positions were marked by epimysial sutures for subsequent silver histology. The types of intrafusal fibre activated were inferred using Boyd's (1981) criteria, and in the case of 9 axons (the β, 7 static γs and a dynamic γ) the silver staining was sufficiently good to allow histological confirmation of their intrafusal distributions.

One experiment yielded potentially 70 motor/primary (10/7) combinations, of which 32 were effective. A dynamic γ activated 5 primaries, and 9 static γs each activated from 1 to 6 primaries, whereas each primary was activated by 3 to 5 static γs. Conduction velocities (c.v.) of the γs ranged from 23·0 m s^{-1} (the dynamic) to 41·1 m s^{-1}. It became apparent when the static motor effects were tabulated according to efferent c.v.s that bag_2 involvement was most common among the faster γs, which also had the widest distribution; chain involvement was most common among the slower γs; and γs of intermediate c.v. showed mixed effects. C.v.s of the γs from the remaining experiments ranged from 22·7 to 41·7 m s^{-1}, and their inferred intrafusal distributions were consistent with this pattern. The remaining dynamic γ had a c.v. of 34·7 m s^{-1} and activated both primaries against which it was tested.

Boyd (1986) has suggested that there may be two types of static γ axon, one always though not exclusively distributed to bag_2 fibres, and one similarly distributed to chain fibres. This has been criticized by Banks *et al.* (1985), who have argued for a single type. The present results offer a resolution in that, when sampled, a single population of static γs of wide c.v. range, whose extremes are preferentially associated with one of the two effectors, would appear to fall into the two populations described by Boyd.

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Paper 36.

R.W. Banks, D. Barker, H.H. Saed & M.J. Stacey (1988)
Innervation of muscle spindles in rat deep masseter.
J. Physiol. 406; 161P.

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Journal of Physiology, **406**, 161*P*, 1988]

Innervation of muscle spindles in rat deep masseter

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Rowlerson *et al.* (1988) report that the muscle fibres in rat deep masseter are all type IIA except for a very few type I and type IIC fibres that surround an anterior cluster of about 40 spindles. Their suggestion that the type I fibres might belong to skeletofusimotor units innervated by slow dynamic β axons prompted us to examine this spindle cluster in teased silver preparations and search for instances of β innervation. We found three examples in which the common origin of the intra- and extrafusal branches were preserved. The intrafusal branch terminated in a small plate on a bag_1 fibre in each case. Similar plates were often seen on the bag_1 fibres of other spindles in the cluster. We therefore conclude that some cluster spindles receive a dynamic β innervation similar to that demonstrated in cat hindlimb spindles by Barker *et al.* (1977).

Whilst searching for β axons, we observed some unusual features of the sensory innervation in the anterior cluster. Altogether 130 afferents were distributed to 37 spindles, the total at spindle entry, 137, being greater due to 7 axons that branched to supply primary endings to two bag fibres, usually bag_1, in separate spindles. There is no previous report of such branching in the afferents from mammalian spindle primary endings. Each spindle received 1–6 afferents, up to 5 of which (total 91) contributed to multiply-innervated primary regions in 34 spindles, though some (total 11) also ended in secondary regions. Bag_1 and bag_2 fibres were often innervated separately in the multiple primary endings. The remaining 43 afferents ended in secondary regions, which were occupied thus: one S_1, 11 spindles; both S_1s, 13 spindles; one S_1 one S_2, 6 spindles; both S_1s one S_2, 2 spindles.

The frequency distribution of the afferent complements in excess of a single Ia followed binomial statistics with parameters $n = 5$, $P = 0{\cdot}51$ (χ^2 observed *vs.* expected = 1·72, n.s.). Characteristic binomial distributions of spindle afferents have been reported for various cat muscles (Banks & Stacey, 1988), and the anterior cluster in rat deep masseter is not unusual in this respect. The extremely high incidence of multiple primaries (92%), and the large proportion of afferents supplied to primary locations (69%), could be the result of unusually high competition for suitable sites in the early stages of spindle development.

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Paper 37.

R.W. Banks & M.J. Stacey (1988)
Quantitative studies on mammalian muscle spindles and their sensory innervation.
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QUANTITATIVE STUDIES ON MAMMALIAN MUSCLE SPINDLES AND THEIR SENSORY INNERVATION

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INTRODUCTION

It is not surprising, in view of the various functional roles played by skeletal muscles, that each muscle should possess a characteristic proprioceptive innervation. Muscle spindles are relatively easy to count and have been the main subject of quantitative studies. In drawing comparisons between different muscles, most authors have used the number of spindles per gram of adult muscle, or spindle density, as a measure of relative abundance. In both man and cat, where sufficient muscles have been examined, smaller muscles have been found usually to have higher spindle densities than larger muscles (reviewed by Hosokawa, 1961; Voss, 1971; and Barker, 1974). This has frequently led to the suggestion that the higher densities are functionally appropriate to small muscles involved in fine postural adjustment or manipulation, yet it has never been demonstrated that it is justifiable to relate spindle number linearly to muscle mass as a simple density.

Spindle counts actually refer to the number of separate encapsulations whose sensory complements are very varied (Barker & Banks, 1986). Each capsule usually encloses a primary sensory ending supplied to all three types of intrafusal muscle fibre (b_1b_2c unit) by a group Ia afferent axon. On either side of the primary up to 3 or 4 secondary endings may also be supplied, mainly to chain fibres, by about as many group II axons. Rarely a b_1b_2c unit may receive two Ia axons that form a double primary ending. Separate encapsulations may be linked in tandem by a continuous bag_2 fibre and, at least in the cat, some of them (b_2c units) lack a bag_1 fibre; the proportion of this type differs from one muscle to another (Bakker & Richmond, 1981; Banks et al. 1982). Their primary endings, which are rarely accompanied by secondaries, are supplied by afferents intermediate in diameter and preterminal branching pattern between those of b_1b_2c primary and S_1 secondary endings (Banks et al., 1982; Richmond et al., 1986; Kucera & Walro, 1987).

In this paper a new measure of relative spindle abundance is proposed and a comparison is made of the different provision of spindle units and their afferents in various muscles of the cat.

RELATIVE SPINDLE NUMBER

The usefulness of spindle density as a measure of relative spindle abundance relies on the assumption that absolute spindle number should be linearly related to muscle size. That this may not be so is suggested by the general occurrence of higher densities in smaller homologous muscles of different species (Table 1) as well as in the smaller muscles within a single species. Moreover, similarly sized muscles of different species may exhibit similar spindle densities: e.g. cat soleus 2.49g, 56 spindles, $23g^{-1}$ (Chin et al., 1962); human abductor pollicis brevis 2.7g, 80 spindles, $29.3g^{-1}$ (Schulze, 1955). These observations led us to question the relationship between spindle number and muscle size, and we have therefore sought evidence for its nature from published data for 75 muscles derived from rat (4 muscles), Arendt and Asmussen (1974); cat (29 muscles), Bakker and Richmond (1982), Barker (1974), Richmond and Abrahams (1975), Richmond and Stuart (1985); and man (42 muscles), Hosokawa (1961), Matthews (1972), von Hoyer (1963).

Logarithmic transformation of both spindle number and muscle weight yielded a linear relationship of the form

$$y = 1.58 + 0.32x$$

where y is $\log_{10}$ spindle number and x is $\log_{10}$ muscle weight in grams (Fig. 1). It is now possible to measure relative spindle abundance by the extent to which any muscle deviates in the richness of its spindle content from the value expected for a muscle of the same size. Some examples are given in Table 2. To those of us long conditioned in the use of spindle density it may come as a surprise to find lumbrical muscles rather poorly supplied with spindles by this measure. Dorsal neck muscles, however, retain their position as having the greatest abundance of spindles, with over five times as many as expected in intertransversarius C2-C3 of the cat.

SENSORY INNERVATION

We have previously shown that the number of secondary endings in b_1b_2c units of cat hindlimb muscles from a mixed sample dominated by tenuissimus and peroneus brevis followed a binomial distribution (Banks et al., 1982). However, it was also apparent that the average number of secondary endings per unit varied somewhat in different muscles. We have therefore analysed the sensory innervation of spindles from a variety of axial and limb muscles of the cat using teased, silver-impregnated preparations.

Table 1. Spindle-capsule Density in Homologous Muscles of Rat, Cat and Man.

Species	Capsule Density (g^{-1})		
	Lumbrical III (Hand)	Soleus	Gastrocnemius (Total)
Rat		310.5	40.0
Cat	173	23	6.5
Man	12.2	0.94	0.4

Data from Voss, 1971; Arendt & Asmussen, 1974; Barker 1974

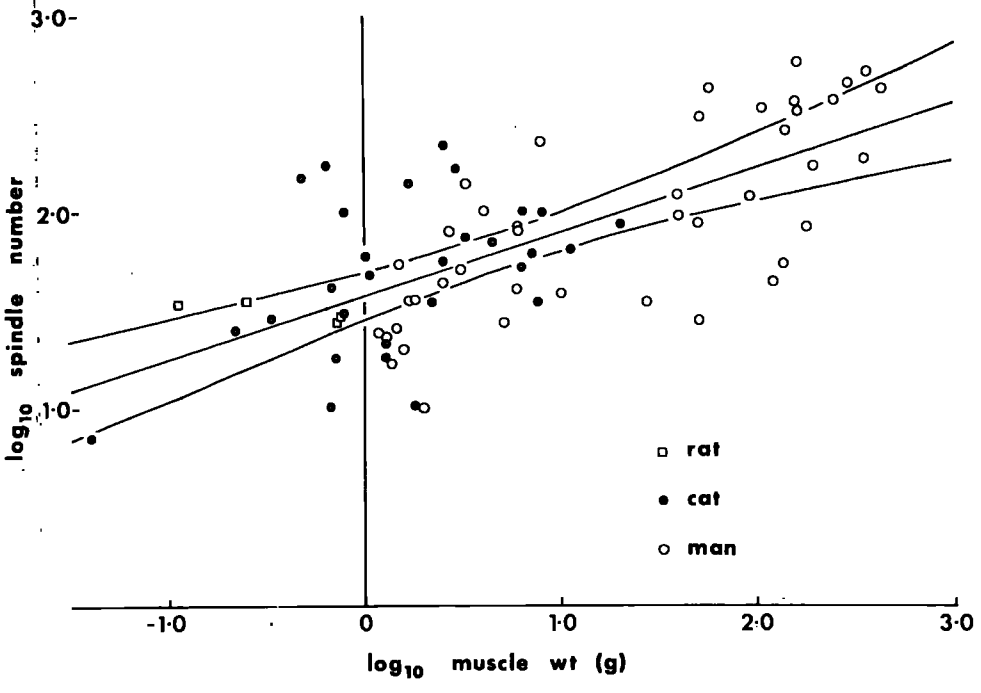


Fig. 1. Logarithmic transformations of spindle number and muscle weight are highly correlated (r=0.69). The linear regression is shown with 95% confidence limits.

To avoid biasing the sample, it was important whenever possible to use as many well-stained spindles as could be obtained from individual muscles.

The composition of the sample is given in Table 3a together with data on the proportion of all units that were of b_2c type, the proportion of b_1b_2c units that possessed double primary endings, and the average number ($\bar{a}$) per b_1b_2c unit of afferent fibres in excess of a single Ia fibre. The last feature is expressed in this way to take account of the double primary endings and also a small number of II fibres that had terminals in the S_1 positions on both sides of some primary endings from extensor digitorum longus and popliteus. Values of $\bar{a}$ ranged from 0.56 in extensor digitorum lateralis of the forelimb to 3.5 in complexus of the neck; however, the greatest number of afferents and endings occurred in 2 spindles from popliteus (1 from each muscle sampled) with complements of $S_2S_1PPS_1S_2S_3$ (7 afferents) and $S_2S_1PPS_1S_2S_3S_4$ (8 afferents).

Frequency distribution of the number of afferents in excess of a single Ia differed for each type of muscle, but when several complete muscles of a single type (e.g. peroneus brevis) were examined the individual distributions were all similar. For each muscle the value of $\bar{a}$ was used to calculate the corresponding Poisson distribution and a set of binomial distributions from which one could be selected that presented the greatest overlap with the observed frequency distribution. Whenever there were sufficient degrees of freedom a χ^2 goodness-of-fit test was performed to determine if the observed distribution differed from the calculated one. The results are shown in Table 3b. In general, the binomial distributions fitted the observed data better than did the Poisson distributions, as is particularly clear in the case of peroneus brevis which was the type most extensively sampled in that we were able to analyse virtually all the spindles from 5 muscles. The complexus sample was slightly different

Table 2. Deviations of Actual Spindle-capsule Abundances from Values Expected in Terms of Muscle Weight.

Species/Muscle	Number		Deviation	
	Expected	Actual	Linear	Log
Cat/Intertrans	32.8	176	5.4	+0.73
Man/Obl cap inf	73.6	232	3.2	+0.50
Cat/Biv cervicis	45.1	140	3.1	+0.49
Rat/Soleus	18.8	34	1.8	+0.26
Man/Bic brachii	194.3	320	1.6	+0.22
/Soleus	265.3	408	1.5	+0.19
Rat/Plantaris	24.1	35	1.5	+0.16
Cat/Rect femoris	75.0	104	1.3	+0.14
/Soleus	50.9	56	1.1	+0.04
/Int V (hand)	23.1	25	1.1	+0.03
/Gastr medialis	71.9	62	0.86	-0.06
Rat/Gastr medialis	34.0	28	0.82	-0.08
Cat/Lumb III (hand)	13.6	7	0.51	-0.29
Man/Plantaris	80.6	39	0.48	-0.32
/Lumb III (hand)	44.2	20	0.45	-0.34
Cat/Occipitoscap	33.3	11	0.33	-0.48
Man/Infraspinatus	183.5	54	0.29	-0.53
/Thyreohyoideus	47.4	12	0.25	-0.60
Cat/Infrahyoideus	45.9	6	0.13	-0.88

Abbreviations: Bic(eps); Biv(enter); Gastr(ocnemius); Int(erosseus); Intertrans(versarius); Lumb(rical); Obl(iquus) cap(itis) inf(erior); Occipitoscap(ularis); Rect(us).

from the best-fitting binomial distribution, but this may well have been due to sampling bias since the number of spindles analysed was small compared to the number present in a single muscle, largely because of the poor staining which is a common problem with dorsal neck muscles.

We feel justified in concluding, therefore, that the observed data are well described by binomial statistics, so that each muscle may be classified two-dimensionally by the parameters n, p (Fig. 2). Where p is very small (<0.1) the distributions reduce to Poisson form, but as p increases the advantages of the binomial distribution become more apparent. This should be borne in mind when considering interosseus ($p = 0.41$) for which there were insufficient degrees of freedom to test the binomial distribution, but whose observed and Poisson distributions differed only slightly.

DISCUSSION

In attempting to interpret these results it must be recalled that the spindle is a structure that is not entirely purpose-built from unique

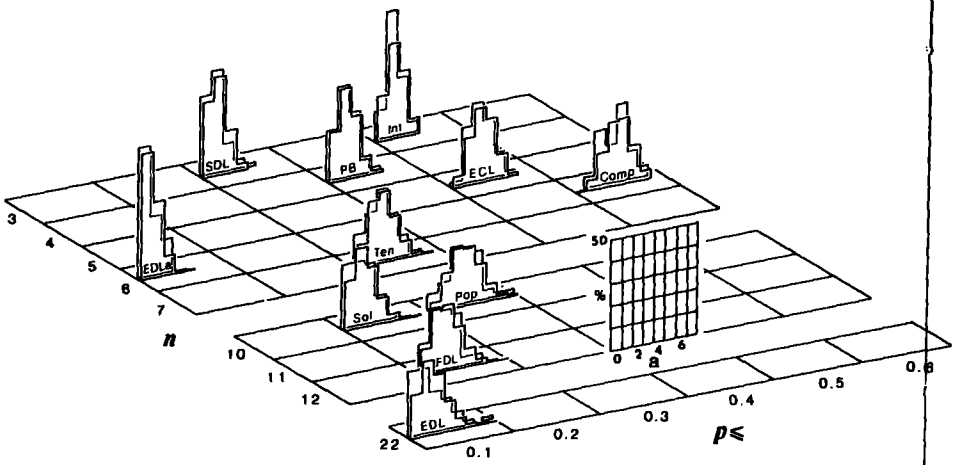


Fig. 2. Pairs of histograms showing observed (rear) and best-fitting binomial (front) distributions of numbers of afferents (a) in excess of a single Ia. The histograms are positioned on the grid according to their binomial parameters (n ,p). Abbreviations: Comp(lexus); ECL, Extensor caudae lateralis; EDLa, Extensor digitorum lateralis; Int(erosseus); Pop(liteus); Sol(eus); SDL Superficial and deep lumbrical; Ten(uissimus); and as Table 3.

components, but is a makeshift structure assembled from what is available. In order to understand which features are functionally important in the adult we must recognize that the significance of some features may relate principally to the way that the spindle is constructed.

Our results indicate that in the case of the muscle spindle the proprioceptive needs of functionally diverse muscles are met in two mainly independent ways: i) variations in the abundance of spindles (and hence Ia afferents), and ii) variations in the proportional balance of Ia and II afferents. The association of II with Ia afferents is clearly a random process, but it does not seem to depend only on the average number of afferents present. One obvious factor that may also be important is the period of time during which developing afferents continue to arrive at the presumptive spindle.

The other features considered here, double primary endings and b_2c units, might not be independently produced, but might arise as inevitable consequences of the developmental programme in which relative timing of events such as myotube formation, regional maturation, and neurite arrival are crucially important (e.g. Milburn, 1984). For example, if Ia and II afferents differentiate only during spindle development, b_2c units would be more likely to occur if there is high competition among neurites (either sensory/sensory or sensory/motor) for available target sites on primary myotubes early in development. This can be brought about if most of the afferents arrive at an early stage (which perhaps gives rise to a distribution with high n and low p, e.g. extensor digitorum longus); or if there is simply a very large number of afferents (e.g. complexus). This offers an alternative explanation for the occurrence of b_2c units in cat muscles to the specific functional role that has been postulated (Richmond et al. 1986), and that could just as easily be played by secondary endings.

Table 3. a) Composition of the Sample of Cat Muscles Used in the Analysis of Spindle Sensory Innervation, Together with Some Basic Observations. b) Comparisons of Observed Frequency Distributions of Afferent Axons in Excess of One Ia with Distributions Calculated According to Poisson and Binomial Statistics. χ^2 Goodness-of-Fit Test.

a						b					
Muscle		All Units		b_1b_2c Units		Poisson			Binomial		
Type	Number Sampled	Total Number	$\%b_2c$ Type	% with Double P	$\bar{a}$	χ^2	df	P	χ^2	df	P
EDLat	1	32	16	0	0.56	-	0	-	-	0	-
S&DLumb	14	52	4	0	0.9	2.34	1	NS	-	0	-
PB	5	164	7	0.7	1.22	9.11	3	$0.5 \gtrsim P > 0.1$	0.18	1	NS
Interosseus	5	39	0	0	1.23	8.68	2	$0.5 \gtrsim P > 0.1$	-	0	-
Soleus	3	47	11	0	1.36	0.48	2	NS	1.2	1	NS
EDL	1	71	20	12	1.58	3.38	3	NS	3.82	1	NS
Tenuissimus	14	70	7	6	1.86	1.82	3	NS	1.47	2	NS
FDL	2	81	7	0	1.89	3.61	3	NS	1.93	2	NS
ECLat	2	64	10	0	2.26	7.54	4	NS	0.11	1	NS
Popliteus	2	68	13	8	2.72	4.49	3	NS	1.27	2	NS
Complexus	2	64	34	0	3.5	10.7	4	$0.5 \gtrsim P > 0.1$	4.85	1	$0.5 \gtrsim P > 0.1$

Abbreviations: ECLat, extensor caudae lateralis; EDLat, extensor digitorum lateralis; EDL, extensor digitorum longus; FDL, flexor digitorum longus; PB, peroneus brevis; S&DLumb, superficial and deep lumbricals.

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Paper 38.

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SPECIFICITIES OF AFFERENTS REINNERVATING CAT MUSCLE SPINDLES AFTER NERVE SECTION

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SUMMARY

1. We have made quantitative assessments of the sensory reinnervation and recovery of peroneus brevis muscle spindles following section and epineurial repair of the common peroneal nerve. After 6–50 weeks recovery, single-unit, dorsal-root recordings were made of the responses to ramp-and-hold or sinusoidal stretch of the reinnervated spindles, which were subsequently examined in teased, silver preparations.

2. Assessments of recovery used data obtained from cross-union experiments in which foreign afferents (including Ib) were given the opportunity of reinnervating spindles in the absence of their native (Ia, spindle II) afferents; and from an examination of tenuissimus spindles reinnervated by Ia and spindle II afferents in the absence of Ib afferents. These studies revealed: (i) that regenerating Ib afferents can terminate in sites originally occupied by the endings of Ia or spindle II afferents, and respond to stretch like normal Ia and spindle II afferents; (ii) that Ib and spindle II afferents reinnervating spindles are histologically identical apart from diameter range; and (iii) that some cutaneous afferents can reinnervate spindles and give highly abnormal, phasic stretch responses.

3. Recovery of afferents reinnervating spindles was marked by increases in conduction velocity and proportions firing tonically, but their firing rates at the three phases of ramp-and-hold stretch were considerably lower than normal and showed no tendency to increase.

4. Some relatively fast afferents that gave spindle II-type responses were identified as Ib afferents reinnervating secondary-ending sites; conversely, some relatively slow afferents that gave Ia-type responses were identified as spindle II afferents reinnervating primary-ending sites.

5. The estimated loss of spindle afferents from tenuissimus after nerve section (52% Ia, 49% spindle II) was considerably less than the estimated loss of these afferents from peroneus brevis after section of the common peroneal nerve (79% Ia, 86% spindle II). The proportion of spindles in tenuissimus reinnervated by free-ending afferents was also much lower (22%) than in peroneus brevis (73%). These differences are partly attributed to the greater size and degree of afferent complexity of the common peroneal nerve.

6. Similar proportions of spindles in peroneus brevis were reinnervated by Ia and

Ib afferents after both partial (27% Ia, 20% Ib) and complete (21% Ia, 20% Ib) section of the common peroneal nerve.

7. In discussing our results in relation to the work of others we conclude that when Ia, spindle II and Ib afferents regenerate after nerve section, some make random and functional reconnections, which, by virtue of their dual cell specificity for intrafusal muscle fibres and collagen, can be with either spindles or tendon organs.

INTRODUCTION

The afferents that innervate mammalian skeletal muscle terminate in muscle spindles (as primary and secondary endings), tendon organs, and paciniform corpuscles, and end freely around blood vessels and in fat and connective tissue. Free-ending afferents are the most common; among the rest, secondary (spindle II) afferents occur more frequently than either primary (Ia) or tendon-organ (Ib) afferents, and paciniform afferents are always scarce (Barker, 1974). The ability of afferents to regenerate after nerve section raises questions about their specificity that can only be answered on the basis of a substantial knowledge of their normal histological and physiological properties, such as now exists for spindle afferents. Does the spindle have to be reconnected with the same types of afferent it received before the injury to function successfully? Can 'foreign' afferents be distinguished histologically from 'native' afferents in the reinnervated receptor? If foreign afferents can reinnervate, can they also substitute functionally for native afferents? If so, to what extent?

We were confronted by such questions when trying to assess the reinnervation and recovery of spindles in peroneus brevis after section and suture of the common peroneal nerve. We sought answers in two ways: by devising nerve cross-union experiments in which foreign afferents (Ib, free-ending, pacinian, cutaneous) were given the opportunity of reinnervating spindles in the absence of their native afferents (Ia, spindle II); and by examining spindles reinnervated by their native afferents in a muscle that lacked a supply of Ib afferents. The main answers were: (i) that foreign afferents are able to reinnervate spindles, but only with their own kinds of terminal; (ii) that regenerating Ib afferents can terminate in sites originally occupied by the endings of Ia or spindle II afferents, and respond to stretch like normal Ia and spindle II afferents; (iii) that Ib and spindle II afferents reinnervating spindles are histologically identical apart from diameter range; and (iv) that some cutaneous afferents can reinnervate spindles and respond to stretch.

This information helped us to understand the stretch–response behaviour of peroneus brevis spindles reinnervated after nerve section, and enabled us to make quantitative assessments of their reinnervation by native and foreign afferents. Since the common peroneal nerve is musculocutaneous we were also able to make comparisons between recoveries achieved after its complete section and suture, with those achieved after section of two muscle–nerve fascicles only.

While this work was in progress, Collins, Mendell & Munson (1986) were studying the specificities of afferents reinnervating spindles after nerve section by recording their central actions. By ascertaining whether the reinnervating afferent was able (Ia, some spindle II) or unable (Ib) to generate field potentials in the appropriate

motoneurone pool, they showed that Ib afferents were involved in spindle reinnervation, and thus confirmed our preliminary report of this finding from cross-union experiments (Banks, Barker & Stacey, 1984). Preliminary reports of some of the other results have also been published (Banks & Barker, 1983; Banks, Barker & Brown, 1985; Banks & Barker, 1986; Banks, 1987; Banks, Barker & Stacey, 1988).

METHODS

Fifty-six 1·5–3·7 kg adult cats of either sex were used, ten in various types of control experiment, the rest at recovery times ranging from 6 to 50 weeks after preparative surgery. For all surgical and experimental procedures anaesthesia was induced with sodium pentobarbitone (Sagatal, 45 mg kg^{-1}, I.P.) and was maintained during surgery with halothane (Fluothane) by inhalation or, during acute experiments, by Sagatal given intravenously as required.

Surgery. Five types of surgical procedure were applied unilaterally as follows (number of animals and recovery times in parentheses after each): (i) complete section of the common peroneal nerve about 4 mm proximal to its point of entry into gastrocnemius, followed immediately by repair with about ten epineurial sutures, care being taken to preserve the fascicular alignment as far as possible (23; 6–50 weeks); (ii) section at the same site of only those fascicles that supply the peroneus brevis and tertius muscles, followed immediately by repair with one or two epineurial sutures (13; 6–50 weeks); (iii) section at the same site of the same fascicles as (ii) together with one of the large cutaneous fascicles that constitute the superficial peroneal nerve, followed immediately by cross-union with two sutures of the proximal cutaneous and distal muscular stumps and by resection of the two remaining stumps (3; 16–22 weeks); (iv) section within a branch of the tibial nerve of the fascicles that form the interosseous and flexor digitorum longus (FDL) nerves, followed immediately by cross-union with one suture of the proximal interosseous and distal muscular stumps and by resection and ligation of the remaining two stumps (7; 10–20 weeks); (v) section without repair of the fascicle that forms the tenuissimus nerve just proximal to its point of exit from the sciatic nerve (6; 23–24 weeks). All sections were made with fine scissors, and all sutures were made with 10/0 polyamide.

The early results from the interosseous–FDL cross-union experiments showed that there was reinnervation from the proximal stump of the FDL nerve in addition to that from the interosseous nerve, presumably because of the size disparity between the cross-united fascicles. This was actually beneficial for the physiological aspect of the experiments, since the muscle would twitch on stimulation of the FDL nerve distal to the lesion due to the restored motor innervation, and any FDL afferents that might have returned could be effectively removed by sectioning the proximal stump of the FDL nerve. But for the histology such 'contaminating' FDL afferents could only be removed by a second operation in which an attempt was made to section the FDL nerve proximal to the cross-union. The animal then recovered for 1 week to allow time for any restored FDL afferents to degenerate. This had to be done at the original site, now obscured by scar tissue, and was consequently much more difficult than during the radical dissection for physiology. It is therefore possible that in order to avoid damage to the interosseous nerve not all of the 'contaminating' reinnervation was removed. The histological data include results from two cats in which the second operation was carried out.

Neurophysiology. For the acute part of each experiment the reinnervated peroneus brevis (procedures (i)–(iii)), or FDL muscle (procedure (iv)), was exposed and the rest of the hindlimb extensively denervated. Recordings were made from filaments of the appropriate dorsal and ventral roots, which were cut close to the spinal cord. Surgically-exposed surfaces were covered with pools of warm mineral oil, and the animal's core temperature was maintained at 37 ± 1 °C. The muscle was attached by its freed distal tendon to a servo-controlled electromagnetic puller (Ling Dynamics), which was used to apply ramp-and-hold stretches of 1·8 mm amplitude at three different rates (2·5, 5 or 10 mm s^{-1}) up to maximum physiological length. Initial muscle length was set in this way rather than by reference to spindle output, since the method was accurately reproducible at the start of each experiment, whereas the effects of denervation and reinnervation on spindle responses could not be predicted. In some cases sinusoidal stretches of 100 μm maximum

amplitude at 100 Hz were also used. Responses of more than three or four spikes were recorded on magnetic tape for later analysis. Whenever possible, afferents were identified as of tendon-organ or muscle-spindle type according to whether they tended to fire during or after a muscle twitch evoked by stimulating electrodes in contact with the muscle nerve. Direct responses of the afferent to this stimulation were used to obtain its latency and to verify that it was functionally single. At the end of each experiment the nerve length between the recording electrodes on the dorsal root and the stimulating electrode on the muscle nerve was measured post-mortem. Normal preparations of peroneus brevis (5), FDL (1), flexor hallucis longus (3), and the superficial peroneal nerve (1) were used as control experiments.

Spindle afferents (Ia, II) of control muscles normally fired tonically at the initial muscle length. It is convenient to refer to the mean rate of such firing as 'the tonic discharge'. In general, the firing rate reached a local peak at the end of the ramp phase of stretch when the muscle reached maximum physiological length. For normal afferents this peak always increased with greater rates of stretch, and usually, but not invariably, did so for regenerated afferents. The range of dynamic index, referred to in the Results, was therefore taken as the difference between the maximum and minimum dynamic indices irrespective of rate of stretch. During the hold phase of stretch the firing rate declined rapidly, then more slowly, to settle eventually at a maintained level higher than the tonic discharge. It is convenient to refer to the level of maintained firing 0·5 s after the end of the ramp as 'the static discharge', this value being obtained by interpolation of instantaneous frequency plots. The difference between the local peak level at the end of the ramp and the static discharge is then a measure of the dynamic index as defined by Matthews (1972).

In some experiments intrafusal motor effects were sought by stimulating ventral-root filaments at 50 Hz while recording from afferents identified as reinnervating spindles at various recovery stages from 16 to 50 weeks. When a possible effect occurred the filament was subdivided until a functionally single efferent was present, and its effect was then examined more systematically.

Histology. The experimental muscle was removed post-mortem and processed for silver staining using the technique of Barker & Ip (1963) with the modifications reported by Barker, Scott & Stacey (1985). The reinnervation of spindles was then examined in teased preparations. Preliminary observations of the endings regenerated by afferents reinnervating peroneus brevis spindles after section of the common peroneal nerve showed that their form was either free or annulospiral. When the source of the reinnervating afferents was restricted to cutaneous free-ending afferents (surgical procedure (iii) above) the spindles contained regenerated free endings only. This suggests that the regenerated form of an afferent ending resembles its normal form. On this basis we have assumed that regenerated annulospiral endings reinnervating peroneus spindles after section of the common peroneal nerve belonged to annulospiral muscle afferents (Ia, Ib or spindle II).

The interosseous–FDL cross-union and tenuissimus experiments established the ability of regenerating Ib afferents to terminate in spindles in sites originally occupied by primary (Ia) or secondary (II) endings, and showed that only Ia afferents were able to regenerate primary endings with transverse terminal bands around the nuclear bags. This feature, taken together with axon diameter measured near spindle entry after 12 weeks or more recovery, made it possible to identify primary endings regenerated by Ia afferents in peroneus brevis spindles reinnervated after nerve section, and to distinguish them from primary endings regenerated by Ib or spindle II afferents. The diameter range of thirty-seven regenerated afferents identified as Ia was 3·5–7·5 μm, mean 5·4 μm; a sample of sixty-six normal peroneus brevis Ia afferents had a diameter range of 4·0–12·5 μm, mean 7·0 μm.

The similarity of location and terminal form of Ib and spindle II afferents meant that they could be identified histologically in the reinnervated peroneus brevis spindles only on the basis of their diameters. The diameters of normal peroneus brevis Ib and spindle II afferents measured near to receptor entry fall mostly into two groups: large/medium (Ib) and medium/small (II), with partial separation at around 3·5–4·0 μm (fig. 11 *A* and *C*). Thus, 77·7 % of sixty-three spindle II afferents (diameter range 1·7–6·8 μm, mean 3·0 μm) had diameters $\leqslant$3·5 μm, as against only 9·3 % of forty-three Ib afferents (diameter range 2·5–8·5 μm, mean 5·5 μm). Our interosseous–FDL cross-union and tenuissimus experiments showed that after nerve section and full recovery the intramuscular diameters of regenerated Ib and spindle II afferents decreased in range and increased in overlap (Fig. 11 *B* and *D*). Hence, 44 % of regenerated interosseous Ib afferents (diameter range 1·75–7·25 μm, mean 3·9 μm, $n = 50$) had diameters $\leqslant$3·5 μm as compared with 76 % of regenerated tenuissimus spindle II afferents (diameter range 1·6–4·8 μm, mean 3·0 μm, $n = 45$).

It follows that Ib/spindle II afferents with diameters > 3·5 μm are more likely to be Ib than II, and those ⩽ 3·5 μm are more likely to be II than Ib. In order to obtain approximate estimates of the numbers of the two types of afferent engaged in peroneus brevis spindle reinnervation, we have identified all afferents with diameters ⩽ 3·5 μm as spindle II, and all above that as Ib, thus producing fifty Ib and eighty-two II from a total of 132 Ib/II afferents. The alternative of adopting 3·0 μm as a segregating diameter would have resulted in identifying sixty-nine as Ib and sixty-three as II, but it seems unlikely that more Ib than spindle II afferents would have succeeded in reinnervating spindles, especially since spindle II afferents outnumber Ib afferents in normal peroneal nerves by 1·5–1·75:1 (Scott & Young, 1987).

In using these criteria for the identification of regenerated Ia, Ib and spindle II afferents we acknowledge that any Ia afferent that may have reinnervated a secondary site would have been identified as II if its diameter was 3·5 μm (only one of the thirty-seven afferents identified as Ia was of this diameter), or Ib if its diameter was > 3·5 μm. The results given for the afferent reinnervation of peroneus brevis spindles after complete or partial section of the common peroneal nerve represent data pooled from several experiments, as follows: complete section, nine animals with recovery periods of 12 (2), 16 (2), 20, 30, 40 and 50 (2) weeks; partial section, four animals with recovery periods of 20, 30, 40 and 50 weeks. The analysis was restricted to spindles with all three types of intrafusal muscle fibre (b_1b_2c units). Doubt about the ending form of a regenerated annulospiral afferent, or inability to measure its axon diameter, resulted in eleven (6% of the total) remaining unidentified.

RESULTS

Complete or partial section of the common peroneal nerve

Sequence of recovery

Afferents were present in the nerve to peroneus brevis at the earliest time (6 weeks) investigated after the operation. No attempt was made to count those giving only a direct response to electrical stimulation, but initially they were very much more numerous than those responding to muscle stretch or contraction, and there were always some present. The total number of afferents responding with at least one spike to muscle stretch or contraction at all stages of recovery amounted to 566 (see Table 1A). They increased from an average of seven per muscle at 6 weeks to eighteen per muscle at 7 weeks, and by 8 weeks had reached the same number, twenty-six per muscle, as the pooled average for all the subsequent recovery stages. There was no significant difference, in this respect, between recovery following section of the whole common peroneal nerve and that following section of only the fascicles supplying peroneus brevis and tertius (although the highest number from a single experiment, sixty, was obtained 40 weeks after such a partial section).

Conduction velocities recovered most rapidly in the earliest post-operative periods, and the rate of recovery declined progressively until the 20–30 week period when the maximum attainable velocity was reached, as illustrated by the conduction velocities of the fastest afferent present at each stage (Table 1B). In this case the maximum velocity was 85% of the corresponding control value.

There was considerable variation in the proportions of unidentified afferents as against those identified as reinnervating spindles or tendon organs, so that the only clear trend to emerge in the later stages of recovery was the increase in the proportion of afferents identified as reinnervating spindles at the expense of unidentified afferents (Table 1C–E). Following complete nerve section at least 7% of afferents were always unidentified, but after partial section there were no unidentified

TABLE 1. Aspects of the sequence of mechanosensory afferent reinnervation of cat peroneus brevis following (i) complete and (ii) partial section of the common peroneal nerve

	Weeks after operation									Control
	6	7	8	12	16	20	30	40	50	
(A) Total no. afferents										
(i)	6	47	44	42	80	50	25	33	38	
(ii)	15	8	34	21	13	29	21	60	—	412
(B) Conduction velocity of fastest afferent ($m\ s^{-1}$), (i) and (ii)	44·1	55·2	58·0	63·1	68·9	75·6	83·9	84·6	81·0	98·4
(C) Percentage afferents reinnervating spindles										
(i)	0	21	23	43	33	32	56	79	61	
				pooled 46						
(ii)	33	13	35	62	77	44	38	50	—	73
				pooled 54						
(D) Percentage afferents reinnervating tendon organs										
(i)	50	17	41	21	41	34	28	12	29	
				pooled 30						
(ii)	33	38	53	33	23	56	62	45	—	27
				pooled 44						
(E) Percentage unidentified afferents										
(i)	50	62	36	36	26	34	16	9	11	
				pooled 24						
(ii)	33	50	12	5	0	0	0	5	—	0
				pooled 3						
(F) Percentage tonically firing spindles, (i) and (ii)	0	27	23	19	31	45	50	55	48	91

afferents in three of the later recovery stages. The increase in the proportion of afferents identified as reinnervating spindles was probably due to a corresponding change in their excitability, shown, for example, by the greater number that were tonically firing later in recovery (Table 1F). In the pooled results from the 12–40 week recovery stages after partial section 98% of afferents were identified, 54% as reinnervating spindles, 44% as reinnervating tendon organs. This represents a considerably lower ratio of spindle: tendon-organ afferents than would be expected from a normal muscle (control values, 73% spindle, 27% tendon organ).

Tonically firing, regenerated afferents in spindles had lower mean firing rates than a sample of normal Ia and spindle II afferents under the same conditions of muscle stretch in relation to maximum physiological length of the muscle. They showed no tendency to improve in this respect throughout the recovery period (Fig. 1*A*).

Distribution of conduction velocities at later recovery stages

The distribution of conduction velocities for all mechanosensory afferents present at recovery stages 30–50 weeks is shown in Fig. 1*B*. It is unimodal with a peak at about 50 m s^{-1}. Unidentified afferents mostly conducted at less than 60 m s^{-1}. Afferents identified as reinnervating tendon organs, and those identified as reinnervating spindles, are shown in Fig. 1*C* and *D*, respectively. The distribution for afferents reinnervating tendon organs is very peculiar, with a large majority conducting at less than 60 m s^{-1} and perhaps a small separate group conducting at more than 70 m s^{-1}. Conduction velocities of afferents reinnervating spindles were slightly bimodal with a major peak at 60–70 m s^{-1} and a subsidiary peak at 40–50 m s^{-1}. In control experiments (Fig. 1*E*) group Ia peaked at 80–90 m s^{-1} and group II at around 30 m s^{-1} with a very clear distinction between the two groups.

Responses of afferents reinnervating spindles to ramp-and-hold stretch

Examples of responses to ramp-and-hold stretches of normal Ia and spindle II afferents, and of afferents reinnervating spindles, are shown in Fig. 2. Normal Ia afferents characteristically show a large initial burst soon after the start of the ramp, fire irregularly, and fall silent at the onset of release (Fig. 2*A*); whereas spindle II afferents have a small initial burst often seen only as an inflexion in the rising phase, fire regularly, and often maintain firing during release (Fig. 2*B*). Corresponding types of response were obtained from some reinnervated spindles (Fig. 2*C* and *D*), but many regenerated afferents exhibited various abnormalities, the most common of which were: lack of a tonic discharge (Fig. 2*E*); a spindle II-type response associated with a relatively fast conduction velocity (Fig. 2*F*); a Ia-type response associated with a relatively slow conduction velocity (Fig. 2*G*); and the lack of a sustained static response during the hold phase of stretch (Fig. 2*H*). In the early recovery period (6–12 weeks) 31% of all afferents identified as reinnervating spindles were of this last type. Their proportion fell to less than 2% in the later period (16–40 weeks) after partial nerve section; however, after complete nerve section the proportion remained rather high (13%, 16–50 weeks) and showed no tendency to fall throughout the later period. Other, rare types of abnormality were: firing in rather regularly repetitive bursts, unconnected to arterial pulse; very high

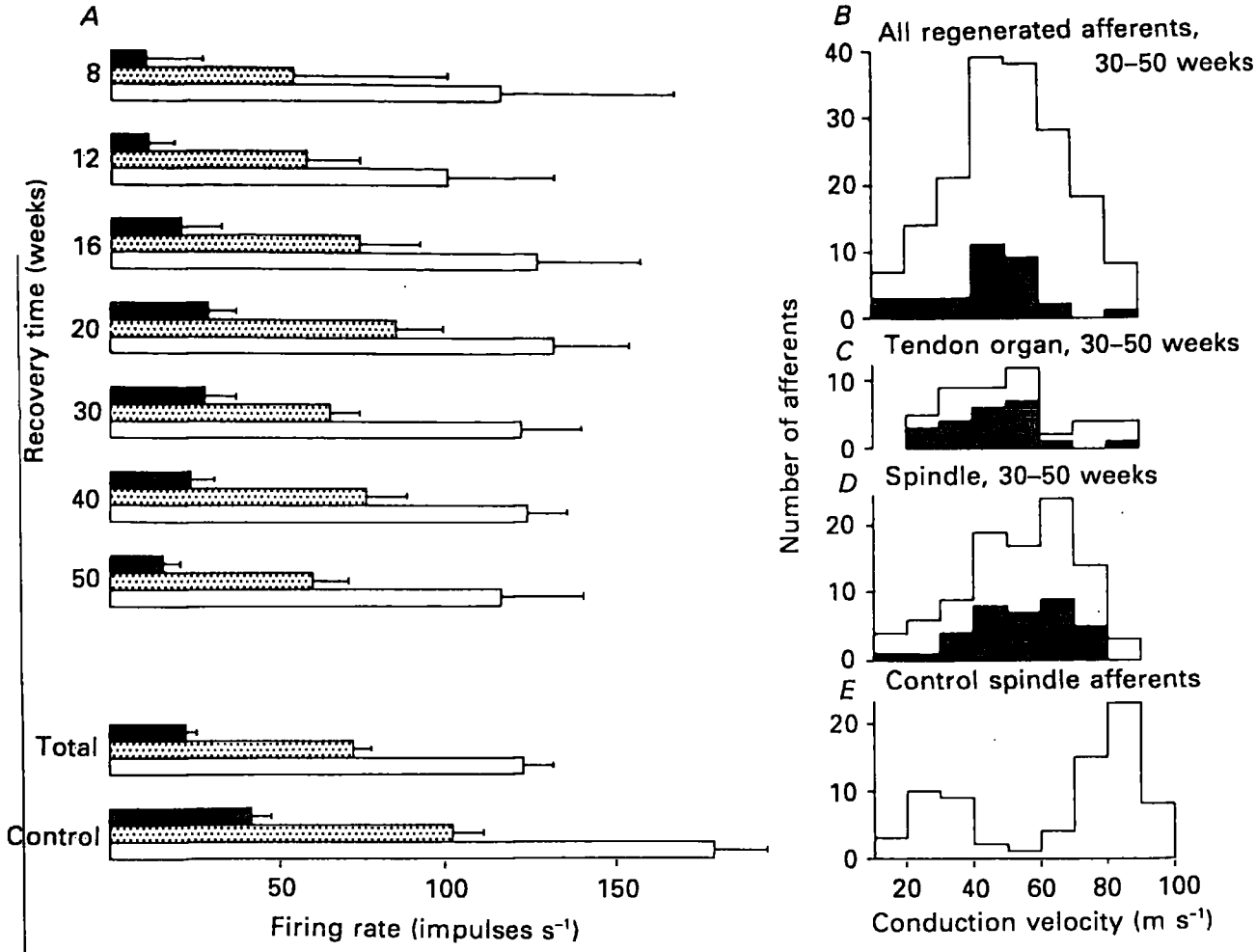


Fig. 1. *A*, mean firing rates (+95% confidence limits) of tonically firing peroneus brevis spindle afferents at various recovery stages 8–50 weeks after complete or partial section of the common peroneal nerve. Mean rates of combined total shown below compared with those of control sample. Muscles were stretched by 1·8 mm up to maximum physiological length. Filled bars, tonic discharge; stippled bars, static discharge after 0·5 s at extended length; open bars, dynamic peak discharge at end of 5 mm s^{-1} ramp. *B–E*, distribution of afferent conduction velocities. *B*, all regenerated mechanosensory afferents from 30–50 week recovery stages innervating peroneus brevis after complete or partial section of the common peroneal nerve. Filled area, afferents of unidentified type. *C* and *D*, afferents responding as from tendon organs (*C*), and those responding as from spindles (*D*). Filled areas, afferents from the partial-section experiments. *E*, control sample of peroneus brevis spindle afferents.

length sensitivity associated with a small dynamic index; and the occurrence of a brief burst of firing at the onset of release.

Despite these abnormalities, certain properties of the dynamic and static phases of the responses of regenerated afferents in spindles were essentially similar to the corresponding properties of normal spindle afferents, whether Ia or II. Thus in both cases although the range of dynamic index varied considerably, it was strongly correlated ($r = 0{\cdot}85$ control, 0·73 regenerated) with the dynamic index of medium velocity (5 mm s^{-1}) ramps (Fig. 3*A*). It is not self-evident why this should occur, so the fact that the slopes of the calculated regression lines differed highly significantly ($P < 0{\cdot}01$) from zero, whilst those of the regenerated and control afferents did not

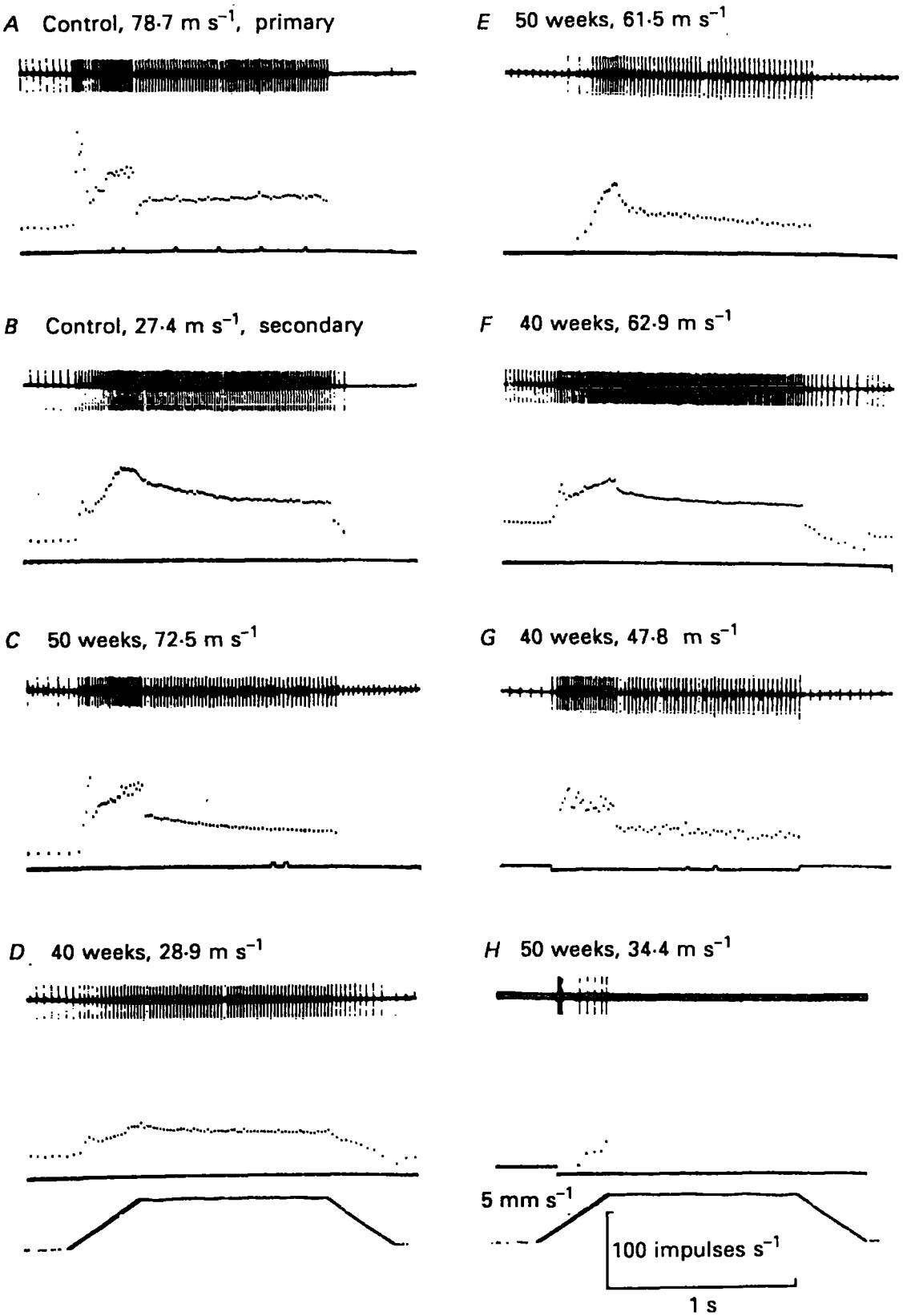


Fig. 2. Examples of responses to ramp-and-hold stretches applied at 5 mm s^{-1} (base of each column) of normal peroneus brevis spindle afferents (*A* and *B*), and of afferents reinnervating peroneus brevis spindles after section of the common peroneal nerve at the recovery stages indicated (*C–H*). Each example shows the activity recorded from the dorsal-root filament; below this, its instantaneous-frequency transformation and, at the bottom, the baseline zero of instantaneous frequency (lowest level of heavy line; upward shifts are artifacts). *A*, control Ia. *B*, control spindle II. *C*, Ia type. *D*, spindle II type. *E*, without tonic discharge. *F*, relatively fast afferent with spindle II-type response. *G*, relatively slow afferent with Ia-type response. *H*, phasic type responding to ramp only.

differ significantly from each other, may be taken as evidence of an essentially normal mechanosensory transduction in the regenerated afferents. Note, however, that highly abnormal regenerated afferents are excluded from consideration since the existence of a dynamic index implies a maintained response for at least 0·5 s during the hold phase of stretch. For tonically firing afferents the static and tonic discharges were also strongly correlated ($r = 0{\cdot}70$ control, 0·49 regenerated, Fig. 3*B*) and again the slopes of the fitted linear regressions differed highly significantly

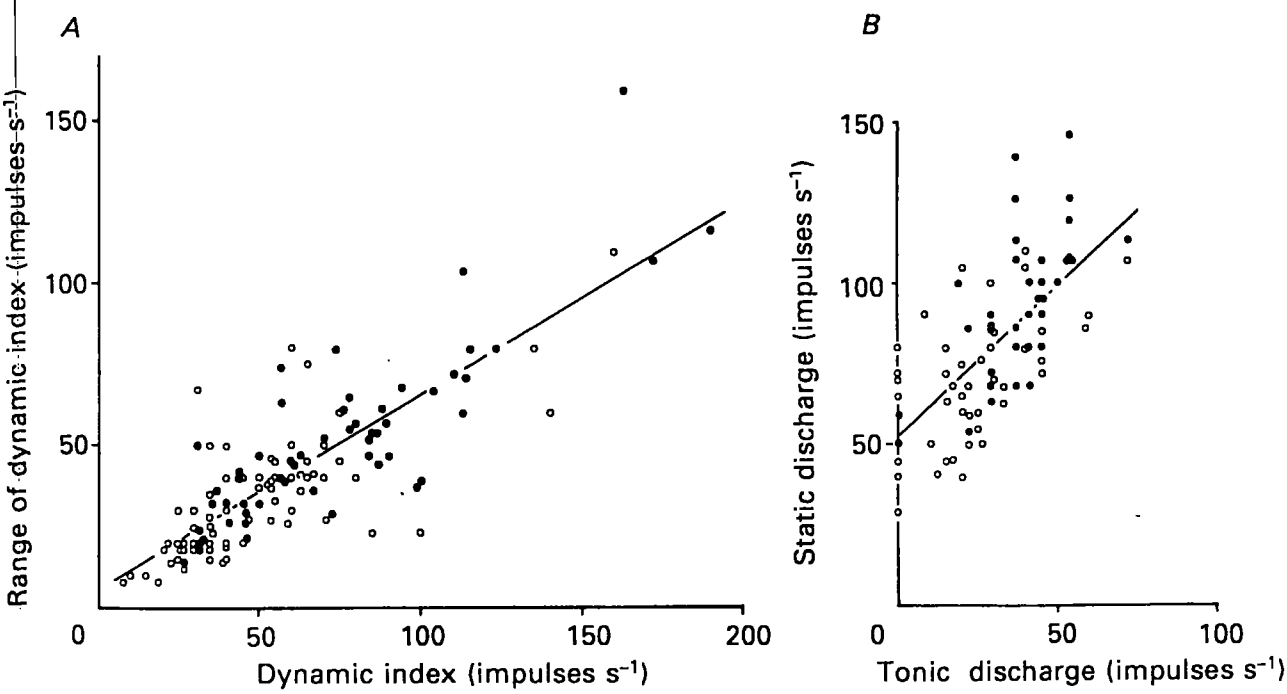


Fig. 3. Plots of certain response characteristics of normal peroneus brevis spindle afferents (●), and those of afferents reinnervating peroneus brevis spindles 40–50 weeks after section of the common peroneal nerve (○). Regression lines are shown for the combined normal and regenerated populations. *A*, plots of range of dynamic index for 2·5, 5 and 10 mm s^{-1} ramp stretches against dynamic index of 5 mm s^{-1} stretches. *B*, plots of static discharge after 0·5 s at extended length against adapted tonic discharge before stretch.

($P < 0{\cdot}01$) from zero, whilst those of the regenerated and control afferents were not significantly different.

In three-dimensional plots of dynamic index, range of dynamic index, and conduction velocity, control Ia and spindle II afferents were clearly segregated by velocity, but the dynamic response properties of the group II afferents coincided with the low end of the Ia range (Fig. 4*A*). Regenerated afferents showed no clear segregation by conduction velocity, and there was a predominance of low-dynamic responses even among the faster-conducting afferents (Fig. 4*B*). It was therefore necessary to examine other properties of the responses of the tonically firing regenerated afferents to assess more precisely their similarity to Ia- or spindle II-type responses and the extent to which the relationship with conduction velocity was abnormal.

The difference between the amplitudes of the initial burst and the static discharge of an afferent was found to be a particularly sensitive indicator of Ia- and spindle II-type responses in normal spindles, being generally greater for Ia than for II afferents,

and having a maximal segregation at about a difference of 35 impulses s^{-1} (Fig. 5*A*). Afferents from reinnervated spindles exhibited the full range of initial burst–static discharge difference, but the distribution with conduction velocity was grossly abnormal (Fig. 5*B*). Arbitrarily dividing them into fast (> 50 m s^{-1}) and slow (< 50 m s^{-1}) groups, it was found that 35% of the fast group had differences < 35 impulses s^{-1}, and 45% of the slow group had differences > 35 impulses s^{-1}. This cannot be attributed merely to a blurring of the distribution at intermediate

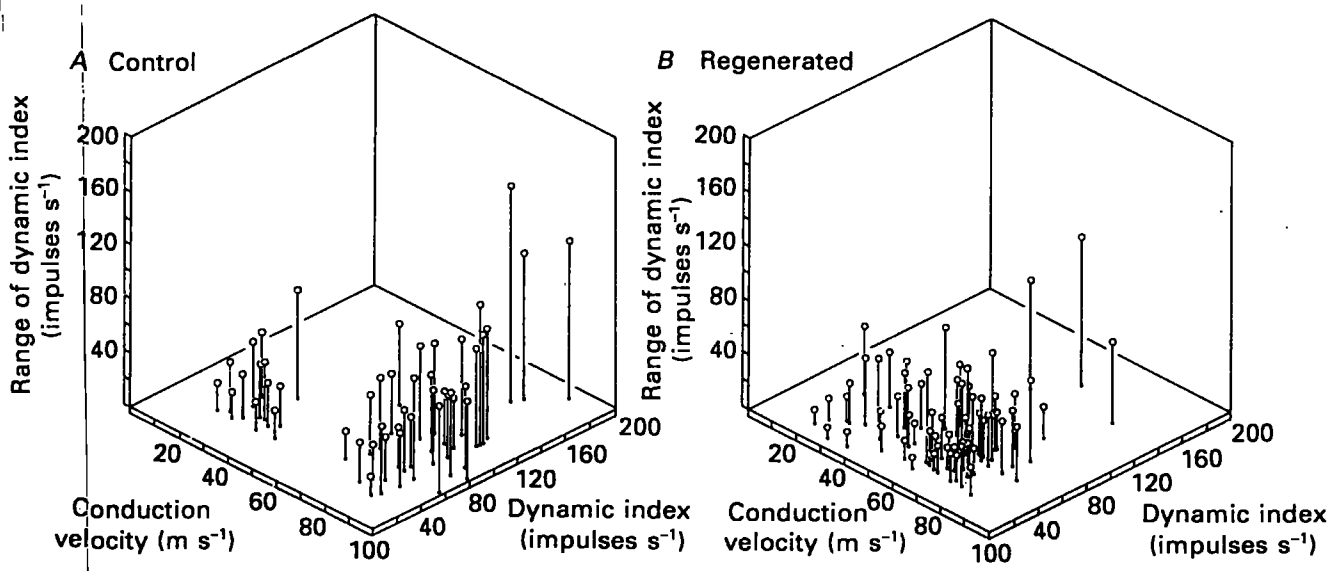


Fig. 4. Three-dimensional plots of range of dynamic index for 2·5, 5 and 10 mm s^{-1} ramp stretches against conduction velocity and dynamic index for: *A*, normal peroneus brevis spindle afferents; and *B*, those reinnervating peroneus brevis spindles 40–50 weeks after section of the common peroneal nerve.

conduction velocities, since the same effect was found if only the extremes (< 30, > 70 m s^{-1}) of the conduction velocity range were considered.

Responses of afferents reinnervating spindles to small-amplitude sinusoidal stretch

Confirmation of the existence of the abnormal association of response properties with conduction velocity in regenerated afferents was sought using sinusoidal stretch. Brown, Engberg & Matthews (1967) showed that normal Ia afferents can be selectively driven to fire at the same frequency as the stimulus by very small sinusoidal stretches. In a control experiment we found that twenty-five of thirty spindle afferents were driven in this way and twenty-three were probably Ia, conducting at more than 60 m s^{-1}. All five of the afferents that were not driven conducted at less than 60 m s^{-1}. Following reinnervation after complete section of the common peroneal nerve fifty-three of eighty-two low-threshold afferents reinnervating spindles were driven, but twenty of them conducted at less than 50 m s^{-1} and of these eleven had initial burst–static response differences of at least 35 impulses s^{-1}. Conversely, of eight afferents that conducted at more than 50 m s^{-1} and were not driven, six had initial burst–static response differences of less than 35 impulses s^{-1}.

Motor reinnervation

Although the main purpose of these experiments was to study sensory reinnervation, some observations were made on regenerated motor fibres. The distribution of their conduction velocities in four experiments from recovery stages 30 and 40 weeks was bimodal with sharp peaks at 20–25 and 60–65 m s^{-1}.

Of eighteen efferents that on stimulation elicited possible intrafusal effects, seven

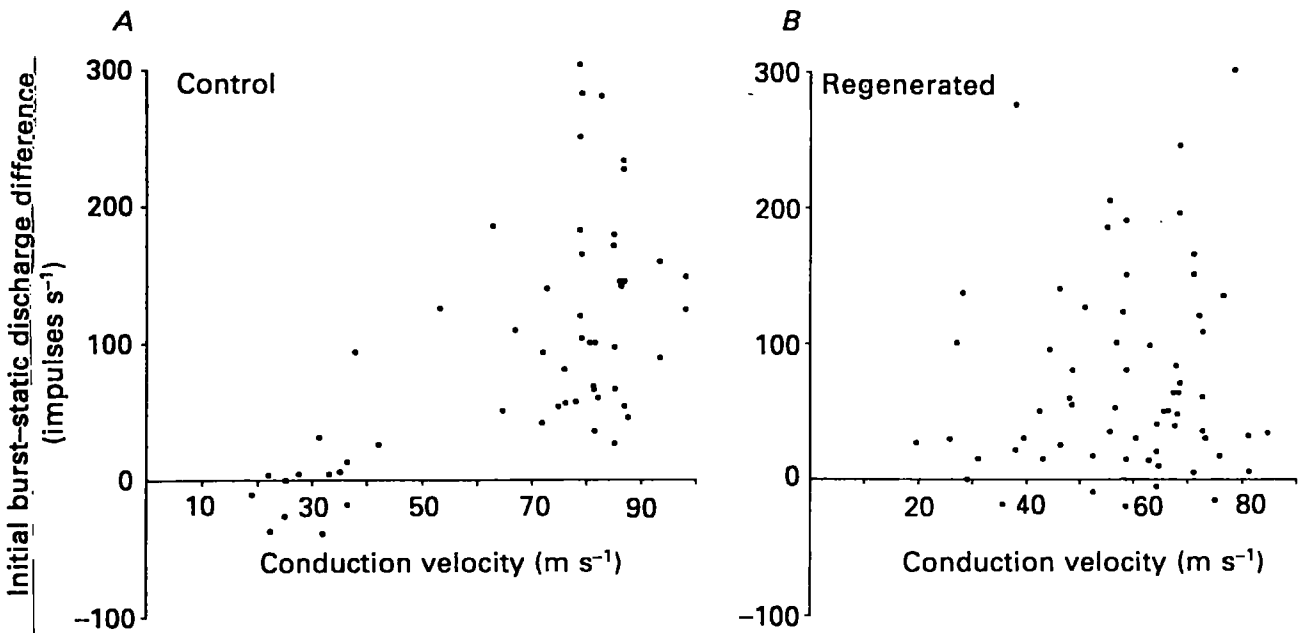


Fig. 5. Plots of the difference between the peak firing rate of the initial burst of the response to a 10 mm s^{-1} ramp and the static discharge, against conduction velocity, for: *A*, normal peroneus brevis spindle afferents; and *B*, afferents reinnervating peroneus brevis spindles 40–50 weeks after section of the common peroneal nerve.

had slow (< 40 m s^{-1}) conduction velocities and did not produce visible contraction of the muscle. They all had static or mixed effects (Fig. 6*A–C*); no slow dynamic efferents were found, despite a specific search for them in three experiments. The remaining efferents had fast (> 40 m s^{-1}) conduction velocities and usually produced some visible contraction of the muscle. This could be abolished by tetanizing the motor unit for 1 min at 200 Hz, after which a dynamic effect persisted in four cases (Fig. 6*D–F*). Static effects were always abolished by this treatment so it was not possible to attribute them specifically to extra- or intrafusal contraction.

Preliminary histological observations

The endings of normal spindle and tendon-organ afferents are mainly annulospiral in form. Regenerated annulospiral endings were present in some spindles and tendon organs from the earliest recovery stage, but in addition there were many of wholly abnormal appearance which ramified freely in tendon organs or in the equatorial and polar regions of spindles (Plate 1*A*). Following complete nerve section all spindles contained one or both types of regenerated ending from the 7 week recovery stage onwards, whereas many tendon organs remained completely denervated (Banks *et al.* 1985). Annulospiral endings were present in 49% of these spindle capsules (n = 139),

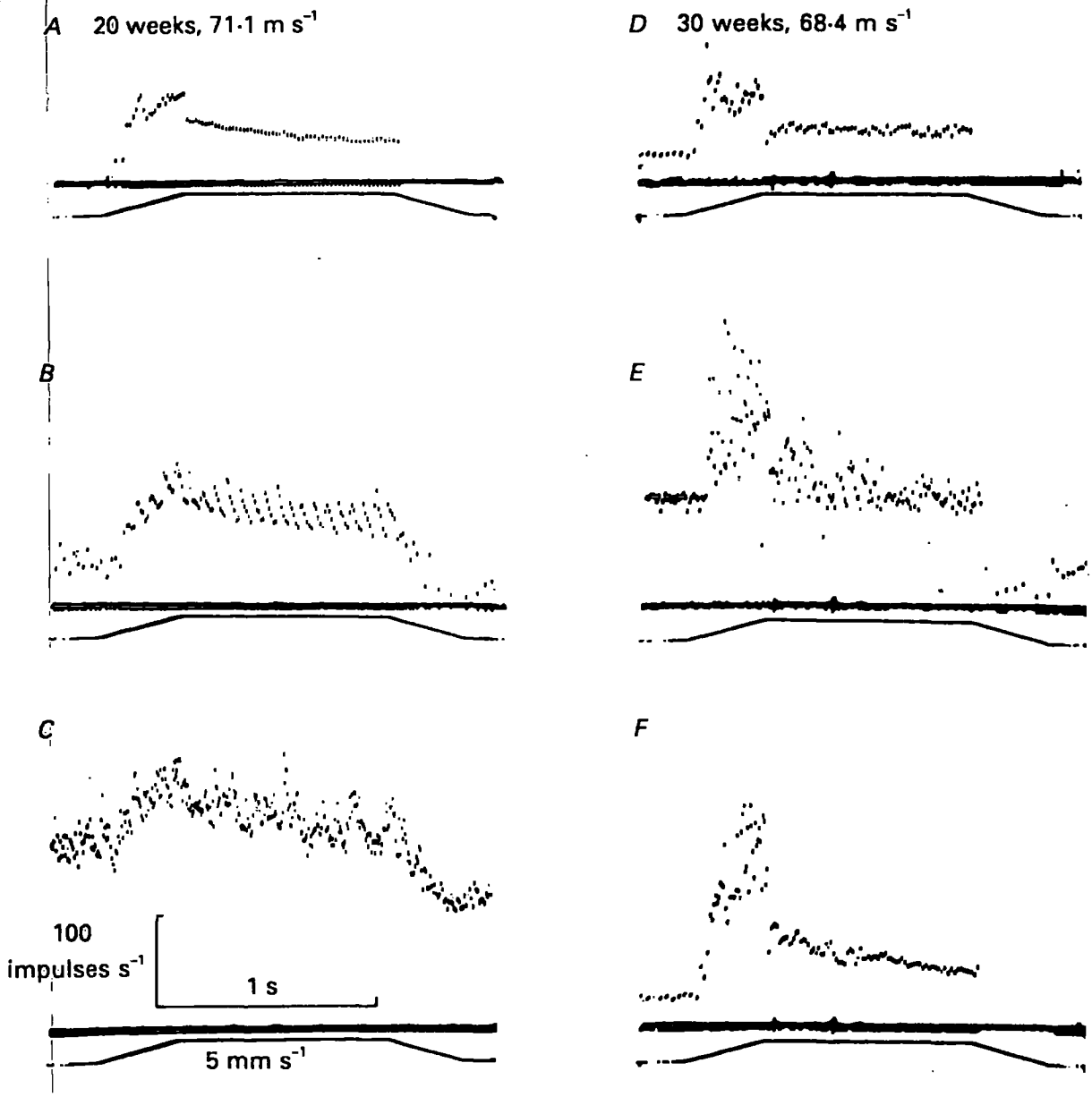


Fig. 6. Responses to ramp-and-hold stretch of two relatively fast Ia-type afferents (*A–C* and *D–F*) reinnervating peroneus brevis spindles (recovery periods after nerve section and conduction velocities as indicated) with concurrent motor stimulation (*B*, *C*, *E* and *F*), and without (*A* and *D*). *B* and *C*, with stimulation of a static γ-axon (conduction velocity 16·3 m s^{-1}) at 10 Hz (*B*) and 100 Hz (*C*). *E* and *F*, with stimulation of a β-axon (conduction velocity 68·4 m s^{-1}) at 100 Hz before (*E*) and after (*F*) tetanization for 60 s to expose the intrafusal dynamic effect.

and free endings in 73%. Following partial nerve section the number of capsules with annulospiral endings was higher (68%, $n = 109$), whereas the number with free endings was much less (38%).

Cross-union experiments

Cross-union of common peroneal nerve fascicles

Since free endings were particularly common in spindles after complete nerve section, it seemed likely that they were mostly of foreign origin, probably from the fascicles supplying the cutaneous superficial peroneal branch of the common peroneal

nerve. This was tested in three cats by cross-uniting one of the cutaneous fascicles with the fascicles supplying peroneus brevis and tertius.

In a control experiment most of the mechanoreceptors in the normal superficial peroneal nerve were found to be various types of hair-follicle afferent together with some slowly adapting skin afferents. There were some tonically firing units that gave no response to mechanical stimulation. The receptive field of the posterior fascicle was the hairy skin of digits III, IV and V, and the dorsum of the foot. Receptive fields of individual afferents were mostly on the edge of the hairy skin adjacent to the foot pad.

Peroneus brevis was investigated 16–22 weeks after the fascicular cross-union. The muscles were much reduced in size and did not twitch in response to stimulation of their nerves. The distribution of conduction velocities of 120 regenerated afferents was unimodal with a broad peak around 40 m s^{-1}. There was some evidence of functional specificity, since most of the afferents responsive to muscle stretch conducted at 40 m s^{-1} or above, whereas most non-responsive ones conducted at < 40 m s^{-1}. Ramp-and-hold stretch (5 mm s^{-1}, 1·8 mm) elicited one or more spikes in forty afferents. Additionally, four units responded to localized probing of the muscle belly, but not to stretch. Multiple-spike responses were mostly phasic; when tonic responses were present they were usually weak (Fig. 7).

On subsequent histological examination of the muscles, motor endings and annulospiral afferent terminals were absent and spindles contained freely ending afferents of various diameters and terminal form.

Cross-union of interosseous and FDL nerves

We therefore assumed that the annulospiral endings restored to some peroneus brevis spindles in the nerve section experiments had been regenerated by annulospiral muscle afferents (Ia, Ib or spindle II), but there remained the possibility that some were of a type inappropriate to their new locations. In order to test whether tendon-organ (Ib) afferents could regenerate annulospiral endings in spindles we cross-united the interosseous and FDL divisions of the tibial nerve.

According to Hunt & McIntyre (1960) the group I and II afferents in the cat's interosseous nerve are derived mainly from tension and vibration receptors. Barker (1962) identified these as tendon organs and pacinian corpuscles, respectively, and found that the nerve contained 150–200 myelinated afferents. These supplied an average of eighteen tendon organs, sixty pacinian corpuscles, three spindles and an indeterminate number of free endings in the interosseous membrane and flexor hallucis longus muscle. The proportion of spindle afferents present was challenged by Yeo (1976), who claimed to have found thirty-eight in a sample of 207 fibres. However, Harrison & Johannisson (1983) could find no evidence for this when recording monosynaptic EPSPs from FDL motoneurones, and they concluded that the interosseous nerve contained 'a very small number' of Ia afferents.

In three control experiments we recorded from L6 and L7 dorsal-root filaments in normal cats with the left hindlimb extensively denervated except for the interosseous and flexor hallucis longus branches of the tibial nerve. Of 132 groups I and II afferents forty-three were from spindles and all were located in the flexor hallucis longus nerve; fifty-eight were from tendon organs, thirty in the flexor hallucis longus

nerve and twenty-eight in the interosseous nerve; and twenty-two were from pacinian corpuscles, all in the interosseous nerve. The remaining nine afferents were of unidentified type, usually giving no response to mechanical stimulation, and mostly (8) located in the interosseous nerve. The distribution of conduction velocities of all these afferents is shown in Fig. 8*A–D*.

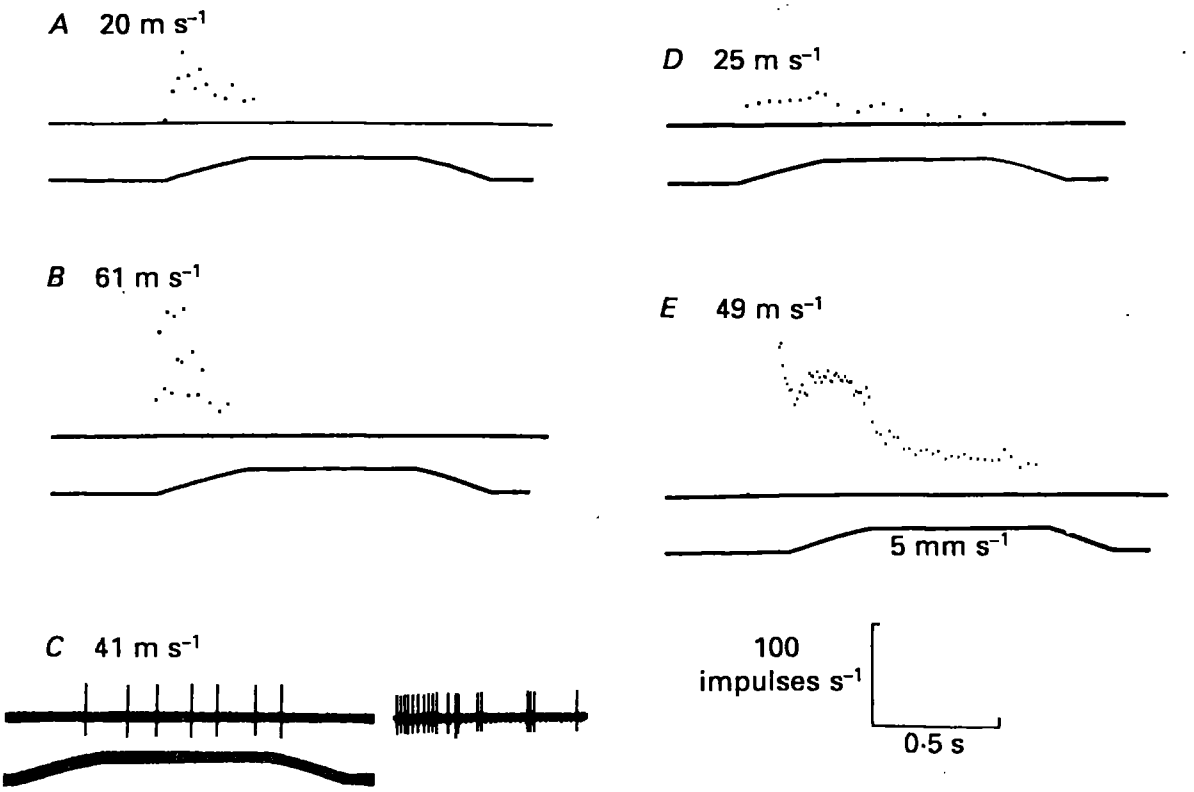


Fig. 7. Examples of responses of regenerated superficial peroneal afferents to ramp-and-hold stretch of peroneus brevis following partial section of the common peroneal nerve and cross-union of the cutaneous and muscle fascicles. Most were of the phasic type (*A* and *B*); examples shown are from the extremes of the conduction-velocity range. Afferent *C* showed a weak tonic response to stretch (left), but a brisk response to localized probing of the muscle (right). Several afferents showed a weak phasic–tonic response, as in *D*, but only one (*E*) gave a response resembling that of a normal spindle afferent.

We therefore confidently proceeded with the interosseous–FDL cross-unions, but found that, perhaps because of the disparity in size between the cross-united nerves, FDL reinnervation occurred from the proximal stumps of both the interosseous and FDL nerves. This necessitated the later removal of the homonymous reinnervation 1 week before histological examination, or its interruption at the time of the acute physiological experiments (see Methods).

Composition of the cross-united interosseous and FDL nerves

As judged by their responses, many (31/79) interosseous afferents appeared to have reinnervated FDL spindles after cross-union, and presumably were in functional contact with intrafusal fibres. The remaining afferents included some that were identified as reinnervating tendon organs or pacinian corpuscles, but most did not respond to ramp or sinusoidal stretch or muscle twitch. The distribution of the

conduction velocities of all these afferents is shown in Fig. 8*E* and *F*. All the afferents that appeared to have reinnervated intrafusal fibres were relatively fast (mean 73·7 m $s^{-1} \pm 8{\cdot}18$ S.D.)and therefore had presumably previously innervated tendon organs (mean conduction velocity of interosseous Ib afferents 77·2 m $s^{-1} \pm 6{\cdot}98$ S.D.; pacinian II afferents, 51·5 m $s^{-1} \pm 5{\cdot}22$ S.D.).

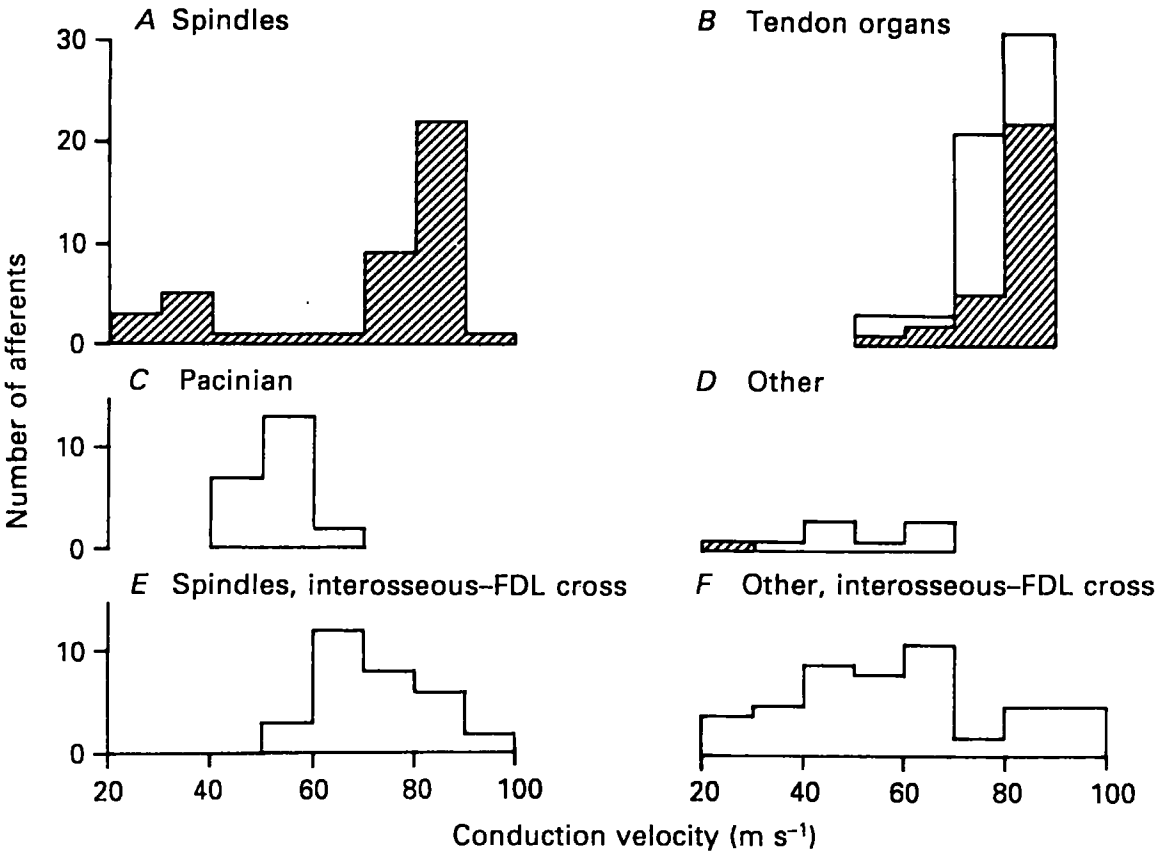


Fig. 8. Histograms of afferent conduction velocities. *A–D*, afferents in normal flexor hallucis longus (shaded) and interosseous (unshaded) nerves innervating spindles (*A*), tendon organs (*B*), pacinian corpuscles (*C*) and other mechanoreceptors (*D*). *E* and *F*, regenerated interosseous afferents reinnervating receptors in FDL after cross-union of interosseous and FDL nerves. *E*, afferents reinnervating spindles and giving Ia-type or spindle II-type responses to ramp-and-hold stretch. *F*, other afferents, including those that reinnervated tendon organs or pacinian corpuscles.

Responses of FDL spindles reinnervated by presumed former Ib afferents

Examples of responses to ramp-and-hold stretches obtained from FDL spindles reinnervated by afferents presumed formerly to have terminated in tendon organs are shown in Fig. 9*A* and *B*. Both Ia-like and spindle II-like responses occurred, as demonstrated by the values obtained for the difference between initial burst and static discharge of each ending (Fig. 9*C* and *D*). The Ia-like responses often showed a tendency to maintain firing during release of stretch, which was not found in control Ia responses. The static and dynamic components of the responses were entirely normal as assessed by plots of static against tonic discharges, and of range of dynamic index against dynamic index of 5 mm s^{-1} ramps, respectively.

Histological analyses

In order to find out whether it was possible to distinguish between regenerated Ia, Ib and spindle II afferents and their endings in peroneus brevis spindles reinnervated after nerve section, it was first necessary to examine other reinnervated spindles in which the nerve sectioned lacked one or more of these types of afferent. Opportunities

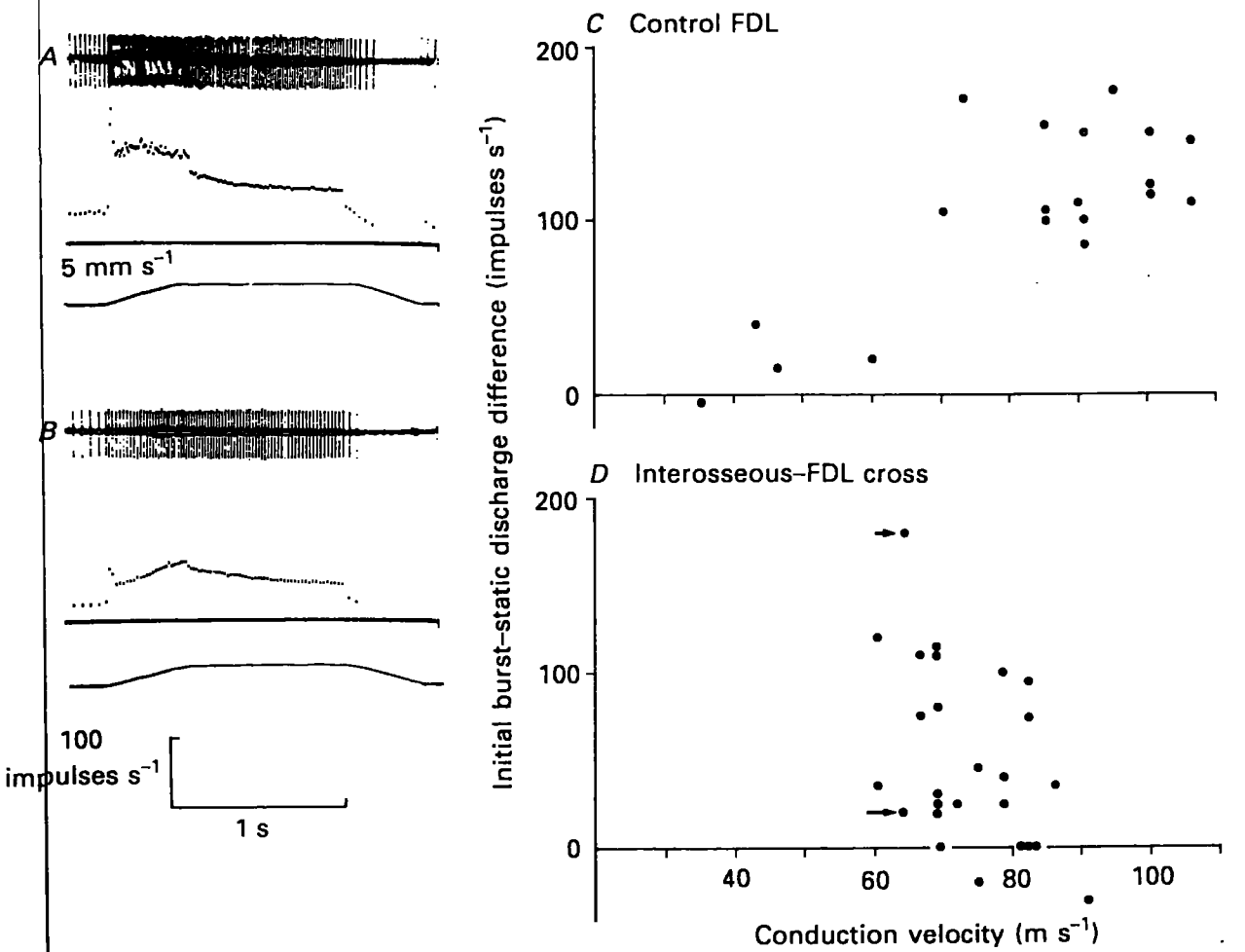


Fig. 9. Responses of regenerated interosseous Ib afferents innervating FDL spindles after cross-union of interosseous and FDL nerves. *A* and *B*, Ia-like response (*A*) and spindle II-type response (*B*) to ramp-and-hold stretch; conduction velocities 64·6 and 64·3 m s^{-1} respectively. *C* and *D*, plots of the difference between the peak firing rate of the initial burst of the response to a 10 mm s^{-1} ramp and the static discharge against conduction velocity for: *C*, normal FDL spindle afferents; and *D*, regenerated interosseous Ib afferents innervating FDL spindles. Note that all the Ib afferents in *D* are relatively fast. Arrows in *D* indicate plots for the responses shown in *A* (short arrow) and *B* (long arrow).

for Ia and spindle II afferents to reinnervate spindles in the absence of Ib afferents arise when tenuissimus spindles are reinnervated after nerve section, since this muscle usually lacks tendon organs (Palmer & Stilwell, 1958; Boyd & Davey, 1968). Conversely, opportunities for Ib afferents to reinnervate FDL spindles in the absence of Ia and spindle II afferents were provided in our interosseous–FDL cross-union experiments.

Afferent reinnervation of tenuissimus spindles after nerve section

In the five tenuissimus muscles examined, annulospiral endings were present in 76% of the spindle capsules ($n = 58$) and free endings in 22% (see Table 2). Most of the annulospiral endings that formed in primary regions resembled normal primary endings in having regular transverse terminal bands around the nuclear bags and

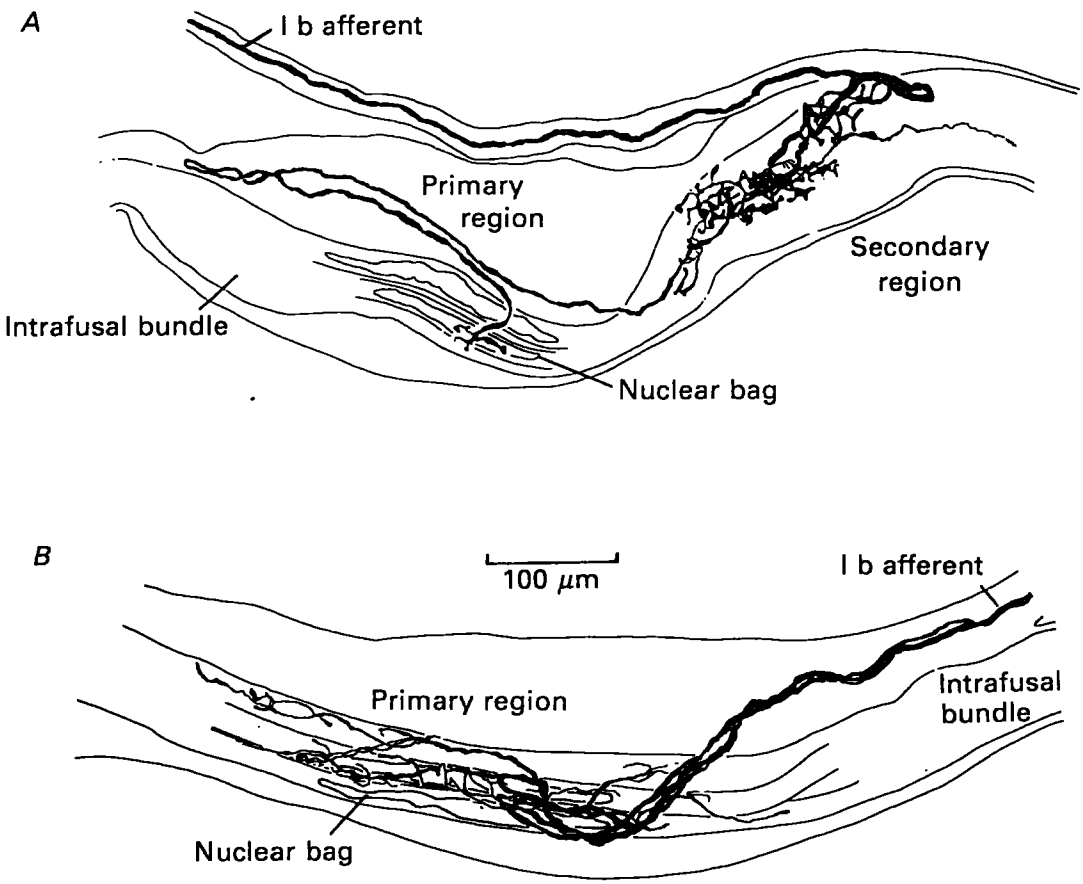


Fig. 10. Tracings of teased, silver preparations of FDL spindles innervated by regenerated interosseous Ib afferents after cross-union of interosseous and FDL nerves. Axons and terminals traced in detail; intrafusal bundle, nuclear bags and capsule traced in outline only. *A*, Ib afferent, diameter 4·0 μm, innervates site previously occupied by S_1 secondary (II) ending; a branch has grown beyond this and finally peters out in a few terminals on a nuclear bag in the primary region. *B*, Ib afferent, diameter 4·75 μm, innervates site previously occupied by primary (Ia) ending. Terminals are diffuse and irregular and lack the transverse terminal bands around the nuclear bags characteristic of primary endings formed by normal and regenerated Ia afferents (cf. Plate 2*A* and *B*).

spiral terminals around the chain fibres. However, the endings were generally shorter than normal and had fewer transverse terminal bands, as in primaries regenerated after nerve-crush injury (Barker *et al.* 1985). Occasionally the bands were restricted to one bag, and sometimes the regenerated ending extended into a neighbouring S_1 secondary region. The twenty-nine afferents supplying these endings had diameters of 3·8–7·5 μm, mean 5·0 μm, and were identified as Ia; fifty-one normal tenuissimus Ia afferents had diameters of 4·8–12·1 μm, mean 7·9 μm. A further five afferents innervated primary regions in which the nuclear bags had been replaced by cross-

striated myotubes. The regenerated endings lacked terminal bands around the bag fibres and spirals were restricted to the chain fibres. These afferents had diameters of 2·3, 2·8, 2·8, 3·0 and 3·6 μm and were assumed to be spindle II.

Endings restored to the S_1 (29) and S_2 (6) secondary regions resembled normal secondary endings and those that regenerate after nerve-crush injury (Barker *et al.* 1985), but a further ten such endings were abnormally located in intracapsular polar

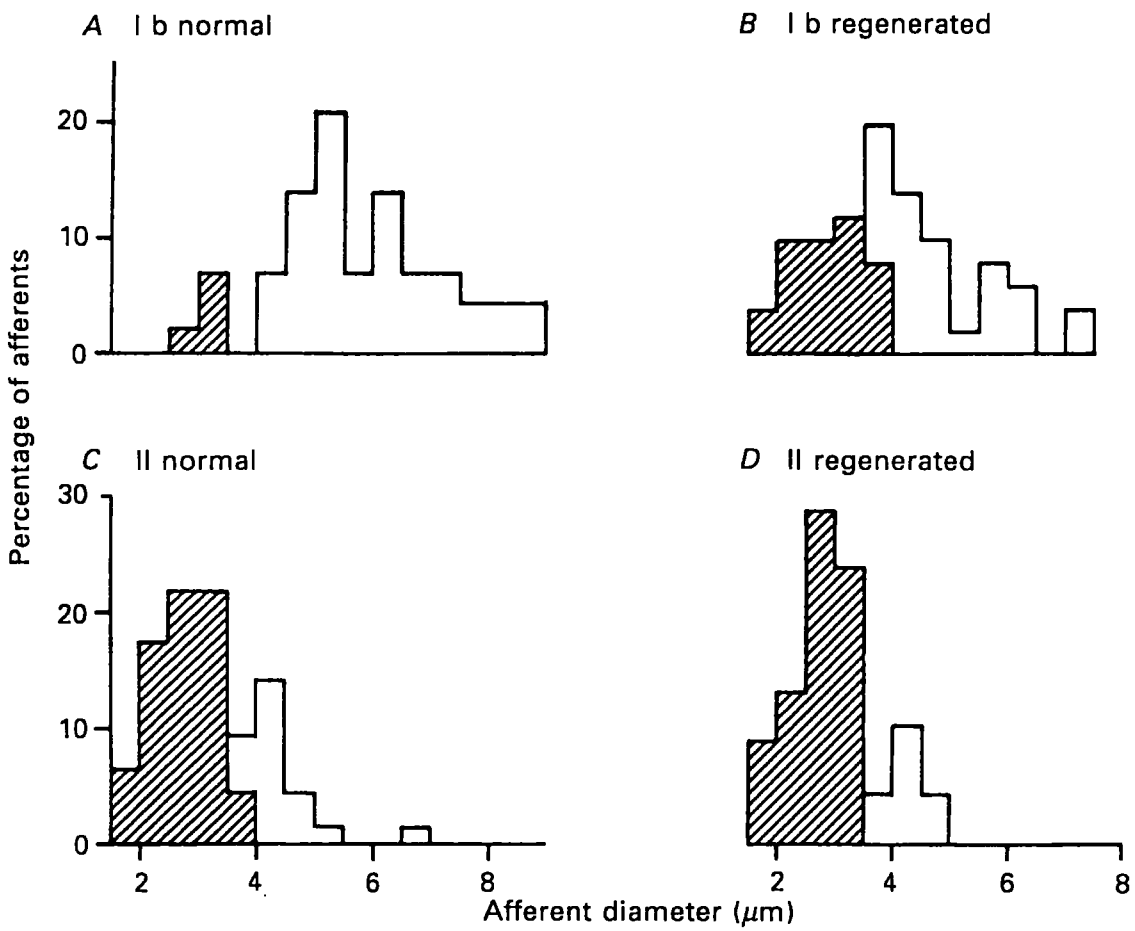


Fig. 11. Histograms of the diameters of normal and regenerated cat muscle afferents as measured near receptor entry; shading indicates afferents with diameters measuring $\leqslant 3{\cdot}5$ μm. *A*, forty-three normal peroneus brevis Ib afferents. *B*, fifty interosseous afferents identified as Ib that innervated FDL spindles in the interosseous–FDL cross-union experiments. *C*, sixty-three normal peroneus brevis spindle II afferents. *D*, forty-five tenuissimus afferents identified as spindle II that innervated secondary-ending and polar sites in tenuissimus spindles after nerve section.

regions, one ending extending well beyond the capsule limits. The forty-five afferents supplying these endings had diameters of 1·6–4·8 μm, mean 3·0 μm (Fig. 11*D*). We identify all these as spindle II afferents, though acknowledge that among the largest (20% had diameters of 4·0–4·8 μm) there may have been a few Ia afferents that reinnervated S_1 sites. The diameters of 101 normal tenuissimus spindle II afferents measured 1·5–7·7 μm, mean 3·6 μm.

On the basis of these identifications we estimate that, as compared with the normal supply of spindle afferents, 52% Ia and 49% of II afferents were lost in the reinnervation process (see Table 3).

Reinnervation of FDL spindles by interosseous afferents

Eighty-two spindles from four muscles were examined. The 188 myelinated afferents that reinnervated them terminated either in an extensively branched network of free endings (84), annulospiral endings (58), or tapers and bulbs resembling pacinian terminals (46). The free endings and pacinian-like terminals were intracapsular and appeared to be located mainly in the axial sheath of the intrafusal bundle. The free-ending afferents had diameters of 1·25–2·75 μm (mean 1·9 μm, $n = 39$), the presumed pacinian afferents 1·5–4·0 μm (mean 2·7 μm, $n = 46$).

Among the fifty-eight afferents that formed annulospiral endings, four were identified as Ia since they formed endings with regular transverse terminal bands around the nuclear-bag regions, as in normal primaries. Three of these occurred in one muscle and one in another; the diameters of the only two that could be measured were 4·5 and 5·5 μm. These were most probably 'contaminating' primary afferents that had regenerated from the FDL nerve (see Methods).

Most of the fifty-four other afferents formed annulospiral terminals in regions previously occupied by primary (fourteen) or secondary (twenty-six) endings. A few (five) terminated in both regions, and the remainder (nine) terminated mainly in intracapsular polar regions and made only minor extensions into a neighbouring primary or secondary region. Reinnervation of secondary regions was usually restricted to S_1, but occasionally the regenerated ending was distributed over two or three adjacent secondary regions. Both bag and chain fibres were reinnervated, the annulospiral nature of the terminals being most evident among those distributed to the chain fibres in secondary regions (Fig. 10*A*; Plate 1*B*). These afferents had diameters of 1·75–7·25 μm, mean 3·9 μm (Fig. 11*B*). We may safely assume that most were Ib afferents, but must allow for the possibility of there being some 'contaminating' II afferents among those with diameters of 3·5 μm or less (44%).

Afferent reinnervation of peroneus brevis spindles after nerve section

In view of the above findings, we concluded that Ib and spindle II afferents reinnervating peroneus brevis spindles would have the same regenerative specificities (i.e. a preference for reinnervating secondary-ending sites; an ability to innervate polar regions; an inability to regenerate primary endings with regular transverse terminal bands as in Fig. 10*B*), and could only be distinguished by diameter. For reasons given in the Methods, we decided on 3·5 μm as a segregating diameter, identifying those $> 3{\cdot}5$ μm as Ib and those $\leqslant 3{\cdot}5$ μm as II. We recognized Ia afferents as those with diameters $> 3{\cdot}5$ μm that formed primary endings with regular transverse terminal bands, and acknowledged the inability to identify any Ia afferent that may have reinnervated a secondary site.

The results of analysing reinnervated peroneus brevis spindles on this basis, 109 after partial section of the common peroneal nerve, 139 after complete section, are summarized and compared with the tenuissimus analysis in Tables 2 and 3. These show that the reinnervation was most successful in tenuissimus; and that partial section of the common peroneal nerve gave better results in peroneus brevis than complete section, the estimated deficit in spindle afferents, for example, being, respectively, 52, 73 and 79% in Ia afferents, and 49, 56 and 86% in II afferents

TABLE 2. Percentage occurrence of spindles in tenuissimus and peroneus brevis reinnervated by different types of afferent after nerve section compared with their normal afferent innervation

	Number		Percentage								
	Muscles	Spindles	Ia	II	Ia and II	Ib	?AS	All AS	?Non-AS	FE	Nil
			Nerve section								
Tenuissimus	5	58	43	59	31	—	5	76	2	22	10
Peroneus brevis: CPN											
Partial cut	4	109	27	53	6	20	3	68	16	38	4
Complete cut	9	139	21	17	4	20	6	49	8	73	4
			Normal								
Tenuissimus	14	65	100	85	85	(Banks, Barker & Stacey, 1982)					
Peroneus brevis	5	183	100	78	78	(Scott & Young, 1987)					

?AS, unidentified annulospiral afferents; CPN, common peroneal nerve; FE, free ending; ?Non-AS, unidentified non-annulospiral afferents; II, spindle II afferent.

(Table 3). Table 2 also shows (i) that Ia and Ib afferents participated in the reinnervation of similar proportions of peroneus brevis spindles; and (ii) that the normal proportion of spindles innervated by both Ia and spindle II afferents was reduced in the reinnervated spindles by over 50% in tenuissimus and over 70% in peroneus brevis.

TABLE 3. Numbers of Ia and spindle II afferents reinnervating spindles in tenuissimus and peroneus brevis after nerve section

	No. spindles	Regenerated		Estimated normal*		% deficit	
		Ia	II	Ia	II	Ia	II
Tenuissimus	58	29	50	61	99	52	49
Peroneus brevis: CPN							
Partial cut	109	29	58	109	131	73	56
Complete cut	139	29	24	139	167	79	86

* Estimated from the following afferent: spindle ratios; tenuissimus, Ia 1·06:1, II 1·7:1 (Banks *et al.* 1982); peroneus brevis, Ia 1:1, II 1·2:1 (Scott & Young, 1987). CPN, common peroneal nerve.

Altogether 190 spindle and tendon-organ afferents (fifty-eight Ia, eighty-two II and fifty Ib) reinnervated peroneus brevis spindles in the nerve-section experiments. Among 112 primary-ending sites fifty-eight (52%) were reinnervated by Ia afferents, twenty-two (20%) by II afferents, and thirty-two (28%) by Ib. Ten of the Ia afferents formed primary endings that extended into an adjacent secondary-ending site, and three of the other afferents (one II, two Ib) also formed terminals in an adjacent polar region. Among ninety-six secondary-ending sites, sixty-two (65%) were reinnervated by II afferents, and thirty-four (35%) by Ib. Twenty of these afferents (five II, fifteen Ib) formed secondary endings that encroached upon a primary site, and a further thirteen (five II, eight Ib) formed secondaries that extended into a polar region. Four afferents (one II, three Ib) regenerated endings that were exclusively polar. An example of a peroneus brevis spindle reinnervated by spindle II and free-ending afferents is illustrated in Plate 1*C* where it is compared with an FDL spindle similarly reinnervated by interosseous Ib and free-ending afferents (Plate 1*B*). Normal and regenerated primary (Ia) and secondary (II) endings are illustrated in Plate 2*A–D*.

No systematic study was made of motor reinnervation, but it can be said that whereas p_1 plates were commonly observed in the poles of spindles from both series of experiments, p_2 plates were only rarely seen. The restoration of trail innervation was occasionally obvious, but more usually this could not be discerned with confidence because of profuse polar reinnervation by free endings.

DISCUSSION

Specificities of regenerating muscle afferents

An afferent engaged in muscle reinnervation after nerve injury is regarded as showing specificity if it reconnects with the same type of receptor it normally innervates (Brown & Butler, 1976; Collins *et al.* 1986). Reinnervation after nerve

crush is highly specific since the injury leaves most of the endoneurial tubes intact (Sunderland, 1978) and the regenerating axons are thus guided back to their original receptors. In these circumstances muscle spindles regain their motor (β, γ) and sensory (Ia, II) innervation (Ip & Vrbová, 1973; Ip, Vrbová & Westbury, 1977; Barker & Boddy, 1980; Barker *et al.* 1985), and the responses of the regenerated spindle afferents to stretch, and to fusimotor stimulation, eventually return to normal (Brown & Butler, 1976; Hyde & Scott, 1983). But our concern has been to determine whether there is any specificity in the afferent reinnervation of spindles after nerve section, when presumably any type of muscle afferent may participate in the process.

The same question has been addressed by Brown & Butler (1976) and Collins *et al.* (1986); Gregory, Luff & Proske (1982) have also studied the reinnervation of muscle after nerve section, but their concern was to compare the response characteristics of self-reinnervated soleus receptors with those that had been cross-reinnervated by afferents from a fast twitch muscle. Brown & Butler (1976) concluded that some specific reinnervation did occur because some afferents reinnervating spindles had response characteristics and conduction velocities similar to normal spindle afferents; and because some regenerated efferents produced stimulating effects on afferents that were both typical of normal static or dynamic γ-efferents, and consistent when tested on more than one afferent. Collins *et al.* (1986) approached the problem centrally by recording the presence or absence of field potentials generated by activated stretch afferents, and concluded that random populations of both specific (Ia, spindle II) and non-specific (Ib) afferents reinnervated spindles after nerve section. They also found that tendon organs were reinnervated by afferents that were both specific (Ib, eight examples) and non-specific (Ia, one example).

The fact that a non-specific afferent can functionally reinnervate a receptor suggests that the specificities of regenerating muscle afferents should be considered in terms of the cells they reinnervate as well as the receptors. Prenatally, the Ib afferent is specified to terminate on bundles of collagen fibres in the developing tendon organ as well as around the ends of the myotubes that insert into it (Zelená & Soukup, 1977). After adult nerve section this same dual specificity thus enables the regenerating Ib afferent either to reinnervate its own receptor as a native afferent, or to terminate on intrafusal muscle fibres as a foreign afferent. The results of our interosseous–FDL and tenuissimus experiments show that Ib and spindle II afferents reinnervating spindles may be regarded as members of a single population distinguished from each other only by diameter and conduction velocity. Conversely, it seems likely that some regenerating spindle II afferents terminate in tendon organs after nerve section, thus accounting for the group of slow afferents reinnervating tendon organs noted by Gregory *et al.* (1982) and ourselves (Fig. 1*C*). We have seen the terminals of a normal spindle II afferent distributed partly as a secondary ending in a very short spindle pole, and partly as an ending in its tendinous insertion. If we accept the finding by Collins *et al.* (1986) of a Ia afferent reinnervating a tendon organ, the conclusion emerges that all three stretch afferents (Ia, Ib, spindle II) possess the cell specificities required to make functional reconnections with either spindles or tendon organs when regenerating after nerve section.

Since intrafusal muscle fibres are of three types and show regional differences in structure and nucleation, we may ask whether a regenerating stretch afferent is not only specified to terminate on these fibres, but is further specificied as to fibre type and region. The histological results indicate that regenerating Ib and spindle II afferents show a preference for innervating S_1 secondary-ending sites, though they may also terminate in primary-ending sites, and, occasionally in polar regions. They form secondary endings that appear normal and primary endings that are abnormal, since they lack the transverse terminal bands formed around the nuclear bags by normal and regenerating Ia afferents. Nevertheless, the physiological evidence is that the endings formed by Ib and spindle II afferents in these primary- and secondary-ending sites have similar response characteristics to normal primary (Ia) and secondary (spindle II) endings. Abnormal connections reveal themselves in single-fibre recordings chiefly by abnormal conduction velocities, spindle II primary afferents being slower, and most Ib secondaries being faster, than their normal counterparts. Morphological abnormalities in regenerated primary endings do not affect their pattern of response to ramp-and-hold stretch, as has been shown by Barker *et al.* (1986) with respect to the grossly abnormal primaries that reinnervate spindles after long-term denervation. In this study the primary endings formed by regenerating Ia afferents resembled normal primary endings, though they sometimes extended into a neighbouring secondary-ending site. We had no means of determining whether any Ia afferents exclusively reinnervated secondary-ending sites, since they would have been indistinguishable from Ib secondaries.

The foreign afferents that terminated as free endings in spindles after nerve section were probably exercising a cell specificity for fibrocytes, hence the location of their ramifying terminals in the axial sheath and intrafusal endomysium. Stacey (1969) has shown that such innervation sometimes occurs in normal spindles, though very rarely.

Process and quality of recovery

The physiological and histological results from different animals were very variable, so that only broad generalizations can be made about the recovery process. Reinnervation was rapidly established during the early recovery periods (6–8 weeks), and there was no evidence of any reorganization of non-specific afferent reconnections. The conduction velocities of regenerated afferents continued to increase until 30 weeks, as judged by that of the fastest afferent at each stage. There was some tendency for the proportion of afferents identified as having reinnervated spindles to increase at the expense of unidentified afferents, and for there to be increasing proportions of these firing tonically (means of 17, 32, and 51% at 6–8, 12–20 and 30–50 weeks, respectively). Presumably both trends are due to progressively increasing afferent excitability. However, despite the increase in the proportion of tonically firing afferents in spindles, the actual firing rates at the three phases of ramp-and-hold stretch responses showed no such tendency and were considerably lower than those of normal spindle afferents. This may have been due to the reduced size and complexity of the regenerated endings (see Plate 2).

The histological analyses summarized in Tables 2 and 3 suggest that the quality of the afferent reinnervation of spindles achieved after nerve section is partly related

to the nerve's size and degree of afferent complexity. Obviously the nature of the repair effected, the degree of fascicular alignment achieved, and the extent to which the nerve stumps retract, are also relevant factors. Hence the relatively good quality of the afferent reinnervation of spindles achieved after sectioning the tenuissimus nerve (unifascicular; muscle afferents without Ib, no cutaneous afferents; minimum retraction of nerve stumps, no stitching required), as compared with that of peroneus brevis spindles following complete section of the common peroneal nerve (multifascicular; muscle and cutaneous afferents present; epineurial stitch repair effected to counteract retraction of nerve stumps and achieve fascicular alignment). In the tenuissimus experiments 43% of spindles ($n = 58$) were reinnervated by Ia afferents as compared with 21% of peroneus brevis spindles after complete section of the common peroneal nerve (Table 2). In the histological analysis by Ip, Luff & Proske (1988) of the soleus muscles that were self- or cross-reinnervated in the experiments by Gregory *et al.* (1982) only 3% of spindles were observed to have regenerated annulospiral primary endings (presumably Ia) among 180 examined. This may reflect the fact that in these experiments the nerve stumps were simply tied together with silk thread without attempting epineurial repair or fascicular alignment.

Concluding remarks

The regeneration of stretch afferents after nerve section cannot be fully understood without reference to their development. The first (and largest) afferents to arrive in the muscle primordium initiate spindle development by chance contact with a primary myotube, and are recognized as Ia (Zelená, 1975; Milburn, 1984; Barker & Milburn, 1984). Those arriving later (Ib, spindle II) initiate the development of tendon organs and terminate in spindles as secondary endings (Zelená & Soukup, 1977; Milburn, 1984). This sequence of arrival and termination is reflected in the adult by a decrease in diameter of the different types of stretch afferent in the order Ia, Ib, spindle II. The afferents that innervate spindles in excess of the initial Ia appear to be randomly distributed among spindle populations since their frequency distribution in various adult cat muscles has been shown by Banks & Stacey (1988) to be binomial.

We see muscle stretch receptors as being established during a critical period of muscle development by randomly distributed afferents of dual myotube–collagen specificity that enter sequentially with decreasing diameters. When these afferents regenerate after nerve section many are lost, but some make random and functional reconnections, which, by virtue of their dual cell specificity, can be with either spindles or tendon organs. The deficit of Ia and spindle II afferents that participate in spindle reinnervation is considerable (Table 3), yet about 60% of normal reflex contraction tension may be restored (Barker & Young, 1947).

Does any kind of specificity operate at the site of the lesion? We have noticed that regenerating efferents only occasionally enter spindles via afferent endoneurial tubes, despite regenerating more quickly than afferents (Ip & Vrbová, 1973; Barker & Boddy, 1980) and perhaps reaching the distal stump earlier. This suggests that regenerating efferents may preferentially enter efferent tubes, as concluded by Brushart & Seiler (1987) from experiments in which regenerating motor axons were given equal access to distal motor and sensory nerve stumps. Presumably

regenerating afferents also selectively reinnervate their own tubes. Future work may reveal whether such selectivity is mediated by the regenerating axons preferentially relating to different types of Schwann cell, or different types of tube, or both. Our results, and those of Collins *et al.* (1986), appear to rule out the possibility of there being any further specificity that preferentially caters for type of afferent.

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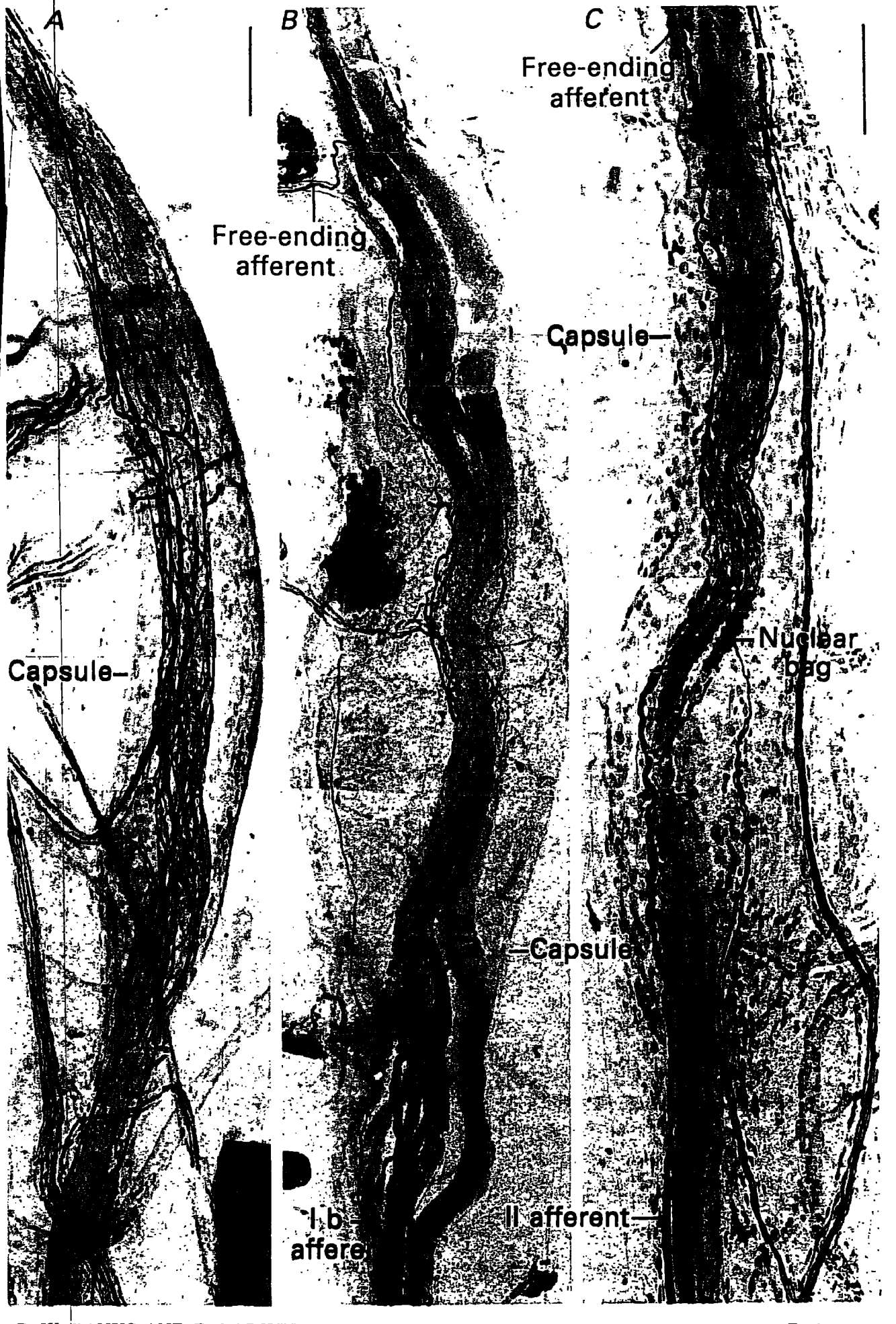
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EXPLANATION OF PLATES

PLATE 1

Photographs of teased, silver preparations of regenerated afferent endings in cat peroneus brevis or FDL spindles. *A*, equatorial region of a peroneus brevis spindle exclusively reinnervated by free-ending afferents after complete section of the common peroneal nerve followed by 16 weeks recovery. *B*, equatorial region of reinnervated FDL spindle 10 weeks after cross-union of interosseous and FDL nerves. Length of periaxial space enclosed by capsule, and mode of afferent entries into it, indicate that the normal afferent innervation was S_1PS_1, i.e. an S_1 secondary ending on each side of a primary ending. A myelinated free-ending afferent enters at top from endoneurial tube previously occupied by a spindle II afferent, and branches to reinnervate upper S_1 and P sites. Reinnervation of P site is contributed to by non-myelinated free-ending afferents entering on left from endoneurial tube previously occupied by a Ia afferent. Myonuclei normally present in P site have now been replaced by myofibrils. Lower S_1 site has been reinnervated by a Ib afferent, diameter 6·25 μm, entering at bottom from endoneurial tube previously occupied by spindle II

afferent. *C*, equatorial region of peroneus brevis spindle after complete section of the common peroneal nerve followed by 30 weeks recovery. Afferent reinnervation of previous S_1PS_1 provision shows a very similar pattern to that seen in *B*. The main differences are that the lower S_1 site is reinnervated by a spindle II afferent, diameter 3·5 μm; and that the P site retains its myonuclei and is partially reinnervated by both II and free-ending afferents. Scale bars indicate 100 μm; *B* is at same scale as *A*.

PLATE 2

Photographs of teased silver preparations of normal and regenerated peroneus brevis primary and secondary endings. *A*, normal primary ending. *B*, primary ending regenerated by Ia afferent after complete section of the common peroneal nerve followed by 16 weeks recovery. The ending is smaller and shorter than normal and has fewer transverse terminal bands. *C*, normal S_1 secondary ending. *D*, S_1 secondary ending regenerated by spindle II afferent, diameter 3·0 μm, after complete section of the common peroneal nerve followed by 16 weeks recovery. Scale bar in *A* indicates 50 μm; *B–D* at same scale.

Paper 39.

M.N. Adal & R.W. Banks (1990)
Ultrastructural observations on native and foreign reinnervation of muscle spindles in relation to mechanosensory function in the anaesthetized cat.
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Ultrastructural observations on native and foreign reinnervation of muscle spindles in relation to mechanosensory function in the anaesthetized cat

By M. N. Adal and R. W. Banks. *Department of Biological Sciences, University of Durham, Durham DH1 3LE*

Following nerve section, muscle spindles may be reinnervated by functional sensory endings derived from Ia, spindle II, and Ib afferents that formerly supplied spindle primary, spindle secondary and tendon-organ endings respectively (Banks & Barker, 1989). Sensory endings of normal spindles are inserted into intrafusal-fibre sarcolemma between basal lamina and plasmalemma, and Banks (1986) has suggested that this relationship is important in mechanosensory transduction. In our studies on reinnervation, we have not previously examined the ultrastructure of the sensory neuro-muscular junctions.

Experiments were carried out on four adult cats, using sodium pentobarbitone anaesthesia (Sagatal, 45 mg kg^{-1}, I.P. with I.V. supplements) for all invasive procedures. Section of the left tenuissimus nerve just proximal to its exit from the sciatic (2 cats) was used to provide a practically pure native reinnervation. The responses of reinnervated spindles to ramp-and-hold stretches were obtained by single-unit dorsal-root recording 53 or 68 weeks later. Spindle locations were marked by epimysial stitches. The muscles were initially immersion-fixed under light tension before all spindles were teased out prior to embedding in epoxy resin.

The interosseous nerve contains many Ib (as well as Pacinian II and free-ending) afferents but practically no spindle afferents. It was used to introduce foreign afferents into the left flexor digitorum longus (FDL) of 2 cats. The proximal FDL nerve stump was sectioned 16 or 18 weeks later to remove contaminating native reinnervation, which was allowed to degenerate for 1 week before the animals were fixed by ventricular perfusion. As many spindles as could be found were teased from the muscles prior to embedding.

All spindles were examined for evidence of reinnervation while in unpolymerized resin. In tenuissimus, endings were found on sites recognizable as those of former primary or secondary endings. Intrafusal fibres that did not receive sensory terminals were striated throughout these sites. In FDL, endings of large diameter (presumably Ib) afferents were located predominantly in old secondary sites. The intrafusal fibres contained central nuclei as they would with normal spindle endings. Terminals of all these native and foreign afferents occupied the usual intrasarcolemmal position. However, small diameter axons (probably Pacinian II) that terminated in the sensory regions of FDL spindles did not form intimate contact with intrafusal fibres but were associated with connective tissue of the inner capsule.

Financial support from the Wellcome Trust is gratefully acknowledged.

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A quantitative analysis of the sensory innervation of cat tenuissimus muscle spindles

By R. W. Banks and M. J. Stacey. *Department of Biological Sciences, University of Durham, Durham DH1 3LE*

Ten Tenuissimus muscles were removed post-mortem from nine adult cats killed by sodium pentobarbitone overdose. The muscles were silver-impregnated and all spindles were teased out so as to preserve their linear sequence and nerve supply. In each case the nerve divided unequally at least once before entering the muscle, the largest branch supplying the distal portion of approximately $\frac{2}{3}$ total muscle length. The number of capsules ranged from 14 to 20 (mean 16·7), but in a muscle of this size (0·28 g average wet weight of 2) about twenty-five would be expected, representing a relative spindle abundance of only 0·64 (Banks & Stacey, 1988). Capsules containing only bag_2 and chain fibres (b_2c units) occurred in eight muscles, but most capsules (95%) possessed the full complement of intrafusal fibres (b_1b_2c units).

Each b_1b_2c unit received an afferent axon that ended equatorially and from 0 to 5 afferents that usually ended juxta-equatorially. The mean number of these latter afferents ($\bar{a}$) ranged from 1·05 to 2·13 per muscle with an overall mean of 1·45; and the frequency distribution of the various sensory complements did not differ significantly from binomial form ($n = 4$, $P = 0{\cdot}36$), suggesting that the afferents are distributed randomly among the capsules (Banks & Stacey, 1988). Capsules close to the nerve entry might then be expected to receive more afferents than average, if the greater availability of afferents were reflected in an increased likelihood of association during development. For b_1b_2c units supplied by the large distal nerve branch or by the small proximal branch(es) $\bar{a} = 1{\cdot}57$ and 1·22 respectively, but the difference was not significant (ANOVA). However, within the distribution of the large branch, the more proximal capsules received significantly more afferents than did the more distal ones ($\bar{a} = 1{\cdot}83$ and 1·30 respectively; $F = 5{\cdot}495$, $P < 0{\cdot}05$, ANOVA). That this effect was related to the nerve rather than to location within the muscle was indicated by analysis of the distribution of afferents among the sequentially ordered capsules aligned either on muscle origin or nerve entry. At the level of individual capsules the distribution did not depart significantly from the overall mean in either case (χ^2 test), but grouping the capsules into sequential pairs revealed a significant departure only for nerve alignment ($\chi^2 = 13{\cdot}27$, 6 d.f., $P < 0{\cdot}05$).

Most b_2c units received only a single afferent and were linked in tandem with b_1b_2c units that received on average slightly more afferents ($\bar{a} = 1{\cdot}63$) than the overall mean. Though the difference was not significant (ANOVA) for the tenuissimus alone, combination of this with eight similar results from various different muscles revealed an overall significance (paired comparison, $t = 3{\cdot}28$, $P < 0{\cdot}02$).

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Paper 40.

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A quantitative analysis of the sensory innervation of cat tenuissimus muscle spindles.
J. Physiol. 429; 129P.

Paper 41.

R.W. Banks (1991)
A quantitative histological analysis of the intrafusal motor innervation of the cat tenuissimus muscle.
J. Physiol. 438; 284P.

A quantitative histological analysis of the intrafusal motor innervation of the cat tenuissimus muscle

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The left tenuissimus was removed post mortem from 3 adult cats killed by sodium pentobarbitone overdose. The muscles formed part of a larger sample previously used to analyse the sensory innervation (Banks & Stacey, 1990). They were silver-impregnated and all spindles (total 51 capsules) were teased out so as to preserve their nerve supply.

The distributions of 268 intrafusal branches of motor axons were traced as follows: bag_1 (b_1), 103; long chain (lc), 20; $b_1$lc, 2; bag_2 (b_2), 46; chain (c), 51; b_2c, 45; ?b_1b_2, 1. All branches to lc and $b_1$lc, most to b_1, and one to b_2 were identified as those of β axons, mainly by the form of their intrafusal end-plates (Banks *et al.* 1985). The remainder, mainly to b_2, c, or b_2c, were identified as branches of γ axons, some of whose distribution among different spindles has been reported previously (Banks, 1988). Of 58 poles the b_2 and c fibres were supplied only jointly in 19, completely separately in 21, not at all in 1, and with various degrees of segregation in the rest.

The mean numbers of motor-axon branches per spindle in each muscle were 6.0, 7.7, and 8.5; those of β axons were 2.0, 2.8, and 3.9; and of γ axons were 3.2, 4.5, and 4.7. In each case the frequency of occurrence of various numbers of branches supplied to each spindle did not differ significantly (χ^2 test) from calculated binomial distributions (β, overall mean 3.24; $n = 12$, $P = 0.27$: γ, overall mean 3.83; $n = 8$, $P = 0.48$: total, overall mean 6.95; $n = 20$, $P = 0.35$).

In one muscle it was possible to analyse the number of motor branches received by spindles with different sensory complements. Spindles with more afferents tended to receive more γ branches ($P < 0.01$, χ^2 test) but not more β branches.

The present results confirm the importance of random factors in the innervation of muscle spindles. The correlation between numbers of afferent axons and γ branches but not β branches may reflect differences in the necessity for pathway guidance during development.

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Paper 42.

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THE DISTRIBUTION OF STATIC γ-AXONS IN THE TENUISSIMUS MUSCLE OF THE CAT

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SUMMARY

1. The distribution of static γ-axons within and among muscle spindles of the tenuissimus muscle has been studied in the anaesthetized cat, on the basis of the effects on the responses of primary endings when bag_2 or chain fibres or both are activated by static γ-stimulation.

2. Locations of spindles were marked for subsequent histological analysis using teased, silver-impregnated preparations.

3. Static effects were classified into: (i) biassing; (ii) driving; or (iii) indeterminate categories.

4. Critical correlations established that the biassing type was produced by bag_2 activity, either alone or in combination with chain fibres, whereas the driving type was produced by chain fibres active alone. Indirect evidence suggested that indeterminate effects were produced by bag_2 and chain fibres active together.

5. The static γ-axons showed some differential distribution according to their conduction velocities: faster-conducting axons were likely to be more widely distributed among spindles but less likely to innervate chain fibres alone than were more slowly conducting axons.

6. The results are discussed in terms of their possible functional and developmental significance.

INTRODUCTION

Mammalian muscle spindles regularly contain three types of intrafusal muscle fibre that differ structurally, metabolically and functionally, and are known as bag_1, bag_2 and chain fibres (Barker & Banks, 1986).The distribution of fusimotor axons to these muscle fibres at the level of individual spindles is now well established, at least for the tenuissimus muscle of the cat. Fusimotor axons are usually branched intrafusally, but each axon commonly supplies only one type of intrafusal fibre in individual spindle poles, as shown by reconstructions from serial sections carried out independently in three laboratories (Banks, 1981; Banks, Barker & Stacey, 1981; Arbuthnott, Ballard, Boyd, Gladden & Sutherland, 1982; Kucera, 1985). Kucera (1985) reported a higher proportion of axons that were distributed exclusively to a single type of muscle fibre than did the other two groups; nevertheless all three found that a small proportion (3–4%) supplied both the bag_1 and one chain fibre, usually

the longest, whereas a much larger proportion (up to 29%) supplied the bag_2 and at least one chain fibre. No axon was found to innervate all three types of muscle fibre, nor the bag_1 and bag_2 together. This pattern of innervation has been related to the functional subdivision of fusimotor neurons into dynamic and static categories by a series of experiments, reviewed by Barker & Banks (1986), which demonstrated that dynamic axons always activated bag_1 fibres whereas static axons activated bag_2 fibres, chain fibres, or both together.

Each purely fusimotor- or γ-axon generally innervates several spindles in a single muscle, and the constancy of the effects of stimulating a single γ-axon on the response of the primary sensory ending, whether dynamic or static, has been amply confirmed since first described by Crowe & Matthews (1964). It follows that dynamic γ-axons are highly selective, probably innervating only the bag_1 fibres in all the spindles supplied by them. Static γ-axons, on the other hand, are less selective, because at least some innervate both bag_2 and chain fibres; nevertheless, there remains the possibility of some degree of selectivity among them, as was originally suggested by Boyd, Gladden, McWilliam & Ward in 1977 and has since been consistently supported by Boyd, Gladden and their colleagues.

In the fullest development of his ideas, Boyd (1985, 1986) proposed that there are two kinds of static γ-motoneuron: (i) the 'static bag' type that always innervates bag_2 fibres, as identified chiefly by its biassing action on the output of the primary sensory ending; and (ii) the 'static chain' type that always innervates chain fibres and often drives the primary output at the stimulus rate over a wide range of frequencies. He (Boyd, 1986) regarded those axons that supply both bag_2 and chain fibres in a single spindle as belonging to one or other of the above types according to whether, in other spindles, they are distributed exclusively to the bag_2 or chain fibres. Any axon that supplied bag_2 and chain fibres separately in different spindles was seen as a rare aberration of this pattern. Gladden & Sutherland (1989) have recently suggested that there may, in addition, be a third type of static γ-motoneuron that supplies chain fibres exclusively.

The segregation of static γ-axons into two or three types was based on two main lines of evidence; firstly, a histological description of different types of intrafusal motor ending, especially two types of neuromuscular junction on chain fibres (the m_a and m_c plates of Arbuthnott *et al.* 1982; Arbuthnott, Sutherland, Boyd & Gladden, 1985; Sutherland, Arbuthnott, Boyd & Gladden, 1985); and, secondly, the similarity of the effects of stimulating a single static γ-axon on the responses of more than one spindle (Boyd, Gladden & Ward, 1983; Boyd, 1986). The use of this evidence to support the case for subdivision of static γ-axons has been criticized previously (Banks, Barker & Stacey, 1985*a*), when it was shown that variation in quantifiable features of intrafusal motor endings was correlated with polar location of the endings (Banks, Barker & Stacey, 1985*b*; Kucera & Walro, 1986); and that the methods used by Boyd and his colleagues (1983; 1986) would result in unconscious bias if, as is quite possible, the actual distribution of a single group of γ-axons among the two different effectors, bag_2 and chain fibres, is subject to random variation in different spindles.

This paper describes a series of experiments whose purpose was to describe the distribution of fusimotor axons within and among tenuissimus spindles of the cat, by

inference from the effects of stimulating the axons separately on the responses of as many afferents of primary endings as could be obtained. Whenever possible, histological correlations were provided by silver-impregnated, teased preparations of the spindles previously recorded. This was particularly important in some key experiments that identified the intrafusal fibres responsible for producing characteristic effects such as driving. The approach to the problem is similar in principle to that used by Boyd and his colleagues (1983; 1986) but differs in a number of important respects, mainly in the method of histological analysis, but also in details of the physiological preparation. Preliminary results of some of the experiments have been published (Banks, 1988), on the basis of which it was concluded that there exists a single population of static γ-axons which shows some differential distribution related to conduction velocity. This conclusion is reaffirmed here on the basis of a full description of the results that now include an especially productive experiment, C883.

METHODS

Five adult cats (1·8–2·8 kg) of either sex, anaesthetized with sodium pentobarbitone (Sagatal, 45 mg kg^{-1} I.P. with I.V. supplements), were used. After extensive denervation of the left hindlimb, the tenuissimus muscle was exposed by removal of the biceps femoris. Its distal part, approximately two-thirds of the total length from the point of entry of the nerve's main branch to the crural insertion, was freed from surrounding tissues to be reflected into a bath through which circulated an artificial interstitial fluid (Bretag, 1969, as modified by Fink, 1984; experiments C689, C700, C870), or to be immersed in a pool of mineral oil formed from skin flaps (experiments C876, C883). In each case the temperature of the immersion medium and the animal's core temperature were maintained at 37 ± 1 °C. The blood supply of the tenuissimus muscle is provided by about three inputs to an anastomosing system of intramuscular vessels, which allows an adequate blood flow through an inactive muscle if one of the inputs is removed. When the muscle was reflected into the bath, it was necessary to remove the mid-muscle popliteal supply; however, in the pool-type experiments a pedicle was formed so as to retain this supply whilst allowing the muscle to be stretched as far proximally as the nerve input.

In order to accommodate the muscle in the bath or skin-flap pool it was usually necessary to discard the most distal part of approximately 1 cm. The muscle was then attached at its distal end to a servo-controlled electromagnetic puller (Ling Dynamics) which was used to apply ramp-and-hold stretches of approximately 2 mm amplitude at 5 mm s^{-1}. Initial muscle length was set so as to be in the upper part of the length–tension relationship but without using accurately reproducible criteria.

All physiological observations were restricted to the part of the muscle distal to the nerve entry that had been freed from surrounding tissues, since the stretch was not effective more proximally. In the bath-type experiments the proximal portion of the muscle was further mechanically isolated by locating the muscle below a pair of stiff copper wires at the proximal end of the bath. The wires also served as stimulating electrodes in order to establish action potential latencies; in the pool-type experiments they were replaced with platinum hook electrodes in contact with the tenuissimus nerve. The left dorsal and ventral roots L7 and S1 were cut, and functionally single afferents, especially from primary endings, were sought by splitting the dorsal roots. As each was isolated the position of the corresponding spindle was found by localized probing and stretch of the muscle, then marked with an epimysial stitch (5-0 silk). With care it is possible to identify the proximal–distal position of the spindle's sensory equatorial region with considerable accuracy in this way. In experiments C689 and C700 the ventral roots were split into 20–30 filaments and each was stimulated at 50 Hz while the output of a single primary afferent was monitored. When an effect on the primary response was found the motor axon responsible was isolated by further splitting of the ventral-root filament. In the remaining experiments the ventral roots were first subdivided to produce filaments containing functionally single motor axons, and those conducting at 60 m^{-1} or less were retained to study the effects of stimulating them on each afferent in turn. In

all the experiments each effective motor/sensory combination was characterized by stimulation of the motor axon with a range of fixed frequencies (typically 10, 30, 50, 70, 100, 200 and 300 Hz) during which ramp-and-hold stretch was applied. The frequencies were those nominally supplied by the variable output of a Devices Digitimer D4030 to the isolated stimulator and are those quoted in the results. Calibration of the output showed the actual frequencies to be about 10% lower than the nominal values.

The experimental muscles were removed post-mortem and processed by silver staining by a modified Barker & Ip method (Barker, Scott & Stacey, 1985). The intramuscular nerve and all spindles were teased out, the epimysial stitches serving to identify those spindles investigated physiologically. The silver impregnation was adequate or good for histological analysis in experiments C689, C700 and C883. With such material it is now possible to identify the three types of intrafusal muscle fibre in virtually all cases, principally by details of the primary sensory ending (Banks, Barker & Stacey, 1982), but also by the differences in polar length and diameter and by the distribution of elastic fibres (Gladden, 1976). The bag fibres can be traced individually from end to end, whereas chain fibres usually cannot; however, in locating motor nerve endings there was no doubt when one was dealing with a chain fibre. Some chain fibres had a pole similar in length and diameter to those of the bag fibres and were therefore identified as long chain fibres (Barker, Banks, Harker, Milburn & Stacey, 1976; Kucera, 1980). Individual motor axons were traced from within, or as close as possible to, the intramuscular nerve trunk using the high resolution and narrow depth of focus of a 100× oil-immersion objective on a Nikon Optiphot. A drawing-tube attachment was used to make low-power plans of all the spindles and the intramuscular nerve, and high-power detailed drawings of most motor endings including all those in the physiologically observed spindles of C883. Schematic diagrams of the intrafusal distribution of all the motor axons were prepared on the basis of the detailed histological analysis. It is important to emphasize that unless otherwise stated the connections shown in such diagrams have been positively established, and all connections are represented although the axonal branches, particularly those to the chain fibres, are simplified. Motor endings are identified as trail, p_1, or p_2 plates according to the criteria established by Banks *et al.* (1985*b*).

RESULTS

Characteristic fusimotor effects and their correlation with intrafusal motor axonal distributions

The results in this section establish the criteria to be used in inferring the intrafusal distribution of fusimotor axons from the effects of their stimulation on primary-ending output. They are presented in the order that most emphasizes the logical independence of the whole series of experiments.

C700. In this experiment three γ-axons were isolated that had static effects on a single primary ending, together with a fast (85 m s^{-1}) axon that had a specific and powerful static effect on the same primary and was provisionally identified as a skeletofusimotor-, or β-axon (Fig. 1). In addition to their extrafusal distribution, β-axons have been shown to innervate long chain and bag_1 fibres, usually separately, and to have corresponding static and dynamic effects on the primary response (Emonet-Dénand, Jami & Laporte, 1975; Barker, Emonet-Dénand, Harker, Jami & Laporte, 1977; Harker, Jami, Laporte & Petit, 1977; Jami, Lan-Couton, Malmgren & Petit, 1978, 1979; Kucera & Hughes, 1983; Kucera, 1984*a*, *b*; Kucera, Hammar & Meek, 1984; Banks *et al.* 1985*a*, *b*). In C700 the β-axon drove the primary ending at the stimulus frequency (1:1) between about 50 and 70 Hz, subharmonic (1:2, 1:3, etc.) driving occurring at higher or lower stimulus rates. Similarly one of the γ-axons drove the primary ending 1:1 between about 70 and 100 Hz, driving being enhanced by static stretch at the peak of the ramp-and-hold stretch, but giving way to highly irregular firing when the axon was stimulated at 200 Hz. (Notice that this γ-axon

had the slowest conduction velocity.) Stimulation of each of the two remaining γ-axons (1 and 2) biassed the primary response with no evidence of driving, though the response was more irregular during stimulation of $\gamma 1$ than $\gamma 2$. The size of the biassing effect was dependent on the phase of the stretch cycle, being greatest during release and least during application of stretch, thus reducing the dynamic index.

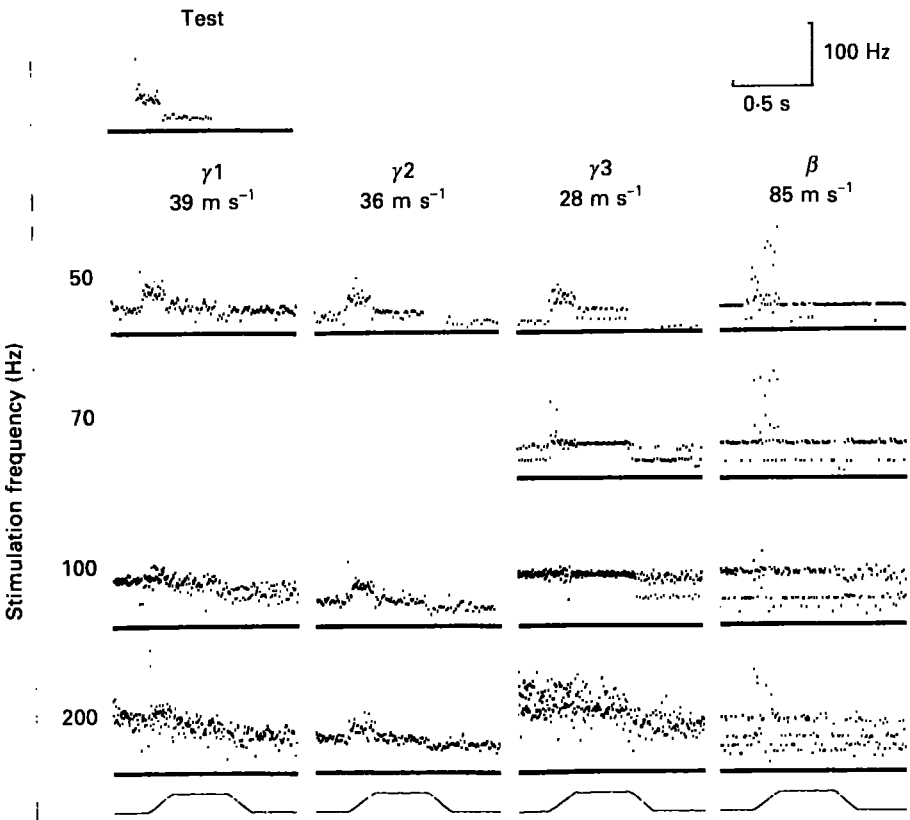


Fig. 1. C700, unit E, 92 m s^{-1}. Instantaneous frequency displays showing the responses to ramp-and-hold stretch, indicated at the bottom of each column, of a primary ending in the absence (test) and the presence of fusimotor stimulation at different frequencies. Each response plot includes a continuous baseline representing zero frequency. The motor axons all had static effects on the primary response but only two, including the β, drove the primary.

Subsequent histological analysis revealed that one of the chain fibres possessed a long distal pole (Fig. 2). The axon supplying this pole may be confidently identified with the presumed static β-axon previously isolated. Its terminals were in the typical form of a closely adjacent pair of small p_1 plates, indicated by a single symbol in the schematic diagram because of their proximity. The effect of stimulating this axon confirms a previous observation by Jami, Petit & Scott (1985) that even a single chain fibre, albeit a large one, can drive the primary ending when it is active alone.

The static fusimotor supply (i.e. that to the bag_2 and chain fibres) was completed by three, possibly four axons, only one of which innervated the remaining chain fibres, forming three endings in each pole. The simplest conclusion, shown in Fig. 2, is that this was the driving γ-axon ($\gamma 3$), whereas the non-driving γ-axons ($\gamma 1$ and 2) each supplied one pole of the bag_2 fibre.

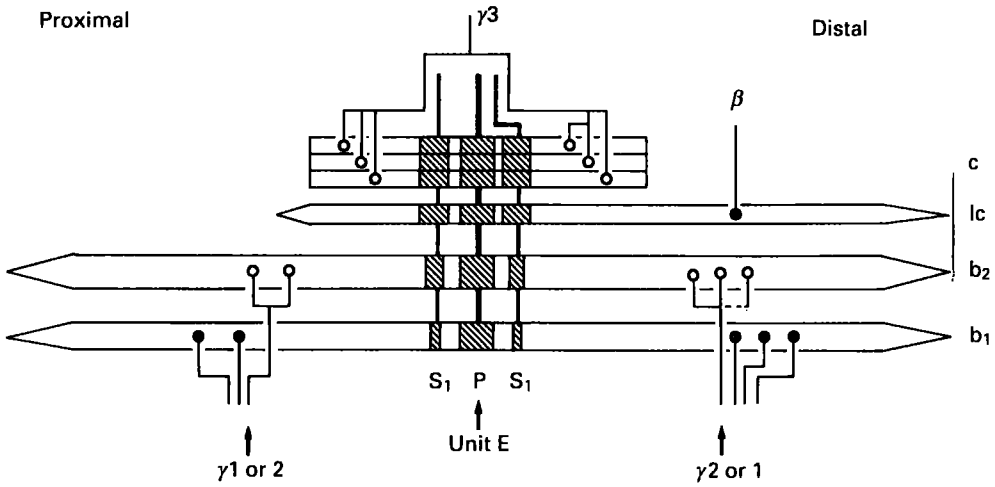


Fig. 2. C700. Schematic diagram of the innervation of spindle 12 that contained the primary ending whose responses are shown in Fig. 1. There were probably four static motor axons (the connection shown by the dashed line is uncertain), all of which were isolated in L7 ventral root. Note that the bag_1 fibre received only β innervation, as judged by the nature of its end plates. ○, trail plate; ●, p_1 plate (as defined by Banks *et al.* 1985*b*). In this and following figures, abbreviations are: P, Primary ending; S, secondary ending; b_1 bag_1 fibre; b_2, bag_2 fibre; c, chain fibre; lc, long chain fibre.

C689. In this experiment, three γ-axons were found during a search of ventral root filaments while recording from a primary-ending afferent (unit D). $\gamma 1$ was dynamic, $\gamma 2$ had a biassing static effect, and $\gamma 3$ drove 1 : 1, though ramp stretch interfered with the driving (Fig. 3*A*). At 100 Hz stimulation and above, $\gamma 3$ produced high-frequency, highly variable primary firing. In the same dorsal root filament and from the same spindle as unit D there was a secondary-ending afferent (unit E) that was activated by $\gamma 2$ but not by $\gamma 3$. Histological analysis revealed that the spindle (spindle 12, Fig. 4) possessed two secondary endings both on the distal side of the primary, and that in the distal pole the bag_2 and all the chain fibres were innervated by a single motor axon allowing it to be identified with the non-driving $\gamma 2$. In conformity with its lack of effect on the secondary ending, $\gamma 3$ is therefore seen to have been supplied to the proximal pole of the spindle. It may be identified with the axon that innervated the chain fibres exclusively in that pole, by its driving effect on the primary ending.

In the same experiment, the responses of a primary-ending (afferent unit F) from a closely adjacent spindle were also studied during ventral-root filament stimulation (Fig. 3*B*). $\gamma 1$ again had a dynamic action, but $\gamma 2$ drove this primary whereas $\gamma 3$ had no effect. In addition two static γ-axons ($\gamma 4$ and $\gamma 5$) were isolated that had similar

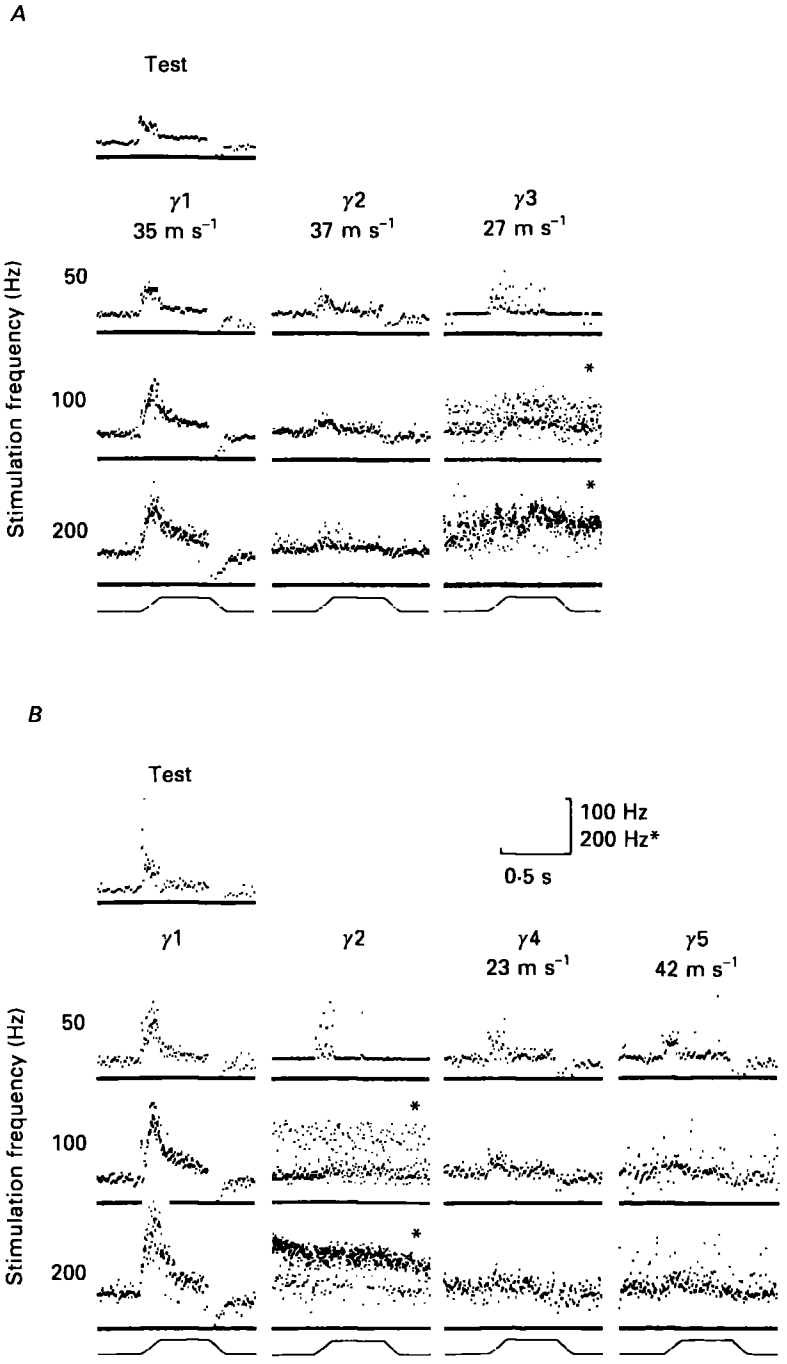


Fig. 3. C689. Instantaneous frequency displays showing the responses to ramp-and-hold stretch of two primary endings of adjacent spindles in the absence and the presence of fusimotor stimulation. Arrangement as in Fig. 1. *A*, unit D. 71 m s^{-1}. The effects of activation of three γ-axons on one primary. γ1 is dynamic; γ2, biassing static; and γ3, driving static. A secondary ending (unit E), present in the same spindle, was only excited

biassing effects on the primary. Histologically, a maximum of five fusimotor axons were present in the spindle (11, Fig. 4), whose poles appeared to be supplied separately. In the proximal pole the bag_1 fibre was innervated by one axon, the bag_2 fibre by two axons and the chain fibres by one axon. The remaining axon innervated the bag_2 and chain fibres in the distal pole. If the results from units D and E, spindle 12, are applicable to this spindle, the driving γ ($\gamma 2$) may be identified with the axon to the proximal chain-fibre poles. Furthermore, on any consistent interpretation, at least one of axons $\gamma 4$ and $\gamma 5$ may be identified with an axon to the bag_2 fibre alone in the proximal pole.

C883. The correlations found in experiments C700 and C689 were confirmed by additional examples from experiment C883, but whereas the interpretation of the first two experiments depended to some extent on the identification of the static β-axon with the innervation of the long chain fibre, no such assumption was necessary for C883. Only the correlative examples are listed in this section, together with schematic diagrams showing the results of the histological analysis of the relevant spindles (Fig. 5). The complete results of experiment C883 are described in the next section (see Tables 1 and 4).

Concerning the biassing response, the following direct correlations may be made: afferent J/spindle 13, where a biassing effect must have been produced by an axon that supplied both bag_2 and chain fibres, and where a second biassing effect must have involved the bag_2 fibre, either alone or in combination with chain fibres; and afferent F/spindle 17, where at least one biassing effect must have been due to the bag_2 active alone.

Concerning the 1:1 driving response: for afferent J/spindle 13 both a pure chain input and 1:1 driving were certainly absent; whereas in two cases a driving efferent was present when at least one (afferent D/spindle 15), or only one (afferent H/spindle 19), of the isolated efferents innervating those spindles must have supplied chain fibres only. Although none of these last three correlations, when considered in isolation, conclusively associated 1:1 driving with chain fibres active alone, taken together they are suggestive, and all of them *must* have occurred for the association to be valid. Moreover, if bag_2 and chain fibres active together do not produce 1:1 driving, afferents D and H must have been driven by motor axons supplying chain fibres only (see Table 1).

In summary, among the intrafusal fusimotor branches that exclusively supplied bag_2 fibres from experiments C700, C689 and C883, of which four are confirmed and at least one is probable, none elicited any primary driving, either fundamental or subharmonic, and even at high rates of stimulation the primary response was comparatively low, quite unlike the highly variable, high-mean-frequency responses often produced when chain fibres were exclusively activated at such rates. Of the two intrafusal branches confirmed to supply bag_2 and chain fibres together in these experiments, neither produced 1:1 driving. Finally, on the basis of these correlations

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by $\gamma 2$. *B*, unit F, 83 m s^{-1}. The effects of activation of four γ-axons on the second primary. $\gamma 1$ and $\gamma 2$ are the same as in Fig. 3*A*, $\gamma 1$ again having a dynamic effect, but $\gamma 2$ now drives this primary. $\gamma 4$ and $\gamma 5$ are biassing static axons. Asterisks indicate that vertical calibration is 200 Hz rather than 100 Hz, as shown by calibration bars in *B*.

and the identification of the β-innervated long chain fibre in C700, six intrafusal fusimotor branches (including that of the β-axon) that produced 1:1 driving in the three experiments may be positively identified as supplying chain fibres only.

The distributions of γ-axons to several muscle spindles

The intention in experiments C870, C876 and C883 was to establish the distributions of most of the γ-axons present in the large distal branch of the

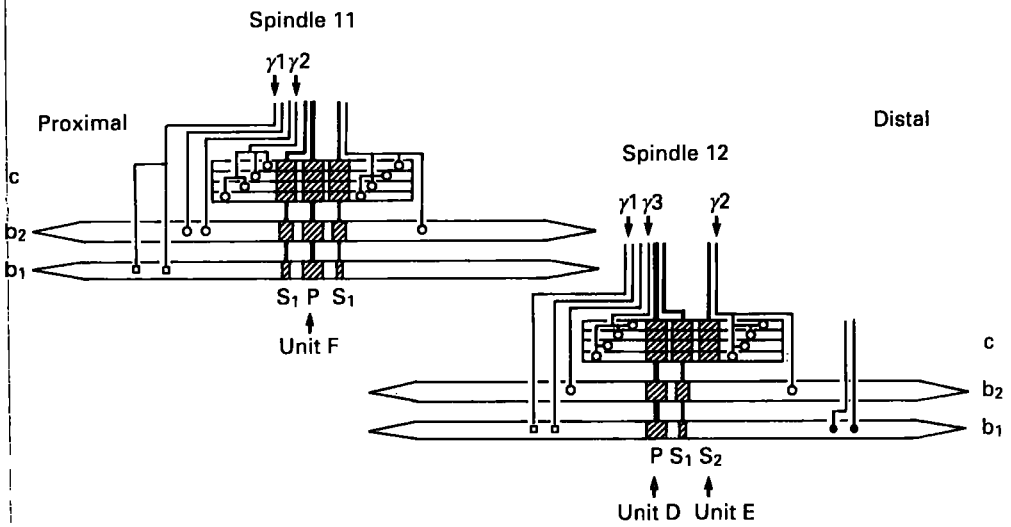


Fig. 4. C689. Schematic diagram of the innervation of the spindles that contained the primary endings whose responses are shown in Fig. 3. The distal pole of spindle 11 overlapped with the proximal pole of spindle 12. Note that: the dynamic $\gamma(1)$ innervated only one bag_1-fibre pole in each spindle (though in spindle 12 it might be represented by one or both of two axons); unit E is identified as S_2 in spindle 12 because of its slow conduction velocity (30 m s^{-1}); and γ-axons 4 and 5 in spindle 11 are not individually identified, but presumably both supplied the proximal pole of bag_2, or only one did so, whereas the other supplied the distal pole together with the distal poles of the chain fibres. ○, trail plate; ●, p_1 plate; □, p_2 plate.

tenuissimus, by inference from the primary responses to fusimotor stimulation, using as many combinations of primary-ending afferents and γ-efferents as possible. Altogether twenty-two primary-ending afferents and twenty-one γ-efferents were isolated and tested in combination; only one of the γ-efferents was dynamic. Among the effects produced by stimulation of the remaining static axons, examples of both biassing and driving types occurred, similar to those illustrated above for C700 and C689. In addition, there were indeterminate effects that could not be classified into either the biassing or driving categories, since they combined features of both, as shown in the examples of Fig. 6.

On the basis of the correlations established above, the combination of features that occurred in the indeterminate responses indicates that they were produced by bag_2 and chain fibres together. If so, there should never have been more efferents that elicited indeterminate responses from a single afferent than there were intrafusal

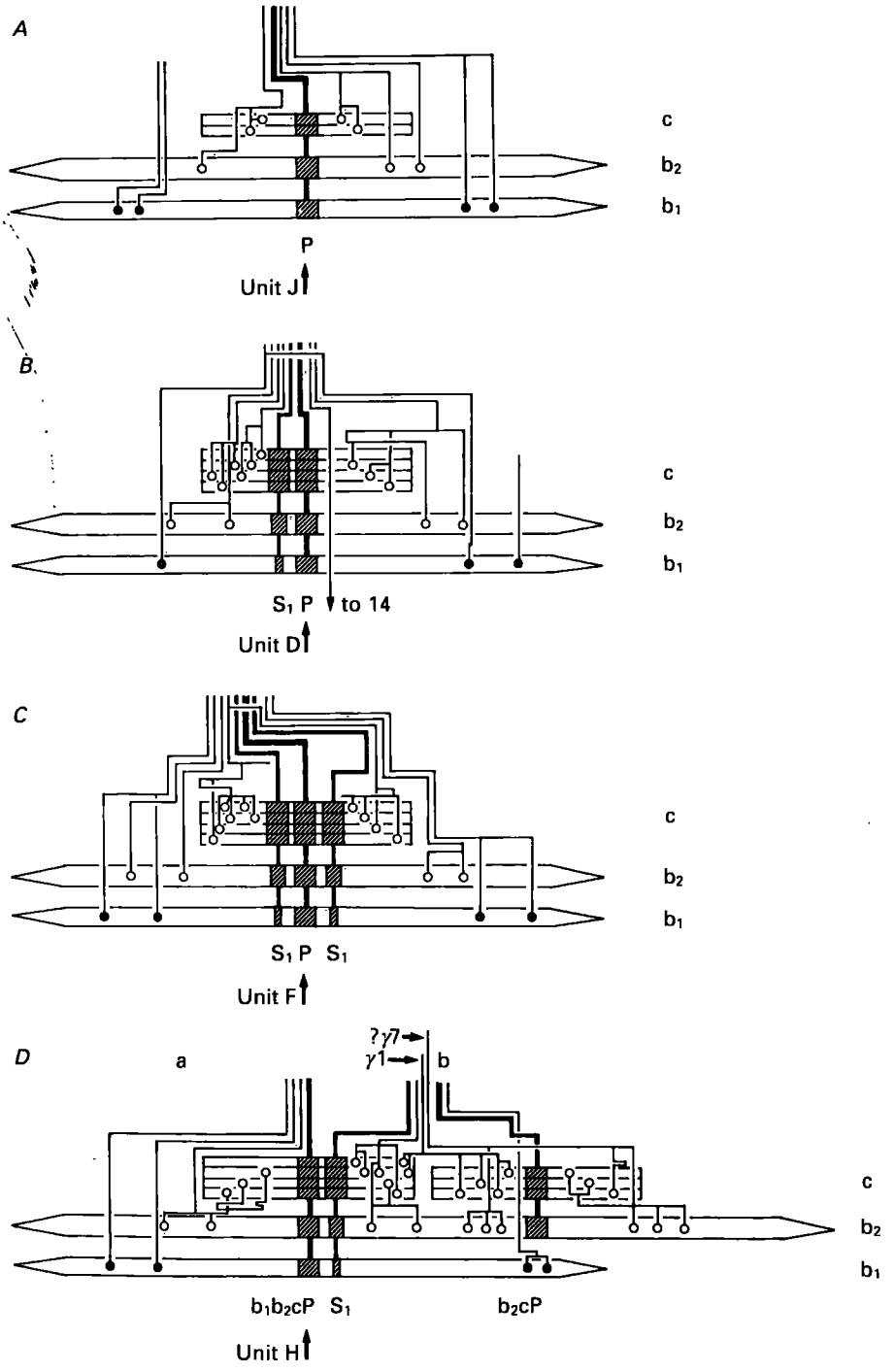


Fig. 5. Schematic diagrams of the innervation of selected spindles from experiment C883. ○, trail plate. ●, p_1 plate. *A*, in spindle 13 only two efferents innervated the bag_2 and chain fibres: one branched to supply both poles, but it could not be established which of the two distal branches was derived from the same parent axon as the proximal branch. Both efferents were isolated and had biassing effects on the primary ending (unit J). *B*,

branches of motor axons supplying bag_2 and chain fibres together in the corresponding spindles. This was generally true in C883, and specifically for the five afferents from which indeterminate responses were obtained (see Tables 1 and 4). Furthermore, no indeterminate response should have been obtained from a spindle receiving only a segregated motor input. This is not contradicted by either C700 or afferent F/spindle 17 (C883), the only examples where completely segregated inputs occurred, though in the latter case only two efferents were isolated from a possible maximum of four.

C870. The results of this experiment are summarized in Table 2, showing symbolically the effects of ten γ-efferents on seven primary-ending afferents. All seventy combinations were tested, and thirty-two (46%) were effective. Each efferent activated from one to six of the afferents, whereas each afferent was activated by three to six efferents, including a dynamic γ in five cases. Among the twenty-seven static effects only six were of the driving type; and in each case 1:1 driving occurred at a restricted range of frequencies somewhere between 50 and 100 Hz. Only four primary endings could be driven in this way; however, the static effects that could be elicited from each primary ending always included the biassing type and either the driving or indeterminate type. All three types of response were produced by two primary endings.

C876. Of the three experiments described in this section, C876 yielded the greatest proportion (16/21, 76%) of effective combinations of γ-efferents and primary-ending afferents. However, one afferent (F) died after its response to only the first efferent had been tested, and another afferent (A) was present in a very small filament along with interfering afferent responses from sources other than tenuissimus, so that its responses could not be characterized. Moreover, only four γ-efferents were isolated, and their conduction velocities encompassed a very limited range that was close to the middle of the ranges of the other two experiments. The nature of the effects of the γ-efferents on the primary-ending afferents are shown symbolically in Table 3; all were static and included driving, indeterminate and biassing types.

C883. This was the most complete experiment, in which seven γ-efferents and ten primary-ending afferents were isolated, and a full histological analysis was possible,

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in spindle 15 a maximum of four efferents innervated the bag_2 and chain fibres, two of the branches supplying chain fibres exclusively in the proximal pole. Three efferents were isolated, each one producing a different category (biassing, indeterminate, and driving) of effect on the primary ending (unit D). (The branch labelled 'to 14' was a β-axon that innervated a long chain fibre in spindle 14.) *C*, in spindle 17 the chain fibres were all supplied, in both poles, by a single fusimotor axon. Two efferents were isolated, both having a biassing effect on the primary ending (unit F). At least one of these must have innervated the bag_2 fibre alone. *D*, spindle 19a consisted of a b_1b_2c component containing the recorded afferent (unit H) in tandem with a b_2c component. The bag_2 and chain fibres of the entire complex were supplied by four fusimotor axons all of which were isolated. Only one (γ1, 46 m s^{-1}) drove the primary ending: this was therefore identified with the only intrafusal branch that supplied chain fibres exclusively. Chain fibres of the two components are shown separately but may have been in mechanical, or even direct, continuity. One efferent (γ7, 42 m s^{-1}) had a mixed dynamic/static effect on the primary (see Table 4 and Figs 7 and 8) but could not have innervated the bag_1 fibre. It may have been the axon that supplied the distal and intermediate poles of the bag_2 fibre, since activity here, in the much longer bag_2 fibre, could conceivably stretch the bag_1 through mechanical linkages.

TABLE 1. Comparison of the numbers of static γ-efferents that activated individual primary endings in the tenuissimus of cat C883 with the maximum numbers of γ-axons found histologically to supply the corresponding spindles

	No. of efferents by response category					Max. no. of γ-axons supplying			
Afferent	Biassing	Indeterminate	Driving	Total	Corresponding spindle no.	b_2	b_2c	c	Total
A	2	2		4	8	2	2	2	6
G				0	10	1	1	1	3
K	1	1	1	3	12	2	1	2+1*	5+1*
J	2			2	13	1	1		
						or	2		2
B	1		1	2	14	1+1†	1	2	4+1†
D	1	1	1	3	15	1	1	2	4
C	1	1‡	1	3	16	2	1	1	4
F	2			2	17	3		1	4
H	2	1‡	1	4	19	1§	2	1‖	4
Subtotals	12	6				14+1†	10		
						or 13+1†	11		
Totals		18	5			24+1†		12+1*	

*; very small axon with minute terminals, possibly not somatic.
†; ?β.
‡; mixed static-dynamic.
§; also chains to distal side of tandem-linked b_2c unit.
‖; chains only to intermediate pole.

relevant details of which are included here. The physiological results are shown symbolically in Table 4. Afferent E was initially isolated and its ending located, but it died before any motor effects could be tested. All of the remaining sixty-three possible combinations were tested and twenty-three (37%) were effective. Single

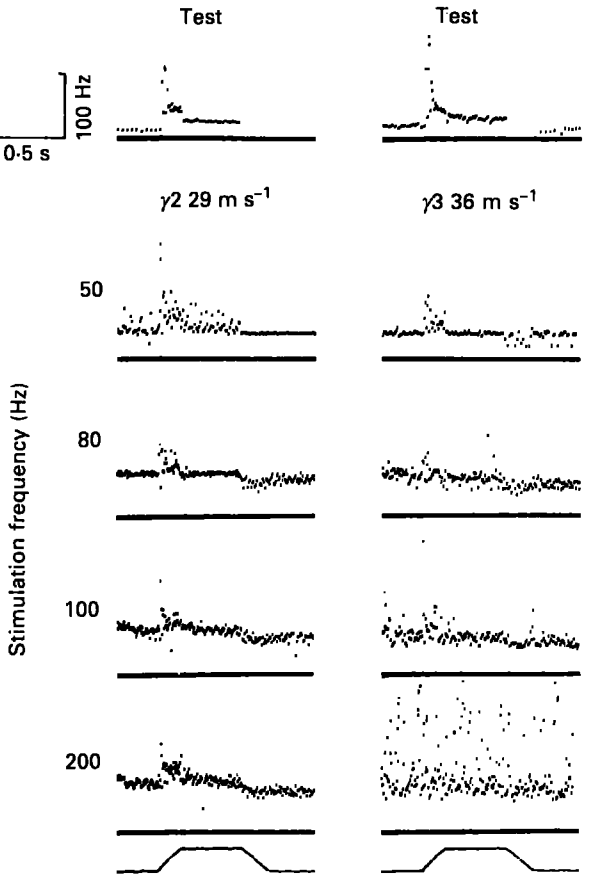


Fig. 6. C876. Instantaneous frequency displays showing the responses to ramp-and-hold stretch of two primary endings, unit D, 57 m s^{-1} (left) and unit E, 67 m s^{-1} (right) in the absence and the presence of fusimotor stimulation having static effects of indeterminate kind. Arrangements as in Fig. 1. In the example on the left, driving at low stimulus rates is replaced by biassing at higher rates; in the example on the right, biassing occurs at intermediate rates of stimulation in an otherwise typical driving effect.

efferents activated from one to five of the afferents, each of which, apart from afferent G, could be activated by two, three or four efferents.

No dynamic γ was found, nor did one appear to be present histologically, since the bag_1 fibres were supplied exclusively by p_1 plates (see the examples in Fig. 5). Static effects obtainable from each afferent during γ-stimulation included the biassing type, and usually included the driving, or indeterminate types, or both. One of the efferents (7) produced a marked increase in the dynamic response in addition to its static effect on two of the afferents (C and H, Fig. 7). Histological evidence indicated

TABLE 2. Symbolic representation of the effects of stimulating γ-efferents on the responses of primary endings in the left tenuissimus muscle of cat C870

Efferents by cv	Afferents: proximal → distal D	E	A	J	B	H	C	No. of spindles
41	●	●	●	●	○	—	○	6
40	—	—	●	●	●	●	●	5
33	●	○	●	—	○	▲	▲	6
31	—	—	○	○	—	—	—	2
30	—	▲	—	—	—	—	—	1
28	—	—	—	●	—	—	—	1
27	▲	—	—	—	—	▲	—	2
27	▲	—	●	—	○	—	—	3
24	—	—	—	●	—	—	—	1
23	□	□	□	□	□	—	—	5
No. static	4	3	5	5	4	3	3	
No. dynamic	1	1	1	1	1	0	0	
Total	5	4	6	6	5	3	3	

Each row shows the effects of a single efferent on the several primaries activated by it. —, no effect; ●, biassing; ○, indeterminate; □, dynamic; ▲, driving. The afferents are arranged in the proximal to distal sequence of their corresponding spindles and are identified alphabetically according to the order in which they were isolated. Efferents are arranged by conduction velocity (cv, in m s^{-1}) as shown in the column at the left; the total number of spindles supplied by each one is given in the column at the right. The numbers of efferents supplying each spindle are summarized at the bottom of the table in the corresponding column.

TABLE 3. Symbolic representation of the effects of stimulating γ-efferents on the responses of primary endings in the left tenuissimus muscle of cat C876

Efferents by cv	Afferents: proximal → distal G	H	E	A	D	No. of spindles
36	○	▲	○	+	—	4
35	●	—	▲	+	—	3
35	●	●	●	+	—	4
29	○	○	▲	—	○	4
Total	4	3	4	3	1	

Details as in Table 2 with the addition of: +, positive but unclassified effect. Afferent F (not shown) was located between afferents A and D and was activated by the efferent with cv 36 m s^{-1}, but died before the other efferents could be tested against it.

that in neither case was this due to direct innervation of the bag_1 fibre, whilst in both cases it revealed peculiarities in the mechanical arrangement of the bag_1 fibres that could conceivably have been involved (for afferent H, see Fig. 5). If so, these mixed effects may be included with the indeterminate static type, as in Table 1 which is a numerical summary of the distribution of static effects by type, compared with the distribution of the branches of fusimotor axons revealed histologically to supply bag_2 alone, bag_2 and chain together, or chains alone in the corresponding spindles.

Although expected, it is nevertheless important to point out that the number of motor axonal branches seen in each spindle was always sufficient to account for the

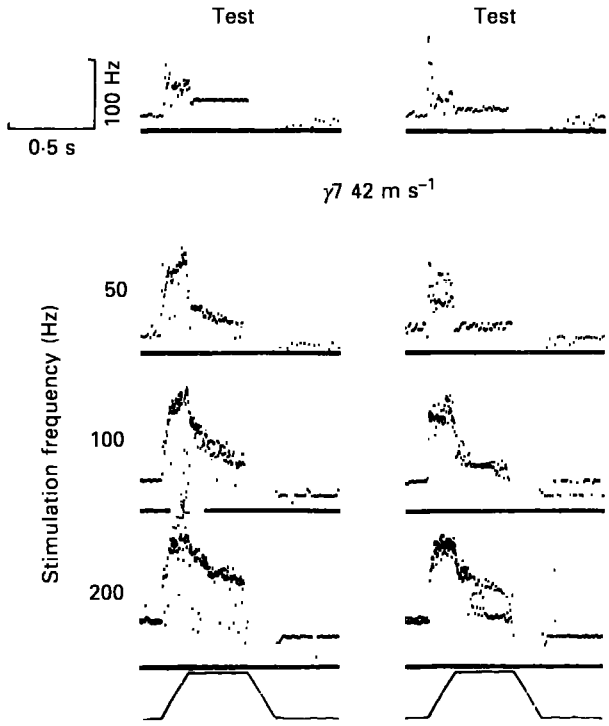


Fig. 7. C883. Instantaneous frequency displays showing the responses to ramp-and-hold stretch of afferents C (79 m s^{-1}; left) and H (88 m s^{-1}; right) in the absence and the presence of stimulation of γ7. The responses show mixed static-dynamic effects, but the bag_1 fibre was not innervated by the motor axon in either case.

TABLE 4. Symbolic representation of the effects of stimulating γ-efferents on the responses of primary endings in the left tenuissimus muscle of cat C883

Efferents by cv	Afferents: proximal → distal A	C	K	J	B	D	C	F	H	No. of spindles
51	○	—	—	●	●	●	—	—	●	5
46	—	—	○	—	—	—	●	—	▲	3
42	●	—	—	●	—	—	○	●	○	5
39	○	—	—	—	—	○	—	●	●	4
37	—	—	▲	—	—	—	▲	—	—	2
22	●	—	—	—	—	—	—	—	—	1
19	—	—	●	—	▲	▲	—	—	—	3
Total	4	0	3	2	2	3	3	2	4	

Details as in Table 2.

efferents that activated the corresponding primary ending. This number, which ranged from two to six, takes account of two branches that subdivided to supply both poles of their spindles, one whose complete distribution remained unresolved but which included bag_2 and chain fibres in spindle 13 (see Fig. 5), and one that

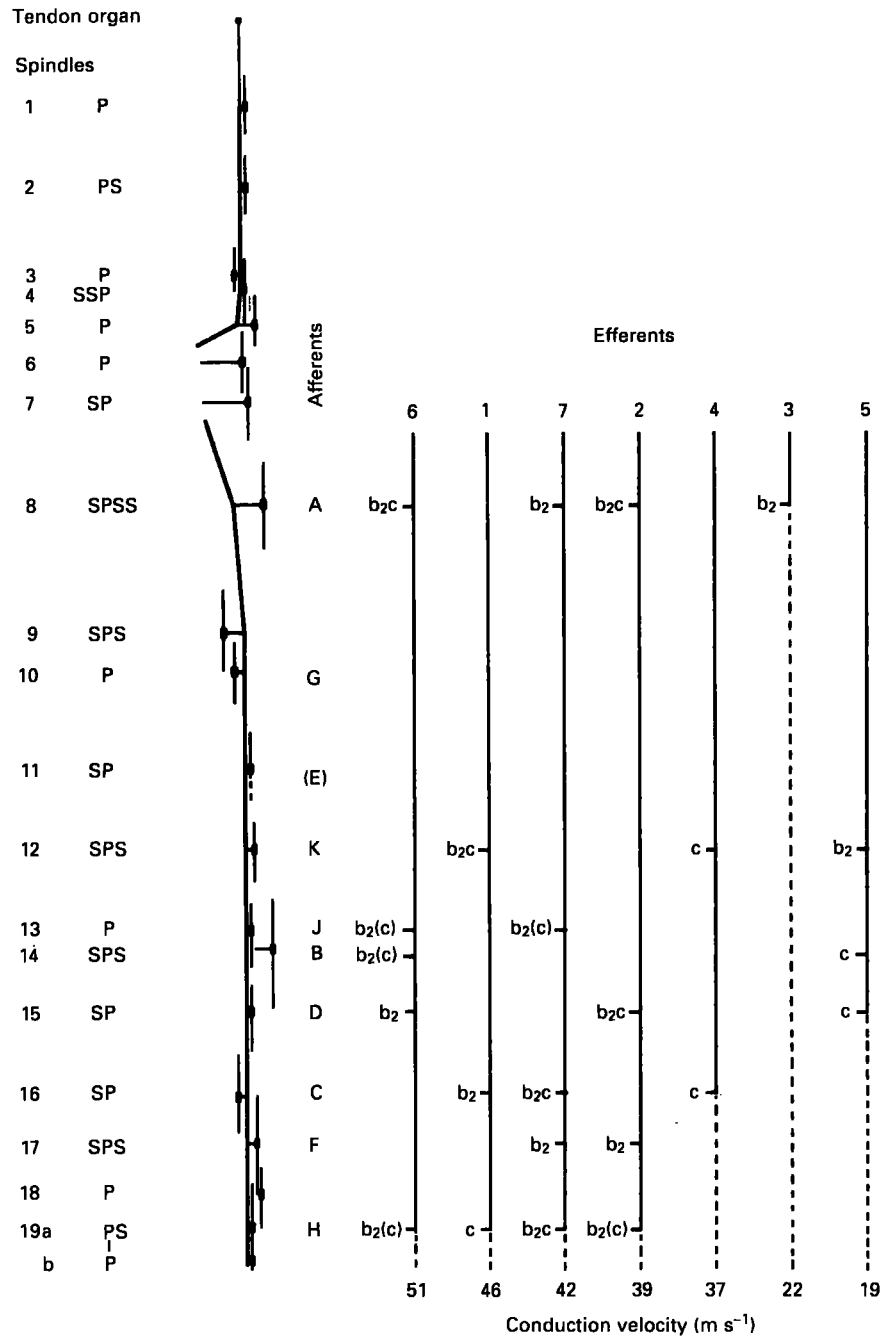


Fig. 8. Graphic representation of the distribution of γ-efferents among the spindles supplied by the distal nerve branch in the left tenuissimus muscle of cat C883, together with a schematic diagram of the arrangement of the spindles and their nerve supply in the whole muscle. At the left, the spindles are identified by number according to their proximal-to-distal sequence, and the sensory complement of each is given. Spindles 3 and 19b lacked bag_1 fibres. In the diagram, the short vertical lines represent the overall length of the bundle of intrafusal muscle fibres. Recorded afferents were all from primary

supplied only chain fibres in spindle 17. All other axonal branches were seen to supply only a single pole each, and were usually traced separately into the intramuscular nerve trunk; but the possibility remains that some were derived from parent axons that supplied both poles.

The histophysiological correlations made above (biassing/bag_2(chain); indeterminate/bag_2chain; 1:1 driving/chain) may be extrapolated to the complete results

TABLE 5. Analysis of the numbers of spindles supplied by each static efferent in the cat tenuissimus in relation to the conduction velocities of the efferents

	Rank									P	Q	S	τ
C870													
cv	1	2	3	4	5	6	7	8	9				
Distribution	1	3	1	5	7	7	5	4	7				
										24	7	17*	0·55
C883													
cv	1	2	3	4	5	6	7						
Distribution	1	4	1	3	6	7	4						
										15	4	11†	0·58

Rankings are of conduction velocity (cv), fastest = 1, and distribution, widest = 1. Taking each pair of ranks in turn, P is the total number of times that both the cv and distribution are exceeded in rank by the remaining pairs, whereas Q is the total number of times that the cv is exceeded, but the distribution is smaller, in rank. The overall score, $S = P-Q$. τ is Kendall's rank correlation coefficient (Sillitto, 1947). For C870, $\tau = 2S/(n(n-1)-2p_2-6p_3)$, where p_2 is the number of paired ties and p_3 is the number of triplet ties. For C883, $\tau = 2S/(n(n-1)-2p_2)$.

* For $n = 9$ with $p_2 = 2$ and $p_3 = 1$, $P < 0{\cdot}05$.

† For $n = 7$ with $p_2 = 2$, $P < 0{\cdot}1$ ($P = 0{\cdot}063$).

of C883 in an entirely consistent manner (Fig. 8), therefore serving to increase the confidence with which the generality of the correlations may be accepted. Notice that, on this interpretation, whereas the four fastest-conducting efferents almost always supplied bag_2 fibres, they also supplied chain fibres extensively. In addition, efferents 1 and 5 each supplied bag_2 and chain fibres separately in different spindles, indicating that this is not a rare occurrence.

General observations on the distribution of static γ-axons especially in relation to their conduction velocity

In C870 and C883 there appeared to be a tendency for the faster-conducting static γ-efferents to be more widely distributed among spindles than the slower ones. Rank correlation coefficients were similar at 0·55 and 0·58 respectively, but only in the case of C870 was this statistically significant (Table 5).

Moreover, the faster efferents were less likely to produce driving-type effects than the slower ones. Thus among the twenty-seven static γ-axons from all five

endings; they are identified alphabetically according to the order in which they were isolated in dorsal-root filaments, and are positioned opposite their corresponding spindle. Efferents are identified according to their order of isolation in ventral-root filaments, and are positioned according to their conduction velocities. Apart from afferent E all combinations were tested, and the probable intrafusal distribution of the motor axons, based on the afferent responses, are given. P, primary ending; S, secondary ending; b_2(c) signifies that the axon supplied either bag_2 alone or bag_2 and chain fibres together.

experiments, thirteen produced driving of at least one afferent, but only one conducted at 40 m s^{-1} or more, whereas five efferents that conducted at those speeds produced only biassing or indeterminate effects. The likelihood that this would have occurred by chance can be estimated as follows: overall 44/61, or 72% of all static efferent/primary-ending afferent combinations were of biassing or indeterminate

TABLE 6. Assessment of the probability that axons having a driving effect on a spindle primary should, by chance, have the slowest conduction velocity of all the static axons supplying the spindle, as often as observed in the cat tenuissimus

Experiment	Afferent	Total no. of static γ-axons	No. driving	Probability of being slowest	Relative cv (fastest = 1)	No. where slowest drives
C700	E	3	1	1/3	3	1
C689	D	3	1	1/3	3	1
	F	4	1	1/4	2	
C870	C	3	1	1/3	3	4
	D	4	2	1/2	3, 4	
	E	3	1	1/3	3	
	H	3	2	2/3	2, 3	
C876	E	4	2	1/2	2, 4	1
	H	3	1	1/3	1	
C883	B	2	1	1/2	2	3
	C	3	1	1/3	3	
	D	3	1	1/3	3	
	H	4	1	1/4	2	
	K	3	1	1/3	2	
	Total 14		Average 0·31			Total 10

Overall probability $= {}^{14}C_{10}(0{\cdot}31)^{10}(0{\cdot}69)^{4}$
$= 3{\cdot}1 \times 10^{-3}$

kinds; and twenty-five combinations involved γ-axons that conducted at 40 m s^{-1} or more, but included only one example of driving. If each combination, irrespective of conduction velocity, had an equal and independent chance of producing driving, the probability that the observed distribution occurred by chance is $25 \times (0{\cdot}72)^{24} \times 0{\cdot}28$ or less than one in 350.

Moreover, an axon that produced 1:1 driving in a spindle was likely to be the slowest-conducting or, when appropriate, next-slowest static axon supplied to the spindle. Table 6 lists those primary-ending afferents from which a driving effect could be elicited by at least one efferent, together with the total number of efferents working each of them, and the number and ranking by conduction velocity of the driving axons. Assuming that they had an equal chance of appearing in any of the available conduction velocity ranks, the probability is less than one in 300 that the efferents that occurred in the slowest ranks did so by chance as often as observed.

This is particularly remarkable in view of the widespread occurrence of driving. Thus of twelve static γ-axons that conducted at less than 40 m s^{-1} and activated two or more primary endings, nine produced a driving-category effect in at least one primary. Within this sample, eight activated three or more primary endings, and six of them produced a driving-category effect in at least one primary. Conversely, of ten

driving axons that worked two or more primary endings, eight had biassing or indeterminate effects on at least one of the primaries, as did all seven γ-axons that worked three or more primaries within this sample.

DISCUSSION

Correlations between muscle-fibre and static-response types

It is proposed that the responses to static fusimotor stimulation may be considered as a sequence ranging from pure biassing to 1:1 driving through indeterminate types; and that this may be correlated with the morphological range of fusimotor innervation in individual spindles from exclusively bag_2 to exclusively chain distributions, through various degrees of common distribution to both types of fibre. The end points of the sequence have been established by direct and indirect correlations, but only biassing responses have been positively associated with shared innervation. However, if these correlations are consistently applicable, as they were in C883, then the indeterminate responses can be confidently ascribed to common activation of bag_2 and chain fibres.

It should be emphasized that whereas the biassing effect can be produced either by the bag_2 acting alone or in combination with chain fibres, the driving-category effect may be produced virtually exclusively by chain fibres. This is not contradicted by any example from the present experiments and is further supported by the similarity between the proportion of driving-category effects (overall 28% of static responses due to γ-stimulation) and the proportion of chain-selective, presumed static γ-axonal branches found in spindles in this study and several others (C883 30%; overall 35%; Barker, Emonet-Dénand, Laporte, Proske & Stacey, 1973; Banks *et al.* 1981; Arbuthnott *et al.* 1982; Kucera, 1985; R. W. Banks, unpublished observations).

The correlations imply that chain-fibre driving can be suppressed, partially or wholly, by bag_2 activity. Since 1:1 driving may appear to be quite powerful in comparison with the response produced by the bag_2 alone, some explanation for this is required. Two factors seem to be particularly important. Firstly, driving is length dependent, as may be seen, for example, in the responses of C700 unit E to $\gamma 3$ and C689 unit D to $\gamma 3$. Subharmonic driving gives way to 1:1 driving, and then irregularity of firing, as muscle length is increased. This has subsequently been confirmed in an unpublished experiment (C891).

Secondly, the mechanical arrangements of the equatorial and polar regions of the bag_2 and chain fibres differ significantly. Elastic fibres surrounding the equatorial region of the bag_2 fibre insert into the surface of the fibre juxta-equatorially (Banks, 1984) and presumably absorb some of the tension generated by the contractile pole, thereby reducing the primary response to bag_2 activation. This does not occur on the chain fibres, where instead the equatorial region of the fibres remains relatively extended even when their poles are short, presumably due to juxta-equatorial tension transmission by some as yet unidentified mechanical linkage (Banks, 1986). The occurrence and location of chain-fibre kinking (Boyd, 1976; R. W. Banks, unpublished observations) indicates that some equatorial tension persists in the chain fibres even when their poles are completely slack.

These mechanical arrangements are consistent with the possibility that con-

comitant bag_2 activity *in the same pole* as active chain fibres could unload the chain fibres so as to prevent them from generating sufficient tension to exceed that transmitted from some external source to the chain-fibre equatorial region, particularly if the bag_2 fibre itself contributes to that source. Such a mechanism has recently been proposed to explain the occasional inhibitory effect of static γ-axons on secondary endings (Gioux, Petit & Proske, 1990). Moreover, Boyd (1976), using the semi-isolated spindle preparation, directly observed unloading of chain fibres by bag-fibre activity in the same pole.

The bag_2/biassing and chain/driving correlations described above are in broad agreement with those of Boyd (1981, 1986), but the remainder of the correlation conflicts with his conclusions. Prior to 1986, Boyd does not appear to have recognized the existence of non-selective static γ-axons whose activation produced biassing, non-driving primary responses; rather, non-selective axons were always described as enhancing an otherwise typically chain-produced driving (Boyd & Ward, 1982; Boyd *et al.* 1983; Boyd, 1985). Furthermore, driving at only subharmonics of the stimulus rate, such as occurred in some of the present indeterminate responses, was described as peculiar to selective chain-fibre activation (Boyd *et al.* 1983). If the mechanical analysis presented above is correct, it is difficult to see how bag_2 activity could ever enhance chain-fibre driving, and though it may be supposed that observation of intrafusal-fibre movements may, through its directness, be particularly reliable, there have been several instances of contradictory conclusions drawn from it (Barker & Banks, 1986). It is relevant here that Boyd (1986) attributed to exclusively chain-fibre activity a much higher proportion (48%) of inferred static γ-distributions within individual spindles than is likely on the basis of the known histology.

Estimation of the completeness of the results from C870 and C883

In terms of the numbers of afferent and efferent axons isolated and tested in combination, experiments C870 and C883 are probably the most complete so far described, each representing sixty-three spindle/static γ-combinations. Recordings were made from almost all of the spindle primaries supplied by the distal branch of the tenuissimus nerve in each case, and the histological results from C883 can be used to estimate the completeness of the isolated motor supplies.

On the basis of the total number of presumed static γ-axonal branches that supplied the spindles from which recordings were made, a maximum of thirty-eight positive responses would have been expected, fifteen more than were actually obtained. Since each isolated static axon activated on average 3·3 of the spindles, the deficit in responses could be accounted for by four or five axons, though only three axons would be required if the proportion supplying both poles of a spindle was as high as that found by Barker *et al.* (1973). Thus the mean number of static γ-axons innervating each spindle would be between 3·6 and 4·2, whereas the observed value was 2·6. However, in C870 an average of 3·9 static γ-axons activated each spindle, so that in this case the motor supply is unlikely to be missing more than one axon and may be complete. In each of these two muscles, therefore, there were probably between ten and twelve γ-motoneurons, values that may be compared with the total numbers of motoneurons found by Lev-Tov, Pratt & Burke (1988) to range from eight to thirty-one, approximately half of which were estimated to be γ-neurons.

These conclusions demonstrate that the accuracy of the quantitative results concerning the distribution of the static γ-axons can be accepted with confidence.

Static γ distribution among spindles

Despite the small number of muscles involved, the quantitative results clearly demonstrate the existence of a differential distribution of static γ-axons according to conduction velocity and hence, presumably, neuronal size. This allows the possibility of a limited amount of separate central control of bag_2 and chain fibres, or of sequential recruitment according to the size principle (Henneman, 1981).

Faster-conducting axons are more widely distributed and, as judged by their scarce 1:1 driving effects, much less likely to innervate chain fibres exclusively than are slower fibres (see also Boyd *et al.* 1977). However, the separation of biassing, indeterminate and driving responses is so imperfect, particularly among the slower axons, that it cannot be taken as evidence of more than one type of γ-neuron. Conversely any static γ-axon with a sufficiently wide distribution seems to innervate both bag_2 and chain fibres with varying degrees of segregation in the several spindles supplied by it.

This is consistent with the results of Barker *et al.* (1973) in which single static γ-axons were studied in muscles whose remaining motor innervation had degenerated after ventral root section. All of the six axons whose distribution is tabulated in detail in that study (Barker *et al.* 1973) included both bag and chain fibres in their motor units, and three of them each supplied bag and chain fibres separately in different spindles. The present results are also consistent with the distributions inferred from glycogen-depletion experiments (Brown & Butler, 1973; Barker, Emonet-Dénand, Harker, Jami & Laporte, 1976) provided that the probably spurious bag_1 depletions are ignored.

All these experiments were carried out on tenuissimus so it is particularly important that Brown, Crowe & Matthews (1965) noted that driving was produced more frequently by slow static γ-axons than by fast ones in the tibialis anterior. Moreover, as may be seen by reference to their Fig. 14, Emonet-Dénand, Laporte, Matthews & Petit (1977) found that in peroneus brevis 1:1 driving occurred in only ten of seventy-six static effects on primary responses; that nevertheless these were produced by eight of the twenty-five γ-axons involved; and that individual axons might produce different effects in different spindles.

The existence of differential distribution of a single population of static γ-neurons among two effectors raises interesting functional and developmental problems. Thus, whereas it might provide sufficient segregation to account for the observations, based on cortical, brainstem or reflex activation that have been taken to support the subdivision of static γ-neurons into two kinds (Gladden & McWilliam, 1977*a*, *b*; Wand & Schwarz, 1985), functional segregation of bag_2 and chain-fibre activation is far from complete.

In the absence of a full understanding of the separate roles of the intrafusal muscle fibres one cannot exclude the possibility that the observed pattern of innervation is positively adaptive rather than a maladaptive condition that is insufficiently harmful to be eliminated through evolution. That it may be adaptive is made more likely by the probable existence of developmental mechanisms which could bring

about virtually complete segregation, as suggested by the general occurrence of motor-unit homogeneity in the adult (Kugelberg, 1981). Even the skeletofusimotor, or β-motor units may not breach this generality in the same way as static fusimotor units, because, of course, only their intrafusal component receives sensory as well as motor innervation.

But perhaps the most remarkable observation concerning the distribution of the static γ-axons is that those that drive primary endings 1:1 over a range of stimulus frequencies, and hence are probably distributed exclusively to chain fibres, almost always have the slowest conduction velocity of all the static axons supplying the spindle, even though they may not be the slowest-conducting axons in the muscle as a whole. This distribution might make sense in terms of a strict size-principle activation, if it has an adaptive functional origin. It may be brought about even if individual static γ-axons do not inherently prefer bag_2 or chain fibres but rather if their conduction velocities in the adult were to reflect the time of axonal entry into the muscle during development (as may be implied for the afferents; Milburn, 1984), since chain fibres are the last intrafusal fibres to be formed.

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CHAPTER 26

Neuronal specificities in the reinnervation of muscle spindles

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Introduction

In most skeletal muscles the specialized intrafusal fibres of muscle spindles contribute a very small proportion of the total muscle fibres comprising the muscle, either by volume or by number, but they receive by far the largest proportion of the myelinated axons in the nerve supply. Most of these are afferent, whose sensory endings occur in the middle (equatorial) and adjacent (juxta-equatorial) regions of the intrafusal fibres (Fig. 1*A*), where they are protected by a complex capsule composed of perineurium and connective tissue (Barker and Banks, 1986). Here, especially in the equatorial region, the myofibrils are attenuated and replaced by vesicular nuclei. In development the intrafusal fibres seem to arise from the same population of myotubes as do the ordinary (extrafusal) muscle fibres, differentiation being initiated after contact by a sensory neurite which, in the mature spindle, gives rise normally to a single, equatorial, primary ending (Milburn, 1984; Kucera and Walro, 1990). Subsequently, other sensory neurites may arrive to form juxta-equatorial secondary endings, whose number follows a binomial frequency distribution, indicating that they associate randomly with the developing spindles (Banks and Stacey, 1988). The only other encapsulated sense organ that occurs commonly in skeletal muscles is the tendon organ (Fig. 1*B*), in which the sensory ending lies among modified tendon fibres after a transient contact with the ends of muscle fibres during development (Zelená and Soukup, 1977).

Spindle primary endings and tendon organ sensory endings are supplied by rapidly conducting afferent axons of groups Ia and Ib, respectively, whereas spindle secondary endings are supplied by more slowly conducting axons of group II. Although the spindle and tendon organ endings all have a low threshold and are slowly adapting in response to suitable stimulation, their positions relative to the extrafusal muscle fibres result in opposite responses to muscle contraction. Furthermore, the three types differ in the importance of the phasic, or dynamic, component of their responses (Fig. 1*C–E*), which is probably related to the diverse mechanical properties of the underlying muscle or tendon fibres. Centrally, they have quite different reflex effects that must be important in their roles in motor control (Brooks, 1986). Matching the central and peripheral specificities of the afferent axons in reinnervation is, therefore, essential for the normal restoration of function after nerve lesion.

Reinnervated muscle spindles

After peripheral nerve lesions in the adult, resulting in degeneration of the sensory endings, the gross structure of the muscle spindle persists so that sites of former primary or secondary endings remain recognizable for a considerable time. Moreover, a lesion affecting only the axons, such as nerve crush,

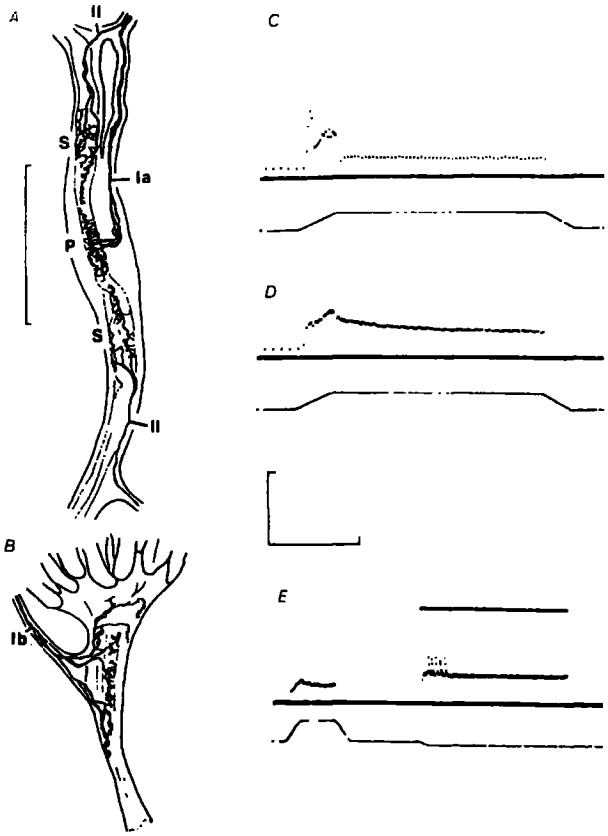


Fig. 1. Representative encapsulated muscle afferents and their responses from normal hindlimb muscles of the cat. *A, B.* Tracings of teased, silver-impregnated preparations of the sensory region of a muscle spindle (*A*) and of a complete tendon organ (*B*) to the same scale (bar: 500 μm). The spindle contains a primary (P) and two secondary (S) endings supplied by group Ia and II afferent axons, respectively; note the obvious difference in form between the primary and secondary terminals. The tendon organ has a single ending supplied by a Ib afferent. *C–E.* Reciprocal-interval or 'instantaneous-frequency' displays (dot rasters above zero frequency baseline) of the sensory discharges of a primary ending (*C*), a secondary ending (*D*) and a tendon organ (*E*). Muscle length is shown by the lowest trace in each case. Calibrations – vertical: 100 impulses s^{-1}; horizontal: (*C, D*) 0.5 s; (*E*) 2.5 s. The spindle afferents are stimulated by ramp-and-hold stretch (upward displacement of muscle length trace); the tendon organ by a similar stretch followed by ventral root stimulation at 50 Hz (horizontal bar). Note that the spindle afferents are tonically firing prior to stretch. The response has a rapid onset shown most markedly as an initial burst of impulses from the primary. Also the dynamic index (difference in firing rate at the end of ramp and that of the static discharge 0.5 s later) is greatest in the primary ending. Conduction velocities of afferents ($m\,s^{-1}$): (*C*) 86; (*D*) 36; (*E*) 95.

is followed by reinnervation leading to virtually complete structural and functional restoration of the intrafusal nerve endings in their normal locations (Hyde and Scott, 1983; Barker et al., 1985). Presumably the regrowth of the axons is guided by their uninterrupted endoneurial sheaths, since disruption of the sheaths by nerve section results in incomplete and abnormal reinnervation (Gregory et al., 1982; Banks et al., 1985), although individual endings can appear normal.

Initially our observations were made on the peroneus brevis muscle of the cat after section of the mixed musculo-cutaneous common peroneal nerve (Banks and Barker, 1983). It soon became apparent that the cutaneous afferents could reinnervate muscle spindles, but they never behaved like normal spindle afferents, most of them not responding to muscle stretch at all; and their often extensive terminal branches were associated with connective tissue rather than with the intrafusal muscle fibres. For these reasons they will not be considered further here.

Regenerated afferents that responded to muscle stretch and were identified as having reinnervated muscle spindles showed, by the nature of their responses, essentially normal mechanosensory transduction, although mean firing rates were significantly depressed (Fig. 2). Nevertheless, unlike normal primary and secondary endings, they failed to segregate into two populations (Fig. 3); in particular, there were some that conducted with group II velocities but showed primary-like responses, and rather more that conducted with group I velocities but responded like secondary endings (Fig. 4) (Banks and Barker, 1989).

These observations indicated that some of the afferents had formed functional endings in inappropriate locations. We were able to test the possibility that they might include Ib afferents previously supplied to tendon organs by exploiting the peculiar composition of the interosseous branch of the tibial nerve in the cat. This branch innervates the interosseous membrane and the adjacent origin of the flexor hallucis longus muscle, its group I and II components being almost exclusively derived from

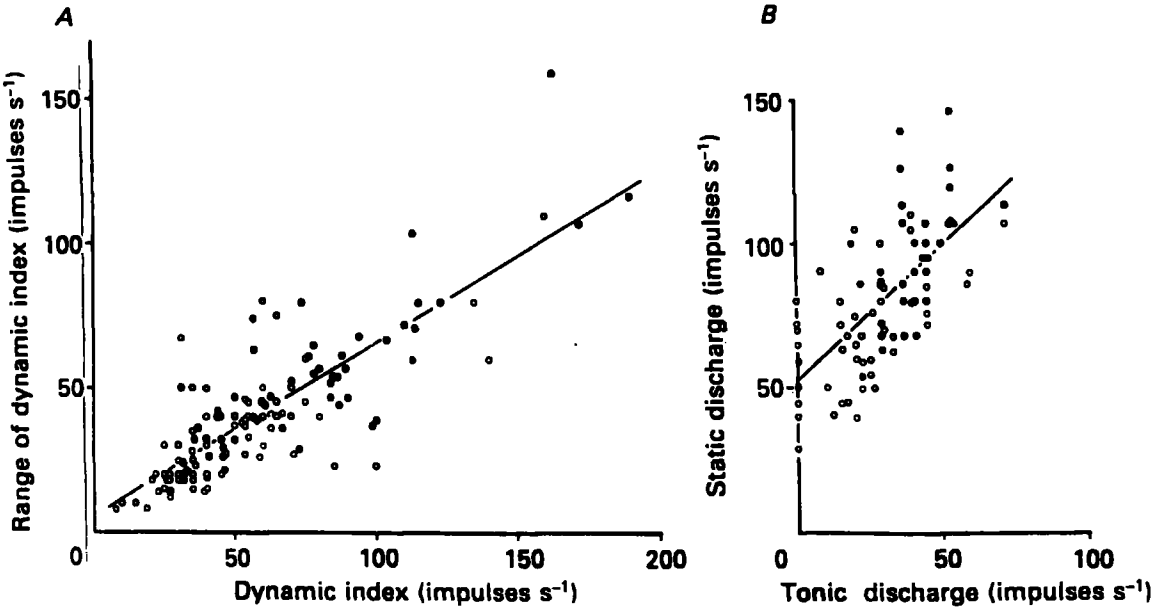


Fig. 2. Plots of certain response characteristics of normal peroneus brevis spindle afferents (filled circles), and those of afferents reinnervating peroneus brevis spindles 40–50 weeks after section of the common peroneal nerve in the cat (open circles). Mean firing rates of regenerated afferents are significantly lower than those of the normal ones, but their regression relationships do not differ. Regression lines are shown for the combined normal and regenerated populations. *A*. Plots of range of dynamic index for 2.5, 5 and 10 mm s^{-1} ramp stretches against dynamic index of 5 mm s^{-1} stretches. *B*. Plots of static discharge after 0.5 s of extended length against adapted tonic discharge before stretch.

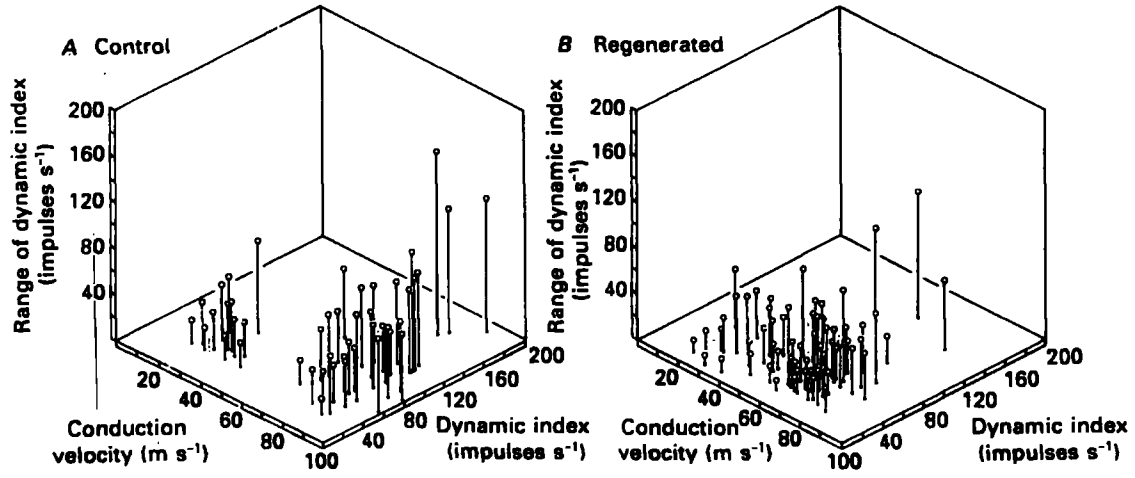


Fig. 3. Three-dimensional plots of range of dynamic index for 2.5, 5 and 10 mm s^{-1} ramp stretches against conduction velocity and dynamic index for (*A*) normal peroneus brevis spindle afferents, showing separate populations of primaries and secondaries; and (*B*) those reinnervating peroneus brevis spindles 40–50 weeks after section of the common peroneal nerve, where separate populations, if present, are not differentiated.

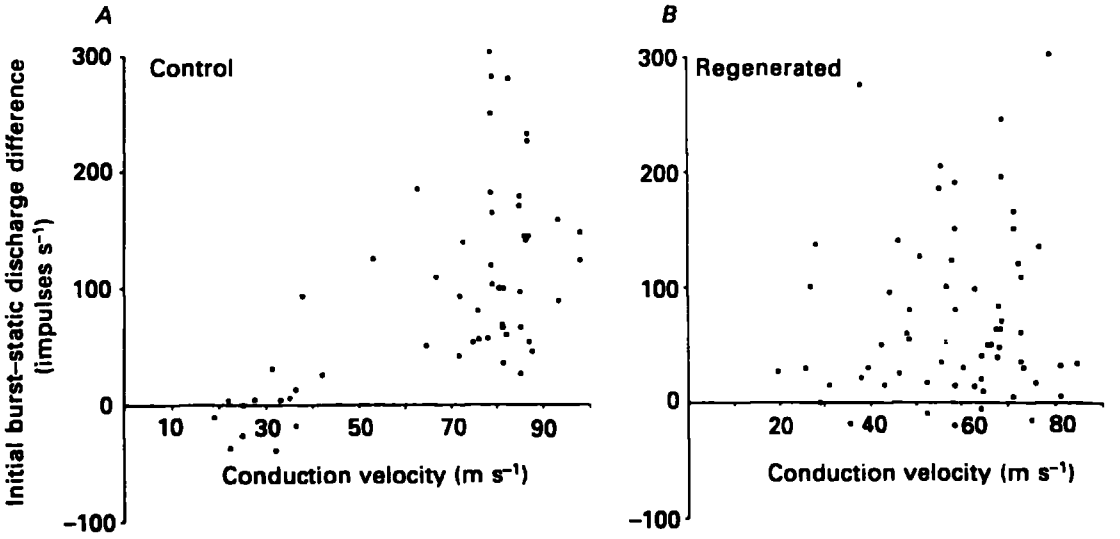
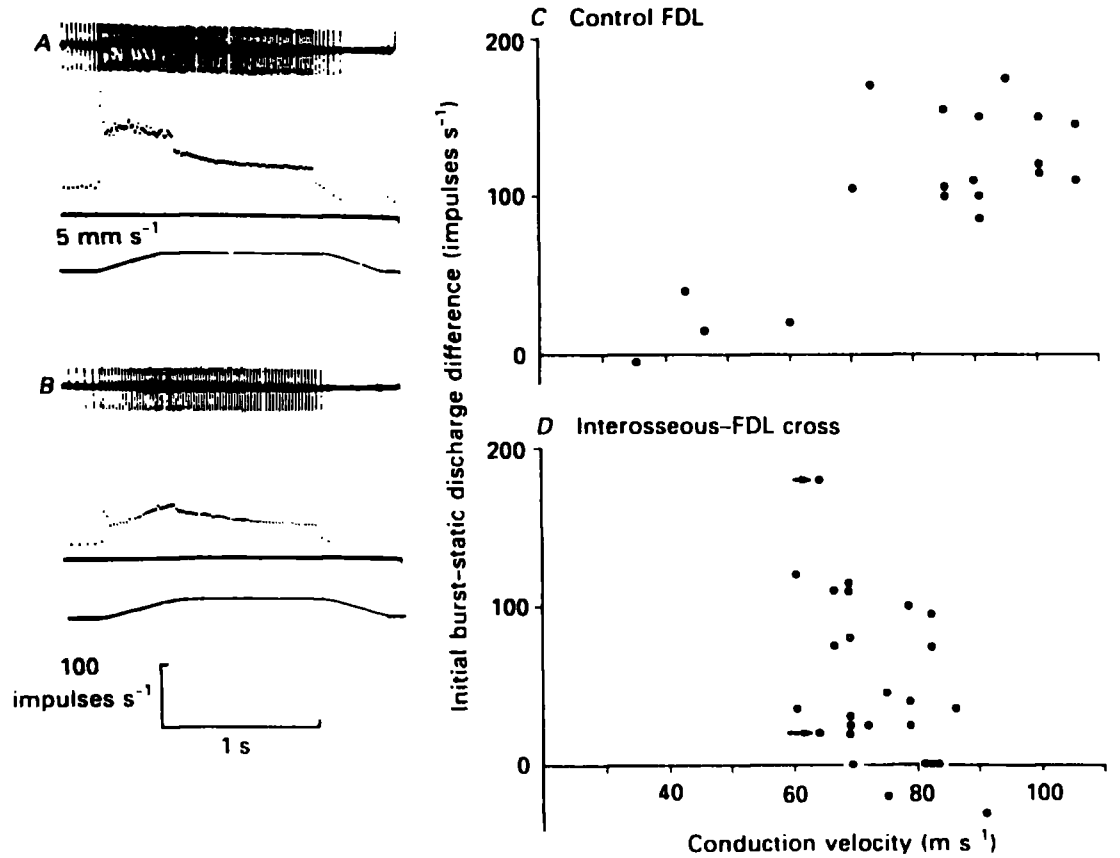


Fig. 4. Plots of the difference between the peak firing rate of the initial burst of the response to a 10 mm s^{-1} ramp and the static discharge, against conduction velocity, for (*A*) normal peroneus brevis spindle afferents; and (*B*) afferents reinnervating peroneus brevis spindles 40–50 weeks after section of the common peroneal nerve. In (*A*) maximum segregation into two groups occurs at 35 impulses s^{-1} and 60 m s^{-1}, providing a conduction-velocity-free criterion for differentiating primary and secondary responses. In (*B*) application of this criterion shows that primary- and secondary-type responses are no longer closely correlated with conduction velocity.



tendon organs and pacinian corpuscles, respectively. Reinnervation of spindles in the flexor digitorum longus (FDL) muscle after section and cross-union of the adjacent interosseous and FDL branches of the tibial nerve demonstrated that Ib afferents could indeed form functional intrafusal endings (Banks et al., 1984; Banks and Barker, 1989). Collins et al. (1986) came to a similar conclusion after measuring field potentials in the motoneuron pool of medial gastrocnemius muscle

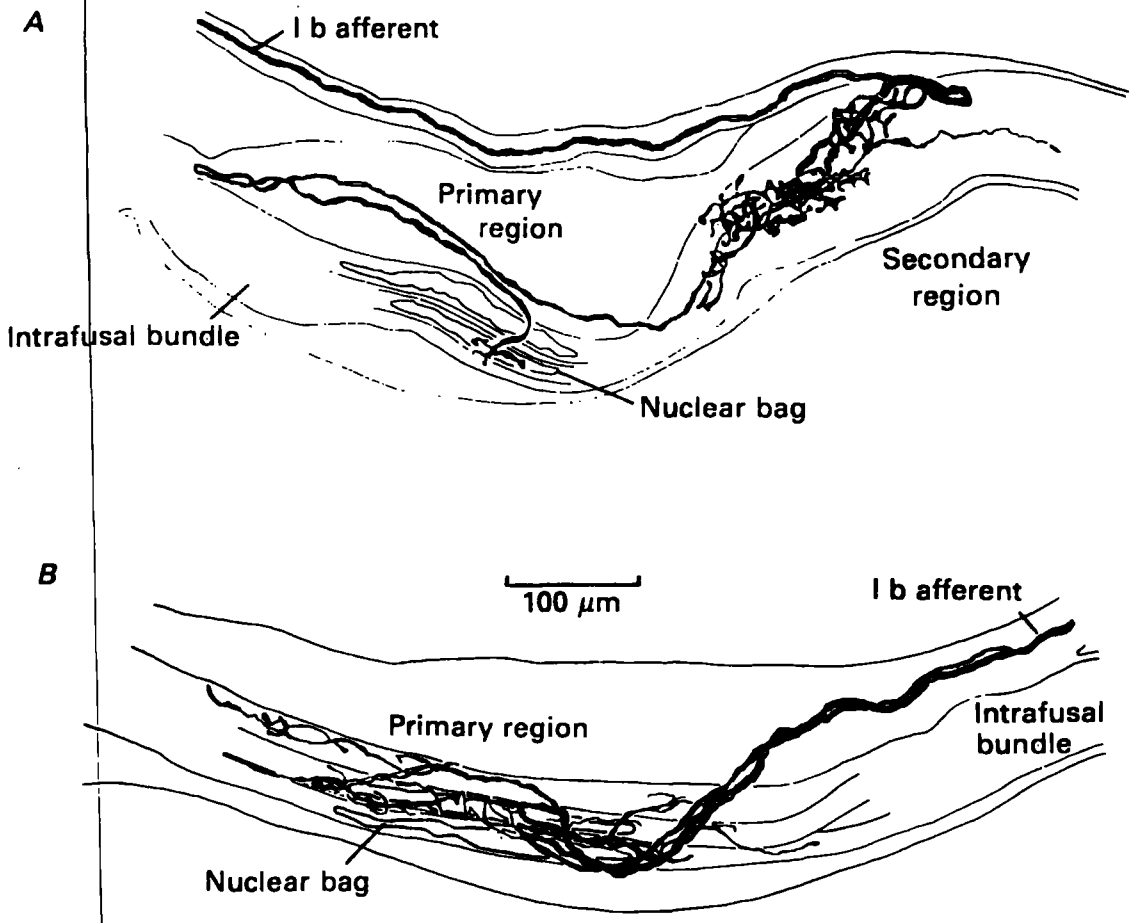


Fig. 6. Tracings of teased, silver preparations of FDL spindles innervated by regenerated interosseous Ib afferents after cross-union of interosseous and FDL nerves. *A*. Ib afferent, diameter 4.0 μm, innervates a site previously occupied by an S_1 secondary ending: a branch has grown beyond this, made a hairpin bend in the old Ia pathway, and finally petered out in a few terminals in the primary region marked by collection of equatorial nuclei (nuclear bags). *B*. Ib afferent, diameter 4.8 μm, innervates a site previously occupied by a primary ending. Terminals are diffuse and irregular and lack the transverse terminal bands around the nuclear bags characteristic of primary endings formed by normal and regenerated Ia afferents.

Fig. 5. Responses of regenerated interosseus Ib afferents innervating FDL spindles after cross-union of interosseous and FDL nerves. *A*, *B*. Primary-like response (*A*) and secondary-like response (*B*) to ramp-and-hold stretches; conduction velocities 65 and 64 $m\,s^{-1}$, respectively. Both impulse trains and reciprocal-interval plots are shown. *C*, *D*. Plots of difference between the peak firing rate of the initial burst of the response to a 10 $mm\,s^{-1}$ ramp and the static discharge against conduction velocity for (*C*) normal FDL spindle afferents; and (*D*) regenerated interosseous Ib afferents innervating FDL spindles. Note that all the Ib afferents in (*D*) are relatively fast. Arrows in *D* indicate plots for the responses shown in (*A*) (short arrow) and (*B*) (long arrow).

reinnervated after nerve section. Responses of the endings showed virtually the complete range of characteristics as the combined population of normal primary and secondary endings, although all the regenerated Ib afferents were relatively fast-conducting (Fig. 5). Histologically, the endings were found to be located equatorially, juxta-equatorially, or in both regions, thus corresponding to the broad range of their responses (Fig. 6). They appeared to prefer sites formerly occupied by secondary endings, where they would produce terminals closely similar to those of normal secondary endings, but if such a site was not available they ended equatorially, forming irregular terminals wholly unlike those of the normal primary ending (compare Figs. 6 and 1*A*).

Typically, the innervation of skeletal muscles includes both spindle and tendon organ afferents so that at least some of the inappropriate reinnervation that we observed in peroneus brevis muscle spindles could be confidently attributed to Ib afferents. Consequently, it was necessary to enquire what would be the nature of the reinnervation of spindles by their own afferents in the absence of the Ib input. The answer was provided by the tenuissimus muscle of the cat which receives about 40 spindle afferents distributed among some 17 capsules, whereas it frequently lacks tendon organs and rarely possesses more than one. Reinnervation of

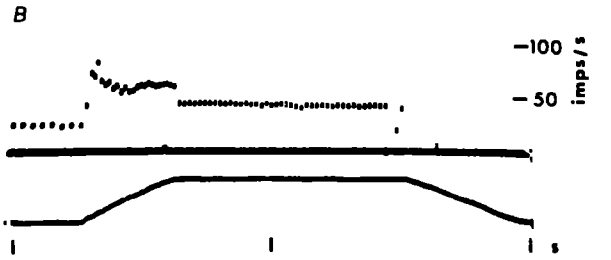


Fig. 7. *A*. Photomicrograph of the equatorial region of a tenuissimus spindle reinnervated by a presumed group II afferent (conduction velocity 26 m s^{-1}) forming an ending in the old primary site (P). *B*. The response of the ending in (*A*) to ramp-and-hold stretch showing primary-like dynamic behaviour.



Fig. 7*A*

the tenuissimus muscle after section of its nerve resulted in a much more normal pattern than that seen in peroneus brevis with relatively large-diameter axons (mean 5.0 μm close to the spindles) almost exclusively ending in primary-like bands and spirals in the equatorial regions, whereas smaller axons (mean 3.0 μm) ended predominantly in old secondary sites with virtually normal terminal form. These were, therefore, identified as group Ia and II afferents, respectively. Abnormalities remained, however, in that group II afferents occasionally ended equatorially (Fig. 7), or even in polar regions where there could not previously have been any sensory endings; and there might be multiple innervation of single spindles by Ia axons, whereas the majority of spindles (57%) lacked any Ia innervation (Banks and Barker, 1989). Furthermore, for regenerated Ia afferents it was possible to determine whether they had followed the original Ia pathways or some others, usually old II pathways. In 18 examples where both possibilities were available only 8 had returned via the Ia pathways.

In normal muscle spindles the sensory terminals are intrasarcolemmal, lying between the plasma membranes of the intrafusal fibres and their basal laminae, in contrast to the extrasarcolemmal motor terminals. The arrangement may be important in mechanosensory transduction, so for this reason, as well as the question of target recognition, it was of interest to see whether regenerated afferents occupied the same position. We have confirmed that this is so both for native- and Ib-reinnervated spindles of tenuissimus and FDL, respectively (Adal and Banks, 1990) (Fig. 8). The equatorial nuclei, also probably important in transduction and, therefore, an important feature of intrafusal fibre differentiation, were only maintained in the presence of sensory terminals, whether Ia, II or Ib. In permanently deafferented fibres they were replaced by well-developed cross striations.

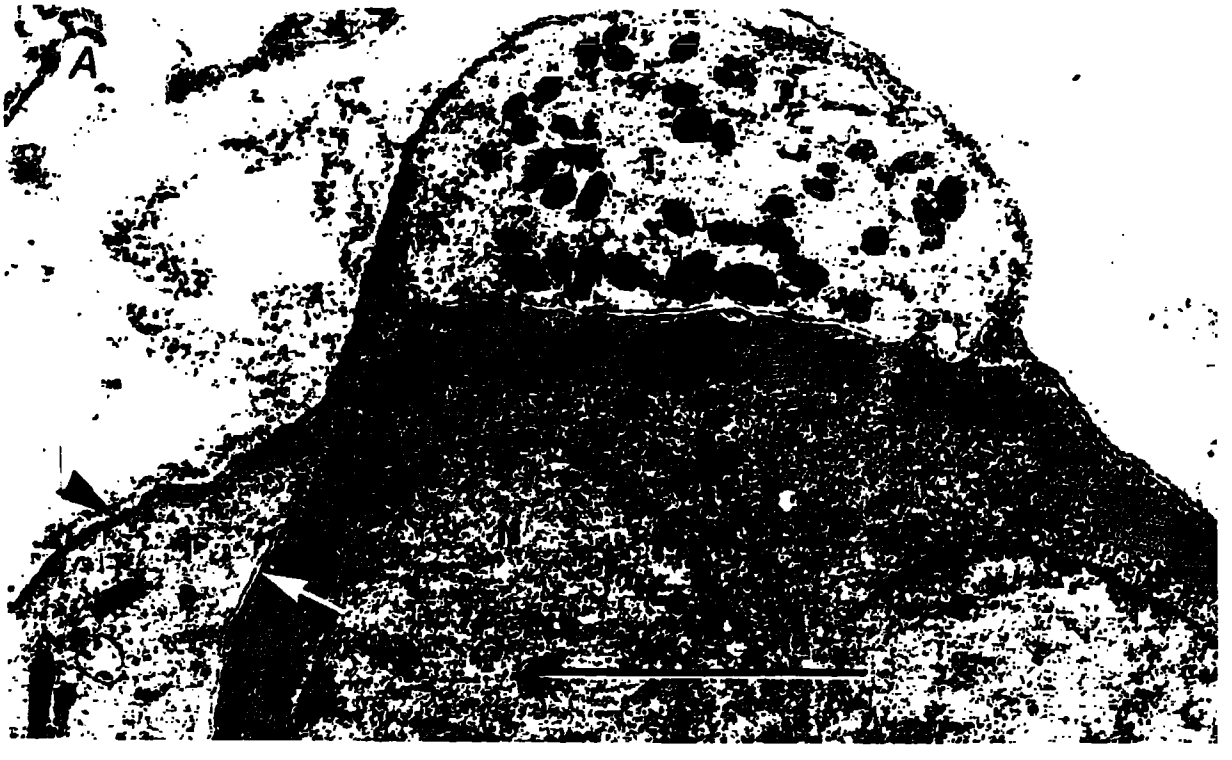


Fig. 8*A*. For legend see following page.



Fig. 8. Electronmicrographs of transverse sections through the sensory regions of FDL spindles showing the intrasarcolemmal location of sensory terminals (T) between the basal laminae (arrowheads) and plasma membranes (arrows) of intrafusal muscle fibres. N: equatorial nucleus; bar: 2 μm. *A*. Part of a large (nuclear bag) fibre of a normal spindle. *B*. Small (nuclear chain) fibres reinnervated by a Ib afferent after cross-union of the interosseous and FDL nerves.

Conclusion

Reinnervation after nerve section involves the regenerating neurites in two crucial choices, pathway selection and target recognition, whose outcome determines the quality of recovery. For the muscle afferents described here the evidence clearly indicates that, whereas they may be able to recognize afferent pathways as such, they are unable to discriminate between them in terms of their destinations; consequently, the afferents distribute themselves randomly among potential targets whether appropriate or not. The likelihood of misrouting increases with the complexity of the composition of the nerve at the site of the lesion, and many pathways might lead to sites without recognizable targets. However, in muscle spindles all three types of muscle afferents are able to form functional endings

the locations of which indicate that they have similar, and rather precise, specificities in target recognition. Nevertheless, some differentiation is possible as shown, for example, by the preference of Ib afferents for old secondary sites.

Inevitably, the contrast between the outcome of innervation in normal development and that of reinnervation after nerve lesion calls for an explanation. As yet several possibilities are feasible; for example, developing afferents may be much more specific in pathway and target selection than regenerating ones; conversely, the choice of pathway and target might be just as random in development as in reinnervation, but might be followed by a period of initial differentiation in a pluripotential system. Whichever is correct will have important consequences for our future approach to the problems arising from nerve injury.

Acknowledgements

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Paper 44.

R.W. Banks (1992)
Studies on the motor innervation of the cat's muscle spindle.
In Muscle Afferents and Spinal Control of Movement, ed. L. Jami, E. Pierrot-Deseilligny & D. Zytnicki. pp.31-36 Pergamon Press: Oxford.

Studies on the Motor Innervation of the Cat's Muscle Spindle

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Although mammalian muscle spindles regularly contain three types of intrafusal muscle fibre, the organization of their motor innervation varies considerably, even within a single muscle. Since it is only through knowledge of the nature and extent of the variability that the organizational principles governing the motor innervation can be discovered, this knowledge is likely to be important in understanding the role of the muscle spindle in motor control. Furthermore, it is likely to have implications for theories of neuromuscular pattern formation in general, since intrafusal and extrafusal muscle fibres seem to be derived from the same population of primary myotubes (Barker, this volume). It is therefore chastening to realize that our knowledge of the intrafusal motor innervation may be considered to be anywhere nearly complete for only one muscle, the tenuissimus of the cat.

The description that follows is a summary of my recent observations based largely on teased, silver-impregnated preparations, supplemented with serial-section reconstructions from previously published work (Banks 1981, 1991a; Banks *et al.* 1981; Kucera 1984; Kucera *et al.* 1984; Kucera & Hughes 1983). It concerns the provision of motor axons to individual spindles of the tenuissimus and of their distribution within those spindles; I have dealt elsewhere with the distribution of static γ axons among several spindles (Banks 1991b).

The elements involved are illustrated in Fig. 1, which is a schematic diagram showing the innervation of one particular spindle (the second in proximal-to-distal sequence from experiment C883 of Banks, 1991b). The criteria used to identify the bag_1 (b_1), bag_2 (b_2), and chain (c) fibres, and the motor endings, have been described previously (Banks *et al.* 1982, 1985); some chain fibres possessed a pole of similar length and diameter to those of the bag fibres, and were thus identified as long chain (lc) fibres.

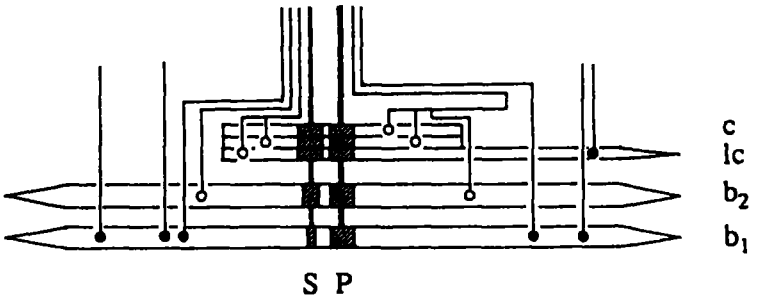


Fig. 1. Schematic diagram of the innervation of a representative spindle, illustrating the elements concerned: muscle fibres (b_1, bag_1; b_2, bag_2; c, chain; lc, long chain), sensory endings (P, primary; S, secondary) and intrafusal motor branches (filled circles, p1 plates; open circles, trail plates).

The intrafusal branches of motor axons, such as those shown in the diagram of Fig. 1, were traced as far into the nerve as possible, and often into the main intramuscular nerve. In 43 spindles the number of motor-axon branches entering each one ranged from 2 to 13, with a mean of 6.95. The frequency distribution of different numbers of branches was not significantly different (χ^2 test) from a binomial distribution with parameters n = 20, p = 0.35, indicating that the branches were randomly distributed with respect to the spindles. In most cases it was possible to differentiate the intrafusal branches of β and γ axons. They numbered from 1 to 6 (mean 3.2) and from 1 to 7 (mean 3.8) per spindle respectively. Again they appeared to be randomly distributed to their spindles, the frequency distribution of the γ branches not differing significantly from binomial form with parameters n = 12, p = 0.27, nor that of the β branches from binomial form with n = 8, p = 0.48.

It may be recalled, however, that the number of afferent axons additional to a single Ia also follows a binomial frequency distribution (Banks & Stacey 1988). It is possible, therefore, that the frequency distributions of the numbers of both afferent and efferent axons could be randomly determined with respect to their spindles, yet be correlated with each other. This possibility could be tested in one case, C883, where the results were particularly complete: both χ^2 and regression analyses showed a very clear tendency ($P < 0.01$) for spindles with more afferents to receive more γ branches, but there was no relationship between the numbers of β branches and afferents.

Three silver-impregnated muscles that were complete, or virtually so, yielded 51 spindles supplied by 330 branches of motor axons. The intrafusal distributions of 269 of the branches were traceable to their destinations on bag_1, bag_2, chain, or long chain fibres (Table 1). All branches to lc and $b_1$lc, most to b_1, one to b_2 and that to ?b_1b_2 were identified as belonging to β axons. The query relates to the identity of a bag fibre, co-innervated with the bag_2, in a spindle containing three bag fibres. The remaining branches, mainly to b_2, c, or b_2c, were identified as belonging to γ axons.

Table 1

The distributions of intrafusal branches of motor axons to tenuissimus muscle spindles of 3 cats

	b_1	b_1 lc	lc	?b_1 b_2	b_2	b_2 c	c	Unknown	total
C637	21		6		10	12	12	46	107
C700	35	1	6	1	16	7	19	12	97
C883	47	1	9		20	26	20	3	126
total	103	2	21	1	46	45	51	61	330

The proportion of motor branches supplying b_1 fibres was similar in each of the three muscles (34-41%, mean 38%). In C883 the entire b_1 motor supply appeared to be derived from β axons. As with the total population of β branches (including those to $b_1$lc and lc fibres), regression analysis showed no relationship between the number of branches to b_1 and the number of afferents.

The b_2 and c fibres also received similar proportions of the motor branches in each of the three muscles (49-56%, mean 53%), but within this group the proportion that co-innervated both types of fibre (b_2c) was more variable, ranging from 17% in C700 to 40% in C883. The b_2 and c fibres in each spindle pole might receive a completely segregated motor input as in the proximal pole of the spindle of Fig. 1, a completely mixed input as in the distal pole of that spindle, or an input variously segregated such as to b_2 and b_2c. Of 58 poles, the b_2 and c fibres were supplied only jointly in 19, completely separately in 21, not at all in 1, and with various degrees of segregation in the rest. The three polar types (ignoring the rarity without innervation) therefore occur with about equal frequency, and if they associate randomly in

complete spindles the six possible combinations should also occur about equally often. The deviations that there were – fewer mixed/partially segregated and more mixed/wholly segregated than expected – did not depart significantly from the overall random expectation (χ^2 test).

Nevertheless, as with the numbers of static γ branches supplied to spindles, so with their intrafusal distribution, an apparent randomness in the association of differently segregated poles could conceal a relationship with the afferent supply, since that itself is subject to random variation. In order to examine this possibility, individual spindle poles were first scored as follows:

Static γ supply	*Score*
unsegregated	0
partially segregated	1
segregated	2

Of 74 poles, 36 lacked secondary endings and had a mean motor-supply score of 0.64, whereas the 38 that possessed one or more secondary endings had a mean motor-supply score of 1.34, clearly indicating that the degree of segregation of input to the bag_2 and chain fibres is correlated in some way with the presence of secondary endings. Adding the scores for both poles of complete spindles showed that the static γ supply is increasingly segregated as first one pole and then both receive secondary endings. The motor-supply scores departed significantly ($P < 0.01$, χ^2 test) from values that would be expected if no such relationship were to exist.

It might be supposed that this is due simply to a progressive segregation as the number of static γ branches to a spindle increases in step with the number of afferent axons. But the relationship was not actually so straightforward, since there was a tendency for segregated poles to be supplied by fewer (mean 2.2) branches than partially segregated poles (mean 2.5 branches), even though unsegregated poles were almost always supplied by a single branch (mean number 1.1). This effect, combined with the increased numbers of static γ branches in spindles with more afferents, resulted in a much greater degree of segregation of static γ input to spindles with secondary endings in one pole as compared to those with only primary endings (difference of scores, 2.14–0.88 = 1.26), than of spindles with secondary endings in both poles as compared to only one pole (difference of scores, 2.88–2.14 = 0.74).

Finally, one further possibility concerning the intrafusal distribution of static γ branches remains to be examined: whether, in spindles with secondary endings in only one pole, the static γ innervation is more segregated in that pole than in the other. Fifteen spindles were relevant to this question of which 11 supported the proposed relationship whereas 4 contradicted it. Assuming that the more segregated static γ innervation is equally likely to be associated with either pole, the probability that the observed numbers occurred by chance is $^{15}C_{11}\ (0.5)^{11}(0.5)^4 = 0.042$. This is sufficiently low to confirm the relationship.

The results presented in this paper demonstrate the interplay of random and deterministic factors in the organization of the innervation of mammalian muscle spindles, and they have revealed some surprising subtleties concerning the intrafusal distribution of static γ axons. In contrast to β axons, the number of γ branches entering spindles is related to the number of afferent axons, and this may reflect basic differences in their requirements for guidance during development. It is worth noting that γ axons almost always enter spindles in company with afferent axons whereas β axons often do not (see Fig. 1 for example).

The number of static γ axons that supply a spindle whose sensory innervation consists only of a primary ending is typically 2, as shown by the regression relationship between the number of static γ axons (y) and the number of afferents (x):

$$y = 0.73 + 1.25x$$

In principle this would allow complete segregation of the motor supply to the bag_2 and chain fibres, yet it is the spindle poles rather than the different intrafusal fibres that are normally innervated separately. When a secondary ending is added, the corresponding increase in the number of static γ axons entering the spindle is sufficient to allow one pole to receive a wholly or partially segregated motor supply. It is remarkable enough that this does usually occur – 59% of poles with 2 static γ branches are wholly segregated and 34% are partially segregated - but it is particularly remarkable that the pole with the secondary ending normally (73%) receives the more segregated input (again, see Fig. 1, for example).

This amounts to an impressively complex organization, but any functional benefit that it provides is obscure, since the only sensory ending with a proportionately large contribution to the bag_2 is the

primary (Banks *et al.* 1981, 1982), and we have seen that a segregated motor input is most commonly absent from spindles that possess a primary ending alone. So far as the secondary ending is concerned it would seem to be virtually irrelevant whether its motor input was segregated or not; but it may be important that those axons which in part provide a segregated input to chain fibres (and hence are likely to excite secondary endings) tend to have the slowest conduction velocity of the static γ axons supplying the spindles concerned (Banks 1991b).

Perhaps the most important functional requirement is for the provision of several distributed, exclusively fusimotor, static inputs to the whole spindle complement of a muscle. It is then conceivable that the developmental programme, presumably involving contact guidance by afferents and known to involve the sequential appearance of the chain fibres after the bag_2, inevitably results in a degree of intrafusal segregation which is itself of little functional significance.

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Paper 45.

R.W. Banks, M. Hulliger, B. Otten & K.A. Scheepstra (1993)
The Ia afferent of the muscle spindle as an example in the study of pacemaker interactions in branched myelinated axons, both model and real.
Proc. XXXII Congress IUPS 322.3P.

322.3/P

THE Ia AFFERENT OF THE MUSCLE SPINDLE AS AN EXAMPLE IN THE STUDY OF PACEMAKER INTERACTIONS IN BRANCHED MYELINATED AXONS, BOTH MODEL AND REAL

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Myelinated sensory axons typically possess several receptor terminals, whose encoded outputs converge on the main transmitting axon through a system of preterminal branches. This presents an important theoretical problem since each pacemaker could be influenced by electrotonic spread of potentials and antidromic invasion by spikes generated at distant sites, in addition to the local receptor potential. During simultaneous encoder activity, the output of the first common node would generally not be a linear summation of those of the individual pacemakers acting alone. Moreover, the interaction should be influenced by the preterminal branching pattern. The spindle's Ia afferent provides an excellent example of such a system since: (i) it contains at least two pacemakers ('static' and 'dynamic') with separate access to the first common node, each under separate motor control, and (ii) the precise pattern of preterminal branching is very variable. We have studied the interactions of these pacemakers during static, dynamic and combined fusimotor activation of Ia afferents of the cat tenuissimus. Individual spindles were localized to determine their Ia branching patterns. The data were compared with computer simulations based on a modified Frankenhaeuser-Huxley model of mechanoreceptor terminals and the actual branching patterns. In general, highly complex branching patterns were paralleled by very highly competitive interactions. The model could account for this by limited spread of receptor potentials and significant competition between pacemakers based on antidromic invasion and probabilistic mixing of concurrent pulse trains from separate encoder sites. Support: MRC Canada and Wellcome Trust.

Paper 46.

D. Barker, R. W. Banks & R. B. Berry (1993)
Comparison of muscle-receptor recovery after nerve repairs using neural and non-neural grafts of two lengths.
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Comparison of Muscle-Receptor Recovery After Nerve Repairs Using Neural and Non-Neural Grafts of Two Lengths

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Summary

We have examined the sensory reinnervation of muscle spindles and tendon organs in teased, silver preparations of cat peroneal muscles after graft repairs of 2–15 mm defects in the muscle nerves in order to compare recovery after different lengths (2 mm and 10–15 mm) and different types of graft. The grafts were either neural (same or sural nerve) or non-neural (collagen-glycosaminoglycan matrix or freeze-thawed muscle). The significant ($p < 0.05$) factor affecting the outcome of the sensory reinnervation was not the type of graft used, but its length. In terms of annulospiral endings restored by stretch afferents, grafts 2 mm long gave, on average, results that were from 3.0 to 3.6 times better than grafts 10–15 mm long. Restoration of primary (Ia) endings to spindles averaged from 3 to 15%. Despite fair motor recovery, application of small-amplitude vibratory stimulation showed that the monosynaptic (Ia) stretch reflex was absent in peroneus brevis after 10–15 mm same-nerve grafts. This is attributed to insufficient primary endings and mismatched, but functional, afferent reconnections.

Keywords: Muscle afferents; muscle nerves; muscle receptors; muscle spindles; myotatic reflex; nerve grafts; stretch reflex.

Introduction

It is well established that the sensory feedback from limb muscles plays an important part in their motor control. When this feedback is impaired, as in patients with large-fibre sensory neuropathy, accurate maintenance of posture and the fine control of movement become heavily dependent on visual guidance[14,16,17]. The large fibres involved are the proprioceptive muscle afferents that innervate muscle spindles (Ia and spindle II afferents) and tendon organs (Ib afferents); their annulospiral endings monitor muscle stretch and tension and provide the sensory feedback required for the reflex control of muscle contraction and its co-ordination.

Banks *et al.*[1] and Banks and Barker[2] have shown that after section and microsuture of the cat common peroneal nerve large muscle afferents are able to regenerate and functionally reinnervate some spindles and tendon organs

with annulospiral endings. The number of such reinnervated receptors observed in any given muscle, expressed as a percentage of the total examined, provides a measure of the amount of annulospiral afferent reinnervation achieved. This is referred to as the spiral index. Such assessments of afferent reinnervation of muscle may be used to compare different methods of nerve repair, or to evaluate the results of varying different factors that may affect the course of normal regeneration following the same method of nerve repair. The value of spiral index assessments has already been demonstrated by us in other work in which the reinnervation and recovery of muscle receptors has been quantified after immediate and delayed nerve repair[6], after temporary ischaemia in muscle grafts[18], and after muscle devascularization[7].

In this investigation we have used the spiral index to compare the afferent reinnervation of spindles and tendon organs achieved in peroneal muscles after nerve repairs in cats made with neural and non-neural grafts of two lengths. In the case of the reinnervated spindles we have also determined the number of primary endings that have been regenerated by Ia afferents. Such endings, though smaller than normal, can easily be distinguished from primary endings regenerated by Ib or spindle II afferents[2]. We refer to the percentage of spindles with Ia primaries in a reinnervated muscle as the Ia index. Establishing the Ia index provides some indication of the degree of myotatic recovery that might be expected of a reinnervated muscle since it is the Ia afferent that excites the monosynaptic stretch reflex.

The neural grafts used were either a portion of the same nerve sectioned (the common peroneal) or a portion of the sural nerve, whereas the non-neural grafts were either composed of the biodegradable collagen-glycosaminoglycan (CG) matrix developed by Yannas *et al.*[19] or strips of freeze-thawed muscle as used by Ide[12] and Glasby *et al.*[10]. In some experiments we were able to measure the maximal tetanic tensions developed by the reinnervated peroneus brevis (PB) muscle, both directly in response to the electrical stimulation of its nerve, and reflexly in response to small-amplitude vibratory stimulation.

Some of the results reported here were briefly mentioned in a review lecture given by one of us at a recent symposium[3].

Materials and Methods

The left common peroneal nerve of adult cats was used for the graft repairs, anaesthesia being induced with sodium pentobarbitone (Sagatal, 45 mg kg^{-1}, I.P.) and maintained with halothane (Fluothane) by inhalation. The nerve was either completely or partially sectioned about 4 mm proximal to its entry into the gastrocnemius lateralis muscle, and the grafts were stitched in place with 10/0 nylon epineurial sutures. The animals were then maintained for not less than 20 weeks, the minimum period required to allow for optimum afferent recovery[2] and changes in the properties of motor units, which continue to occur during this time[11]. At the end of each experiment (average recovery period 26 weeks), PB and, usually, peroneus tertius (PT) were removed post mortem and processed for silver staining using the technique of Barker and Ip[14] with the modifications recommended by Barker *et al.*[5]. The spiral index and Ia index

were then determined for each muscle by teasing spindles and tendon organs from it and examining them for reinnervation by annulospiral afferents.

Twenty-nine animals were used to perform 3 series of experiments in 8 groups, as follows.

Series I

In these experiments the CG matrix was used to repair a 2 mm defect in the common peroneal nerve in two cats, and the results compared with a 2 mm autograft achieved by double neurotomy in 5 cats, this being taken to represent the optimum possible repair. These autografts were among 9 carried out by Banks *et al.*[1]. They were able to make only preliminary observations on the subsequent afferent reinnervation, since the experimental work necessary for the identification of the regenerated afferents had then still to be done. In the present analysis we have included those autografts from 5 cats in which there was a minimum recovery period of 20 weeks. The CG matrix repairs were effected using a modified version of the technique devised by de Medinaceli *et al.*[9]. About 5 mm of the common peroneal nerve was frozen and a 2 mm segment cut out using a cold vibrating blade. After thawing, the proximal and distal stumps were sutured to a rubber sheet with epineurial stitches about 1 mm from their cut surfaces thereby eliminating all mechanical tension between them. The CG matrix was cut into sufficient 2 mm lengths to replace the missing nerve segment to its full diameter, and the rubber sheet was then folded over the graft material and sutured on to the lateral surface of the nerve. The free edges of the tube thus formed were also sutured. Three weeks later the rubber sheet was removed.

Series II

The disadvantage of using the whole common peroneal nerve for such experiments is its musculocutaneous composition, which results in the reinnervation of muscle by both muscle and skin afferents thereby increasing the complexity of the subsequent histological analysis. In the second series of experiments we therefore used only two fascicles of the nerve, namely those that innervate the PB and PT muscles. In 2 cats the CG matrix was used to repair a 2 mm defect in these fascicles, and the results compared with those from 4 cats in which the defect was repaired with a 2 mm autograft consisting of a portion of the left sural nerve. Both matrix and sural grafts were held in place at each end with one or two epineurial sutures.

Series III

In this series of experiments the same peroneal fascicles were used, but the length of the grafts was increased to 10–15 mm. The aim was to compare the results of using non-neural grafts composed of CG matrix or freeze-thawed tenuissimus muscle with those using autografts of the PB and PT nerves or the sural nerve, 4 cats being allocated for each type of graft. Unfortunately poor silver staining prevented analysis of the muscles in 5 experiments (2 muscle

autografts, 1 CG matrix graft, 1 PB/PT nerve autograft and 1 sural nerve autograft). Also in 2 CG matrix experiments the muscles atrophied, presumably because the grafts did not take. We attribute this to our not enclosing the matrix and the two nerve stumps within a silicone tube, as is recommended by Yannas[19]. It was not practicable to do this in these experiments.

At the end of some experiments an intercollicular decerebration was carried out under halothane anaesthesia, which was then discontinued. About 3 h after the decerebration, the PB tendon was freed from its insertion in both operated and control limbs and attached to an electromagnetic puller (Ling Dynamics). Small-amplitude sinusoidal stretches were then applied to the tendon at 200 Hz; any development of reflex tension was measured by a tension transducer (Harvard) in series with the puller. Such stimulation selectively excites the primary endings of Ia afferents in normal spindles[13], and presumably likewise activates regenerated Ia primaries. Finally the animal was re-anaesthetized in order to measure the maximum tetanic tensions of the PB muscles in both limbs in response to direct stimulation of the common peroneal nerve.

Results

Histology

The histological results are summarized in Table 1; the results for each experimental group have been pooled, and the percentages given for the spiral and Ia indexes for each group represent the averages for the number of animals used. In a statistical analysis of the results the data used consisted of the individual values for each animal. One-way analysis of variance revealed no significant differences in the extent of the annulospiral afferent innervation achieved among the eight neural and non-neural experimental groups, but did show that there were significant differences between the results of the three series of experiments in some cases, as shown in Table 2.

We draw two main conclusions from these results. First, that the increase in graft length significantly reduced the extent of the annulospiral afferent reinnervation. Thus increasing it from 2 mm in the Series II experiments (short graft repairs of two muscle-nerve fascicles belonging to the common peroneal nerve) to 10–15 mm in the Series III experiments (long graft repairs of the two muscle-nerve fascicles) reduced the average spiral index for spindles by 41%. Second, that a significantly smaller proportion of spindles were reinnervated by annulospiral afferents after short graft repairs of the whole common peroneal nerve (Series I experiments) than after similar repairs had been made using only two of its muscle-nerve fascicles (Series II experiments). The reverse appeared to be true with respect to the annulospiral afferent reinnervation of tendon organs, though in this case the difference was not statistically significant (see Table 2).

Physiology

Only two of the Series III decerebrate preparations gave complete and decisive results owing to various technical problems and variability in the level

Table 1. *Afferent Reinnervation of Stretch Receptors in Cat Peroneal Muscles After Repairs of the Common Peroneal Nerve Using Neural and Non-Neural Grafts*

Type and length of graft	n^1/n^2	A. Muscle spindles			B. Tendon organs		C. Total A + B	
		no.	spiral index (%)	Ia index (%)	no.	spiral index (%)	no.	spiral index (%)
Series I								
CPN; 2 mm								
CPN autograft	5/5	123	35	6	55	51	178	40
CG matrix	2/2	43	29	3	21	54	64	35
Series II								
PB, PT nvs; 2 mm								
SN autograft	4/7	100	47	10	41	35	141	43
CG matrix	2/4	58	65	15	31	24	89	51
Series III								
PB, PT nvs; 10–15 mm								
PB, PT nvs, autograft	3/6	96	30	3	32	14	128	26
SN autograft	3/6	72	28	11	20	51	92	27
CG matrix	1/2	19	21	5	12	33	31	26
Muscle autograft	2/4	46	41	15	10	8	56	33
single neurotomy data for comparison								
CPN	9/9	139*	49*	21*				
PB, PT nvs	4/4	109*	68*	27*	75	37	184	55

* Data from Banks and Barker[2]. Abbreviations: *CG* collagen-glycosaminoglycan; *CPN* common peroneal nerve; *PB* peroneus brevis; *PT* peroneus tertius; *SN* sural nerve; n^1 number of cats; n^2 number of muscles; *nvs* nerves.

Table 2. *Comparison of the Results of the Three Series of Experiments (Summarized in Table 1) by One-Way Analysis of Variance*

Series	n	A. Muscle spindles spiral index (%) mean S.E.	Ia index (%) mean S.E.	B. Tendon organs spiral index (%) mean S.E.	C. Total A + B spiral index (%) mean S.E.
I whole nerve	7	33.13 ± 4.37	5.33 ± 2.13	51.68 ± 10.84	38.61 ± 4.63
II 2 fascicles, short graft	6	52.97 ± 6.56	11.78 ± 3.99	31.12 ± 3.26	45.98 ± 3.86
III 2 fascicles, long graft	9	31.02 ± 5.30	8.49 ± 2.15	27.21 ± 9.88	12.77 ± 4.26
F		4.489, $p < 0.05$	1.278, N.S.	1.796*, N.S.	4.072, $p < 0.05$
t series I–II		−4.354, $p < 0.001$			−1.121, N.S.
series I–III		0.284, N.S.			1.686, N.S.
series II–III		2.829, $p < 0.05$			2.795, $p < 0.05$

Values of t for individual pairs of series are calculated when the variance ratio (F) between series: within series is sufficiently large. n number of cats in each series. * Calculated after square-root transformation of data and modified test for unequal variances, t student's test.

of reflex activity. Both experiments were PB/PT nerve autograft repairs produced by double neurotomy. The spiral indexes of the two reinnervated PB muscles were, respectively, 52.5% and 26.0%, and their Ia indexes 7.9% and zero. Direct stimulation of the common peroneal nerve produced maximum tetanic tensions in these muscles that were, respectively, 33% and 53% of those produced by the two control muscles, but when reflexly excited they produced no detectable tension. Reflex excitation of the control muscles produced maximum tetanic tensions that were less than half their directly evoked tensions (respectively, 27% and 40%).

Discussion

The results show that for grafts 2–15 mm long the significant factor affecting the outcome of the reinnervation of stretch receptors by annulospiral afferents was not the type of graft used, but its length. Thus the average spiral index for spindles and tendon organs after 2 mm grafts repairs in the Series II experiments was 72% greater than after repairs using 10–15 mm grafts in the Series III experiments (Table 2, column C). It is known from clinical experience[15] that longer grafts give poorer recoveries of motor function and cutaneous sensibility in patients, and in the light of our findings it seems likely that this also applies to proprioceptive recovery. It is not clear why an increase in graft length should have this effect. The fact that neural and non-neural grafts gave similar results in our experiments suggests that the factor or factors responsible do not operate within the grafts themselves. It may be that because regenerating axons take more time to grow through long grafts than short ones, the delay in their arrival at the second suture line and distal stump leads to a deterioration in the conditions awaiting their reception, which adversely affects the subsequent reinnervation.

The experiments showed that more spindles were reinnervated by annulospiral afferents after short graft repairs of two muscle-nerve fascicles of the common peroneal nerve (Series II experiments) than after short graft repairs of the whole nerve itself (Series I experiments). We attribute this to the absence of competition from cutaneous afferents. Banks and Barker[2] obtained a similar result after comparable single neurotomy experiments (see Table 1). The fact that, under the same circumstances, the reverse appears to be true of the annulospiral afferent reinnervation of tendon organs may be due to the participation of annulospiral cutaneous afferents, i.e. Ruffini-ending afferents. In an experiment performed in connection with other work[2], we cross-united the cutaneous superficial peroneal nerve with the PB fascicles of the common peroneal nerve in order to examine the responses and innervation of spindles reinnervated by cutaneous afferents. We recently examined the tendon organs from this experiment and found that 25% (n = 12) had been reinnervated by afferents with annulospiral terminals, presumably Ruffini-ending afferents. These formed endings in tendon organs that were indistinguishable from normal Ib endings. Such afferents appear to be unable to reinnervate spindles.

We have shown that there was no return of the stretch reflex in PB in two 10–15 mm PB/PT nerve autograft repairs. Small-amplitude vibratory

stimulation applied to their tendons failed to produce any detectable reflex contraction, though such stimulation is known to produce a monosynaptic stretch reflex in normal muscle by selectively exciting Ia afferents[13], and did so in the controls. Motor reinnervation had occurred since direct stimulation of the CPN evoked contraction of the muscle. These observations were made on decerebrated unanaesthetized animals. They agree with the recent finding by Carrick *et al.*[8] that there was no reflexly generated electrical (EMG) or mechanical response to sinusoidal stretching of the triceps surae tendon in the anaesthetized rat after a 10 mm muscle autograft repair of the sciatic nerve.

This failure of myotatic recovery is due to the poor quality of the afferent reinnervation. Banks and Barker[2] showed that after section and microsuture of the PB and PT nerves many stretch afferents failed to return to spindles and tendon organs, whilst others made random and functional connections that were either specific (the Ia index for PB was 27%) or non-specific (20% of spindles were reinnervated by tendon-organ afferents, and 38% by free-ending afferents). When two suture lines are involved, as in graft repairs, the quality of the afferent reinnervation is further reduced. In the present experiments the average Ia index ranged from 3 to 15% (see Table 1). Given this low level of specific Ia reconnection, and the fact that many afferents make functional connections with the wrong receptors, it is hardly surprising that myotatic recovery fails.

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Chapter 11

The Muscle Spindle

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Intrafusal Muscle Fibers
Capsule and Vascular Supply
Types of Spindle Unit
Number and Distribution
Sensory Innervation
Motor Innervation
The Spindle as a Receptor
Reinnervation and Recovery
Autonomic Innervation
Effects of Sympathetic Stimulation

Muscle spindles are mechanoreceptors sensitive to muscle length and changes in muscle length. They are composed of small (*intrafusal*) muscle fibers that lie as bundles in parallel with ordinary (*extrafusal*) muscle fibers, their ends attached to connective tissue, tendon, or extrafusal endomysium. They receive both a motor and a sensory innervation. The sensory innervation, which responds to active and passive changes in muscle length, is protected by a fusiform, fluid-filled capsule and occupies the *equatorial region* of the intrafusal bundle, whereas the motor innervation is distributed to the *polar regions* that extend on each side. Activation of the motor innervation elicits contractions in the polar regions that modify the sensory discharge.

Muscle spindles occur in the somatic muscles of vertebrates. They were first noticed and described in frog muscle by Weismann[1] in 1861. It is generally supposed that the receptor first appeared in early tetrapods in antigravity muscles associated with posture and locomotion, but Maeda et al.[2] have recently suggested an earlier appearance in the jaw-closing muscle of fish and claim to have found monofibral spindles in this muscle in salmon. The report has not been confirmed; meanwhile, Saed[3] has made a thorough search for the receptor in the jaw-closing muscle of trout without success. No one has yet searched lungfish muscle, which might prove more rewarding.

The nonmammalian spindle is supplied with one sensory ending and receives its motor innervation from branches of axons that also innervate extrafusal muscle fibers. Mammalian spindles are supplied with a *primary* sensory ending (the homologue of the sensory ending in nonmammalian spindles) and may, in addition, be supplied with one or more *secondary* sensory endings. Two kinds of motor system are involved in their motor innervation: a *fusimotor* (γ) system, which is exclusively intrafusal, and a *skelefusimotor* (β) system, in which intrafusal and extrafusal muscle fibers share a common innervation, as in nonmammalian spindles. Each system contains two functionally different types of motor axon whose stimulation produces different effects on the dynamic response of the primary ending. (The *dynamic response* may be defined as the alteration in the rate of impulses discharged by the ending that is related to the rate of change of muscle length. It contrasts with the *static response*, which is the alteration in discharge that arises as a result of the muscle's changing from one steady state length to another.) Stimulation of dynamic γ motor axons increases the dynamic sensitivity of the primary ending, whereas stimulation of static γ axons decreases it[4]; there is the same functional difference between dynamic and static β axons.[5–7] The static response is increased by stimulating either static or dynamic axons, γ or β.

This chapter is about mammalian spindles. It is mostly about those of the cat, because far more is known about them than any others. Such information as there is about human spindles indicates that they do not differ in any radical respect from those in the cat.

A typical spindle in a cat's hindlimb muscle (see Fig. 11-1) consists of a 7- to 10-mm-long bundle of six to nine muscle fibers that is richly vascularized, partly encapsulated (generally the middle third), and innervated by a spindle nerve that leaves a nearby intramuscular nerve trunk to enter the equatorial region. Two kinds of muscle fiber can be recognized on the basis of differences in length, diameter, and equatorial nucleation. The longest and thickest are called *nuclear-bag fibers* because, for a short length in the equatorial region, they contain few myofibrils and are full of round vesicular nuclei, thus forming what Barker described as a *nuclear bag*.[8] Each bag tapers off on either side into a single row of elongated nuclei within a central core of sarcoplasm to form a *myotube region*.[8] The shortest and thinnest fibers contain a single central row of nuclei in the equatorial region and are called *nuclear-chain fibers*[9] (see Fig. 11-1*D*). There are usually two bag fibers and four to seven chain fibers.

The site of the nuclear bags, myotube regions, and chains is innervated by a group Ia axon that terminates as an annulospiral primary ending. This is usually accompanied by one secondary ending supplied by a group II axon that distributes less regular rings and spirals predominantly to the chain fibers. The motor innervation consists of a diffuse multiterminal *trail ending*[10] and two types of plate, designated p_1 and p_2.[11,12] The *p_1 plates* are supplied by β axons, the *p_2 plates* and trail endings by γ axons: a trail ending is now regarded as consisting of several trail plates.[13] Apart from this somatic innervation, some spindles also receive autonomic axons that are in neuroeffective association mainly with the intrafusal muscle fibers.[14]

The manner in which the motor innervation is distributed to the bag and chain muscle fibers has been the subject of considerable controversy, mainly because it took some time for it to be established that there were not two types of muscle fiber (bag and chain) but three (bag_1, bag_2, and chain). It is now accepted that dynamic actions are carried out by the bag_1 fiber (the dynamic bag fiber) and static action by the bag_2 (static bag fiber) and chain fibers. The original work involved in this switch from a dual- to a triple-spindle model is referred to in the following sections; these discussions summarize details of the structure, innervation, and development of the cat spindle and give some

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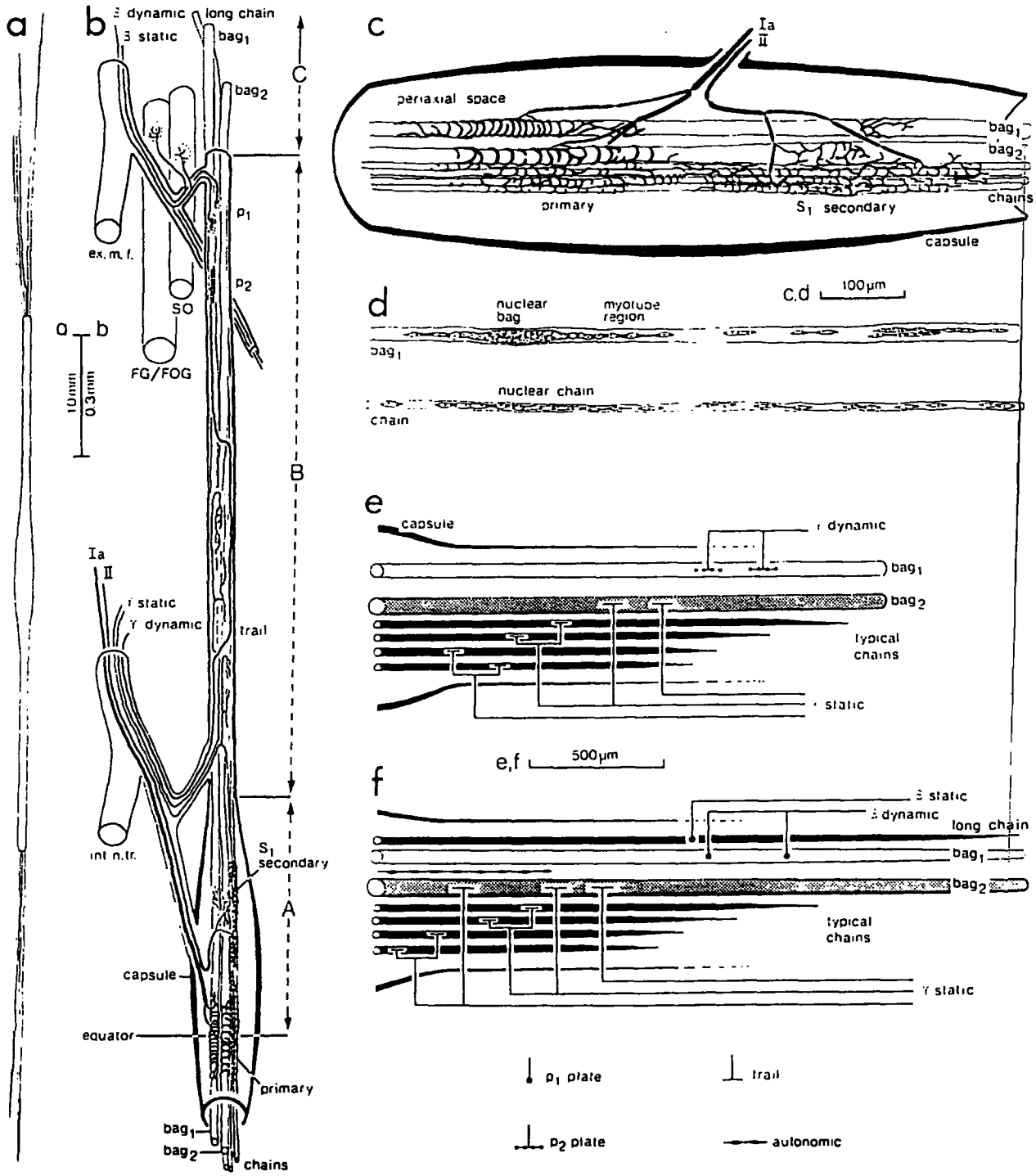


FIGURE 11-1. Schema illustrating the structure and innervation of cat tenuissimus muscle spindles. *A*. The encapsulated bundle of intrafusal muscle fibers that constitutes a spindle. *B*. The equatorial region and part of one pole illustrating regions A, B, C and innervation by Ia and II sensory and β and γ motor axons. ex.m.f. = extrafusal muscle fibers; FG/FOG = fast glycolytic or fast oxidative-glycolytic muscle fiber; SO = slow oxidative muscle fiber. *C*. Sensory innervation comprising a primary ending and an S_1 secondary ending. The distribution of the total terminal contact area of a primary ending is about 35 percent bag_1, 25 percent bag_2, 40 percent chain; of an S_1 secondary 10 percent bag_1, 20 percent bag_2, 70 percent chain. *D*. Nuclear-bag and nuclear-chain intrafusal muscle fibers showing nucleation in primary and S_1 secondary regions. *E,F*. Motor innervation of a typical pole (*E*). The most common variation (*F*) is for static and dynamic β axons to participate in the motor innervation (though seldom of the same spindle). Some spindles receive a nonvascular autonomic innervation. *A–D* depict features drawn to the scale of average dimensions; *C, D* are based on reconstructions;[39] *E, F* are schematic diagrams.

account of how it functions and how it is reinnervated and recovers after nerve injury and ischemia. Recent reviews of work on mammalian spindles include those of Kennedy et al.,[15] Matthews,[16] Boyd and Smith,[17] Hulliger,[18] Barker and Banks,[19] and Hunt[20]; for reviews that deal with both mammalian and nonmammalian spindles, see those of Barker[21] and Hunt.[22] The proceedings of international symposia held in Glasgow (1984),[32] Prague (1987),[24] and Paris (1991)[25] provide useful sources for recent research papers.

Intrafusal Muscle Fibers

The dual model of the mammalian spindle could not satisfactorily account for histochemical observations made in the sixties that distinguished three types of intrafusal muscle fiber. When the histochemical evidence was correlated with observations made on ultrastructure and teased silver preparations, it became clear that there were two kinds of bag fiber (see review by Barker[21]). These were designated as *nuclear bag* and *intermediate* by Barker and Stacey[26] and as *bag*$_1$ and *bag*$_2$ by Ovalle and Smith.[27] The correlation of the histochemistry with the electron microscopy was somewhat conjectural, as the observations reported by various workers had been made on separate preparations of different spindles; for a time, there was some confusion about how the two kinds of bag fiber should be categorized.

The matter was resolved when Banks et al.[28] devised a technique that allowed adjacent sections of the same spindle to be prepared for either histochemical or ultrastructural study. Descriptions by Banks et al.[29] of the ultrastructural and histochemical characteristics of the two types of bag fiber then followed, and Ovalle and Smith's[27] terms *bag*$_1$ and *bag*$_2$ were adopted to designate them.[30] Besides their different ultrastructure and histochemistry, it transpired that the two types could also be histologically distinguished by the abundance (bag$_2$) or scarcity (bag$_1$) of elastic fibers associated with them in the extracapsular polar regions[31] and by the fact that the bag$_1$ fiber often lay apart from the bag$_2$ and chain fibers during its course through the equatorial region.[30]

Barker et al.[30] found it convenient to distinguish three regions, A, B, and C, between the equator and the origin or insertion of a spindle pole, a practice that has since been generally adopted. The regions are defined as follows: A, that part of the equatorial region lying between the equator and the equatorial end of the *periaxial space* (i.e., the space between the intrafusal, or axial, bundle and the capsule wall); B, that part of the pole extending from the equatorial end of the periaxial space to the end of the capsule (often referred to as the *capsule sleeve*); and C, the extracapsular part of the pole (see Fig. 11-1*B*).

In some spindles, one of the chain fibers extends for a considerable distance beyond the capsule and has been called a *long chain* fiber.[30] Kucera[32,33] distinguishes two further subtypes, namely, *typical chain* fibers, which mostly attach within the capsule, and *intermediate chains*, which extend beyond it but not so far as long chain fibers. These subtypes are of functional significance, since there is evidence of selective innervation of long and intermediate chains by static β axons and of typical chains by static γ axons (see "Motor Innervation," below).

Spindles in different muscles are characterized by differences in fiber-type complement. In tenuissimus spindles the number of bag fibers is rarely more than two and long chain fibers are scarce,[32] whereas in superficial lumbrical muscles spindles with more than two bag fibers (usually three, sometimes four or five) are common[34] and there is a high incidence of long chain fibers.[35] Occasionally "mixed" bag fibers occur, i.e., bag fibers whose histochemical characteristics are not the same at both poles. Such hybrid fibers are either bag$_1$/bag$_2$ or bag/chain; they are found in 3.5 percent of tenuissimus spindles[36] and 12.6 percent of superficial lumbrical spindles.[34]

Length, Diameter, and Nucleation

In most spindles, the bag fibers are the longest, the bag$_2$, generally being longer than the bag$_1$. Kucera[33] reports mean polar lengths of 2947 μm for bag$_2$ fibers, 2760 μm for bag$_1$, and 1231 μm for typical chains (tenuissimus, frozen sections). In 77 percent of 313 spindle poles, the bag$_2$ was the longest fiber; in 14 percent, the bag$_1$; in 3 percent, the bag fibers were of equal length; and in 6 percent, the longest fiber was a long chain. The long chain fibers (i.e., those extending 1.0 mm or more beyond the capsule[30]), when considered as a group, proved to be the longest fibers in Kucera's study,[33] their mean polar length being 2990 μm. This compares with a length of 1928 μm for intermediate chain fibers (i.e., those extending for less than 1.0 mm beyond the capsule). Long and intermediate chain fibers usually lie close to the bag$_1$ fiber for much of their course.[37] Long chain fibers are usually only long in one pole, and there may be more than one long-chain pole per spindle.

Mean juxtaequatorial (inner region B) diameters for bag and chain fibers given by Boyd[38] are 16.86 ± 2.35 μm for bag fibers and 8.37 ± 1.85 μm for chains (tenuissimus, Susa fixation, paraffin sections). No systematic study of intrafusal muscle fiber diameters has been made since it was established that there are two types of bag fiber and three subtypes of chain. All types of fiber become thinner as they pass through the equatorial region, and those that extend well into region C tend to become thickest in this region. The presence of a secondary ending adjacent to the primary results in the bag fibers undergoing a marked increase in diameter at the sites where they receive secondary terminals.[39]

The nuclei of intrafusal muscle fibers are located either peripherally underneath the sarcolemma (subsarcolemmal nuclei), as in the polar regions, or internally among the myofibrils (myonuclei), as in the equatorial region. In light microscopy preparations, it is sometimes difficult to distinguish between subsarcolemmal nuclei and those of satellite cells and endomysial fibrocytes. Satellite cells occur mostly in association with bag$_2$ fibers in region C; they are less frequently associated with the bag$_1$ fiber and rarely occur on chain fibers.[40]

The most detailed information about equatorial nucleation is that obtained by Banks et al.[34] from reconstructions of four tenuissimus spindles (see Fig. 11-2). They found that nuclear bags contained 52 to 106 myonuclei, those of

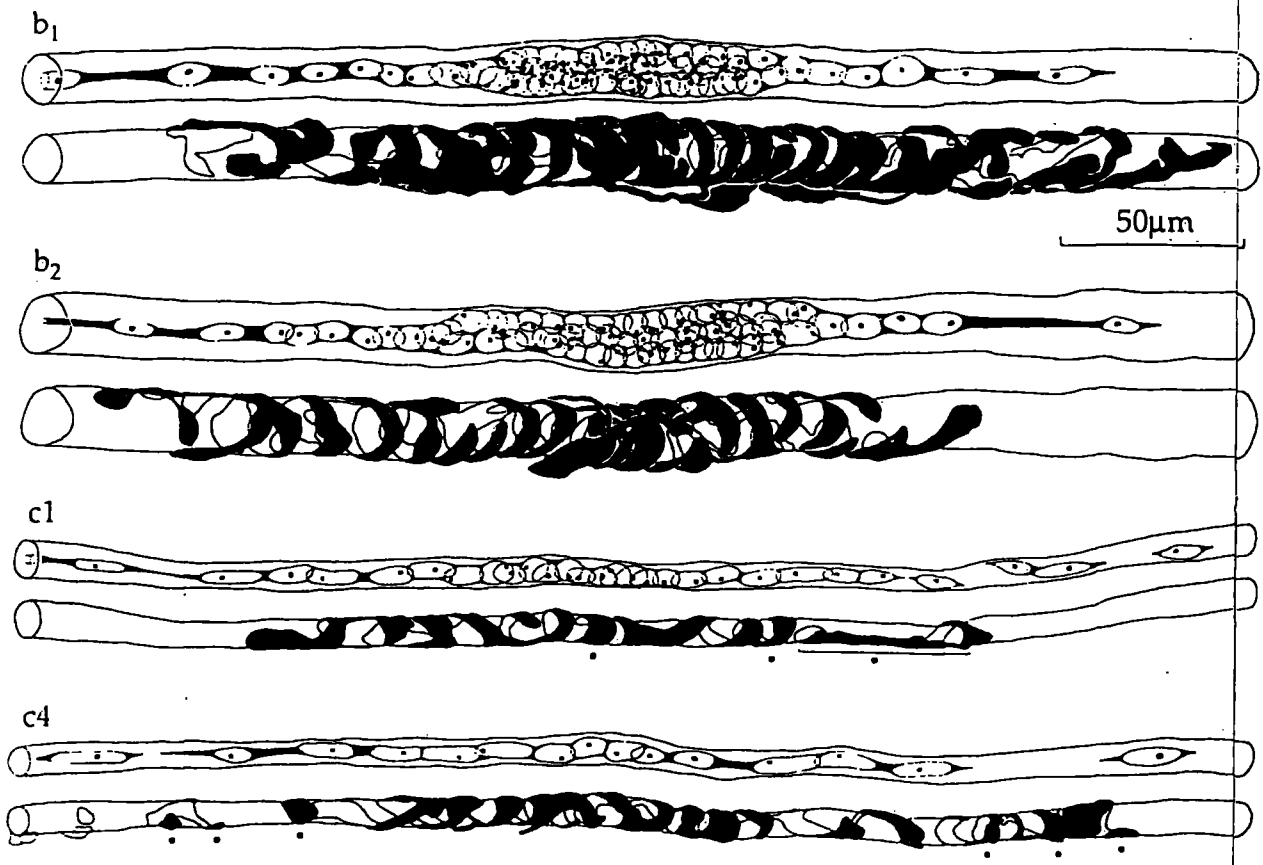


FIGURE 11-2. Schematic representation of parts of a primary ending reconstructed from a cat tenuissimus spindle. The terminals shown are those supplied to the bag_1 (b_1) and bag_2 (b_2) fibers and the longest and shortest chain (c) fibers (numbered 1 and 4). Each fiber is repeated alongside to show its myonucleation and thus demonstrate the relation between nucleation and innervation. Adjustments have been made to the original alignment of each muscle fiber relative to the others, mainly in order to position the center of each nuclear bag on a common midline and thus facilitate comparison between b_1 and b_2 terminal systems. Terminals shown in outline at bottom end of c4 belong to adjacent S_1 secondary ending. Asterisks alongside c terminals indicate positions of sensory cross-terminals with other c fibers. *(Banks RW et al, Philos Trans R Soc Lond 299:329, 1982. Reproduced by permission.)*

bag_1 and bag_2 fibers averaging 68 and 80, respectively. In the myotube regions, there were 6 to 12 myonuclei, average 9; in the nuclear chains, there were 11 to 38, average 24. There was a tendency for the longest chain fibers to be the most densely nucleated and for their myonuclei to aggregate equatorially to form miniature nuclear bags (see also Kucera[37]). Myonuclei occupied 70 to 90 percent of the cross-sectional area of each nuclear bag, 30 to 50 percent of each myotube region, and 40 to 60 percent of the longest chain fibers in the primary region. By contrast, myonuclei in the region of secondary terminals on bag fibers occupied only 10 percent of the cross-sectional area.

Ultrastructure

Observations on the ultrastructure of intrafusal muscle fibers made before the present classification of fiber types was established are reviewed by Barker.[21] It is now apparent that the fibers display two types of myofibrillar ultrastructure, which for convenience have been designated M or dM according to the appearance of the M line.[28,30] In the M condition, the M line crosses the middle of each sarcomere as a single prominent line (low power) composed of five parallel faint lines (high power), whereas in the dM condition the M line either cannot be seen or appears as two parallel faint lines, according to the orientation of the myofibrils.[26]

In the M condition, the myofibrils are packed as discrete units in sarcoplasm that is rich in glycogen and contains many thick, long mitochondria and membranous systems (transverse tubules and sarcoplasmic reticulum) that are well developed at the level of the I and Z bands. Transverse sections at this level show the myofibrils almost completely encircled by membranous elements. By contrast, in the dM condition, there is very little interfibrillar sarcoplasm, little glycogen, and poorly developed membranous systems; the mitochondria are thin, short, and scarce. Transverse sections show the myofibrils to be poorly defined and tightly packed together so as to form a more or less continuous bundle, with the membranous systems only occasionally encountered at the level of the I and Z bands. Chain fibers have the M type of ultrastructure, whereas, remarkably, the bag fibers are a mixture of both M and dM. Observations by us suggest that the transition from M to dM is from a five- to a four-line substructure, the middle line being lost. In the bag_1 fiber, the ultrastructure is dM in region A

and most of region B, then changes to the M condition toward the outer end of region B. The sarcomere length is consistently longer than in the bag_2 or chain fibers.[29] In the bag_2 fiber, the condition is dM in region A and changes to M at level A/B,[29,30] though the membranous systems become progressively less developed toward the polar end in region C.[41]

In the equatorial region, the spaces in between the myonuclei in the myotube regions and nuclear chains are full of sarcoplasm that contains many small mitochondria, ribosomes, Golgi complexes, and occasional lipoid droplets. Corvaja et al.[42] describe how two or three chain fibers (presumably typical chains) may share the same endomysial envelope in the equatorial and juxtaequatorial regions. Here and there these fibers become enclosed within a common basal lamina and *zonulae adhaerentes* (regions of intimate contact between the cells) form between their closely apposed surfaces. Microladders occasionally occur in both bag and chain fibers, usually situated near the surface in the sarcoplasm underneath axon terminals.

According to the descriptions of Cooper and Gladden[43] and Gladden,[31] elastic fibers are most numerous around the bag_2 fiber and anchor the spindle at each end to the elastic-fiber network among extrafusal muscle fibers. In passing through the spindle, they travel alongside muscle fibers, within intercellular spaces in the axial sheath, or between layers of the capsule. Observations by Banks[44] have revealed that the bag fibers have peglike projections on their surface over a length of 300 to 400 μm on either side of the primary region. Each projection slants toward the equator and appears to serve as an anchoring point for an elastic fiber originating from the opposite pole. Such attachments must greatly enhance the elastic properties of the primary region.

Histochemistry

The histochemical profiles of intrafusal muscle fibers are similar to those of extrafusal ones in that they vary according to fiber type, but they are dissimilar in that they are subject to regional variation. The three fiber types—bag_1, bag_2, and chain—differ in their glycogen content and in their profiles of the enzymes myofibrillar adenosine triphosphatase (mATPase), phosphorylase, and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR).[27,29,45,46]

The technique most favored for demonstrating fiber type is that which stains mATPase, as it enables the types to be checked against the different staining reactions that follow alkaline or acid preincubation of the sections. The profiles of the bag fibers show regional variation, but not those of the chains, and the optimum level for distinguishing between fiber types is midregion B (see Fig. 11-3). In transverse sections cut at this level, the staining intensities for alkali-stable mATPase are low for bag_1, medium or high for bag_2, and high for chains, whereas the staining for acid-stable ATPase is low for bag_1, high for bag_2, and low for chains. The three subtypes of chain fiber can be distinguished histochemically by staining for NADH-TR activity, which either increases (intermediate and typical chains) or decreases (long chain) from equator to pole.[46]

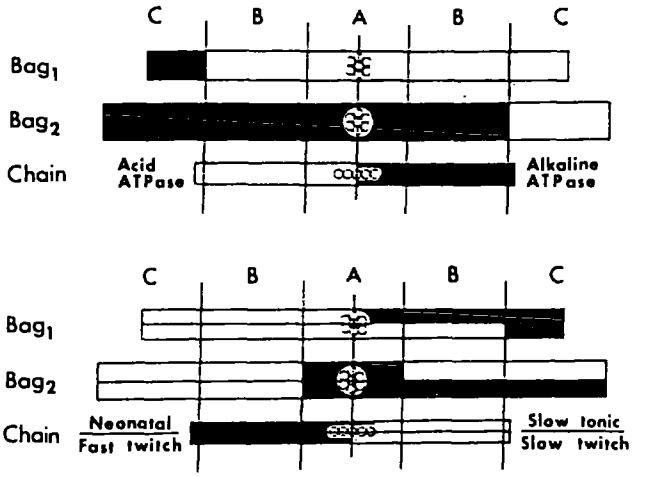


FIGURE 11-3. Schematic summary (top) of the general patterns of mATPase reactivity (black is high) following acid and alkaline preincubation in rat intrafusal fibers; and (bottom) reactivity of the three intrafusal fiber types with the antibodies against slow-tonic MHC, slow-twitch MHC, neonatal MHC and fast-twitch MHC. *(Modified from Pedrosa et al.[50] Reproduced by permission.)*

The use of immunohistological techniques has shown that the differences in mATPase activity between the three types of intrafusal muscle fiber reflect differences in their possession of different forms of myosin heavy chains (MHC). In general, antibodies specific for slow-fiber MHC stain bag fibers and those specific for fast-fiber MHC stain chain fibers. The bag fibers have been shown to contain slow-tonic MHC (mostly in bag_1) and slow-twitch MHC (mostly in bag_2), whereas the chain fibers are characterized by fast-twitch and neonatal MHC (see Fig. 11-3).[47–50] Slow-tonic MHC, such as is found in the slow-tonic muscles of frogs and birds, does not occur extrafusally in mammalian skeletal muscles, though it is present in the slow-tonic portions of the oculorotatory muscles.[51] The bag fibers are also unique in mammalian skeletal muscle in that they react positively to anti-alpha cardiac MHC (mostly bag_2)[52] and anti-embryonic MHC (mostly bag_1).[53] In rat spindles, reactivity to antibodies against M-band proteins and the MM form of creatine kinase[50] is, as might be expected, negative for bag_1 fibers, whose myofibrillar ultrastructure is dM throughout;[30] mixed for bag_2 fibers, which switch from the dM to the M condition; and positive for the chain fibers, which are M throughout. Most of the immunohistological work has been done on rat spindles. When other species have been used, some species-specific differences have emerged—e.g., embryonic MHC is present in cat chain fibers but absent from rat and rabbit chain fibers.[53]

Development

Electron microscopic (EM) investigations of spindle development in rat,[54–56] mouse,[57] and cat[58] have revealed close parallels between intrafusal and extrafusal myogenesis. Both involve the sequential production of myotubes following a stage before innervation when myoblasts fuse to form primary myotubes in the muscle primordium.

The generation of secondary extrafusal myotubes is de-

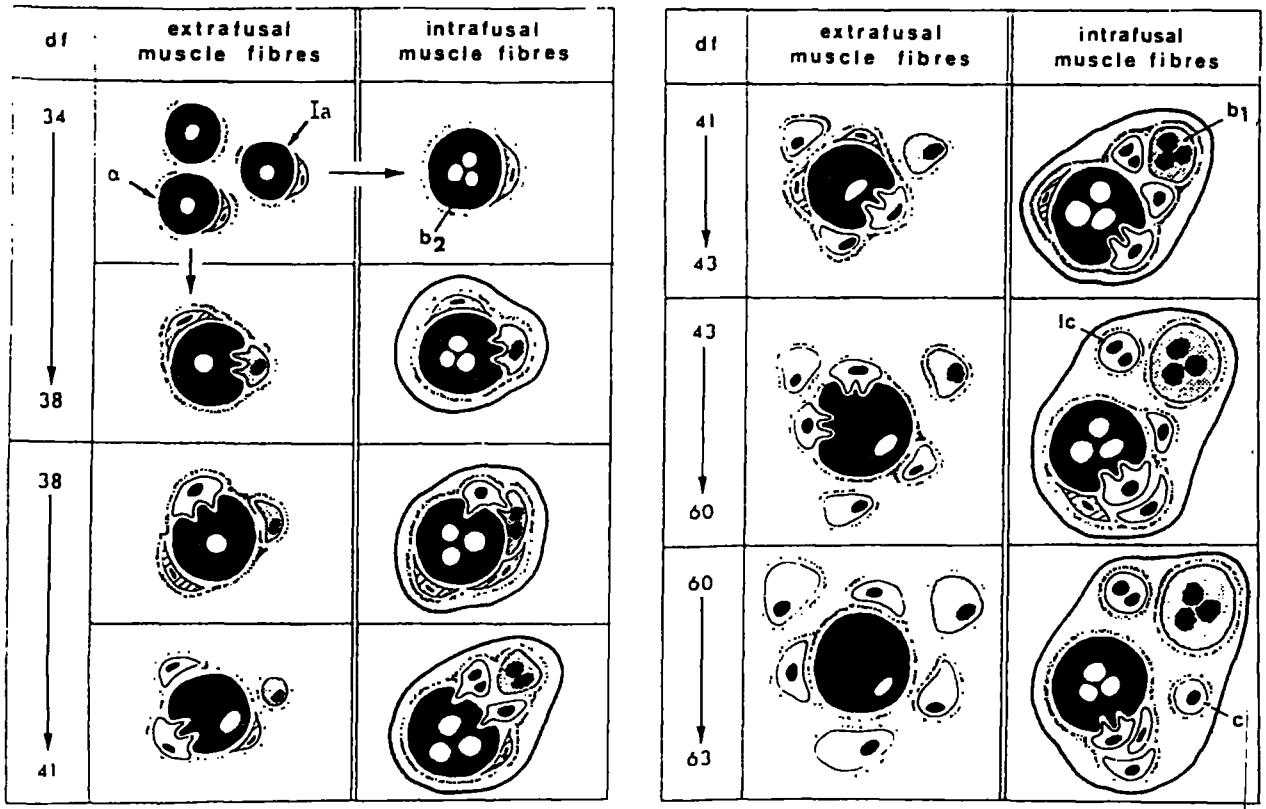


FIGURE 11-4. Schematic diagrams of transverse sections of developing extrafusal and intrafusal muscle fibers in cat peroneal muscles. In the extrafusal fascicle, note how the first-series secondary myotube (stippled) separates from the primary myotube (black) and acquires its own basal lamina (stippled halo) before the assembly of subsequent series of secondary myotubes (white). The thin fusiform cells (hatched) are myoblasts. The diagrams of intrafusal muscle fibers illustrate the process of myogenesis as seen at the spindle equator. α = alpha motoneuron; b_1 = bag_1 fiber; b_2 = bag_2 fiber; lc = long chain fiber; c = intermediate chain fiber (the rest are typical chains); df = days fetal; Ia = Ia afferent. *(Modified from Milburn.[58])*

pendent on innervation and the electrical or contractile activity of the muscle.[59] The secondary myotubes assemble and develop in close apposition to primary myotubes so as to form multicellular muscle clusters from which they subsequently separate, each within its own basal lamina, to form independent muscle fibers.[60-63] According to Milburn,[58] in cat peroneal muscles the sequential process of assembly, maturation, and separation occurs so as to produce successive series of secondary myotubes beginning with the separation of the first series at the 38 to 41 days fetal (df) stage. Meanwhile, subsequent series have begun to assemble in association with primary myotubes, and eventually these also separate, so that by birth (60 to 63 df) primary and secondary muscle fibers are distinguishable only where their separation from fetal muscle clusters is incomplete. Milburn regards the pattern of spindle development as fundamentally similar to that of extrafusal fascicles and correlates the two processes as shown in Fig. 11-4. Her account of the development of cat spindles in peroneal muscles may be summarized as follows.

By the 34 to 38 df stage, motor and sensory axons have grown into the peroneal muscle primordium; primary myotubes have begun to receive the terminals of α motor axons; and secondary extrafusal myotubes have begun to assemble. Those primary myotubes that receive terminals from Ia afferents will ultimately become intrafusal bag_2 muscle fibers. The effects of this initial contact are to produce an accumulation of myonuclei beneath the sensory terminals and to stimulate the formation of a thin capsule that isolates the sensory region from neighboring extrafusal myotubes. Simple motor terminals are also present at this early stage, indicating that both Ia and α motor axons arrive at the site of spindle formation together.

The Ia axon initiates a sequential generation of secondary intrafusal myotubes, which develop in association with the primary myotube within a common basal lamina. Their assembly begins beneath the Ia terminals, which are in contact with the outer surface of the developing intrafusal bundle, and spreads toward the poles. As development proceeds, the successive myotubes are shorter and thinner and contain fewer equatorial myonuclei, so that those formed last become the shortest and thinnest typical chain fibers in the mature spindle. At the 41 to 43 df stage, the presumptive bag_1 myotube (often accompanied by the long chain myotube when present) separates from the primary bag_2 myotube and acquires individual Ia terminals. At the same time most of the nuclear-chain myotubes assemble exclusively in association with the primary bag_2 myotube, from which they subsequently separate, often as a group. Sensory cross terminals between bag_2 and chain myotubes are thereby lost but are retained between chain fibers in the mature spindle.[64]

Separation of the developing myotubes begins in the poles and spreads toward the equator, where the influence of the Ia afferent renders it incomplete. During separation, the Ia innervation is remodeled and the mature form of the primary ending is established. As the intrafusal bundle develops, the capsule increases in girth and length and the motor innervation increases in complexity. By birth, both plate and trail terminals are present. The periaxial space develops during the first two postnatal weeks. The intrafusal muscle fibers thus develop in the order bag_2, bag_1, long chain, intermediate chain, typical chains; their equatorial position in the mature spindle[30,37] reflects the pattern of their assembly and separation.

An EM study of the development of spindles in the rat (21 to 23 days gestation) by Kucera et al.[36] has revealed that there is a transient fetal stage of multiple afferent innervation. The first afferent muscle contacts occur at 17 df, when en passant neuromuscular junctions are formed between primary myotubes and sensory axons located within intramuscular nerves. On 18 df, these contacts are outnumbered by others in which primary myotubes receive clusters of afferent axon terminals. Initially, only one primary (Ia) afferent occupies the contact area, but several follow before the capsule begins to form; for a time, there is a phase of multiple afferent innervation in which seondary (II) afferents; in some spindles, take part, arriving on 19 df. In the soleus spindles studied by Kucera et al.,[36] the supernumerary afferents have withdrawn by 20 df, but they evidently persist in some muscles, since Banks et al.[65] have described mature spindles in rat deep masseter in which a single primary region may be innervated by up to five Ia afferents. Motor endings appear in developing soleus spindles at 20 df on the bag_2 fiber, at 22 df on the bag_1 fiber, and 4 days after birth on the first chain fiber.[36,66] These may be regarded as γ endings, since no β innervation occurs in rat soleus.[67] Most of these perinatal motor endings are multiply innervated. It is assumed that a stage of polyneuronal innervation is followed by the loss of some motor axons and connections, but the details of this, and of the development of the β innervation, are at present unknown. At 18 df, some of the nonencapsulated primary myotubes that have sensory terminals also receive motor terminals from α axons,[36] as occurs at a similar stage in the development of cat spindles.[58] It is conceivable that in certain muscles some of these motor terminals might persist and represent the first stage in the development of a β innervation. In rat soleus, their presence is transitory, since no motor endings occur at 18 and 19 df on primary myotubes that are encapsulated.[36]

An immunohistological study of developing rat spindles by Pedrosa and Thornell[68] has shown that, whereas all primary myotubes contain neonatal and slow-twitch MHC at 17 df, there are some which also contain slow-tonic MHC (see Fig. 11-5). These are presumed to be the precursors of the bag_2 fiber, and their positive antitonic staining serves to indicate the sites of future spindles. The bag_1 fiber contains only neonatal MHC when it first appears at 19 df and does not stain positively for anti-slow-tonic or anti-slow-twitch MHC until 2 days later. When the first chain fiber appears (21 df), it contains only neonatal MHC, according to Pedrosa and Thornell;[68] however, Kucera and Walro,[69] using a different set of antibodies, find that it also expresses both slow-twitch and fast-twitch MHC.

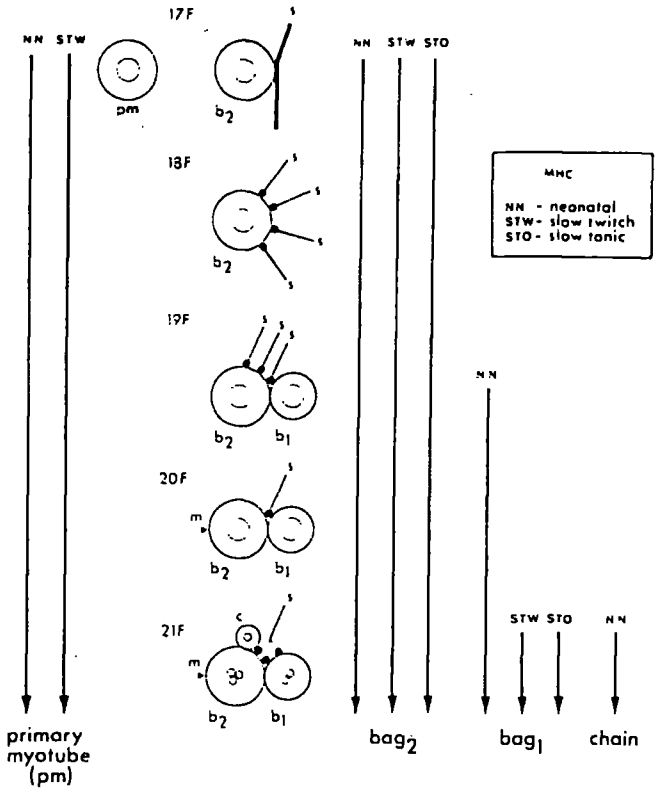


FIGURE 11-5. *Simplified illustration of the development of rat soleus spindles showing the acquisition of sensory (s) and γ motor (m) innervation (based on Kucera et al.[36]) and MHC expression (based on Pedrosa and Thornell[68]). Spindle capsule omitted; intrafusal myotubes/myofibers abbreviated b_1, bag_1; b_2, bag_2; c, chain. (Modified from Pedrosa et al.[32])*

Pedrosa and Thornell[68] have proposed that the three types of intrafusal fiber arise from three different cell lineages commited to differentiate along specific paths, cells in the bag_2 lineage being capable of attracting Ia afferent terminals. They argue that if, as originally suggested by Zelená,[70] Ia afferents make random contacts with undifferentiated myotubes and induce them to differentiate into intrafusal fibers, then the sequence of MHC expression should be the same in all three fiber types. But this does not follow, since the circumstances under which the Ia afferent induces the differentiation of each fiber type, and the timing of each induction, are entirely different and might well lead to differences in the sequence of MHC expression. As Barker[71] points out, the crux of the matter is whether spindles develop from cells that can only form intrafusal muscle fibers, or cells that, according to circumstance, can become either intra- or extrafusal muscle fibers. The evidence available favors the latter. If, for some reason, Ia induction is withdrawn or absent during a critical period of intrafusal differentiation, α motoneurons can take over and induce the differentiation of extrafusal-type fibers. Spindles consisting of encapsulated bundles of such fibers have been produced experimentally in rats as the result of interrupting muscle development by nerve section at a stage when

the spindles are still forming chain fibers.[72] They also occur among spindles that regenerate after prolonged ischemia, in this case being produced as the result of asynchrony between the processes of reinnervation and regenerative myogenesis[73] (see "Reinnervation and Recovery," below).

Capsule and Vascular Supply

The *capsule* is a lamellated structure that encloses the sensory innervation within a fusiform dilation and extends as a sleeve on each side to enclose part of each pole. The width of tenuissimus spindle capsules at the equator is 100 to 150 μm where their walls are 10 to 15 μm thick; their length usually falls within 2.0 to 4.0 mm and varies according to the number of sensory endings present.[21]

The capsule lamellae are composed of layers of thin flat cells arranged in concentric tubular fashion alternating with layers filled with collagenous fibrils. Each *capsular sheet cell*[74] is surrounded by a basal lamina and closely interdigitates with its neighbor to form a continuous layer one cell thick. The outermost capsule layer is composed of thick collagenous fibrils and scattered fibrocytes. The innermost layer is composed of a lining of fibrocytes, some of which cross the periaxial space to join other cells of the same type that form the axial sheath and the endomysial enclosures of the intrafusal muscle fibers.

The capsular sheet cells are continuous with the cells that form the perineurium of the spindle nerve and, according to Low,[76] may confidently be equated with them. He regards the capsule as a modified extension of the perineurium and connective tissue sheaths (epineurium, endoneurium) that enclose the spindle nerve, and we agree with this interpretation. The endoneurial connective tissue space in the spindle nerve is thus continuous with the periaxial space within the capsule, which is, in turn, continuous with the connective tissue space outside the spindle via the open end of each capsule sleeve.

Tight junctions between capsular sheet cells[77] act as a barrier to the diffusion of substances into the periaxial space, in the same way that the perineurium acts as a diffusion barrier in peripheral nerves. Such junctions are presumably located in the inner layers of the capsule, since horseradish peroxidase (HRP) flooded directly onto the living spindle penetrates the outer layers but fails to enter the periaxial space.[78] After systemic injection of HRP, Dow et al.[79] showed that, whereas passive flow of the tracer into the periaxial space was prevented by the capsular cell tight junctions, there was some leakage into the poles through the open end of each capsule sleeve. The small amount of HRP actively transported across the capsular cells via cytoplasmic vesicles was phagocytosed by fibrocytes in the axial sheath.

The periaxial space is full of a highly viscous gel containing the glycosaminoglycan hyaluronate.[80,81] Following long-term deafferentation, the space disappears or is greatly diminished.[82] The origin of the periaxial fluid and its functions are uncertain. Fukami[81] has shown that a transcapsular potential of −15mV is due in part to a relatively high $[K^+]$ in the fluid, which may contribute to the excitability of the endings.

Capillaries course for long distances between capsule layers; they are invariably present in the periaxial space of rabbit spindles[83] but only occasionally so in those of cat. In rabbit tenuissimus spindles, Miyoshi and Kennedy[84] have shown that there is a short, direct pathway from the main muscle artery to the spindle capillaries, which are separate from those supplying extrafusal muscle and different from them in being larger and having intercellular tight junctions. The capillaries supplying intramuscular nerve are similar, and HRP injected intraaortically does not leak from either nerve or spindle capillaries, whereas it leaks rapidly from those supplying extrafusal muscle.[78] A blood/nervous-system barrier, therefore, obtains in both endoneurial and periaxial spaces.

Types of Spindle Unit

Spindles occur singly or may be variously combined in groups or intimately associated with tendon organs. The functional significance of the single encapsulated receptor with its sensory and motor innervation is sometimes stressed by using the term *spindle unit*. Spindle units may be linked in series as *tandem spindles*[85] or combined together in pairs in which the intrafusal bundles either remain separately encapsulated or equatorially share a common capsule. Richmond and her colleagues[86–88] have shown that in cat neck and intervertebral muscles, many spindle units are linked together in tandem and compound fashion to form *spindle complexes*, a type of organization so far previously observed only in the frog's extensor digitorum longus IV muscle.[89,90] In rat deep masseter, some spindles are crowded together to form *spindle clusters* of up to 40 spindle units, a few of which share a common capsule.[91]

The standard spindle unit is provided with one bag_1 fiber, one bag_2 fiber, and about half a dozen typical chain fibers. Variations of intrafusal complement occur with respect to the bag fibers and subtypes of chain fiber. Some spindle units have three, rarely four, bag fibers. In a sample of spindles from various hindlimb muscles, we identified the extra bag fibers from details of their sensory innervation as usually being bag_1.[39] However, in a histochemical study of tenuissimus spindles, Kucera[92] found that extra bag_1 or bag_2 fibers were about equally common and that, more rarely, some were of mixed type.

Absence of the bag_1 fiber occurs in certain tandem spindle units. In the early sixties, Barker and Ip[93] observed that the most common tandem spindle in hindlimb muscles consists of a large and a small capsule linked together by a single bag fiber, the small capsule being supplied with a markedly irregular primary ending. Later studies of the sensory innervation of hindlimb spindles,[39,94] and the mATPase profiles of spindles in neck muscles,[87] established that the continuous bag fiber in such linkages is a bag_2 fiber and that it is the only bag fiber present in the small capsule. Here it is accompanied by a few typical chain fibers and usually has a single row of myonuclei instead of a nuclear bag. These *one-bag spindles*,[87] or *b*$_1$*c spindle units*,[39] typically insert into tendon. Their frequency among spindle units sampled by Banks et al.[39] from various hindlimb muscles was 23.8 percent in extensor digitorum longus, 23 percent

in peroneus brevis, and 6 to 11 percent in the rest. In neck muscles, Bakker and Richmond[87] have found much higher frequencies of 45 percent in complexus and biventer cervicis and 33 percent in splenius.

Number and Distribution

Different muscles possess characteristic numbers of spindles, although there is considerable individual variability, at least in an outbred population.[95] Quantitative comparisons have normally been made using spindle density, which is simply the number of capsules per gram of the adult muscle. On this basis it is often stated (e.g., Cooper[96]) that spindles are relatively common in small muscles involved in fine control, such as the intrinsic muscles of the hand.

Spindle density, however, is only useful as a relative measure of abundance in muscles of different size if, on average, the number of capsules tends to scale directly with muscle mass. Banks and Stacey[97] used data from three species (rat, cat, and human) that range in body size over more than two orders of magnitude to show that this was not so. Logarithmic transformations of the number of capsules (y) and the muscle weight (x) yielded a linear regression of the form $y = 1.58 + 0.32x$, where the muscle weight is measured in grams and y and x are common logarithms. The slope of the regression relationship (0.32) is geometrically significant, since it is virtually identical to that which would be expected for a feature scaling isometrically with dimensions of length. This seems appropriate for the spindle as a length transducer, though it may be fortuitous.

The relative abundance of spindles in a particular muscle, independent of its size, may be expressed as the amount by which the actual number of capsules deviates from the number expected on the basis of the regression relationship. In the sample available to us at present, this varies from 0.13 (or −0.88 log units) for the infrahyoideus to 5.4 (or +0.73 log units) for the intertransversarius C2-C3, both of the cat.

The value of this approach is illustrated by data for the soleus muscle of all three species given in Table 11-1. In particular, notice that in contrast to the deviations from the regression, which are all rather similar, spindle densities decrease by an order of magnitude as muscle size increases. Furthermore, muscles sharing broadly similar functions within and between species have similar distributions about the regression. Thus there are relatively few spindles in shoulder-girdle muscles and relatively many in dorsal neck muscles, whereas hindlimb muscles often have close to average, or typical, numbers. The intrinsic hand muscle, lumbrical III, often regarded as a typical example of a small muscle with high spindle density, actually has only about half as many capsules as would be expected for its size, in both cat and human.

Muscles operating synergistically about a joint often differ considerably in size. The combination of high spindle density with low force output in the small, as compared with the large, muscles of a synergistic group suggested to Peck et al.[98] that the small muscles are functionally specialized as "kinesiological monitors." It is now clear, however, that their relatively high spindle densities are a direct consequence of the small size of these muscles, and that, in general, they are not specialized in respect to their spindle complement. As a specific example, consider the tenuissimus of the cat, which crosses both hip and knee joints yet weighs a mere 0.28 g. With an average of 16.7 capsules, its spindle density (60 g^{-1}) and lack of any obvious mechanical function have led some authors to suggest a sensory role for the muscle. However, the regression relationship shows that a typical muscle of the same size would be expected to have about 25 capsules, thus contradicting the hypothetical sensory specialization.

Table 11-1. ABSOLUTE AND RELATIVE NUMBERS OF SPINDLE CAPSULES IN SOLEUS MUSCLE OF RAT, CAT, AND HUMAN

	Weight, g	No. of Capsules	Spindle Density	Expected No. of Capsules	Linear Deviation
Rat	0.11	34	309	18.8	1.8
Cat	2.49	56	22.5	50.9	1.1
Human	434	408	0.94	265.5	1.5

SOURCE: Data from Banks and Stacey.[97] With permission.

Several detailed maps are available showing the distribution of spindles and other encapsulated receptors in a variety of muscles. Some of the best, together with additional references, are given in van der Wal.[99] Two important features of the distribution of spindles emerge from the maps, as originally described by Gregor[100] and Yellin,[101] respectively: (1) spindles are concentrated in the region of nerve entry and around the subdivisions of the intramuscular nerves and (2) they occur preferentially among extrafusal fibers, with a high proportion of oxidative (SO and FOG) types.

It has been argued that it is functionally appropriate for spindles to be particularly associated with oxidative motor units, since these are recruited first and are thought to be especially important in small, postural movements.[102] One might then reasonably expect spindles to be particularly abundant in muscles with an overall high proportion of oxidative fibers, but there is no evidence for this when comparing the relative abundance of spindles in various cat muscles with the proportion of oxidative fibers in them.

Cameron et al.[103] refer to the differential sensitivity of spindles to surrounding, adjacent, and distant motor units as "sensory partitioning" of the muscle. It is, of course, an inevitable consequence of the mechanical relationships of the spindles and motor units, but it is only likely to be of functional importance if, as Cameron et al. suggest, there are local (intramuscular) reflexes. However, these do not seem to occur,[104] despite increasing evidence for a degree of intramuscular somatotopic organization of both motoneurons and spindles.[105,106] An extensive discussion of sensory partitioning can be found in the review by Windhorst et al.[107] and the commentaries upon it.

It may be, as Richmond and Stuart[108] suggest, that spindles occur among predominantly oxidative fibers, because this allows them to sample the activity of all motor units, including the fast, fatigable (glycolytic) type. If so, it is not



FIGURE 11-6. Photographs and drawings traced from photographs of teased silver preparations illustrating features of the sensory innervation of spindles from cat hindlimb muscles. *A*. Sensory innervation of a b_1b_2c spindle unit from a superficial lumbrical muscle. The primary ending (P) is supplied by a Ia axon that has a mixed distribution, one first-order branch supplying the bag_1 and bag_2 fibers (b_1b_2 br.), the other supplying the bag_2 and chain fibers (b_2c br.). An S_1 secondary ending lies adjacent to it in the lower part of the figure. The equatorial dissociation of the b_1 fiber provides a clear view of its secondary innervation and primary terminal system (b_1 p.t.s.) with characteristic irregular terminals (i.t.). Asterisk denotes point where II axon divides to produce two first-order branches; the branch on the right gives rise to three preterminal axons, two of which travel downward to supply terminals to the b_1 and b_2 fibers, while the third travels upward to supply terminals to b_2 and c fibers. *B*. Sensory innervation comprising one primary and one S_1 secondary ending supplied to a b_2c spindle unit from peroneus brevis. The b_2 fiber lacked a nuclear bag and was accompanied by three c fibers. *C*. Primary terminals supplied to b_1 and b_2 fibers in a superficial lumbrical spindle. Note the

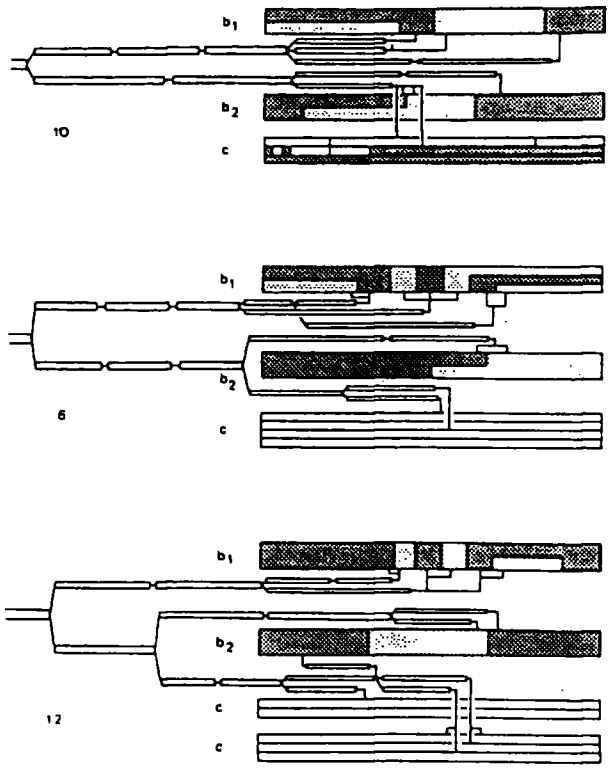


FIGURE 11-7. Diagrams showing the preterminal branches and terminal domains of primary endings innervating cat tenuissimus spindles reconstructed from serial longitudinal (spindle 10) or transverse (spindles 6 and 12) sections. In each case the parent axon (left) divides to form myelinated branches that ultimately give rise to several nonmyelinated branches, which each distributes terminals within a separate domain. The approximate locations and extents of individual terminal domains are shaded; those belonging to the chain fibers in 6 and 12 could not be determined. Despite close similarity in the overall form of all three endings, details of the preterminal branching patterns and terminal domains vary considerably. *(Banks.[109] Reproduced by permission.)*

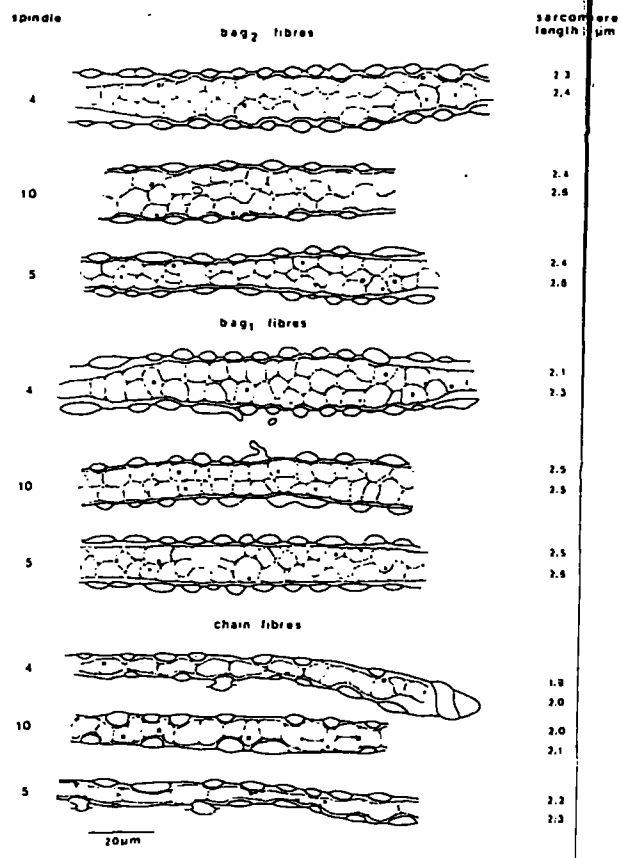


FIGURE 11-8. Tracings of 1 µm-thick longitudinal sections through the equatorial regions of the bag_2 fiber, bag_1 fiber, and one chain fiber from each of three cat tenuissimus spindles. Sections passing closest to the diameter of each fiber were selected. Mean sarcomere lengths of 50 sarcomeres on each side of the primary ending are given for each fiber. They are consistently shortest in spindle 4 and longest in spindle 5, suggesting increasing amounts of static stretch during fixation in the order 4, 10, 5. The terminals are progressively less indented into the bag fibers in the same order, but among the chain fibers only those of spindle 5 are clearly less indented than others. *(Banks.[109] Reproduced by permission.)*

ward the ends of the terminal systems. Dense-core, coated, and complex vesicles occur both in the terminals and in the sarcoplasm beneath them; the terminals also contain populations of large and small clear vesicles (Barker and Saito, unpublished observations). It is probable that these pre- and post-junctional vesicles are engaged in neurosecretory processes that maintain the intrafusal muscle fibers and their equatorial nucleation.

In longitudinal sections of spindles, the terminals are typically lenticular in profile, and it is immediately apparent that they are differentially indented into the three types of muscle fiber: most deeply in the chain and least in the bag_1 fibers (Fig. 11-8). The lenticular profile is consistent with the terminals being deformed from a condition of minimum energy and surface area (circular profile) by longitudinal tension in the intrafusal muscle fibers as well as in the basal laminae that occur continuously over the outer surfaces of both the muscle fibers and the sensory terminals.

If the mechanical properties of the basal laminae associated with the different types of intrafusal fiber are similar, the differential indentation of the fibers by the terminals would be due to the mechanical properties of the fibers. Moreover, increased static stretch of the spindle would be expected to increase standing tension in the basal laminae and the muscle fibers, causing the radii of curvature of the outer and inner surfaces of the lenticular profiles to increase also. A preliminary study by Banks[109] bears this out (Fig. 11-8), and the proposed mechanism for transduction has been supported by Patten and Ovalle's[113] scanning electron microscopy (SEM) study of the terminals and basal laminae.

The primary axons that supply b_1b_2c spindle units are generally thicker than those supplying b_2c spindle units. The manner in which their first-order branches distribute terminals to the three types of intrafusal muscle fiber is either segregated (bag_1 fiber supplied separately from bag_2 and chain fibers, thereby resulting in separation of dynamic and static inputs) or mixed. Distributions are usually segregated in tenuissimus and mixed in the superficial lumbrical muscles; but in most hindlimb muscles sampled by us, neither type of distribution predominated. Mixing was usually

clear why the spindles are still unevenly distributed in cat soleus, which consists only of S fibers, nor why spindles are so often clumped, as is particularly noticeable in muscles especially rich in them (the spindle complexes of Bakker and Richmond,[88] for example).

Intriguing as many of the functional speculations concerning the intramuscular distribution of spindles are, they remain unconvincing. We are left with the clear association between spindles and subdivisions of the intramuscular nerves. If, as seems likely on the evidence from studies on early spindle development (see above), Ia and α axons compete for sites on primary myotubes, the observed distribution of spindles would be expected, especially when it is borne in mind that α axons branch extensively within a muscle whereas Ia axons do not.

Sensory Innervation

The first detailed account of spindle sensory innervation in terms of its distribution to the three types of intrafusal muscle fiber was given by Banks et al.,[39] who used reconstruction, EM, and examination of teased silver preparations. In addition to their description of the form of the primary and secondary endings, they noted that the number of secondary endings in individual spindles followed a binomial frequency distribution. The terminals of the primary ending were further studied by Banks,[109] and the numbers of sensory endings by Banks and Stacey.[97,110] Unless otherwise indicated, what follows is based on these studies.

Primary Endings and Axons

The primary ending terminates on the densely nucleated equatorial parts of the intrafusal muscle fibers (the nuclear bags, myotubes, and nuclear chains) and occupies a length of about 350 μm (see Fig. 11-6*A*). The ending is annulospiral in form,[111] and the terminals consist of spirals, half rings, and a few complete rings. Spirals are more common and more extensive around chain fibers than bag fibers; rings are formed by encircling terminals abutting onto themselves without fusion. The terminal systems supplied to bag fibers consist of a middle portion, in which the terminals are arranged mainly as regular transverse bands, and portions at each end, in which they are disposed as irregular forms in mainly diagonal and longitudinal configurations. Bag_1 terminal systems can be distinguished from those supplied to bag_2 fibers because they have more bands per unit length in the middle and more extensive irregular portions at each end (see Figs. 11-2 and 11-6C). Primary endings supplied to b_2c spindle units are mostly irregular in appearance (see Fig. 11-6*B*).

Reconstructions of three primary endings supplied to tenuissimus spindles showed that bag_1 terminal systems covered the largest amount of muscle fiber surface, the proportional distribution of the total terminal contact areas in two endings being as follows: bag_1 fibers, 33 and 37 percent; bag_2 fibers, 25 and 24 percent; chain fibers, collectively 42 (five chains) and 39 (four chains) percent, individually 5 to 12 percent. The dense innervation of the bag_1 fiber may be adapted to generate a sufficiently large dynamic component in the receptor potential, as considerably more fibers (bag_2 and chain) are involved in the static input.

The reconstructions showed a close association between nucleation and innervation (Fig. 11-2) but no constant relation between number of myonuclei and terminal contact area. Bag_1 fibers received more terminals (on average 5.7 separately derived from nonmyelinated preterminal axonal branches) than did bag_2 fibers (2.7 on average), and both received more than chain fibers (average of 0.7 per fiber). The few terminals on chain fibers were distributed to all the chain fibers in a spindle by sensory cross terminals,[64] which occurred frequently in all three endings. They were formed exclusively among chain fibers, a feature which, taken together with regions of close apposition between fibers of this type (see "Ultrastructure," under "Intrafusal Muscle Fibers," above), may be attributed to their incomplete separation during development (see "Development," under "Intrafusal Muscle Fibers," above). These features correlate with the fact that chain fibers frequently contract as a group on fusimotor stimulation.[112]

Within a primary ending, the terminals are not symmetrically distributed, and the form of each one depends on its location in the complete system. On the bag fibers, for example, pure spirals are confined to those parts of the terminals that happen to overlie the nuclear bag. Despite close similarity in the overall form of the three reconstructed endings, the domains of individual terminals varied considerably between the endings (Fig. 11-7). Thus, underlying the constant overall form of the primary is an independently varying finer level of organization. The terminals appear to be competing among themselves for a limited amount of a suitable contact site on each muscle fiber. Evidence that the necessary site specificity exists has been obtained in our studies of reinnervation (see "Reinnervation and Recovery," below).

Ultrastructural studies of primary axon terminals (reviewed by Barker[21]) have shown that they lie in shallow grooves on the surface of the muscle fibers and form smooth myoneural junctions. In contrast to the motor myoneural junctions, there is no intervening basal lamina, and they are not covered by Schwann cells but rather by basal lamina continuous with that of the muscle fibers. The terminals contain neurofilaments, microtubules, glycogen granules, mitochondria, and a mixed population of vesicles. Degenerating mitochondria tend to accumulate to-

◀ FIGURE 11-6 *(Cont.)* difference in spacing of the bands encircling the regular portions of the b_1 and b_2 systems and also the characteristically irregular portions of the b_1 system. Arrows indicate preterminal axons that have been cut but which in the ending ran on to supply terminals to c fibers. Asterisk indicates position of a sensory cross-terminal with a c fiber. *(Banks et al.[39] Reproduced by permission.)*

restricted to the dynamic input and resulted from the bag_1 fiber sharing a supply of terminals with a few chain fibers.

Quick et al.[114] have shown that the sites for impulse generation in Ia axons are the heminodes and some of the penultimate nodes of the final branches. For Ia axons whose first-order branches have a segregated distribution, there would presumably be separate dynamic and static pacemakers. These have been demonstrated by Hulliger and Noth,[115] and the interaction of spiking nodes in a real preterminal tree has been modeled by Otten et al.[116] The spiking activity of the final branches of spindle afferents is generated by receptor potentials produced by their terminals.[117,118]

Secondary Endings and Axons

Secondary endings terminate on one or both sides of the primary (see Fig. 11-6*A* and *B*). The most we have seen on one side is five and on both sides six. Each occupies a length of about 350 μm and is designated S_1,S_2,S_3, and so on, according to its position relative to the primary. Most secondaries terminate next to the primary in the S_1 position, though the proportion varies from muscle to muscle depending on the parameters of binomial frequency distributions (see "Number of Afferent Axons," below). Of 1250 secondary endings from a variety of mainly hindlimb muscles, the percentages of secondaries in the different locations were S_1, 71; S_2, 22; S_3, 6; S_4, 1; S_5, 0.3 (Banks and Stacey, unpublished results). The proportion of S_1 endings ranged from 56 percent in complexus to 100 percent in extensor digitorum lateralis. Chain fibers are innervated in all secondary endings, but few secondaries are restricted to chain fibers only; most are distributed to all three fiber types. Restriction of terminals to one or two fiber types is more prevalent among secondaries terminating in the more polar positions.

Secondary terminals supplied to chain fibers are annulospiral but generally thinner and more dispersed and irregular than those supplied to chain fibers in primary endings. When bag fibers are included in the innervation, their terminals are generally much more irregular, and the bag_1 fiber usually receives fewer than the bag_2, often in the form of sprays. The ultrastructure of the terminals is very similar to that of primary terminals. Cross terminals occur between chain fibers and also between chain fibers and both types of bag fiber.

The total terminal contact area of a reconstructed S_1 secondary ending was 32 percent less than that of the adjacent primary. The chain fibers received 75 percent (individually 16 to 22 percent); the bag_2 fiber, 17 percent; and the bag_1, 8 percent. Using NADH-TR staining, Kucera[119] reports that long-chain fibers receive less secondary innervation than other types of chain. The secondary innervation of bag_1 fibers appears to be of little functional significance. Stimulation of dynamic fusimotor axons rarely increases the dynamic sensitivity of secondary endings.[120,121] The particularly high dynamic sensitivity of most secondary endings in peroneus tertius, reported by Jami and Petit,[122] is not matched by an unusually extensive secondary innervation of bag_1 fibers in this muscle.

Secondary axons terminating as S_1 endings are generally thicker than those terminating in more polar positions. There is considerable overlap between the intramuscular diameters of Ia and II axons that innervate all three types of muscle fiber.

Number of Afferent Axons

The provision of both Ia and II axons to the spindle complement of a muscle varies characteristically among different muscles. Since a single Ia axon is required to initiate and maintain a differentiated spindle (see "Development," above), the variability can be described by considering the number ("a") of afferent axons supplied to individual spindles, additional to a single Ia. The parameter is expressed in this way, rather than as the number of secondary endings, to take account of those spindles whose primary endings appear to be supplied by two or more Ia axons and of those II axons that branch within a spindle to end in both S_1 positions. These features are rare in most cat muscles so far studied, though in certain muscles the proportion of spindles with primary endings supplied by two Ia axons is quite high (12 to 23 percent in extensor digitorum longus and 8 percent in popliteus).[97,242] In rat masseter, multiply-innervated primary endings are the rule (92 percent) rather than the exception and up to 5 axons may be involved.[65]

For several, mainly hindlimb, muscles of the cat, the mean value of "a" varied from 0.56 in extensor digitorum lateralis to 3.5 in complexus. The greatest number of afferents occurred in a popliteus spindle with a complement of $S_2S_1PPS_1S_2S_3S_4$ (8 afferents). Frequency distributions for "a" are well described by binomial statistics, and they are also characteristic for different muscles (Fig. 11-9). This suggests that, after the initial determinative contact by a Ia axon, the remaining, mostly II, afferents are distributed randomly among the developing spindles. If so, the greater availability of afferents at the point of nerve entry to the muscle might be expected to result in spindles close to this point receiving more afferents than average. This prediction is borne out by a study on tenuissimus, facilitated by its linear spindle arrangement.[110]

These results suggest that any afferent, Ia or II, entering the spindle during development should be able to make an ending capable of persisting into the adult. At present, it is difficult to reconcile this with the observation[56] that transient multiple contacts of the future primary region by several presumed Ia axons occurs in the early development of the rat hindlimb spindle.

Motor Innervation

Analysis of the intrafusal distribution of motor axons and their endings has proved to be one of the most difficult problems in studies of the mammalian spindle and has led to much controversy. Some of the difficulties have arisen because the methods used were either incapable of resolving important details or led through artifact to false conclusions. Another difficulty has been that overlapping variability occurs between some of the categories described,

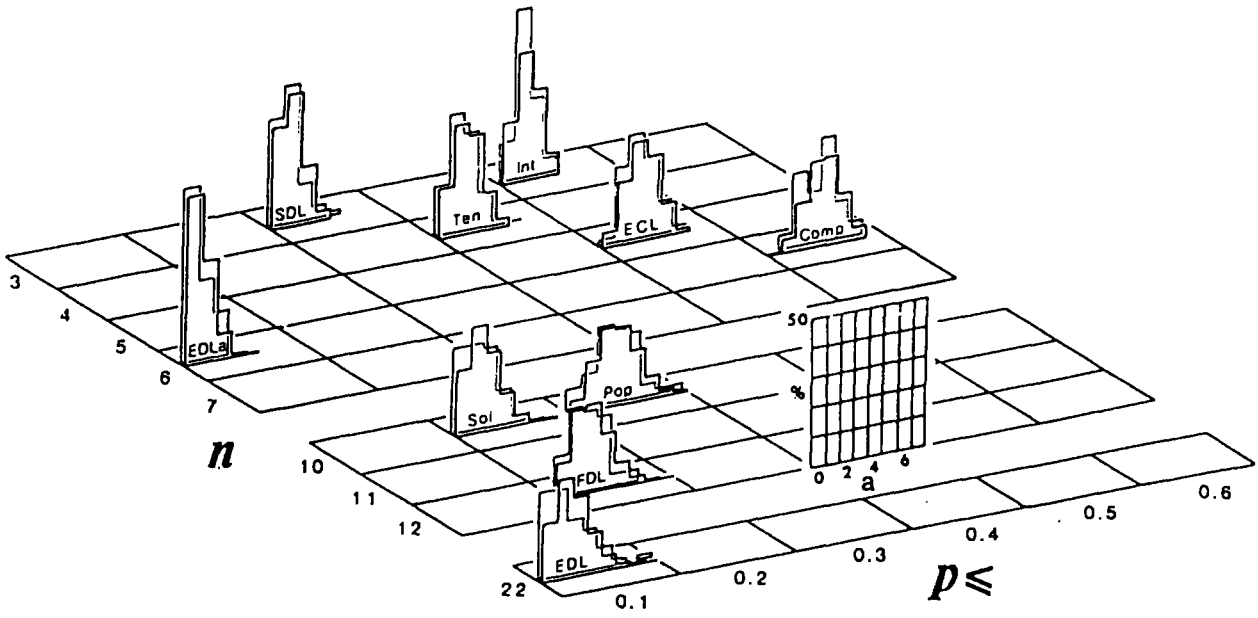


FIGURE 11-9. Pairs of histograms showing the observed distributions (rear of each pair) and best-fitting binomial distributions (front of each pair) of numbers of afferents (a) in excess of a single Ia for various cat muscles. The histograms are positioned on the grid according to their binomial parameters (n, p). Abbreviations: Comp(lexus); ECL, Extensor caudae lateralis; EDLa, Extensor digitorum lateralis; EDL, Extensor digitorum longus; FDL, Flexor digitorum longus; Int(erosseus); Pop(liteus); Sol(eus); SDL, Superficial and deep lumbrical; Ten(uissimus). (*Modified from Banks and Stacey.*[97])

and this allows for a theory to be supported by an unwittingly biased selection of results.

The first controversy arose over the dual model of the spindle proposed by Boyd.[38] Based on a study of teased gold chloride preparations, this distinguished between two systems of γ axons, those that terminated as plates on bag fibers and those that formed *networks* on chain fibers. Such selective innervation of the two types of intrafusal muscle fiber catered admirably for the operation of the two functionally distinct types of γ axon, static and dynamic, that had just been established by Matthews,[4] and this model was generally accepted until well into the seventies,[123] despite increasing histological evidence against it. This came mostly from the findings of Barker and his colleagues, who had devised a technique for producing teased preparations of spindles that had been impregnated with silver.[124] They described the motor innervation of mammalian spindles in terms of a *trail* innervation[10] and two types of plate designated as p_1 and p_2.[11] They concluded[125] that whereas most chain fibers received trail endings only (Boyd's "networks"), the trail innervation was nonselectively distributed to bag fibers, chain fibers, or both. The p_1 plates were recognized as belonging to the β system, since they were similar to extrafusal end plates, and were sometimes seen to be supplied by a branch of a β axon. Moreover, after nerve section, p_1 plates and extrafusal plates degenerated simultaneously, whereas p_2 plates and trail endings persisted for a further 24 to 36 h. The distribution of the plates was also held to be nonselective, the bag/chain ratio of distribution being given as p_1, 75/25; p_2, 90/10.

Boyd[126,127] and Matthews[123] accepted that there was some nonselectivity in the distribution of the trail innervation but did not consider it to be of any functional importance. Such a view became increasingly untenable in the light of convincing evidence obtained from various histophysiological experiments conducted in the early seventies. The first of these, carried out by Barker et al.,[128] entailed the isolation of single static γ axons innervating tenuissimus by cutting all other motor axons supplying the muscle and allowing a few days for them to degenerate. Subsequent examination of the terminals of the surviving static axon in teased silver preparations revealed that they were trail endings and that they were most frequently distributed to both bag and chain fibers. In another series of experiments on tenuissimus carried out by Barker et al.,[129,130] procion yellow dye was injected electrophoretically into intrafusal muscle fibers impaled by microelectrodes and activated by single static γ axons (see Fig. 11-10*A* to *C*). The types of muscle fiber impaled were then determined by fluorescence and EM; they proved to be either bag or chain fibers. In one experiment, two muscle fibers activated by the same static γ axon were impaled and injected in one pole of a spindle; one proved to be a bag fiber, and the other a chain fiber (see Fig. 11-10*C*). Further demonstrations of the nonselectivity of static γ innervation came from glycogen-depletion experiments[131,132] and experiments by Bessou and Pagès[133,134] in which the effects of fusimotor stimulation on intrafusal muscle fibers were directly observed and filmed. A crucial observation made by them with respect to bag fibers was that a static γ axon never activated the same bag fiber that could be activated by a dynamic γ axon.

The dual model of the spindle was finally abandoned in the mid-seventies, when the fact that there were two types of bag fiber became firmly established on histological grounds.[28,29] It was now generally agreed that bag_1 fibers were responsible for dynamic actions and that bag_2 and

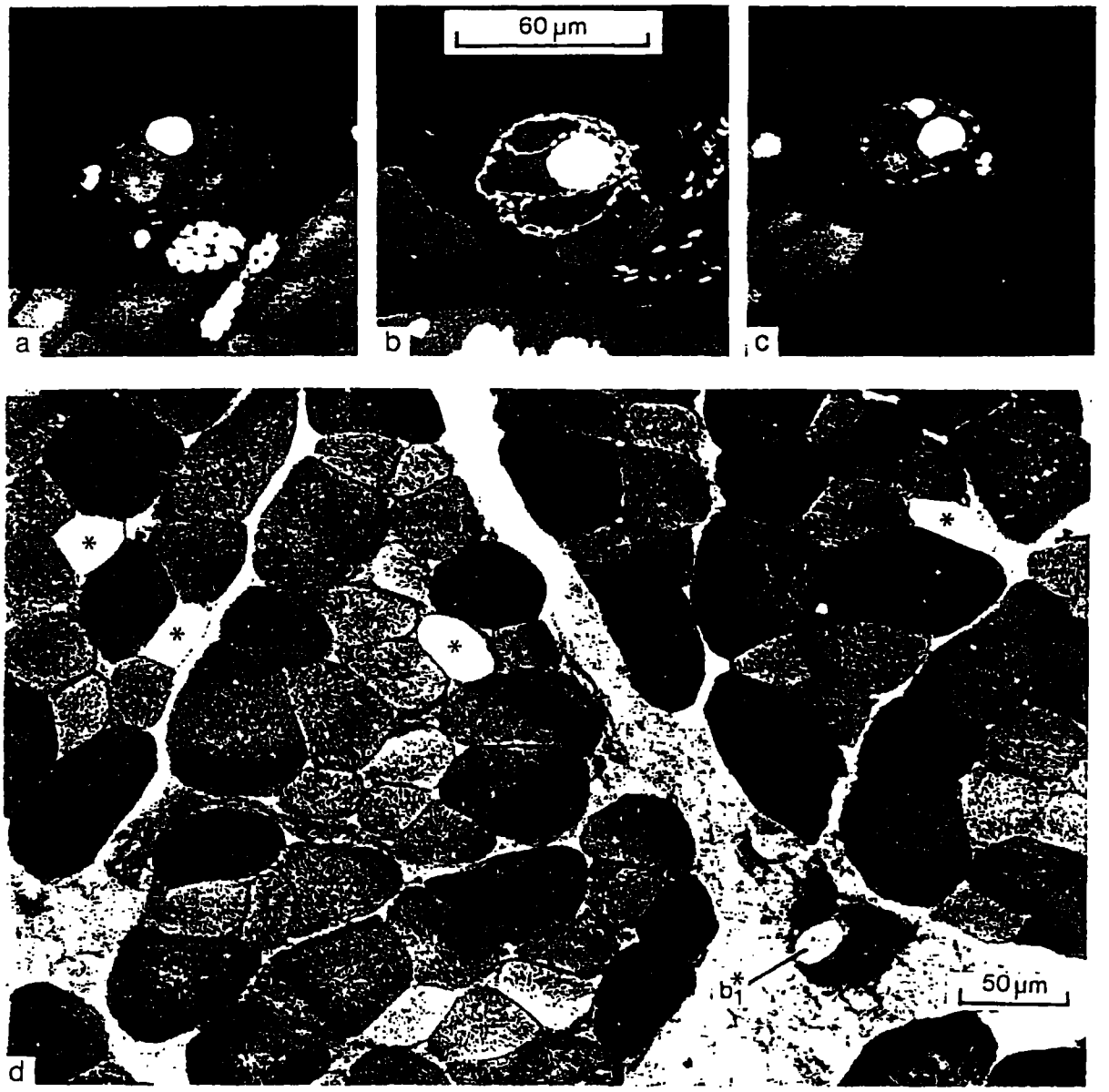


FIGURE 11-10. *A–C.* Transverse sections (10 to 15 μm thick) of cat tenuissimus spindles showing fluorescence of muscle fibers injected with procion yellow following recording of their membrane potentials on activation by single static γ axons. In (*A*) the muscle fiber is a chain fiber from which an action potential was recorded; in (*B*) it is a bag_2 fiber from which a junctional potential was recorded. In (C) two fibers that were activated by the same static γ axon were impaled and injected; the large fiber is a bag_2 fiber from which a junctional potential was recorded, whereas the small fiber is a chain fiber from which an action potential was recorded. *(Barker et al.[129] Reproduced by permission.) D.* A 10-μm frozen transverse section of a cat peroneus brevis muscle stained for glycogen (PAS method) following repetitive stimulation of a single dynamic β axon (conduction velocity 77 m/s) to produce glycogen depletion. Asterisks indicate depleted extrafusal muscle fibers and a depleted bag_1 fiber in the muscle spindle in the lower right quadrant. Histochemical tests applied to adjacent sections showed that the extrafusal muscle fibers were of the slow oxidative type. *(Barker et al.[138] Reproduced by permission.)*

chain fibers were responsible for static actions. This had become evident from more cinematographic analyses of intrafusal contractions[135,136] and further glycogen-depletion studies.[137]

It was clear that in most spindles, dynamic axons, γ or β, exclusively innervated the bag_1 fiber and that such axons seldom supplied other types of fiber. In tenuissimus, the combined results of glycogen-depletion[137] and procion-yellow[130] experiments indicated that dynamic γ axons activated bag_2 or long chain fibers, in addition to the bag_1 fiber, in about one in every four or five spindles. Glycogen-depletion experiments on dynamic β axons[138] showed that these had a very similar distribution (see Fig. 11-10*D*).

What was not clear was whether the bag_1 fiber could also be activated by static γ axons. Glycogen-depletion studies indicated that there was such activation, the frequency of bag_1 depletions produced by stimulating single static γ axons being about 50 percent in tenuissimus.[131,137] More-

over, experiments by Emonet-Dénand et al. (1978),[139] in which they surveyed the effects on the primary-ending response of stimulating single γ axons during stretch, lent support to this conclusion. They recognized categories of response intermediate between "pure" static and "pure" dynamic and showed that these could be mimicked by mixing the effects produced when stimulating two γ axons supplying the same spindle, one purely static and the other purely dynamic in action. They concluded that 33 percent of the responses from peroneus brevis spindles to single γ axon stimulation were produced by an admixture, in various proportions, of static and dynamic actions. Though less than might be expected from the frequency of the glycogen depletion of bag_1 fibers by static γ stimulation in tenuissimus, this compared well with the 38 percent of such depletions subsequently found among peroneus brevis spindles by Emonet-Dénand et al.[140]

Nevertheless the matter remained in doubt because dynamic bag fibers could not be seen to contract in response to static γ stimulation.[134,136] Moreover, although the glycogen-depletion results[137] for tenuissimus suggested that virtually every bag_1 fiber should receive trail plates, reconstructions of 3½ tenuissimus spindles made by Banks[141] from thin serial cross sections failed to reveal any trail innervation on bag_1 fibers. Such reconstructions could feasibly be made of only a few spindles, but fortunately they revealed consistent features of sensory innervation that enabled bag_1 and bag_2 fibers to be identified with confidence in larger samples of teased silver preparations. The frequency of bag_1 trail innervation in such preparations was found by Barker and Stacey[142] to be 8 percent in tenuissimus and 17 percent in peroneus brevis, much less than the glycogen-depletion results had indicated.

These results led to two conclusions. First, that the glycogen-depletion technique produces some activation of the bag_1 fiber that is nonneural; this could be either a stretch-induced contraction[143,144] or some physicochemical factor, e.g., accumulation of potassium ions liberated from contracting bag_2 and chain fibers.[142,145] Second, that innervation by static γ axons only occasionally involved the bag_1 fiber. This implies that the variability of the static response is largely the result of the activation of bag_2 and chain fibers, either on their own or in various combinations in one or both poles of a spindle, with the additional activation of the bag_1 fiber making only an occasional contribution.

Advances in knowledge of spindles since 1970, when Barker et al.[125] gave a detailed description of their p_1, p_2, trail classification of intrafusal motor endings, led them to reexamine it 15 years later. The three categories of ending were based on an analysis of their presynaptic features as seen in teased silver preparations. In the new study,[13] motor endings received by the three types of intrafusal muscle fiber were compared with the endings supplied to spindles by the various functional categories of motor axon. Three forms of motor ending were found that had significantly different presynaptic features; these forms corresponded closely to those previously identified in the literature as p_1 (β), p_2 (dynamic γ), and trail (static γ).

When postsynaptic features are considered, intrafusal motor endings do not fall into categories that correspond to the p_1, p_2, trail classification. The degree of indentation (primary folding) of the intrafusal muscle fibers by motor axon terminals increases with greater distance from the primary ending, irrespective of fiber type,[13,146] whereas the amount of junctional (secondary) folding is related to fiber type and tends to increase in motor endings on bag_1, bag_2, and chain fibers, respectively.[32,46,130,141,146–149] This gradation is matched by the cholinesterase (ChE) content of the endings, there being a parallel increase in the density and thickness of the ChE reaction product.[148] The factor of distance from the primary ending appears to operate regardless of fiber type, such that the greater the distance, the higher the ChE content of an ending[148] and the greater the amount of junctional folding.

The question as to whether there are two physiologically distinct types of static γ axon[150] with ultrastructurally different kinds of plate[151,152] is discussed in the next section.

The γ Motor Innervation

The γ innervation is provided by small motoneurons that have an exclusively fusimotor distribution.[153] Each γ axon is distributed to several spindles, but in a selective manner so as to supply either bag_1 fibers or bag_2 and chain fibers. Exceptions to this dichotomy are rare in the cat but more common in the rat[154] and monkey,[155] though even in these species the overall distribution of the axons concerned may be predominantly of one kind or the other. The functional classification of γ motoneurons into dynamic and static types[4,156] corresponds with their segregated distribution, those having dynamic effects being distributed to bag_1 fibers (see previous section). There is, however, no distinction between the axonal conduction velocities of the two types; both cover a range from 15 to 55 ms^{-1}.[157] In general, static γ axons seem to outnumber dynamic γ axons by about 3:1,[158] but the ratio may be much higher in tenuissimus.[159]

This muscle has proved to be particularly useful in studying the distribution of static γ axons because of its almost linear arrangement of spindles. Individual primary endings can be located and their responses to fusimotor stimulation correlated with directly observed intrafusal contractions and with the pattern of motor innervation subsequently observed histologically. Boyd[120] showed that different types of static effect could be related to either bag_2 or chain fiber activity and went on to use the relationship to infer the distribution of individual static axons in several spindles.[150,160] Some of the spindles were subsequently analyzed by serial section for light microscopy and EM.[151,152,161] Boyd and his colleagues concluded that there are two types of static γ axon whose neuromuscular junctions display different postjunctional characteristics. According to Boyd,[160] one always innervates bag_2 fibers whereas the other always innervates chain fibers, but neither is restricted to its characteristic effector. Gladden and Sutherland[162] later suggested that there may, in addition, be a third type that supplies chain fibers exclusively.

Against this, it has been our experience[13] that postjunctional structure is related to muscle fiber type and to location of motor endings with respect to the primary ending, rather than to axonal type, and Kucera and Walro[146] have described similar relationships (see the introduction to this

section). Furthermore, Banks[159] has used a tenuissimus preparation combined with silver histology to produce an independent correlation of primary responses to static γ stimulation with motor innervation patterns. He found that a biasing effect (see "The Spindle as a Receptor"), attributed by Boyd to the bag_2 alone, requires bag_2 activity but can also be produced when chain fibers are coinnervated with the bag_2. Driving the primary response at the same frequency as the motor stimulation seems to be characteristic of chain fibers active alone, but subharmonic driving, or other indeterminate effects, might again indicate coactivation of bag_2 and chain fibers.

Banks[159] found no evidence for there being two or more distinct kinds of static γ axon but did find that static γ axons were differentially distributed according to conduction velocity (see Table 11-2). Faster-conducting axons were more widely distributed and less likely to innervate chain fibers alone than slower ones. In the innervation of individual spindles, axons that produced 1:1 driving were likely to be the slowest-conducting static axons supplying the spindles concerned, even though they might not have the slowest conduction velocity of all the static axons supplying the muscle.

Experiments involving cortical, brainstem, or reflex activation of intrafusal muscle fibers have been interpreted as supporting the subdivision of static γ axons into two kinds.[163–165] The existence of a differential distribution of a single population of axons might provide sufficient segregation to account for these observations. Nevertheless, functional segregation of bag_2 and chain-fiber activation is undoubtedly far from complete, and further evidence will be presented below (see "The Pattern of Innervation") that raises serious doubts about the existence of functional benefits arising from a segregated bag_2/chain system.

Table 11-2 SYMBOLIC REPRESENTATION OF THE EFFECTS OF STIMULATING γ-EFFERENTS ON THE RESPONSES OF PRIMARY ENDINGS IN A CAT TENUISSIMUS MUSCLE[a]

Efferents by cv	Afferents: proximal → distal D	E	A	J	B	H	C	No. of spindles
41	●	●	●	●	○	—	○	7
40	—	—	●	●	●	●	●	6
33	●	○	●	—	○	▲	▲	5
31	—	—	○	○	—	—	—	6
30	—	▲	—	—	—	—	—	2
28	—	—	—	●	—	—	—	1
27	▲	—	—	—	—	▲	—	1
27	▲	—	●	—	○	—	—	2
24	—	—	—	●	—	—	—	3
23	□	□	□	□	□	—	—	1
No. static	4	3	5	5	4	3	3	5
No. dynamic	1	1	1	1	1	0	0	
Total	5	4	6	6	5	3	3	

[a]Each row shows the effects of a single efferent on the several primaries activated by it: —, no effect; ●, biasing; ○, indeterminate; ▲, 1:1 driving; □, dynamic. The afferents are arranged in the proximal-to-distal sequence of their corresponding spindles and are identified alphabetically according to the order in which they were isolated. Efferents are arranged by conduction velocity (cv, in m/s) as shown in the column at the left; the total number of spindles supplied by each one is given in the column at the right. The numbers of efferents supplying each spindle are summarized at the bottom of the table in the corresponding column.

SOURCE: Banks.[159]

The β Motor Innervation

Physiological evidence for the existence of mammalian skeletofusimotor axons was first demonstrated by Bessou et al.[5,166] in the first deep lumbrical muscle of the cat. They showed that the repetitive stimulation of such axons, later referred to as β,[167] not only produced extrafusal contraction but also activated spindles by increasing the dynamic sensitivity of the primary ending. Histological evidence was provided by Adal and Barker,[168] who traced the intramuscular branching of the motor supply to this muscle in teased osmium tetroxide preparations and showed that it included fibers that had a skeletofusimotor distribution. These had a diameter range of 6.0 to 12.5 μm, which corresponded well with the slow conduction velocities (31 to 61 ms^{-1}) of the skeletofusimotor axons described by Bessou et al.[5] As osmium stains only myelin, no information was obtained on the form and location of the intrafusal β terminals, but Barker et al.[125] recognized these as p_1 plates in their teased silver preparations, with a bag/chain fiber distribution of 75/25 (see the introduction to this section). The fact that β axons terminated in p_1 plates was later demonstrated in spindles deprived of their γ innervation by degeneration.[169]

Barker et al.[125] observed that p_1 innervation was widespread in cat hindlimb muscles and considered it unlikely that the presence of a skeletofusimotor innervation was a vestigial feature, as had been suggested.[5,126] In flexor hallucis longus, they found that 73 percent of spindle poles were innervated by p_1 plates. They argued that this proportion was too high for them all to have been supplied by the collaterals of slow-conducting β axons and suggested that the deficit was made up by fast β axons.

The use of the glycogen-depletion technique, combined with mATPase staining for muscle-fiber type, showed that the intrafusal contraction produced by slow dynamic β (βd) axons was almost exclusively restricted to bag_1 fibers and that the extrafusal contraction was confined to slow oxidative (SO) fibers.[170,171] The predicted existence of fast β axons was confirmed by glycogen-depletion studies on peroneus tertius by Harker et al.[172] and Jami et al.[173] These showed that fast β axons selectively depleted long chain fibers,[172] were static in action, and activated fast oxidative-glycolytic (FOG) fibers.[173]

Jami et al.[7] shed further light on the extrafusal composition of β motor units in peroneus tertius by determining the fatigue index of 24 static β (βs) units and 12 βd units; the conduction velocity range of the βs axons was 69 to 100 ms^{-1}, whereas that of the βd axons was 55 to 91 ms^{-1}. Their results (expressed in terms of Brooke and Kaiser's[174] nomenclature for fiber types and Burke's[175] equivalents for fiber and motor-unit types) showed that, extrafusally, the βd units in this muscle were composed of type I fibers (rarely IIA fibers), and that the βs units consisted of IIA fibers (usually), IIB fibers (occasionally), or type I fibers (rarely). The overlap in conduction velocities between βd

and βs axons results in the extrafusal composition of their units being related more to the conduction velocity of the β axon than to its static or dynamic effect on the spindles.

Barker et al.[176,177] have recently studied the composition of one βd and three βs units in serial cross sections of peroneus tertius using the glycogen-depletion technique combined with mATPase staining for muscle-fiber type. The conduction velocities of the β axons were βd, 72 ms^{-1}; βs_1, 90 ms^{-1}; βs_2 and 3, 95 ms^{-1}. They found that the units were composed of mixed extrafusal fiber types. The βs units consisted of IIB fibers plus varying percentages of IIA fibers, namely, 6.7 (βs_1), 5.8 (βs_2), and 29.9 (βs_3). The βd unit was made up of 93 extrafusal fibers, of which 76 were type I and 18.3 percent IIC (11) and IIC/I (6), the latter changing fiber type during the course of their length. The authors suggest that minimal mixing (around 5 percent) of fiber types is probably common to all motor units; it was, in fact, reported by Edström and Kugelberg[178] in the first study of motor-unit fiber types using the glycogen-depletion technique. Such mixing may be accounted for by small numbers of fibers being relinquished naturally by motoneurons and subsequently incorporated into other motor units. This turnover may be attributed to the continuous process of end plate degeneration and replacement proposed by Barker and Ip[179] in the sixties and now widely accepted to be a feature of normal muscle (see review by Cotman et al.[180]). It is not known, at present, how to account for moderate mixing (around 30 percent).

Skeletofusimotor axons are now regarded as an integral feature of the somatic neuromuscular system in mammals. Estimates by Jami et al.,[7] based on the physiological identification of β axons with conduction velocities of 55 ms^{-1} or more (any slower β axons would have been excluded), indicate that at least 50 percent of the spindles in peroneus tertius receive a β innervation and that one-third of the β supply is dynamic and two-thirds static. In tenuissimus, a glycogen depletion study[181] showed that β axons (about half dynamic, half static) supply at least 40 percent of the spindles. The fusimotor collaterals of β axons provide a positive feedback loop to the spindle in that their activation increases Ia afferent discharge,[171] which then leads to further β activation. This route for spindle excitation extends the range of control of spindle afferent discharge well beyond that provided by γ axons and enables β axons to exert a significant influence in the control of muscle contraction, as in, e.g., the crossed extensor reflex.[182] Apart from the occurrence of β axons in cat spindles, there is physiological evidence for their existence in rabbit,[6] rat,[183] and monkey[184–186] spindles and histological evidence in rat,[187] rabbit,[26] monkey,[185,188] and human[189] spindles. Reconstructions of rat[187] and monkey[188] spindles have shown that the β innervation is selectively distributed to the bag_1 and long chain fibers, as in cat spindles. Some distal muscles of the rat's tail contain spindles that lack any γ innervation and are activated entirely by β axons, dynamic and static.[183,190]

The Pattern of Innervation

It is useful to summarize the present view of how motor axons are "wired up" in cat hindlimb spindles (see recent review by Banks[190a]). Inevitably the view is based on tenuissimus, because most is known about spindles in this muscle. The following summary is based largely on teased silver-impregnated spindles[139,141] supplemented by serial-section reconstructions.[141,191–194] On average, each spindle—containing one bag_1, one bag_2 and four chain fibers—receives branches of nearly 7 motor axons in a range of 2 to 13. The frequency of occurrence of different numbers of branches follows a binomial distribution, indicating that the branches are randomly distributed with respect to the spindles. It is usually possible to identify the intrafusal branches of β and γ axons by their presynaptic form.[13] They number 1 to 6 (mean 3.2) and 1 to 7 (mean 3.8), respectively. In each case they appear to be randomly distributed to their spindles. However, the number of afferent axons additional to a single Ia also follows a binomial frequency distribution (see "Number of Afferent Axons," above), and there is a very clear tendency for spindles with more afferents to receive more γ branches, though there is no relationship between the numbers of β branches and afferents. This may reflect different requirements for guidance during development.

The bag_1 fiber is almost always separately innervated, receiving 38 percent (mean of three muscles, range 34 to 41 percent) of the motor branches. Tenuissimus seems to have very few dynamic γ axons; in some cases they may be absent altogether. Most of the branches to bag_1 fibers in our sample are derived from β axons. Occasionally the bag_1 fiber is co-innervated with a long chain pole, but these account for less than 1 percent of the total motor branches. Static β branches supplied exclusively to long chain poles amount to a further 8 percent of the total, leaving the balance of 53 percent (range 49 to 56 percent) supplied to bag_2 or chain fibers or both. These are almost without exception branches of static γ axons. The proportion that co-innervates both types of fiber in this group is quite variable, ranging in our sample from 17 to 40 percent.

The bag_2 and chain fibers may receive a completely segregated input in each pole, a completely mixed input, or an input variously segregated, e.g., a branch to bag_2 alone plus another to the bag_2 and chain fibers. Examples are shown in Fig. 11-11. When analyzed without reference to the sensory innervation, poles with different degrees of segregated input appear to be randomly associated in complete spindles; but if account is taken of the occurrence of secondary endings in each pole, then a relationship between the sensory and motor systems again emerges. However, the relationship is not what would be expected if a segregated motor supply to the bag_2 and chain fibers were functionally desirable. Thus the degree of segregation in the motor input increases rapidly, then more slowly, as first one pole and then the other receives secondary endings. Ultimately, as more static γ branches enter, the degree of segregation may actually fall, since complete segregation is most likely to occur when only two branches supply each pole. What is remarkable is that when secondary endings are present in only one pole, it is that pole which normally receives the more segregated static γ input, despite the fact that such segregation is virtually irrelevant to the response of the secondary ending.[120] Furthermore, although spindles that possess only a primary ending typically receive two static γ axons, it is the poles rather than the bag_2 and chain fibers that are separately innervated.

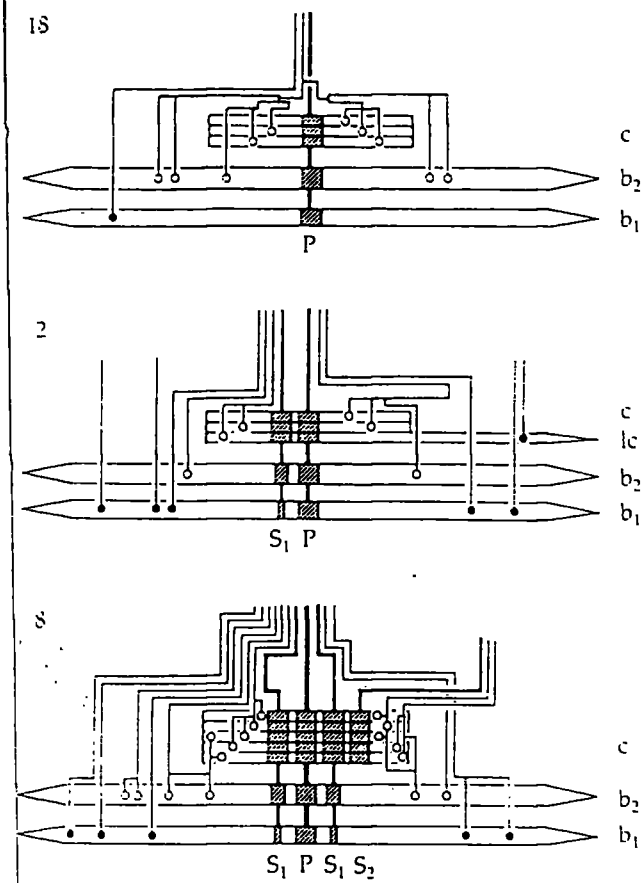


FIGURE 11-11. Schematic representation of the innervation of three cat spindles from a tenuissimus muscle, illustrating the range of complexity. The numbers refer to the order of the spindles in proximal to distal sequence in the muscle, which contained 20 spindles. Static γ axons end as trail plates (open circles) on bag_2 and chain fibers, whereas β axons end as p_1 plates (filled circles) on bag_1 and long chain fibers and are dynamic or static accordingly. Note the concomitant increase in the number of γ axons and afferents but not of β axons; and, in spindle 2, note the occurrence of a segregated input to the bag_2 and chain fibers in the same pole as the secondary endings. *(Data from Banks,[159] experiment C883, in which there was no evidence for any dynamic γ innervation.)*

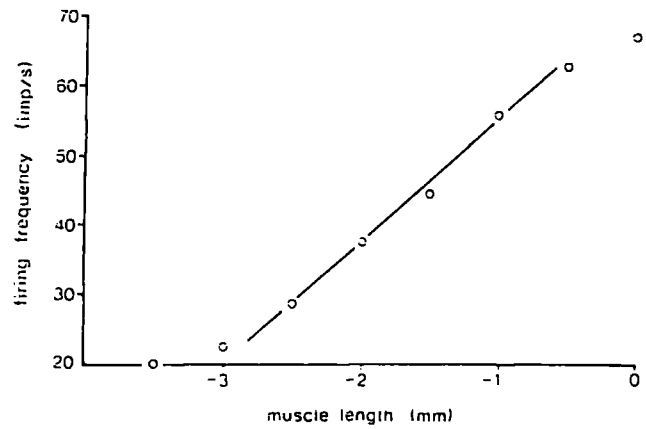


FIGURE 11-12. Length sensitivity of a spindle afferent unit, probably a secondary ending, shown by the slope (18 impulses s^{-1} per mm) of the adapted firing frequency against muscle length. Maximum physiological length corresponds to zero on the abscissa. With the muscle completely slack, the ending had a tonic discharge of 20 impulses s^{-1}. Conduction velocity 63 ms^{-1}; cat peroneus brevis.

Perhaps the most important functional requirement is for a muscle to receive several distributed, exclusively fusimotor static axons that provide both divergence of individual motoneurons to several spindles and convergence of different motoneurons onto individual spindles. The development program may then result in a degree of intrafusal segregation which is itself of little functional significance.

The Spindle as a Receptor

Input-Output Properties

When a ramp-and-hold stretch is applied to an adapted, deefferented spindle, the primary ending fires a short, high-frequency burst of impulses at the start of the ramp; this is followed by a more or less steady increase in the rate of firing until a peak is reached at the end of the ramp. During the held phase, there is an initial adaptation, rapid at first, then slower, until a new maintained level of firing is reached. The relation between this static firing level and the extension of the muscle is approximately linear and has been called *length*, or *position*, *sensitivity*[123,195] (see Fig. 11-12). As the velocity of stretch increases, the frequency of the initial burst becomes higher and the slope of the response during the ramp becomes steeper, leading to higher peak rates at the end of the ramp. However, stretch velocity does not affect length sensitivity, so that the difference between the peak rate and the adapted rate at some arbitrary later time (0.5 s in the case of the widely used dynamic index) is some measure of the dynamic response of the ending. Thus, for an ending with low dynamic sensitivity or one in a muscle stretched at a low velocity, the slope of the dynamic response closely corresponds to the length sensitivity (indeed, Boyd[120] uses the term *length sensitivity* to mean the slope of the dynamic response). All these features are recognizable in secondary endings, although the initial burst and dynamic response are usually much less well developed (see Fig. 11-13).

Although the effects of dynamic fusimotor axons are mediated through the bag_1 fiber, it does not necessarily follow that the dynamic response of passive spindles is due in whole or part to the bag_1. Several authors have now succeeded in identifying b_2c primary afferents from various sources and all are agreed that they show substantial passive dynamic behavior.[196–202] As in secondary endings, the dynamic response cannot be selectively increased by dynamic fusimotor stimulation.

The effects of motor stimulation (β or γ) on the primary-ending response depend on the rate of stimulation and the type of muscle fiber activated (see Fig. 11-13). Stimulation of a dynamic axon activates the bag_1 fiber and produces an increase in the static firing level, though not usually in the length sensitivity. When a ramp-and-hold stretch is applied in addition, the initial burst is abolished and the slope of the dynamic response is increased. Stimulation of a static

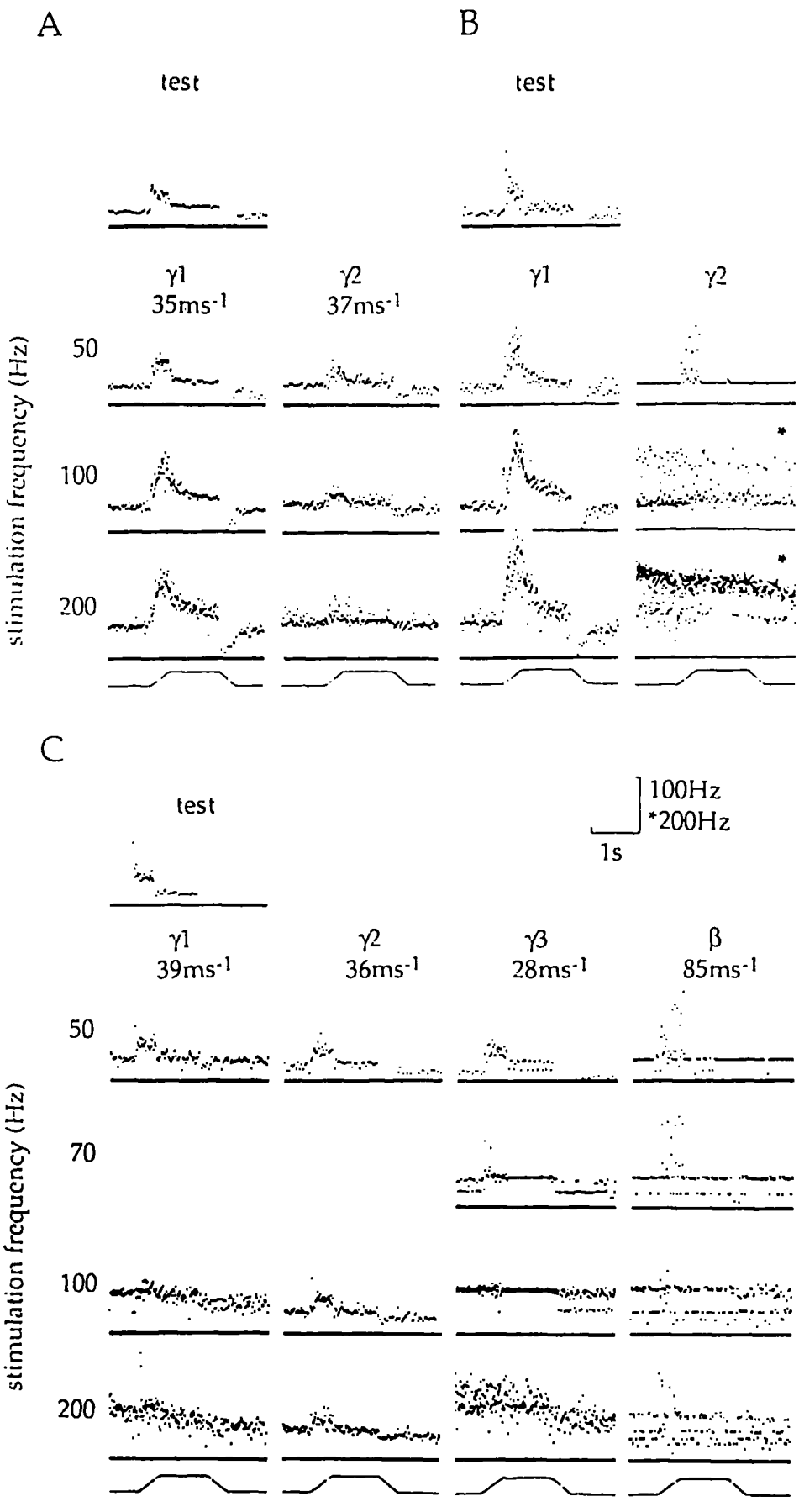


FIGURE 11-13. Responses of primary endings in three cat tenuissimus spindles to ramp-and-hold stretch in the absence (test) and presence (γ, β) of motor stimulation. Responses are shown as instantaneous frequencies above zero baselines; muscle length is indicated at the bottom of each column. *A.* Shows the effects of dynamic (γ1) and static (γ2) fusimotor stimulation on a single primary ending. *B.* Shows the effects of stimulating the same fusimotor axons as in *A*, but acting on a different primary ending. Gamma 1 is again dynamic and γ2 static, but whereas γ2 biases the primary in *A*, it drives the primary in *B* at 1:1. Subsequent histological analysis indicated that the static γ supplied the bag_2 and all the chain fibers of one pole in *A* but only the chain fibers of one pole in *B*; the dynamic γ supplied one pole of the bag_1 fiber in each case. *C.* Shows the effects of stimulating the complete static input to a single spindle, which, on histological analysis, proved to be segregated to the bag_2 and chain fibers, one of which possessed a long pole. γ1 and γ2 were identified with axons that supplied one pole each of a bag_2 fiber. Note the biasing effect as in *A*. γ3 was identified with an axon that supplied all the chain fibers in both poles except for the single long pole; this was supplied by the β axon. Note that both γ3 and the β axon can drive the primary 1:1, and that when only a single chain pole is active, high rates of stimulation lead to preferential primary discharge at a series of subharmonic (1:2, 1:3, 1:4, 1:5) frequencies. *(Banks.[158] Reproduced by permission.)*

axon under these conditions activates the bag_2 fiber, chain fibers (long chain only if static β), or both, and again there is an increase in the static firing level and the initial burst is abolished. In this case, however, the slope of the dynamic response is usually unaffected so that, although the actual dynamic response remains constant during static stimulation, the dynamic index is usually reduced. The length sensitivity is variably affected, but when several static axons are simultaneously stimulated it is generally increased.[123,184]

Some static γ axons elicit 1:1 driving of the primary response over a range of fusimotor stimulus frequencies, typically 50 to 100 Hz, whereas at higher stimulus frequencies very irregular, high mean frequency responses are produced. These effects are undoubtedly due to chain fibers,[120,203] probably active alone.[159] Bag_2 activity results in biasing of the primary response, but driving does not occur, even at subharmonic rates.[159,203] According to Banks,[159] bag_2 activity can partially or completely occlude the effect on the primary ending (though not the secondary) of simultaneous chain action in the same pole.

The effects of static fusimotor stimulation on secondary endings are qualitatively similar to those on primary endings, though driving does not usually occur. Dynamic axons rarely have any effect on secondary endings, reflecting the relatively sparse supply of secondary terminals to bag_1 fibers.

The behavior of the primary ending described above suggests that it is measuring the length (length sensitivity), velocity (dynamic index), and perhaps acceleration (initial burst) of the muscle in which it occurs, and this interpretation is usually found in introductory accounts of the muscle spindle. If this were so, it would be a simple matter to describe the output of the primary ending mathematically by a linear transfer function.[204] However, the sensitivity to velocity declines rapidly with increasing velocity,[205] and for large-amplitude stretches there is no particular response to acceleration.[206] Furthermore, the use of sinusoidal stretches has shown that the primary ending is about 20 times more sensitive to small-amplitude stretches (up to 0.1 mm for cat soleus)[207] than to larger stretches. These complications make the fitting of a linear transfer function more difficult, though various attempts have been made, ranging from power functions[208] to a second-order relation.[209]

Nonlinear functions give better fits to the observed behavior,[210] but it seems necessary to suppose that there is more than one site of impulse initiation, such that their individual outputs may be variously summed or occluded. This phenomenon of *pacemaker switching* is to be expected in a branched axonal system.[211] Evidence that it occurs in mammalian spindles was first presented by Crowe and Matthews;[212] it has since been extended by Hulliger and Noth[115] and has been modeled on the basis of an actual branching pattern by Otten et al.[116]

Input-Output Conversion

How does the spindle transducer work, and what may be the cause of its nonlinearities? Hunt and Wilkinson[118] analyzed receptor potentials recorded from afferent axons whose impulse activity had been blocked with tetrodotoxin. The responses of primary and secondary endings were essentially similar, the main difference being the greater sensitivity of the primary endings. Moreover, the overall tension of these isolated spindles showed similar nonlinearities in response to sinusoidal amplitude and frequency, as did the receptor potentials.

The receptor potentials are generated mainly by an influx of Na^+ into the sensory terminals,[117] and we have suggested[39] that the permeability of the Na^+ channels is affected by an intracellular messenger (probably Ca^{2+}) released from a bound state by the rise in cytoskeletal tension that accompanies bulk deformation of the terminals. As a first approximation, we may further suppose that the receptor potential is linearly related to the longitudinal tension through the terminals, this being locally modified by such elements as the elastic fibers that insert into the juxtaequatorial regions of bag fibers, and thus differs, particularly in phase, from the overall tension (see "Ultrastructure," under "Intrafusal Muscle Fibers," above).

The principal cause of the nonlinearities in the sensory responses may then be sought in the mechanical properties of the intrafusal muscle fibers on the basis of the cross-bridge model of muscle activation, as has now been successfully modeled by Schaatsma et al.[20] (For a counter argument see Kruse and Poppele[212a].) Cross-bridges may be regarded as elements of low compliance whose breakage under tension is manifested as viscosity.[213,214] The number of cross-bridges at any one time will be a function of the rate at which they are being formed, the rate at which they spontaneously break, and the rate at which they break under tension. Each of these factors will have an associated time constant, the actual value of which will vary according to muscle fiber type, particularly in the case of the first two. The bag_1 fiber is especially interesting on account of its peculiar properties, which include the presence of tonic myosin, and the discrepancy between the locations of its motor endings and the convergent sarcomere movements seen during the stimulation of dynamic motor axons.[131] If the bag_1-fiber pole normally has a large number of cross-bridges, activation may produce only a modest shortening restricted to the region with M-line sarcomeres, whereas the stiffness will increase. The time course of the shortening[120] may be much longer than that of the activation giving rise to it[215] because of series viscoelastic components.

The prominent dynamic response of the primary is thus seen as a consequence of the stiffness of the bag_1 fiber. Exposure of potential cross-bridge sites by breakage of cross-bridges under tension will automatically lead to an increased rate of cross-bridge formation, recognizable as stretch activation,[143,216] that tends to maintain the muscle fiber stiffness. The importance of stretch-activation in the generation of the dynamic response is, however, still debated. At the peak of a ramp stretch, the longitudinal tension in the primary terminals on the bag_1 fiber may fall abruptly, perhaps aided by the effectively in-parallel elastic fibers, to be followed by the compensatory length change ("creep"[112,217]) with a longer time course. As the output of the terminals on the bag_1 fiber falls below that of the terminals on the bag_2 and chain fibers, the common pacemaker becomes predominantly influenced by the latter.

The mechanical properties of chain fibers are diametri-

cally opposed to those of the bag_1 fiber in that they show lack of stiffness, low viscosity, and rapid overt contraction. They can be accounted for by assuming that chain fibers have a small number of cross-bridges with a rapid turnover as a result of a high rate of spontaneous breakage. Static fusimotor activation leads to shortening of the chain fiber poles with little, if any, increase in stiffness. Because of the lack of viscosity, small fluctuations in polar length are readily transmitted to the equatorial region and the primary response can be "driven" at the static fusimotor stimulation rate over a wide range of frequencies.[120,159]

Reinnervation and Recovery

Despite the obvious importance of the muscle spindle as a proprioceptor, studies of its reinnervation and recovery after nerve injury were hampered for a long time by insufficient knowledge of the normal receptor. Since this began to be significantly advanced in the seventies, there have been numerous studies, including those concerned with reinnervation and recovery after ischemia.

The reinnervation of skeletal muscle that follows nerve crush results in restoration of function to the spindles[218,219] and complete recovery of stretch reflexes, such as the knee jerk.[220] Recordings of the responses of regenerated spindle afferents to ramp-and-hold stretch, and to fusimotor stimulation, show that they eventually return to normal, though during the early stages of recovery many of the afferents respond only phasically,[218,219] probably because of a temporary increase in pacemaker thresholds.[219] Histological observations have established that both primary and secondary endings are restored to their normal sites in the spindles, though their axons regenerate more slowly than those involved with the motor reinnervation,[221–223] and some secondaries show signs of growth through the primary region apparently designed to establish terminals in the opposite pole.[224]

The primary endings that regenerate after nerve crush are shorter than normal and have fewer transverse terminal bands around the nuclear bags.[224] Even when the interval between nerve crush and the beginning of reinnervation (the denervation period) is reduced to a minimum, the regenerated primary endings are shorter and have about half the normal number of transverse bands.[225] If the nerve crush is repeated at intervals so as to prolong the denervation period up to a maximum of 134 days, the bands become increasingly replaced by tapers, and spirals become increasingly restricted to chain fibers.[226] Remarkably, most of these abnormal endings are capable of giving a normal pattern of response to ramp-and-hold stretch. Barker et al.[226] found that even after a denervation period of 134 days, 73 percent of the regenerated primaries responded normally. These endings had virtually no banding and were restored to severely atrophied spindles in which the periaxial space was greatly reduced or absent. This finding has favorable implications for a patient who has suffered injury to a limb nerve, since the average delay between injury and recovery in such cases is 91 days after crush and 112 days after section and suture.[227] The effects of such denervation periods on the spindles would be unlikely to affect the pattern of the response to stretch of any primary or secondary afferents that succeeded in reinnervating them, though the quality of their response might be impaired. It seems inevitable that the greatly reduced endings that form after such denervation periods should be less sensitive to minute stretches than normal endings. Scott[228] has recently shown that the overall action of their dynamic system is depressed compared with normal.

The afferent reinnervation of spindles after nerve crush is highly specific, since most of the endoneurial tubes are left intact and guide the regenerating axons back to their original sites of termination. But after nerve section, any type of muscle afferent may presumably participate in spindle reinnervation, and this raises the question of whether nonspecific afferents can do so successfully and be distinguished in any way from specific afferents. During the eighties, these problems were addressed by Munson and his colleagues[229–231] and Barker and his colleagues[232–236] in parallel studies on cat hindlimb muscles that used different approaches but reached similar conclusions. Munson and his colleagues recorded the presence or absence of field potentials generated in the appropriate motoneuron pool by activated stretch afferents; they concluded that random population of both specific (Ia, spindle II) and nonspecific (Ib) afferents reinnervate spindles after nerve section.[231] Banks and Barker[234] devised cross-union experiments in which nonspecific afferents (Ib, free-ending, pacinian, cutaneous) were given the opportunity of reinnervating spindles in the absence of their specific afferents. They also found that Ib afferents could reinnervate spindles. They showed that these afferents could form endings in sites originally occupied by the terminals of Ia or spindle II afferents and respond to stretch like normal Ia and spindle II afferents. Cross-unions between cutaneous and muscle nerves showed that free-ending afferents, which are common to both, can also reinnervate spindles. They do so by branching extensively and terminating within the axial sheath. Spindles reinnervated after such cross-unions gave highly abnormal phasic responses to stretch.

There has been no detailed study of the motor reinnervation of spindles after nerve section, though it is known that functional β and γ axons are restored,[218,237] and that the β reinnervation is established before the γ after both crush and section.[218,223] Brown and Butler[218] found that β axons occurred more frequently than normal in peroneus longus after nerve section, but Scott[238] reported that there was no increase after nerve section in the normal proportion of β axons supplied to peroneus tertius. It may be that since β axons arrive back at the spindle earlier than γ axons, they are able to acquire a larger share of the motor innervation. This would account for Banks and Barker's[234] observation that p_1 plates occurred frequently in peroneus brevis spindles after nerve section, whereas p_2 plates were only rarely seen. They were unable to find any functional dynamic γ axons, despite making a specific search for them.

Quantification of the afferent reinnervation of spindles that occurs following nerve section does not encourage the expectation that a good quality of proprioceptive feedback is likely to be restored. After section and microsuture of the musculocutaneous common peroneal nerve, Banks and

Barker[234] found that 21 percent of spindles (n = 139) in cat peroneus brevis were reinnervated by Ia afferents and 20 percent by Ib afferents. Following partial section involving muscle nerves only (those to peroneus brevis and tertius), 27 percent of spindles (n = 109) were reinnervated by Ia afferents and 20 percent by Ib afferents. As compared with the estimated normal afferent innervation of these spindles, the deficits in reinnervation by Ia and spindle II afferents were 79 percent Ia and 86 percent II after complete section, and 73 percent Ia and 56 percent II after partial section. The appropriate afferent reinnervation of spindles is further reduced when grafts are used to repair nerve injuries. Barker et al.[234a] found that after graft repairs of 10 to 15 mm defects in the nerves to cat peroneal muscles, restoration of Ia endings to spindles averaged from 3 to 15 percent. Despite fair motor recovery, application of small-amplitude vibratory stimulation in decerebrate preparations showed that the monosynaptic (Ia) stretch reflex was absent in peroneus brevis after same-nerve autograft repair. This was attributed to insufficient Ia endings and mismatched, but functional, afferent reconnections. Against this background it has to be said that the chances of good afferent control of skeletomotor functions being restored after nerve section are remote. Indeed, in human terms it can be argued that the limb that is accidentally severed from an adult is best not reunited with its owner but replaced artificially.

The prolonged period of ischemia that follows the devascularization of a muscle results in the degeneration of all except the most superficial muscle fibers. Repair and recovery are achieved as revascularization occurs and muscle fibers regenerate. During these events the basal laminae of the muscle fibers remain as intact tubes within which satellite cells become activated and develop into myoblasts and myotubes. Similarly, though there is intramuscular degeneration of motor and sensory axons and their endings, the endoneurial tubes are left intact and serve to direct neural regrowth along the original pathways. Details of these processes as they occur in muscle spindles have been described by Rogers and Carlson,[239] Rogers,[240] and Diwan and Milburn[73] (see review by Barker and Milburn[241]). Since regenerated spindles vary considerably in structure and innervation, Diwan and Milburn[73] classified them into four groups according to degree of abnormality. Afferents establish terminals on intrafusal muscle fibers in groups 1 to 3 but not in group 4, which includes spindles that consist of encapsulated extrafusal-type muscle fibers. The differences between the regenerated spindles are the outcome of the reinnervating axons arriving back at different stages during intrafusal myogenesis.

Barker and Scott[242] investigated the sensory reinnervation and recovery of regenerated spindles in cat extensor digitorum longus at various times following its devascularization. They estimated that about half the original spindle population (53 to 83, mean 69) was lost owing to persistent ischemic necrosis, that 30 percent regenerated and acquired functional afferent connections, and that the total loss of spindle afferents was over 60 percent. Most of the afferents (88 percent) that reinnervated group 1 to 3 spindles responded normally to ramp-and-hold stretch, although their firing rates were lower and the terminal bands in their primary endings were reduced to 70 percent of normal. In other experiments on this muscle,[243] the vascular supply was left intact, but its nerve and tendons were cut and microsutured, thus creating an orthotopic muscle graft. Recovery was compared with that in other experiments in which the muscle had been subjected either to 1.5 h warm ischemia or to 4.5 h cold ischemia while being maintained at 4°C. The intention in the former case was to reproduce the clinical conditions obtaining during an average muscle transplant operation and in the latter case to reproduce the conditions that obtain after the total or near total amputation of a limb and its subsequent reimplantation. In both cases it was found that the periods of ischemia had no significant effect on the quality of recovery compared with that following section and suture of the nerve and tendons alone. In all three cases, about 63 percent of the spindles were reinnervated by afferents that formed annulospiral endings. Physiological tests showed that 75 percent of the functional afferents responded normally to muscle stretch and that static and dynamic γ efferents were also functional.

Autonomic Innervation

For many years it was widely held that skeletal muscle fibers are not innervated by sympathetic axons. It was acknowledged that such axons enter muscle spindles, but opinion was divided as to whether they supplied a vascular or nonvascular innervation. In 1981 Barker and Saito[14] demonstrated unequivocally that an autonomic innervation is distributed to some extrafusal muscle fibers and that it also has a nonvascular presence in some spindles. Earlier claims made in favor of an intrafusal autonomic innervation by Banker and Girvin[244] and Santini and Ibata[245] were thus substantiated and an observation by Ballard[246] confirmed.

Barker and Saito[14] made most of their observations on cat hindlimb muscles deprived of their somatic innervation by degenerative spinal-root section and then prepared for fluorescence, silver staining, or electron microscopy. They found that autonomic axons were not supplied to all spindles; in the muscles sampled, the proportion of spindles receiving autonomic axons was highest in the lumbricals (65 percent) and lowest in peroneus brevis (8 percent). Autonomic axons were absent from 18 tendon organs examined. Fluorescence microscopy revealed a noradrenergic innervation distributed to some spindles by axons supplied either through the spindle nerve or from nearby perivascular nerves, which they left to enter the equatorial and polar regions at various points. Branches of perivascular axons were also occasionally seen ending among extrafusal muscle fibers (Fig. 11-14). It was impossible to determine whether any noradrenergic axons were exclusively distributed to spindles or extrafusal muscle fibers.

On the basis of the vesicle content of varicosities examined by EM, the extrafusal innervation was identified as noradrenergic (32 axons traced) and the spindle innervation as involving noradrenergic, cholinergic, and nonadrenergic axons (14 traced). Varicosities were located within the capsule lamellae, inside the periaxial space, and in neu-

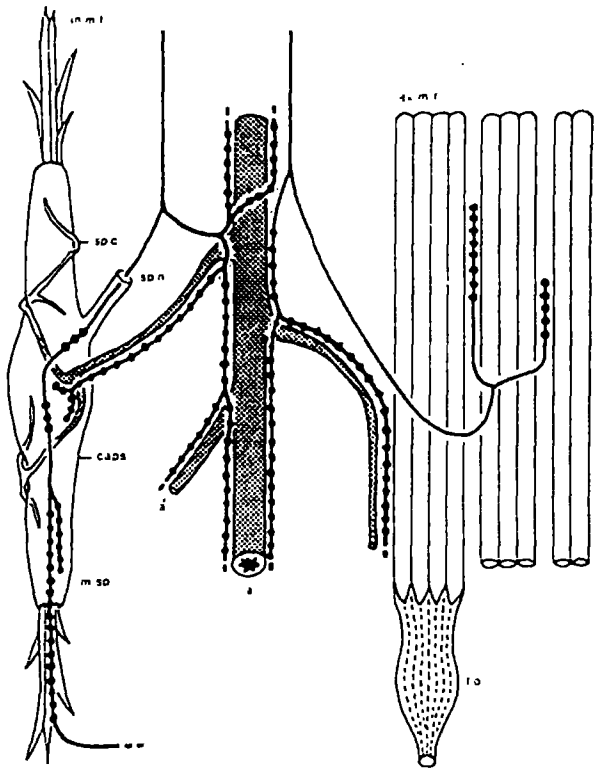


FIGURE 11-14. Schema of the noradrenergic autonomic innervation of cat skeletal muscle. The distribution of the varicosities of two noradrenergic axons is shown. Note varicosities among both intra- and extrafusal muscle fibers as well as those lying between arteriole and muscle fiber. a = artery; a' = arteriole; caps. = capsule; ex.m.f. = extrafusal muscle fibers; in.m.f. = intrafusal muscle fibers; m.sp. = muscle spindle; sp.c. = spindle capillary; sp.n. = spindle nerve; t.o. = tendon organ. *(Barker and Saito.[14] Reproduced by permission.)*

roeffective association with intrafusal muscle fibers (without preference for fiber type) in regions B and C.

In a study of rat deep masseter, Barker and Saed[247] found that about 30 percent of its spindles received an adrenergic innervation. It was always located equatorially within the capsule wall and usually originated from adjacent perivascular axons. Barker and Saed estimated about 40 percent of masseter extrafusal muscle fibers were adrenergically innervated, compared with 55 percent in anterior digastric.

Effects of Sympathetic Stimulation

The histological evidence demonstrating the presence of a nonvascular autonomic innervation in some spindles prompted Passatore and Fillipi[248,249] and Hunt et al.[250] to study the effects of sympathetic stimulation on the discharge of spindle afferents. The responses are variable and complex. At the shortest latency (0.6 to 2.5 s), 40 percent of rabbit jaw-muscle spindle afferents decreased their firing rate during repetitive stimulation of the cervical sympathetic nerve.[249] This does not seem to be due to a reflex inhibition of fusimotor tone[249,250] but could, perhaps, be due to a direct effect on the afferent axons or endings. We have some unpublished evidence in favor of this in the form of a typical autonomic varicosity lying close to a node of Ranvier on a spindle secondary afferent.

Somewhat later (8 to 15 s in rabbit jaw muscles,[248] a few seconds in the cat hindlimb[250]), a weak excitation is shown by some spindle afferents. This is now recognized by all the investigators to be due to a direct effect on the intrafusal muscle fiber.[251] It is simultaneously accompanied by a development of tension in the rabbit jaw-closing muscles, maximally 5 g.[251] Passatore et al.[252] attribute this as being mainly due to intrafusal contraction, but it seems most unlikely, on the basis of studies on isolated spindles,[112] that the number of intrafusal muscle fibers present in rabbit jaw muscles could produce tension of that order.

In the curarized cat hindlimb a reduction in the Aβ component of the compound action potential produced by stimulating the cut sciatic nerve at increasing frequencies was attributed by Grassi et al.[253] to collisions caused by excitation of secondary endings arising from sympathetically activated intrafusal fibers. However, Petit et al.[254] were able to show that this was an artifact and that sympathetic stimulation had no detectable effect on the Aβ wave.

On prolonged stimulation, the direct intrafusal excitation is transitory and is followed after about 30 s by a second excitation of vasomotor origin.[250] Hale et al.[255] and Hale and Kidd,[256] using a preparation in which the rat's tail was maintained independently of its blood supply, found that reflex sympathetic activity, induced by carotid occlusion or nitrogen breathing, led to a long-lasting increase in the regularity and firing frequency of spindle primary afferents[256] and that superfusion with adrenaline produced a similar excitation that lasted as long as the adrenaline was present.[255] The transitoriness of the early excitation seen in cat spindles by Hunt et al.[250] could thus be a further consequence of vasoconstriction. However, all these effects are very weak and unlikely to be functionally significant. Hunt et al.[250] looked for an antifatigue effect of sympathetic stimulation on intrafusal muscle fibers comparable with the classical Orbeli effect on extrafusal muscle fibers, but one was not clearly present. The significance, if any, of intrafusal autonomic innervation thus remains to be elucidated.

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Paper 48.

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Intrafusal motor innervation: a quantitative histological analysis of tenuissimus muscle spindles in the cat

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ABSTRACT

A quantitative analysis of the motor innervation of intrafusal muscle fibres is described, based on teased silver-impregnated spindles of the tenuissimus muscle of the cat. Included in the analysis are the number and distribution of intrafusal branches of both skeletofusimotor (β) and purely fusimotor (γ) axons, and the form of their endings. The number of axonal branches per spindle was found to follow binomial probability distributions, as had previously been shown for the afferent axons. There was a strong correlation between the numbers of γ intrafusal branches and afferent axons, but none for the intrafusal branches of β axons. The degree of segregation of γ input to bag_2 and chain fibres was assessed and was found, among other things, to be related to the presence of secondary sensory endings in the same pole. In this and other respects it did not appear to have the properties that would be expected if independent activation of the bag_2 and chain fibres were to be functionally important. Morphometric analysis of the motor endings supplied to bag_2 or chain fibres by γ axons revealed some differences between those of intrafusal branches with segregated as opposed to unsegregated distributions, but this cannot be taken as evidence of more than one type of static γ motoneuron because of the likely contribution of other influential factors such as fibre size. Finally, the relevance of studies on intrafusal motor innervation to the concept of the motor unit and its development are discussed.

Key words: Fusimotor axons; skeletofusimotor axons; intrafusal muscle fibres; motor unit development.

INTRODUCTION

The almost invariable presence of 3 types of intrafusal fibre in mammalian muscle spindles stands in marked contrast to the highly variable pattern of their innervation (Barker & Banks, 1994). Indeed, virtually the only constant feature of the innervation is the primary sensory ending, which is usually supplied by a single group Ia axon. Secondary sensory endings, derived from group II axons, may or may not be present. Whether these additional afferents occur in a particular spindle, and how many, appear to be randomly determined (Banks & Stacey, 1988). The motor supply may be variously provided by: (1) motoneurons with an exclusively intrafusal distribution, forming a distinct fusimotor or γ system; (2) collateral branches of skeletomotor (α) axons, which, purely for convenience, we may continue to call the β supply (Banks, 1994); or (3) both γ and β axons. Besides variations in their source of supply, the number of motor axons entering a spindle, and their precise intrafusal fibre distribution, may also vary.

Functionally there are 2 classes of intrafusal motor action, defined as dynamic or static according to their effects on primary ending responses to stretch (Matthews, 1962). Each parent axon normally innervates several muscle spindles, and has the same functional effect on them all. Dynamic axons, whether γ or β, invariably supply one type of intrafusal muscle fibre, the bag_1 (b_1), whereas static γ axons supply the 2 remaining types, bag_2 (b_2) and chain (c) fibres, with a differentially distributed input related to axonal conduction velocity. At least in the tenuissimus and other hind limb muscles of the cat, static β axons selectively innervate c fibres, particularly the so-called long chain (lc) fibres whose contractile polar regions extend well beyond the ends of the spindle capsule. Frequently, however, only one pole is long and β-innervated so lc fibres should probably not be thought of as distinct from other, typical, c fibres. A

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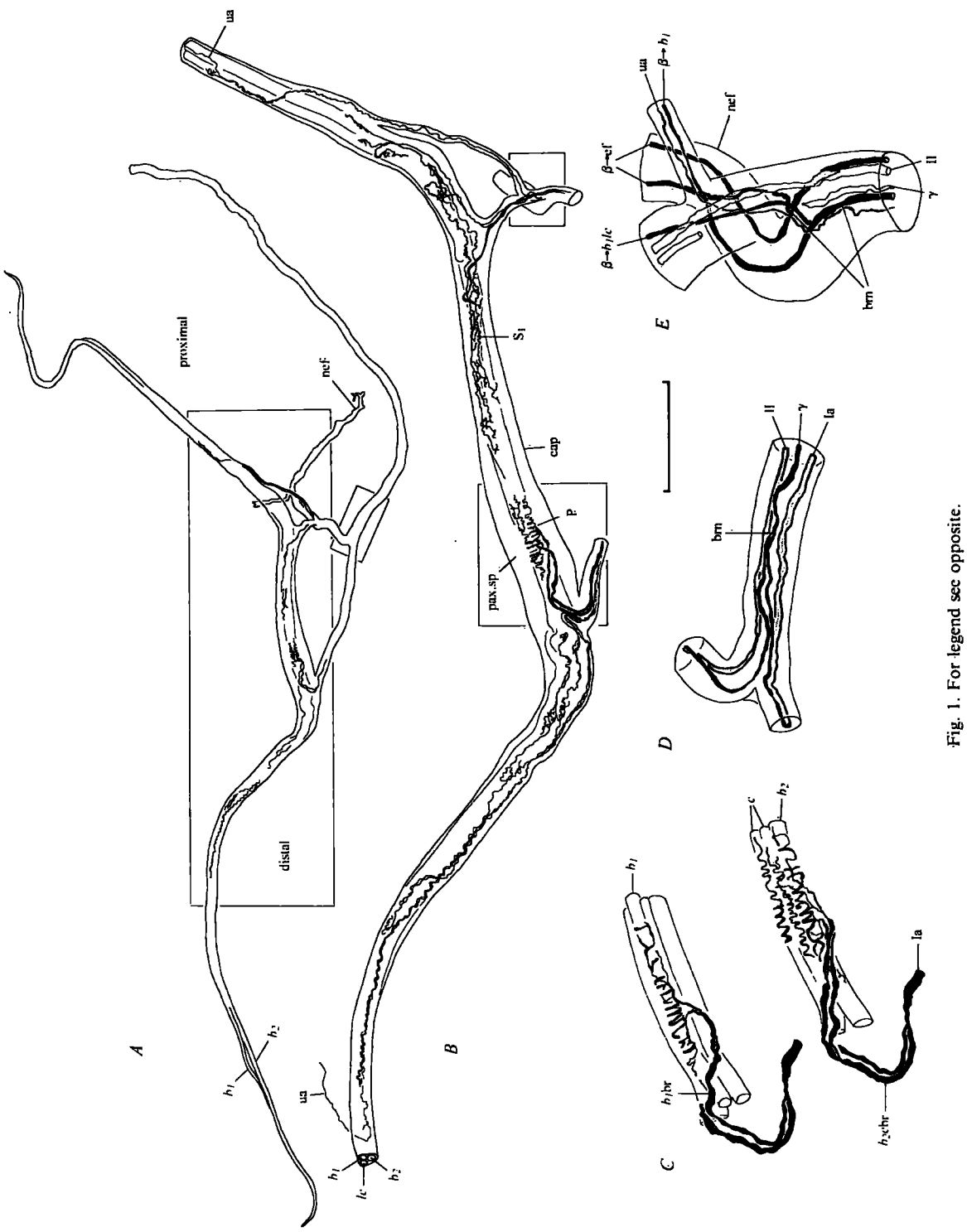


Fig. 1. For legend see opposite.

small, though not insignificant, proportion of β axons is distributed to both b_1 and *lc* fibres in some spindles (see Banks, 1994, for a review on intrafusal motor distribution).

This paper is complementary to a recent study, based on physiological mapping (Banks, 1991*a*), on the distribution of static γ axons within and between muscle spindles of the cat, which was itself a test of Boyd's (1986) proposal that there are 2 types of static γ motoneuron. These he called 'static bag γ-motoneurons' and 'static chain γ-motoneurons', supposing them always (though not exclusively) to supply b_2 or *c* fibres respectively. It was my conclusion, however, that the observed distribution of static γ axons could be accounted for by a single population of motoneurons, though one that showed some evidence of differential distribution in relation to axonal conduction velocity (Banks, 1991*a*).

In that earlier study the interpretation of the physiological results was made possible by a correlated histological analysis, but the importance of the histological data as a whole proved to be such as to warrant their separate description and consideration. In particular, quantitative analysis of the histology revealed: (1) a correlation between the provision of fusimotor (γ) and sensory innervation, but not between that of the intrafusal part of the skeletofusimotor (β) supply and the sensory innervation; and (2) a relationship between the presence of secondary sensory endings and the degree of segregation of the static γ supply within spindles. In addition to their relevance to problems of motor control the results illustrate the interplay of random and deterministic factors that may be presumed to occur in the developmental construction of a spindle and, indeed, of motor units in general.

The quantitative analysis also includes morphometric details of the intrafusal motor endings, for 2 reasons: first, since it was part of Boyd's (1986) proposal that the 'static bag' and 'static chain' γ motoneurons show differences in details of their endplate structure; and secondly because the information was required to help to distinguish between the intrafusal branches of γ and β axons.

The possibility of regional variation in the pattern of innervation within a muscle, already attested in a study of the afferent supply (Banks & Stacey, 1990), indicates that more attention needs to be paid to the sampling of spindles than hitherto. Since one is loath to ignore hard-won data in order to obtain a random sample, the alternative, when possible, of complete analysis of whole muscles is more attractive. This has effectively been achieved for the first time in the present work for one muscle, designated C883. After an overall description of the intrafusal motor innervation of this muscle, and a consideration of the significance of axonal routing and collateral branches to the problem of the identification of the β input, it is necessary for the logical development of the results to examine motor ending morphology in detail before turning to the correlative observations.

Preliminary accounts of the work have already been published (Banks, 1991*b*, 1992) and some of the results have been incorporated in a recent review on the motor innervation of the mammalian muscle spindle (Banks, 1994).

MATERIALS AND METHODS

Most of the data presented below were obtained from the tenuissimus muscle of the left hind limbs of 4 adult cats (1.8–2.8 kg) of both sexes that had been used in acute physiological experiments. When necessary the muscles will be identified individually in this paper by the corresponding experiment, C637, C689, C700 and C883. The physiological preparation has been

Fig. 1. Camera lucida drawings of a spindle (spindle 7, experiment C883), to illustrate the interpretation of teased silver-impregnated material. (*A*) Overall view of the spindle and its nerve supply. Much of the curvature in the specimen is artefactual. (*B*) Enlarged view of the large box in (*A*) showing the entire innervated portion of the spindle. The somatic motor supply was provided by not more than 6 axons, only 1 of which was identified as purely fusimotor (γ). In addition to the somatic sensory and motor axons, each pole was traversed by a branch of an unmyelinated axon, probably sensory, that was principally distributed extrafusally. (*C*) Enlarged view of the large box in (*B*) to show the bag_1 (upper) and bag_2 and chain (lower) portions of the primary sensory ending, including the preterminal branches of the Ia axon. The bag fibres can be traced individually throughout the length of the spindle and, as here, are usually identifiable in this region. Sensory terminals are more closely spaced on bag_1 than bag_2, and are separately supplied by 1 of the 2 main divisions of the Ia axon. (*D*) Enlarged view of the small box in (*A*) to show the division of the single (static) γ axon into branches that entered the proximal and distal poles along with the II and Ia afferents respectively. Other motor axons present here are not shown. (*E*) Enlarged view of the small box in *B* to show the division of each of 2 parent motor axons into an intrafusal and an extrafusal branch thus providing clear evidence of their skeletofusimotor (β) nature. Bar: (*A*) 500 μm, (*B*) 200 μm, (*C*) (*D*) 100 μm, (*E*) 50 μm. Ia, group Ia afferent axon from primary sensory ending; II, group II afferent axon from secondary sensory ending; β, branches of skeletofusimotor axons; γ, a fusimotor axon and its branches; b_1, bag_1 fibre; $b_1$br, preterminal branch of Ia afferent supplying the bag_1 fibre exclusively; b_2, bag_2 fibre; $b_2$cbr, preterminal branch of Ia afferent supplying the bag_2 and chain fibres; brn, branching node of Ranvier; cap, spindle capsule; ef, extrafusal fibres; *lc*, long pole of chain fibre; nef, nerve to extrafusal fibres; P, primary sensory ending; pax.sp, periaxial space between capsule and intrafusal fibres; S_1, secondary sensory ending; ua, unmyelinated axon.

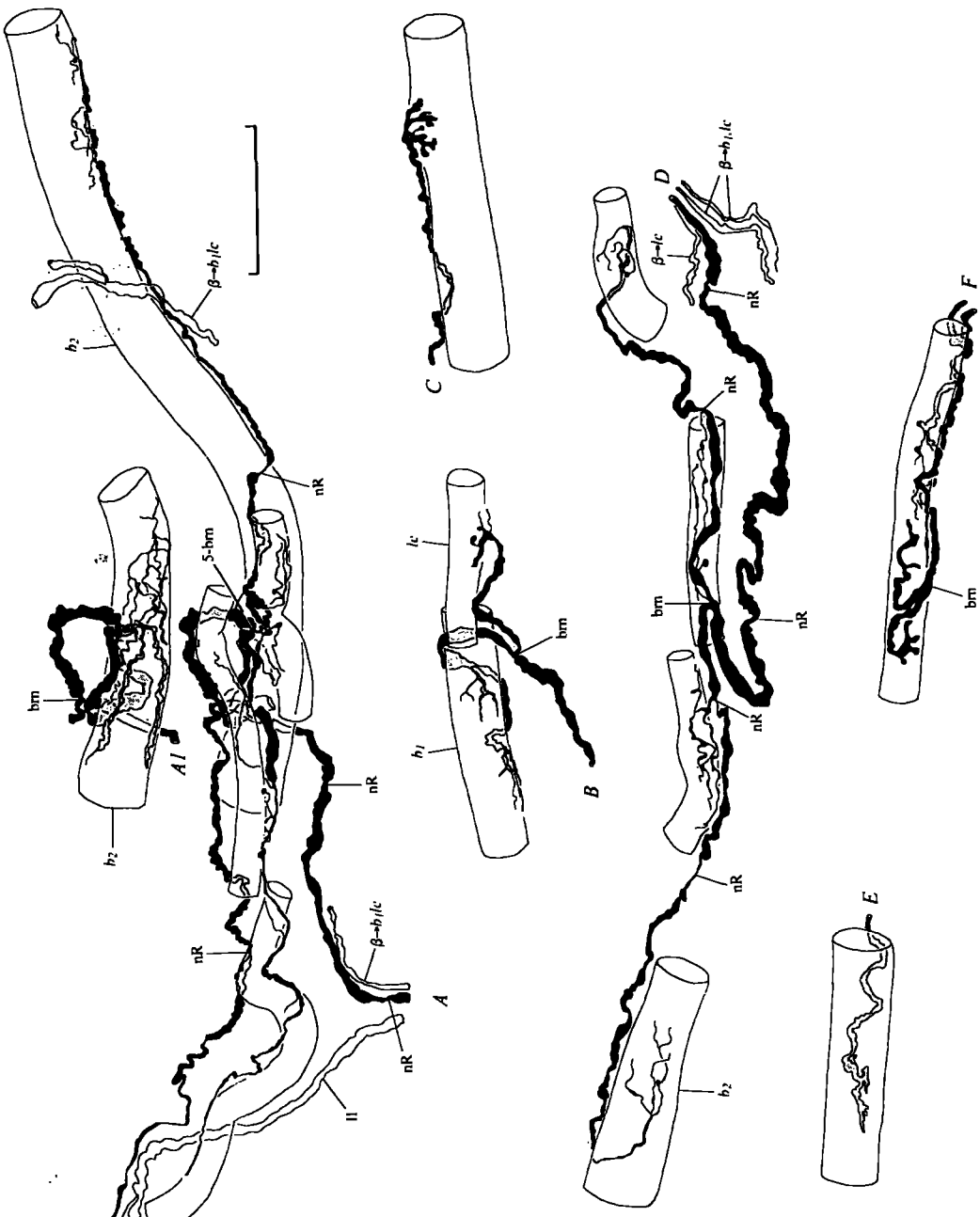


Fig. 2. Camera lucida drawings continuing the illustration of the interpretation of teased silver-impregnated material; details of all the intrafusal motor branches and their endings of spindle 7, C883. Compare with Figure 1 *B*. The letters *A–F* identifying the drawings are placed next to the axonal input of each field of view. (*A*) The proximal branch of the (static γ) axon distributed to bag_2 and chain fibres. There are 2 endings on the bag_2 fibre, one of which, obscured by chain-fibre endings in the main drawing, is shown separately in the inset (*A1*). (*B*) Endings on a bag_1 fibre and the long pole of a chain fibre supplied by a single (β) axon in the proximal pole of the spindle. (*C*) A second (β) axon forming an ending on the proximal pole of the bag_1 fibre. The axons in (*B*) and (*C*) are the same as those with branches to extrafusal muscle shown in Figure 1 *E*. (*D*). The distal branch of the (static γ) axon distributed to bag_2 and chain fibres. (*E*) The ending of the single (β) axon to the distal pole of the bag_1 fibre. (*F*) A group of endings formed by 2 (β) axons on the distal long pole of a chain fibre. Scale bar: 50 μm. 5-brn, pentafurcating node of Ranvier; nR, node of Ranvier; other abbreviations as Figure 1.

described elsewhere (Banks, 1991*a*). At the end of each experiment, the muscle was removed post mortem and processed by silver impregnation according to a modified Barker & Ip method (Barker et al. 1985). The spindles were subsequently teased from the muscle, whenever possible in continuity with the intramuscular nerve. The nerve was also removed in its entirety so as to preserve the relative locations of the spindles, and to provide the maximum opportunity for tracing individual axons.

In those parts of the analysis relating to the degree of segregation of the static γ distribution, additional data were included in order to increase sample sizes for statistical tests. The data were derived from previously published spindles that had been reconstructed from serial 1 μm transverse sections. Only complete spindles whose innervation, including afferents, was fully characterised could be used. Those satisfying this criterion were published by Banks (1981; GS 6, 9 & 12) and by Kucera et al. (Kucera & Hughes, 1983, figs 3, 4; Kucera, 1984, fig. 1; Kucera et al. 1984, fig. 1).

An example of the analysis of the teased spindles

It will be sufficient to illustrate the analysis of the whole-mount preparations with one spindle, so as to demonstrate the confidence that can be placed in the methods used in axonal tracing and recognition of intrafusal fibre types. The spindle selected for this purpose was the 7th in proximal-to-distal sequence from muscle C883. In this, as in silver-impregnated spindles generally, it was possible to identify the 3 types of intrafusal muscle fibre by details of the primary sensory ending (Banks et al. 1982), and by differences in polar length and diameter. The distribution of elastic fibres, which are predominantly associated with b_2 in the extracapsular polar regions (Gladden, 1976) provided additional evidence. Individual bag fibres were traced from end to end, whereas chain fibres were not, not least because they were considerably kinked immediately distal to the sensory endings, as is usually the case. However, in locating motor nerve endings, there was no doubt as to the identification of a chain fibre. Both proximally and distally in spindle 7, C883, one chain fibre pole was much larger than those of the other chain fibres, and was similar in length and diameter to the bag fibre poles. Each was therefore identified as belonging to a long chain fibre (Barker et al. 1976*a*; Kucera, 1980). It was not clear whether they were the 2 poles of a single fibre, though this is entirely possible since the earliest chain fibre to be formed in development tends to be the longest (Milburn, 1984). Some of the features described above are shown in camera lucida drawings of the preparation (Fig. 1 *A–C*).

Spindle 7, C883, was close to the point of entry to the muscle of the main tenuissimus nerve and its branches: it was supplied separately from the other spindles by one of these branches, which also contained skeletomotor axons. For this and for other spindles whose silver impregnation was sufficiently good, motor axons with intrafusal branches were traced individually using the high resolution and narrow depth of focus provided by a ×100 oil immersion objective on a Nikon Optiphot. High power, detailed drawings were prepared of the motor innervation, omitting only long runs of unbranched axons (Fig. 2). Branching of motor axons in the nerves leading to the spindles normally occurs only at the points where the nerves themselves branch. In the example of spindle 7, C883, the single static γ branch to each pole was traced back to a common parent axon at such a branch point (Fig. 1 *D*). In some cases, intrafusal motor axons could be traced to parent axons that also sent branches into nerves apparently with an exclusively extrafusal distribution, and thus provided strong evidence of β innervation. In C883 spindle 7, there were 2 such examples, both in the proximal pole (Fig. 1 *E*); each supplied the b_1 fibre, but 1 supplied a *lc* fibre in addition.

The criteria used in the morphometry of the motor endings, that is to say the neuronal part of the endplates, followed those of Banks et al. (1985). Data were collected on lengths (L) and total terminal and preterminal lengths (PTL) of the endings as defined in that paper.

RESULTS

General features relating to the number, arrangement and motor innervation of the spindles in a complete tenuissimus muscle, C883

It was possible to carry out a virtually complete analysis of 1 muscle, from which only a small distal portion was missing, by virtue of the high, and unusually even, quality of its silver impregnation. It is convenient first to deal with some general features of its organisation, which will help to place the later detailed description in an overall context. The muscle, C883, contained 19 spindles. In the following description they are identified sequentially by number, beginning at the proximal end of the muscle. Each spindle contained 1 b_1, 1 b_2, and probably 2–5 *c* fibres (b_1b_2c units), except spindles 3 and 19*b* both of which lacked a b_1 fibre (b_2c units). Spindle 19*b* formed the

smaller of 2 units (19*a* and *b*) linked in tandem by a continuous b_2 fibre. In several spindles (1, 2, 5, 6, 7, 9, 10, 14) 1 or 2 of the *c* fibres possessed a long pole.

The nerve subdivided before entering the muscle to form 4 separate intramuscular divisions: a small proximal trunk that innervated spindles 5 to 1 together with the proximal tendon, including a tendon organ; a large distal trunk that innervated spindles 8 to 19 (*a* and *b*); and, located between the points of entry of these trunks into the muscle, 2 very small nerves that provided isolated inputs to spindles 6 and 7. The arrangement is shown schematically in Figure 8 of Banks (1991*a*).

The spindles received a total of 118 motor axonal branches, 115 of which were traced to b_1 (45), b_1lc (1), *lc* (9), b_2 (19), b_2c (23), or *c* (18). The destinations of the remaining 3 branches are unknown since the pole they supplied in spindle 11 was not recovered. The number of branches to individual spindles ranged from 2 to 10, with a mean of 6.4 for the single b_1b_2c units, or 6.2 overall. These are maximum values since apparently separate intrafusal branches innervating a single spindle may have been derived from the same parent axon within the nerve trunks. This was perhaps especially likely when the branches entered the spindle through different routes.

Nature of the supplying axons, C883

Skeletofusimotor (β) innervation of b_2 and *c* fibres (other than long *c* poles) in the cat is acknowledged to be rare by all authors of relevant studies (see review by Banks, 1994), thus the parent axons of virtually all the intrafusal branches supplying b_2 and typical poles of *c* fibres in C883 may be safely identified as purely fusimotor (γ). Conversely, β axons seem to be the usual, if not completely exclusive, source of endings on the long poles of *c* fibres. Although the criteria for identification of long poles are necessarily different in teased material from those used in serial section studies, the motor endings on such poles may be safely identified as those of β axons. Motor branches supplying the b_1 fibres, however, present a problem of identification in that b_1 fibres are commonly innervated by both β and γ axons.

In order to resolve this problem, it may first be noted that the intrafusal motor branches approached their spindles either in association with the afferent axons or in separate fine nerves. The latter sometimes contained only a single axon, and were derived from larger nerves with an otherwise exclusively extrafusal distribution. It seems likely that these separate inputs usually carry branches of β axons, though in the teasing process such fine nerves inevitably break off rather close to the spindle, and therefore the origin of the branches cannot be established with certainty.

Of the 60 intrafusal branches that supplied the b_2 and typical poles of *c* fibres, either separately or in common, 57 could be shown to approach the spindles in association with afferent axons. One of the remaining 3 contributed to 2 small endplates, each 29 μm long, in the distal pole of the b_2 fibre of spindle 14. It is quite possible that this was a rare example of β innervation of a b_2 fibre in a cat spindle. The 2nd also provided 2 endings to a b_2 fibre, in this case in the proximal pole of a b_2c unit, spindle 3. The endings were 50 and 107 μm long, the larger of these being well outside the range of size of p_1 plates of Banks et al. (1985). In itself this is not sufficient evidence to deny the possibility that the endplates were supplied by a β axon, though it seems unlikely. The 3rd branch provided the entire static input to the distal pole of spindle 16. In company with 2 branches that supplied the b_1 fibre, it could be traced to a nerve whose distribution undoubtedly included extrafusal fibres, but not into the nerve containing the afferent axons. In this case, however, there was physiological evidence (Banks, 1991*a*) that it was the branch of a γ axon.

In contrast, there were 55 intrafusal motor branches that supplied the b_1 and long poles of c fibres, and 19 of these (14 to b_1, 5 to *lc*) entered their spindles separately from the afferent axons. Moreover, the larger, and therefore more robust, nerves that contained the afferent and much of the efferent innervation of the spindles often gave rise to branches with otherwise purely extrafusal distributions. In some of these instances, parent motor axons were found with branches both to the spindles and to the extrafusal nerves. Within the limitations of the technique, therefore, these may be positively regarded as β axons. There were 6 such parental axons whose intrafusal distribution could be confirmed, and all supplied b_1 and *lc* fibres or both (4 to b_1, 1 to b_1lc, 1 to *lc*).

If dynamic γ axons were present in C883 they would most probably have been represented among the remaining 27 intrafusal branches to b_1 fibres that entered their spindles in association with afferent axons but did not send daughter branches into extrafusal nerves. The endings of this group of axons would then be enriched with p_2 plates, relative to those of the axons that either had branches in extrafusal nerves or that entered their spindles separately from the afferent innervation. The possibility will be further examined in the following section.

Table 1. *The numbers of intrafusal motor-axon branches and their endings supplied to all complete* b_1b_2c *single-unit spindles in a tenuissimus muscle of the cat (C883)*

Distribution	b_1	b_1lc	lc	b_2	b_2c	c	Total
No. of axons	41	1	9	17	19	15	102
No. of endings	57	3	12	35	94	53	254
Mean no. of endings per axon	1.4	—	1.3	2.1	4.9	3.5	

In this and subsequent tables. *c* signifies typical poles of chain fibres. *lc* signifies long poles of chain fibres.

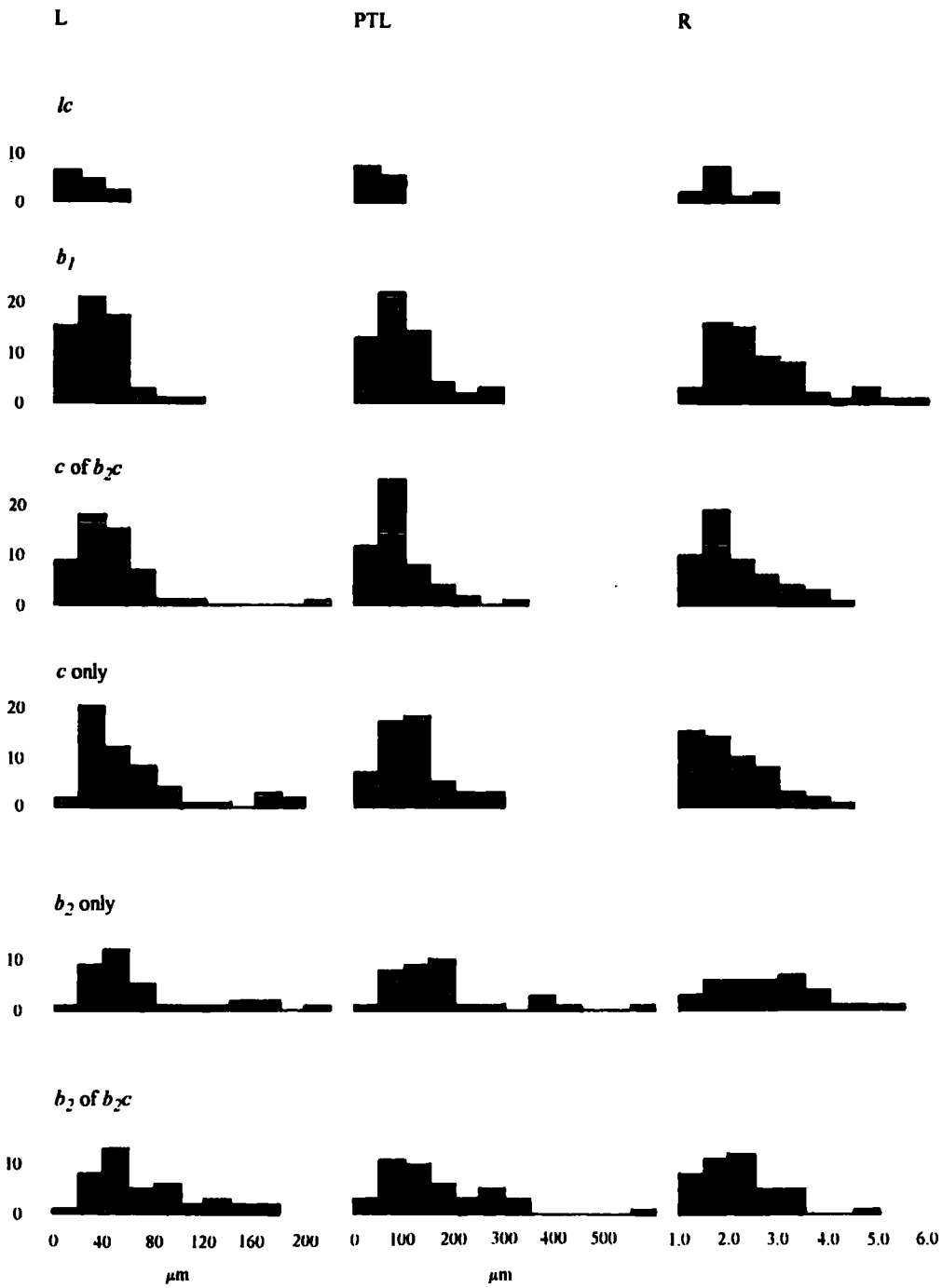


Fig. 3. Histograms showing the lengths (L), total preterminal and terminal lengths (PTL), and ratio (R) of PTL to L of motor endings on the various types of intrafusal fibre. Data relating to endings on bag_2 fibres (b_2) and on typical poles of chain fibres (c) are separately plotted according to the intrafusal distribution of the supplying axons, whether segregated (b_2 only, c only) or unsegregated (b_2 of b_2c, c of b_2c). b_1, endings on bag_1 fibres; lc, endings on long poles of chain fibres.

Table 2. *Summary of morphometric data for 252 intrafusal motor endings of the cat tenuissimus (C883), classified according to parent axon distribution and arranged in order of increasing mean values*

	Range	Mean	Median
Length (L) (μm)			
lc	17–46	25.3	22.0
b_1	9–105	35.9	32.5
c (of b_2c)	8–206	44.3	37.0
c (*c* only)	19–193	63.0	45.0
b_2 (b_2 only)	19–204	68.5	54.0
b_2 (of b_2c)	22–178	72.6	56.5
Total preterminal and terminal length (PTL) (μm)			
lc	25–96	47.3	38.5
c (of b_2c)	19–331	89.0	76.0
b_1	13–287	96.6	83.2
c (*c* only)	38–293	119.2	107.0
b_2 (b_2 only)	32–586	159.9	127.0
b_2 (of b_2c)	38–554	177.0	146.0
Ratio (R = PTL/L)			
lc	1.18–2.63	1.88	1.87
c (*c* only)	1.00–4.17	2.09	2.00
c (of b_2c)	1.00–4.44	2.12	1.87
b_2 (of b_2c)	1.14–4.91	2.20	2.09
b_1	1.11–5.51	2.62	2.40
b_2 (b_2 only)	1.09–5.22	2.76	2.78

Table 3. *Summary of the statistical analysis (ANOVA) of the logarithmically transformed morphometric data given in Table 2. Data are presented here after back-transformation*

	n	Mean	95% confidence limits Lower	Upper
Length (L) (μm)		L		
lc	12	24.0	13.6	42.5
b_1	58	31.2	28.8	33.8
c (of b_2c)	52	36.6	30.3	44.3
c (*c* only)	53	52.5	44.7	61.6
b_2 (b_2 only)	35	56.3	47.0	67.4
b_2 (of b_2c)	42	62.2	53.0	73.0
Total preterminal and terminal length (PTL) (μm)		PTL		
lc	12	43.4	30.3	62.2
c (of b_2c)	52	73.0	60.9	86.5
b_1	58	77.5	66.0	91.8
c (*c* only)	53	102.5	85.6	121.5
b_2 (of b_2c)	42	129.0	116.7	156.0
b_2 (b_2 only)	35	146.9	131.6	181.3
Ratio (R = PTL/L)		R		
lc	12	1.83	1.51	2.23
c (*c* only)	53	1.98	1.80	2.18
c (of b_2c)	52	2.00	1.82	2.19
b_2 (of b_2c)	42	2.07	1.87	2.30
b_1	58	2.48	2.27	2.71
b_2 (b_2 only)	35	2.59	2.33	2.91

Braces connect groups whose means do not differ significantly.

Morphometric analysis of the intrafusal motor endings (C833)

All endings. There were 16 single b_1b_2c units whose innervation was almost certainly complete. They possessed a total of 253 motor endings, 59 on b_1, 77 on b_2, 13 on long poles of *c* and 104 on typical poles of *c* fibres. Each was supplied by a single intrafusal motor branch, except for 1 of the *c* endings (in spindle 8) that was supplied by 2. The mean numbers of endings per pole were thus: b_1, 1.8; b_2, 2.4; long poles of *c*, 1.2; and typical poles of *c*, 0.8. For the typical poles of *c* fibres the value is only approximate, reflecting the uncertainty in the precise numbers of *c* fibres present. The quoted value corresponds to an average of 4.5 fibres per spindle, which is the total value including *lc* fibres given by Kucera (1982).

The numbers of motor axon branches entering these spindles are shown in Table 1, where the branches are classified according to their intrafusal fibre destinations. The total and mean numbers of their endings are also given, from which it may be seen that those branches distributed to both b_2 and *c* fibres supplied the greatest number of endplates both absolutely and per axon.

Morphometric data from virtually all the endings were obtained for each of the 2 measurements of length (L), and total preterminal length (PTL), and for the ratio (R) of PTL/L. Two endings, 1 each on a b_1 and a *lc* fibre, were omitted due to poor silver impregnation, whereas the terminals of the 2 axons supplied to a single *c* endplate were accounted separately. The motor endings were divided into 6 groups corresponding to the distributions of their parent axons: (1) all endings on long poles of *c* fibres, (2) all endings on b_1 fibres, (3) endings of axon branches supplied exclusively to b_2 fibres, (4) endings on b_2 fibres of axon branches supplied to both b_2 and *c* fibres, (5) endings on *c* fibres of axon branches supplied to both b_2 and *c* fibres, and (6) endings of axon branches supplied exclusively to typical poles of *c* fibres. Since only a single intrafusal motor axon branch supplied a b_1 and an *lc* fibre in common, its endplates were included in groups (1) and (2) as appropriate.

The data are presented as histograms in Figure 3 and are summarised in Table 2. In most cases the frequency distributions are significantly different from normal, often showing both skewness and kurtosis; therefore, in order to carry out the statistical analysis, the data were transformed logarithmically. This resulted in distributions that did not differ significantly from normal in all categories except that of length (L)

Table 4. *Correlation analysis of the interrelationships between L, PTL, and R for intrafusal motor endings in cat tenuissimus*

	r	*P*	r^2
Correlation of L and PTL			
lc	0.8	< 0.01	0.64
b_1	0.84	< 0.01	0.71
b_2 (b_2 only)	0.85	< 0.01	0.72
b_2 (of b_2c)	0.85	< 0.01	0.72
c (of b_2c)	0.88	< 0.01	0.77
c (*c* only)	0.85	< 0.01	0.72
Correlation of L and R			
lc	−0.04	n.s.	0.002
b_1	0.13	n.s.	0.02
b_2 (b_2 only)	−0.28	n.s.	0.08
b_2 (of b_2c)	0.01	n.s.	0.0001
c (of b_2c)	−0.22	n.s.	0.05
c (*c* only)	−0.43	< 0.01	0.18
Correlation of PTL and R			
lc	0.54	n.s.	0.29
b_1	−0.15	n.s.	0.02
b_2 (b_2 only)	0.19	n.s.	0.04
b_2 (of b_2c)	0.44	< 0.01	0.19
c (of b_2c)	0.17	n.s.	0.03
c (*c* only)	0.03	n.s.	0.0009

for endings of axon branches supplied exclusively to typical poles of *c* fibres, which retained a small positive skew. One-way analysis of variance (ANOVA) revealed that highly significant differences existed between at least some group means for each of the 3 parameters L, PTL and R. Since the number of groups (6) was very much less than the total number of endings (252) it was possible to assign standard errors and thus 95% confidence limits to the mean value for each group (Bailey, 1981). The results, after back transformation, are summarised in Table 3.

Before attempting to interpret these results it is necessary to examine the independence of L, PTL and R. Table 4 presents correlation coefficients for pairwise comparisons of the 3 parameters for each group. L and PTL are always highly correlated, sharing 64–77% of their variability in common, whereas neither L and R nor PTL and R are ever highly correlated, just 1 group in each case having a significant small correlation that amounted to 18% or 19% of common variability. This result implies that parameters of size (L or PTL) and complexity (R) vary almost independently within each group, although in general, of course, they may not vary independently between the groups.

It is now possible to make some comparisons between the various groups of endings. The following are particularly notable: (1) endings on b_1 fibres showed a unique combination of relatively small size and high complexity; (2) intrafusal motor branches distributed to both b_2 and *c* fibres had endings that were significantly larger, on average, on the b_2 fibres, but were of similar complexity on either type of fibre, (3) endings of axonal branches supplied exclusively to typical poles of *c* fibres tended to be smaller (significantly so for PTL but not for L) and less complex than those of branches supplied exclusively to b_2 fibres; (4) endings on long poles of *c* fibres were the smallest and simplest on average, though the combination of small size and simplicity was not unique, being shared with *c*-fibre endings of axonal branches distributed to both *c* and b_2 fibres; (5) endings on b_2 fibres tended to be large and complex, especially those of axonal branches supplied to b_2 fibres exclusively.

Motor endings on b_1 *fibres.* In a previous study (Banks et al. 1985) the endings of known β axons on b_1 fibres were found by measurement of L and PTL to be indistinguishable from those of presumed β axons on *lc* fibres, both types being similar to the p_1 plates of Barker et al. (1970). In differential degeneration experiments some intrafusal motor endings persisted

Table 5. *Morphometric analysis of the motor endings on* b_1 *fibres in a cat tenuissimus muscle, C883*

	L (μm)			PTL (μm)			R		
	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
(a) Comparison of all endings on b_1 fibres in C883 with those included in the study of Banks et al. (1985)									
C883	9–105	35.9	32.5	13–287	96.6	83.2	1.11–5.51	2.62	2.40
Banks et al. (1985)									
Normal sample	13–107	45.6		15–653	147.2				
p_1 (β)	15–55	36.1		20–150	79.7				
p_2 (γ)	21–115	65.5		48–355	222.2				
(b) Comparison between endings on b_1 fibres in C883 grouped according to degree of axonal evidence for γ supply									
Branch to extrafusal nerve	19–32	23.4	21.8	45–89	66.0	57.5	1.84–4.68	2.93	2.61
Separate entry	10–105	38.8	36.0	19–261	107.2	108	1.23–5.51	2.78	2.50
Remainder	9–83	37.5	36.3	13–287	99.1	89	1.11–4.80	2.48	2.31

Table 6. *Distributions of intrafusal branches of motor axons to tenuissimus muscle spindles of 4 cats. Values in parentheses are the total numbers of intrafusal branches, when each spindle pole is considered separately, in those cases where single axons were observed to supply both poles of a spindle*

	b_1	b_1lc	lc	$?b_1b_2$	b_2	b_2c	c	Total known	Unknown	Overall total
(a) Number of branches										
C689	5				3	2	2	12	—	12
C637	21		6		10	12	12	61	46	107
C700	35	1	6	1	16	7	18 (19)	84	12	96
C883	45 (47)	1	9		18 (20)	23 (26)	19 (20)	115	3	118
Total	106	2	21	1	47	44	51	272	61	333
(b) Proportion of total known (%)										
C689	42				25	17	17			
C637	34		10		16	20	20			
C700	42	1	7	1	19	8	21			
C883	39	1	8		16	20	17			
Mean	39	0.5	6	0.25	19	16	19			
Total sample	39	1	8	< 0.5	17	16	19			

longer than extrafusal or p_1 plates and were therefore presumed to be derived from γ axons. Those on the b_1 fibres were significantly larger than p_1 plates (Banks et al. 1985) and were identified with the p_2 plates of Barker et al. (1970).

The endings on b_1 fibres from C883 entirely encompass the range of size of the p_1 plates of Banks et al. (1985) and extend well into the range of the p_2 plates. However, as shown in Table 5(*a*), the mean values of L and PTL are very much closer to (in the case of L virtually indistinguishable from) those of the p_1 plates than they are to those of the p_2 plates or the normal sample of Banks et al. If any dynamic γ axons had been present, and the arguments presented above are correct, their intrafusal branches would most likely have accompanied the afferent axons into the spindles and they would not, of course, have had branches into extrafusal nerves. The b_1 endings may thus be grouped on axonal criteria into 3 subclasses with different possibilities of β involvement: those whose axons also branched to supply extrafusal nerves; those whose axons entered the spindles separately from the afferent axons; and the remainder, i.e. those whose axons entered the spindles with the afferent axons but were not observed to branch into extrafusal nerves. The data are given in Table 5(*b*). ANOVA (modified when necessary for unequal variances; Sokal & Rohlf, 1981) of the logarithmically transformed data revealed only 1 significant difference ($P < 0.05$), namely that, as measured by L, the endings of the 1st subclass were smaller than those of the other 2. Indeed, by this measure they were smaller, on average, than the p_1 plates of known β axons (Banks et al. 1985) and were as small as the endings on long poles of *c* fibres (see Table 2). Nevertheless, like the whole b_1- ending population in C883, they were significantly ($P < 0.01$ for difference in R) more complex than the *lc* endings.

The 2nd and 3rd subclasses of b_1 endings were remarkably similar in all respects (Table 5(*b*)), so there was nothing to suggest a relative enrichment of the 3rd subclass by the larger p_2 plates that would have been expected if dynamic γ axons had been present. Moreover, in both cases the mean values of L and PTL remained close to those of the p_1 plates of Banks et al. (1985). It seems likely, therefore, that the b_1 supply in C883 was dominated by, if not exclusively derived from, β axons.

Numbers and distributions of intrafusal branches of motor axons in the complete sample of 4 muscles

Altogether, 333 intrafusal branches of motor axons were found to enter 47 spindles, including 3 b_2c units, of 4 tenuissimus muscles, each from a different cat. The distributions were established for 272 of the branches and are given in Table 6. Only 10 (4%) were found to supply more than 1 pole, mostly in C883, which reflected the better quality of staining in this case. For the different muscles, the proportion of branches with known distribution that supplied b_1 alone was fairly constant (34–42%) as was the proportion that supplied b_2 and typical poles of *c* fibres either alone or in combination (48–59%, mean 54%). Most of the remaining branches (8% of total) innervated long poles of c fibres exclusively. Combinations other than b_2c were rare, amounting to just 1% of the total branch distributions, specifically b_1lc (2 branches) and $?b_1b_2$ (1 branch). The uncertainty in the last example is due to the presence of 3 bag fibres in

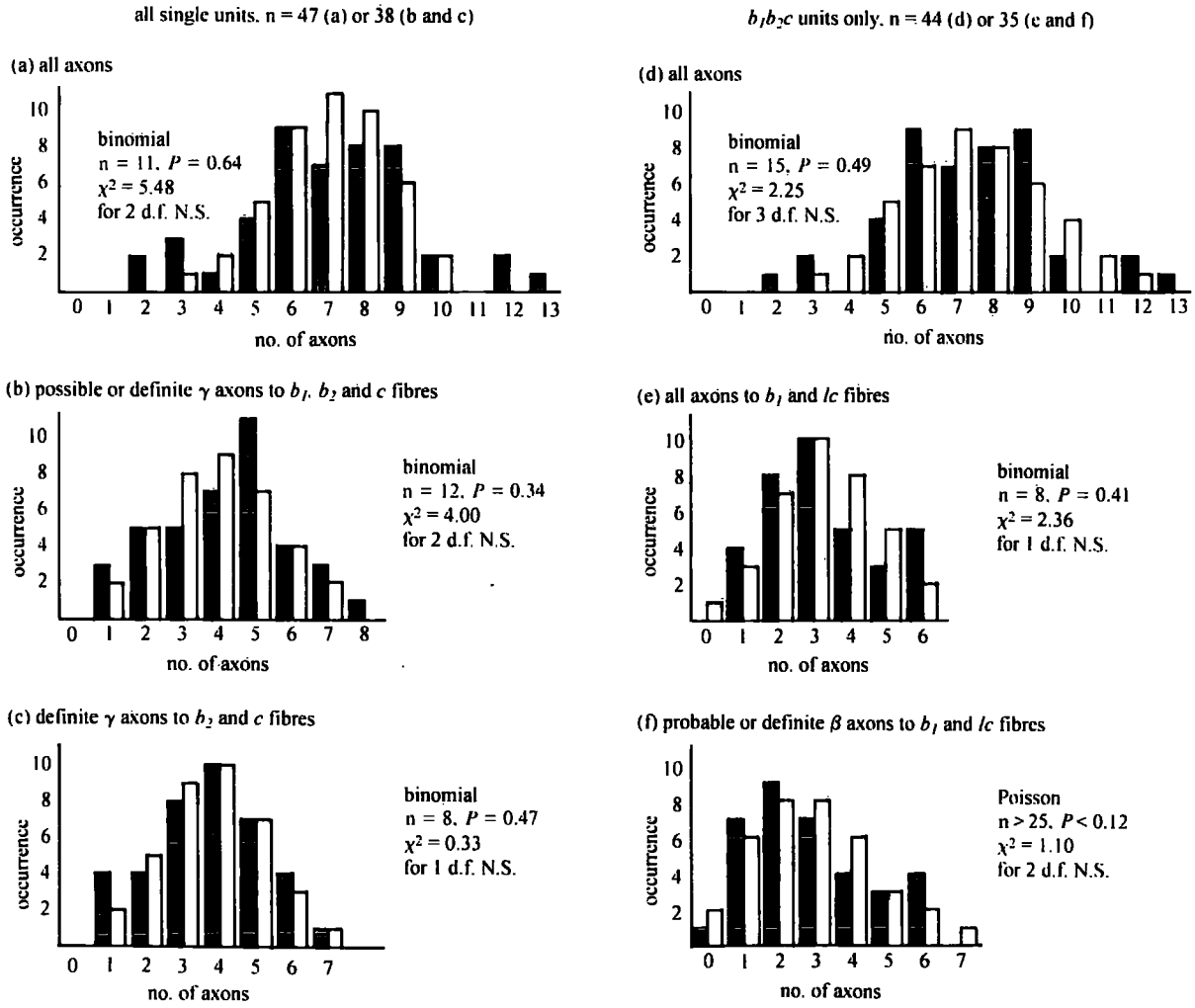


Fig. 4. Observed (filled bars) and best-fitted calculated (open bars) probability distributions of the numbers of motor axons that supplied each spindle, for various more or less inclusive samples. In no case does the observed distribution differ significantly from the theoretical one. The ordinate (occurrence) signifies the number of spindles that received a given number of axons. Abbreviations standard or as in Figure 1.

the spindle concerned (C700, spindle 10). Among the branches that supplied b_2 and c fibres the proportion distributed to both fibre types varied from 17 to 36%.

Each spindle received from 2 to 13 motor branches. The average number, including b_2c units, was 7.09, or excluding them was 7.36. In either case, the frequency of occurrence of spindles with different numbers of motor branches appeared to follow binomial form (Fig. 4*a*, *d*), indicating a random association of branches with spindles. In those spindles (n = 38) whose complete motor distribution had been established, the number of branches that supplied b_2 and typical poles of c fibres ranged from 1 to 7, mean 3.74. They represent a virtually pure sample of γ axons. The number of intrafusal branches that supplied b_1, long poles of c, b_1lc and $?b_1b_2$ ranged from 1 to 6 per b_1b_2c unit (n = 35, mean, 3.29). Based on the criteria of axonal routing and motor ending forms as described above, the large majority was identified as derived from β axons. In both cases the frequency of occurrence of different numbers of branches followed binomial probability distributions (Fig. 4*c*, *e*). Alternatively, those intrafusal motor branches that were probably or quite definitely of β axons (0–6 per b_1b_2c unit; mean, 2.89) could be separated from the remainder that were at least dominated by γ axons (1–7 per spindle; mean, 4.11), irrespective of their intrafusal fibre distribution. In these cases the frequency of occurrence of different numbers followed either binomial (Fig. 4*b*) or Poisson (Fig. 4*f*) form.

Relationships between the numbers of afferent and efferent axons (C883)

Banks & Stacey (1988, 1990) have shown that the numbers of afferent axons additional to a single Ia also follow binomial frequency distributions. These

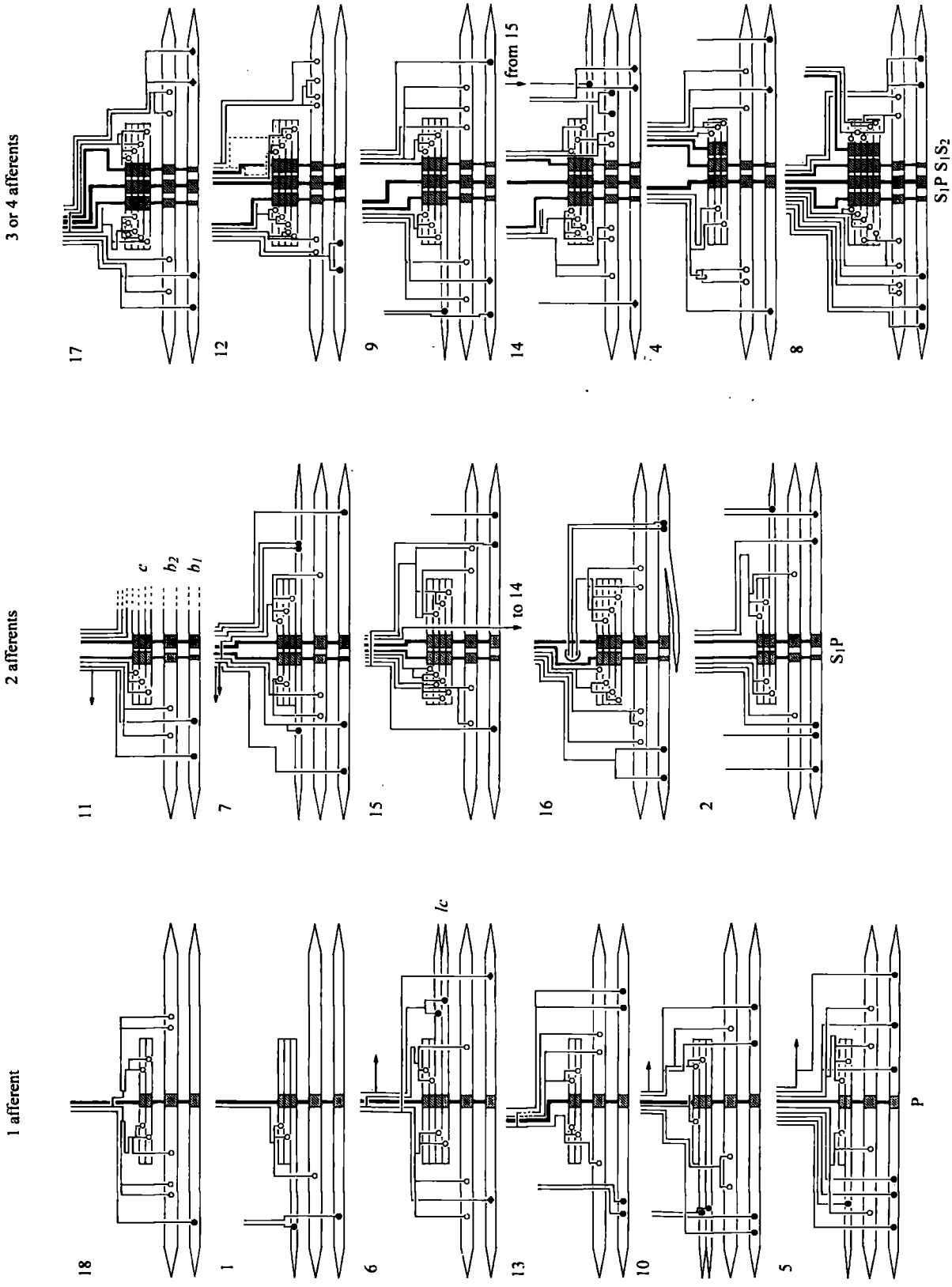


Fig. 5. For legend see opposite.

Table 7. *Numbers of afferent and efferent axons entering muscle spindles of a cat tenuissimus muscle, C883, grouped according to the number of afferents*

Spindle	No. afferents	No efferents						
		probable β				?β/γ		γ
		b_1	lc	b_1lc	Total	b_1	b_2	
1	1	1	1		2			1
3 (b_2c)	1							3
5	1	5	1		6			2
6	1		1		1	1		1
10	1	3	1		4			3
13	1	3			3			2
18	1	1			1			1
19*b* (b_2c)	1							2
Totals	8				17	1		15
2	2	3	1		4	2		3
7	2	2	2	1	5			1
15	2	2			2			4
16	2	3			3			4
19*a*	2	3			3			3
Totals	10				17	2		15
4	3	1			1	2		5
9	3	2	1		3	1		4
12	3	1			1			5
14	3		1		1	2	1	4
17	3	1			1	1		4
Totals	15				7	6	1	22
8	4	4			4			6

axons are usually group II afferents forming secondary endings, but may be group Ia contributing to doubly or multiply innervated primary endings (Banks et al. 1988). It is possible therefore, that the numbers of afferent and efferent axons entering each spindle could be randomly determined with respect to the spindle, yet be correlated with each other. Only in C883 were the results sufficiently complete to test this possibility (Fig. 5, Table 7). Their analysis is complicated by the uncertainty about the origin, whether γ or β, of some of the motor branches. Although the b_1 motor supply was undoubtedly dominated by β axons and could have been entirely provided by them (see above), the size of some endings exceeded the maximum limits of those known to be derived from β axons (p_1 plates) in the analysis of Banks et al. (1985). This raises the possibility that p_2 plates were present, derived from one or more dynamic γ axons. Depending on which measurement of size is used, L or PTL, 2 partially overlapping sets of endings are recognised. The larger set, identified as those with PTL values in excess of 150 μm, is shown in Figure 5 and Table 7.

Both regression (Fig. 6) and χ^2 analyses were applied to these data. The only correlations between the numbers of afferent and efferent axons entering the various spindles involved γ axons, either when

Fig. 5. Schematic diagrams showing the innervation of all bag_1 bag_2 chain, single-unit spindles of experiment C883, numbered 1,2 and 4–18 in proximal-to-distal sequence. Here the spindles are arranged in columns according to the number of afferent axons present, these forming primary (P) and secondary (S_1, S_2) sensory endings. Within each column the spindles are arranged from top to bottom according to the total number of motor axons that they receive. Identification of the nature of individual motor axons is described in the text; in part it relied on features that are indicated in the diagrams—axonal routing, and whether collateral branches entering extrafusal nerves were present (shown as horizontal arrows). Filled circles represent endings positively identified as the p_1 type of Banks et al. (1985) and therefore probably derived from skeletofusimotor (β) parent axons, whether dynamic or static. Filled diamonds represent endings on bag_1 fibres that fall within the criteria of the p_2 type of Banks et al. (1985) and exceed the maximum value of PTL for p_1 endings in that study. The endings could therefore be derived from one or more (dynamic) γ axons, though it is possible that they are intrafusal endings of β axons. Open circles represent the trail endings (see Banks et al. 1985, and Banks, 1994) of purely fusimotor (static) γ axons. Note that each spindle contained 1 bag_1 and 1 bag_2 fibre, but that it was usually possible only to estimate the number of chain fibres present. When long poles of chain fibres occurred, as indicated for example in spindle 6, column 1, their number could be accurately determined. True dimensions of individual spindles and intrafusal fibres are ignored in these diagrams, but axonal routing and relative locations of endings are depicted as accurately as possible :. chain fibres (shown with typical poles in spindle 11, column 2); other abbreviations as in Figure 1.

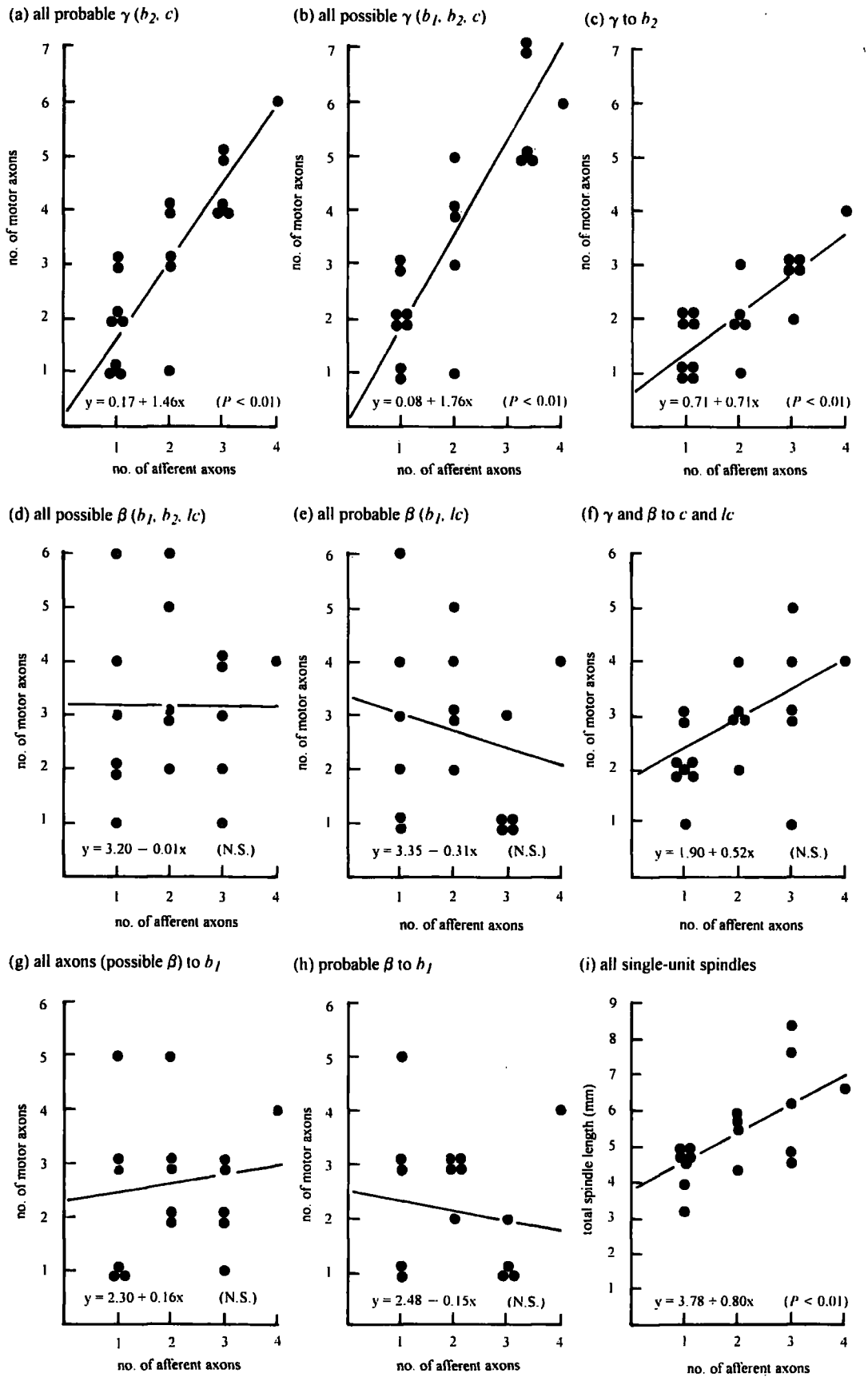


Fig. 6. Scatter plots showing the relationships between numbers of motor and afferent axons for various samples (a–h). The calculated regression of ordinate (y) against abscissa (x) is plotted and given in each case; all those, and only those, involving probable or possible γ axons alone had slopes that differed significantly from 0. The relationship between the number of purely fusimotor (γ) axons to chain fibres and the number of afferent axons (not shown) was similar to the equivalent one for the bag_2 fibres. Also shown is the significant relationship between total recovered spindle length and number of afferent axons (i). Abbreviations standard and as in Figures 1 and 4.

Table 8. *Intrafusal distributions of static γ axons in 32 muscle spindles of cat tenuissimus, grouped according to their degree of segregation*

Segregation	Distribution pole 1 : pole 2	No. observed	Total		Expected		$\frac{(obs-exp)^2}{exp}$
SS	b_2 c : b_2 c	4	4	13	4	11	0.333
MM	b_2c : b_2c	4	4		4		
II	b_2 b_2c : b_2c c	1	5		4		
	b_2c c : b_2c c	1					
	b_2 b_2c : b_2 b_2c c	1					
	b_2c c : b_2 b_2c c	1					
	b_2 b_2c c : b_2 b_2c c	1					
SM	b_2 c : b_2c		10		7		1.286
SI	b_2 c : b_2 b_2c	2	6		7		0.143
	b_2 c : b_2c c	3					
	b_2 c : b_2 b_2c c	1					
MI	b_2c : b_2 b_2c	1	3		7		2.286
	b_2c : b_2c c	2					
							$\chi^2 = 4.048$

Table 9. *Classification of 35 muscle spindles of cat tenuissimus according to the relationships between the occurrence of secondary sensory endings and the relative segregation of the static γ innervation*

	Static γ innervation		
Secondary endings	More segregated in one pole		Neither pole more segregated
Present in both poles or in neither	8 (5)		7 (5)
Present in one pole	15 (5)		5 (2)
	same pole as secondary	opposite pole to secondary	
	11 (5)	4 (0)	

* Values are numbers of spindles (C883 in parentheses).

restricted to the intrafusal motor branches that supplied b_2 and c fibres ($\chi^2 = 8.93$ for 1 d.f., $P < 0.005$) or when including the branches that ended in possible p_2 plates on b_1 fibres ($\chi^2 = 11.75$ for 1 d.f., $P < 0.001$). This was the case irrespective of whatever sample, each at least dominated by intrafusal β branches, was taken to represent the β innervation: all possible β branches, $\chi^2 = 2.93$ for 1 d.f., n.s.; all probable β branches, $\chi^2 = 2.89$ for 1 d.f., n.s.; probable β branches to b_1 only, $\chi^2 = 1.60$ for 1 d.f., n.s.

Static γ axons

Degree of segregation of input to the b_2 *and* c *fibres.* An additional complexity is presented by the static γ axons in that they innervate both b_2 and c fibres, and each intrafusal branch may supply either or both types of fibre. The complete input to each spindle pole might then be entirely segregated (S), entirely mixed (M), or exhibit intermediate degrees of segregation (I), with various combinations occurring in whole spindles. There were 34 muscle spindles whose entire static γ innervation was known. Ignoring 2 poles that had no motor innervation, the 3 polar types were found to occur about equally often: S, 25; M, 22; I, 19. Assuming that they associate randomly in whole spindles, 3 of the 6 possible combinations (SS, MM, II) would then each occur with a probability of about 1/9, whereas the other 3 (SM, SI, MI) would each occur with probability 2/9. the various combinations that were found are given in Table 8. There were considerably fewer of the MI and rather more of the SM types than expected, but this does not appear to be significant ($\chi^2 = 4.048$ for 2 d.f., n.s.).

Relationship of degree of segregation with secondary sensory endings. An apparent randomness in the association of poles, irrespective of their degree of

static-γ segregation, might nevertheless conceal a relationship with the afferent supply, which is itself subject to random variation. This possibility was assessed by first scoring individual spindle poles according to their degree of segregation: M = 0; I = 1; S = 2. The sample from the 4 silver-impregnated muscles was augmented with data from serially sectioned spindles, previously published as described above in Materials and Methods.

Of 74 poles from b_1b_2c spindles, 38 possessed 1 or more secondary endings and had a mean motor-supply score of 1.37, whereas the 36 that lacked secondary endings had a mean score of only 0.64, clearly indicating that the degree of segregation of input to the b_2 and c fibres is correlated in some way with the presence of secondary endings. Adding the motor-supply scores for both poles of 37 complete spindles showed that the static γ supply is increasingly segregated as first one pole and then both receive secondary endings. Mean scores for spindles with primary endings only (n = 8), with secondary endings in one pole (n = 21), and with secondary endings in both poles (n = 8) were 0.75, 2.14, and 3.00 respectively. The corresponding total scores (6, 45, 24) departed significantly from values that would be expected (16, 43, 16) if no such relationship were to exist ($\chi^2 = 10.34$ for 1 d.f., $P < 0.01$).

It might be supposed that this is due simply to a progressive segregation as the number of static γ branches to a spindle increases in step with the number of afferent axons. But the relationship was not actually so straightforward, for although unsegregated poles (n = 25) were almost always supplied by a single branch (mean number 1.04) there was a tendency for segregated poles (n = 26) to be supplied by fewer (mean 2.2) branches than partially segregated poles (n = 23, mean 2.5 branches). It is for this reason that the difference in the degree of segregation of static γ input is greater, on average, between spindles with secondary endings in one pole and those with only primary endings (difference of mean scores, $2.14-0.75 = 1.39$), than it is between spindles with secondary endings in both poles and those with secondary endings in one pole (difference of mean scores, $3.00-2.14 = 0.86$).

One further possibility concerning the intrafusal distribution of static γ axons remains to be examined: whether, in spindles with secondary endings in only one pole, the static γ innervation is more segregated in that pole than the other. The relevant data are presented in Table 9. Fifteen of 35 spindles had secondary endings in only 1 pole, and static γ innervation that was more segregated in one pole than the other. In 11 of these the more segregated pole was the same as that with the secondary endings. Assuming that the more segregated static γ innervation is equally likely to be associated with either pole, the probability that the observed numbers occurred by chance is: ${}^{15}C_{11}(0.5)^{11}(0.5)^4 = 0.042$; or, for C883 alone: ${}^{5}C_{5}(0.5)^{5}(0.5)^0 = 0.0313$. These are sufficiently low probabilities to confirm the relationship.

DISCUSSION

Much of the reasoning to be developed in this discussion depends on a correct identification having been made of the source, whether β or γ, of the intrafusal branches of motor axons. This will therefore be considered first before examining the problem of the segregation, or rather lack of it, in the γ supply of the b_2 and c fibres.

Ever since the conclusive demonstration, in the mid-1970s, that static γ axons have 2 effectors with different mechanical properties (see Barker & Banks, 1994, for review), the problem as to why this should be has remained unresolved. The responses of primary and secondary sensory endings to stretch are known to be modulated in different ways by b_2 or c activity (Boyd, 1981; Boyd et al. 1985*a*, *b*, *c*; Banks, 1991*a*), so it might seem desirable for these effects to be under separate central control. Although there is evidence to support at least a degree of separate control (Gladden & McWilliam, 1977*a*, *b*; Wand & Schwartz, 1985; Asgari-Khozankalaei & Gladden, 1990; Dickson & Gladden, 1990), it is clear from the overall distribution of the static axons that at best only a limited separation is possible (Banks, 1991*a*, 1994). The present results concerning the intrafusal distribution and ending form of the axons invite further consideration of the question as to whether there is more than 1 type of static γ neuron (Boyd, 1986; Gladden & Sutherland, 1989), and this will be dealt with below.

In addition to these problems of motor control, the intrafusal motor innervation, particularly the static γ system, raises questions of potentially more general interest concerning its development which will be considered in the final part of the discussion. The formation of extrafusal motor units has long served as a model for target recognition and synapse elimination in neuronal development (see review by Jansen & Fladby, 1990). Despite their greatly different adult appearances and functional roles, there is a basic similarity in the developmental pattern of intrafusal and extrafusal muscle fibres as shown by the order of assembly and relative positions of the primary and secondary myotubes that give rise to them (Milburn,

1984; Kucera & Walro, 1990). It is therefore of interest to enquire what we can learn from the muscle spindle about the formation of motor units in general.

The identification of the intrafusal branches of γ and β axons and the relationship of their number with that of the afferent axons

It has been argued above, on the basis both of internal evidence and a comparison with the study of Banks et al. (1985), that the motor innervation of the b_1 fibres in the complete tenuissimus muscle, C883, was entirely, or almost entirely, derived from skeletofusimotor (β) axons. In the overall sample, the majority of the b_1 supply was similarly identified as skeletofusimotor. Correlation of the histological and physiological data for C883 in the companion paper to this one (Banks, 1991*a*) made it possible to estimate that the muscle received about 10 static γ axons (7 of which had been isolated in ventral root filaments). It is necessary, therefore, to provide further corroboration for the predominantly β nature of the dynamic input in tenuissimus, since in the larger hindlimb muscles, such as soleus, between a quarter and a third of fusimotor (γ) axons are dynamic (Matthews, 1972) and they may be supposed to contribute a large part of the b_1 input.

Although there has been no systematic study of the proportions of dynamic and static γ axons in tenuissimus, there is evidence for a relative deficiency in the dynamic type. Boyd et al. (1977) reported a dynamic:static ratio of 1:6.5, and in the whole physiological series from which the present work derives only 2 γ axons from a total of 35 were dynamic. Even when dynamic γ axons have been specifically sought in tenuissimus, no muscle seems to have been supplied by more than 2 (e.g. Banks et al., 1978), and it is quite common not to be able to find a single one, despite the undoubted presence of dynamic β and static γ axons (Banks, Hulliger and Scheepstra, unpublished results).

In the case of C883, whereas it should be noted that no dynamic γ axon was isolated, the principal evidence for the β nature of the b_1 innervation was the mean length of the motor endings. At 35.9 μm this was virtually identical to that of the endings of known β axons on b_1 fibres (36.1 μm, Banks et al. 1985) and, despite the different techniques employed, was similar to the mean length (31.6 μm) of known and presumed β endings on b_1 reported by Kucera & Walro (1986). Supplementary evidence was provided by the observation that the b_1 and long poles of *c* fibres, the latter known from glycogen depletion experiments and serial sections to be typically β innervated (Jami et al. 1978, 1979; Kucera & Hughes, 1983), often received their motor supply from otherwise exclusively extrafusal nerves. Direct evidence for the β origin of the axons concerned, in the form of extrafusal collaterals, was lost in teasing the spindles for the present work, but similar axons could thus be positively identified as β in the serial section studies (Kucera & Hughes, 1983; Kucera & Walro, 1986).

Confidence in the general identification of the β intrafusal motor innervation was further increased when collateral branches to extrafusal nerves were subsequently found to arise from several axons which had already been classified as β on the basis of their distribution to b_1 or *lc* fibres and the form of their intrafusal endings. An unpublished earlier version of Figure 5, made before these collaterals had been traced, was included in a poster illustrating a communication given in Paris in 1991 at a symposium in honour of Professor Laporte (Jami et al. 1992). The collaterals are all those in spindles 5, 7, 10, and 11 in Figure 5; those of spindle 7 are also shown in detail in Figure 1 *E*. They were preserved in the teasing process because the parent axons approached the spindles in relatively robust nerves that also contained the afferent axons, in whose company the intrafusal branches of the β axons entered the spindles. Kucera & Walro (1986) do not appear to have recognized β axons in this situation, perhaps because they did not trace the nerves far enough from the spindles. The potential contamination of their γ sample, rather than the difference in techniques, might account for the considerably smaller mean length (43.6 μm) that Kucera & Walro reported for the endings of γ axons on b_1 fibres than that of the p_2 endings (p_2 plates) of Banks et al. (1985).

Before the b_1 motor innervation of the complete muscle, C883, can be accepted as predominantly or entirely skeletofusimotor, it is also necessary to show that a significant γ contribution is unlikely. By removing from the sample those axons and their endings most probably of β origin, a subsample was created that would have been enriched with γ axons, were any present. However, the mean size of the endings in this subsample remained small, thus indicating that β axons still predominated. Even the large size of some endings, which exceeded the maximum values of L or PTL for the p_1 plates of Banks et al. (1985), does not unequivocally mark those endings as p_2 (γ), since 2 of the 4 endings of the confirmed β axon in GS6 of Banks (1981) were equally large (70 and 71 μm long). Nevertheless, it is among the axons supplying those endings that any γ innervation that might have been present is likely to

be represented. In view of the often widespread distribution that has been reported for individual dynamic γ axons in tenuissimus (Barker et al. 1976*b*; Boyd et al. 1977; Banks, 1991*a*), the small number of spindles that contained potential p_2 endings in C883 suggests that probably not more than 1 dynamic γ axon was present.

Once the likely sources of the intrafusal branches of motor axons had been identified, analysis of their number revealed that, whereas they appeared to be distributed at random to individual spindles (though not, of course, within the spindle), the numbers of γ axonal branches and afferent axons were closely correlated. This was not the case for the β branches, the analysis failing to reveal anything other than their random association with spindles. The different behaviour of the 2 types of motor innervation in this respect may be attributed to a specific requirement for the γ axons to be guided to the incipient spindles during development. This could be easily achieved if they were to follow pathways already laid down by the afferent axons, the attractiveness of the pathways for the γ axons being determined by the number of afferents present in them. The β axons, by contrast, seem not to be guided to their intrafusal destinations at all; rather, they appear to have encountered developing spindles by chance. I have argued elsewhere (Banks, 1994) that the β innervation is provided by motoneurons that are indistinguishable from corresponding α motoneurons, a conclusion which, if true, is sufficient to account for the observed distribution of numbers of intrafusal β branches.

The degree of segregation of the static γ supply to the b_2 *and* c *fibres*

We have been so long accustomed to regard extrafusal motor units as homogeneous that we are perhaps conditioned to think of this as the quintessentially normal condition for motor units, deviations from which are due to developmental abberations. In the spindle, the dynamic γ/b_1 units conform to the ideal and so bolster our belief. Even the existence of the β innervation can be accommodated since it might be supposed that the intrafusal components of these units are specially modified by the presence of the sensory endings. The existence of unsegregated or common innervation of b_2 and c fibres by static γ axons is therefore a challenge not only to theories of motor control, but also to the general concept of the motor unit.

It has been argued that despite the lack of complete segregation there are nevertheless 2 (Boyd, 1986) or more (Gladden & Sutherland, 1989) types of static γ neuron. Each would presumably have an intrinsic preference for one or other kind of muscle fibre, but which for unknown reasons, and despite the supposed benefits for motor control, they are often unable to satisfy. One of the observations that Boyd took to support his conclusion was the similarity in the effects on sensory responses attributable to b_2 or *c* activity that were elicited by single static axons in several spindles. Although later studies have failed to confirm such a clear distinction (Banks, 1991*a*; Celichowski et al. 1993, 1994; Dickson et al. 1993), there is a greater than chance similarity in the effects elicited by some axons which Banks (1991*a*) related to conduction velocity. Thus the fastest-conducting axons (> 40 m s^{-1} in tenuissimus), which tended to be most widely distributed, always supplied b_2 fibres. In some spindles c fibres were also innervated by these axons but rarely alone as the b_2 fibres often were. The distribution of slower axons appeared altogether more random, except that there was a tendency for those that supplied only *c* fibres in some spindles to be the slowest among the axons to the spindles concerned. Banks (1991*a*) concluded, therefore, that there was but a single, differentially distributed, population of static γ neurons.

Though there is no clear separation of the b_2 and *c* components of the static system into distinct motor units, it is nevertheless conceivable that there exist 2 (or more) kinds of static γ motoneuron each with a preference to form connexions with either b_2 or *c* fibres. This would then easily account for the observed partial segregation. Alternatively, if there is but 1 type of neuron, capable of making connections equally well on the 2 types of fibre, it is necessary to invoke some additional factor such as the order of neuromuscular contact to explain the presence of a nonrandom component in the distribution of the neurons.

The present results offer a new perspective on this problem. Consider first the case in which a segregated static γ supply is beneficial for motor control, and there are 2 types of neuron. We have seen that the number of γ axons entering a spindle is correlated with the number of afferent axons, which itself appears to be randomly determined. The degree of segregation would then be expected to reflect the increase in the number of axons entering a spindle, and therefore the chance that both types of neuron are represented. This is not so; rather, there is at first an increase then a slight decrease. Single poles show the effect particularly clearly: when 1 axon is present its distribution is entirely unsegregated; when 2 are present segregation is essentially complete; but when 3 are

present, the distribution of 1 of them is often again unsegregated. The hypothesis of 2 types of neuron cannot readily account for this suite of observations: for example, if 2 axons regularly segregate within a single pole, this would imply that they belong to each of the 2 kinds of neuron. The addition of a 3rd neuron, which must show a preference for b_2 or c fibres, should not result in a reduction in the degree of segregation.

Consider now the case where there is only 1 type of static γ neuron. Partition of the b_2 and c fibres in a single pole by 2 or 3 axons from different neurons may be supposed to depend on a competitive interaction of some kind. The outcome of this interaction need not be entirely due to chance, even though the neurons are intrinsically similar; the possibility of bias is strongly indicated by the sequential construction of the b_2 and c fibres over a period of time in which the γ innervation arrives at the developing spindle. Kucera et al. (1988) have shown that, at least in the rat, the presumptive b_2 receives what seems to be a definitive γ supply before the c fibres have begun to form. The adult condition described above shows that if only 1 static γ axon is present or persists, then it normally extends its distribution to include those c fibres not already sequestered by β motor units. When 2 or 3 axons are present it is necessary to suppose that in the competition for intrafusal fibres the outcome is biased in favour of the 1st axon to establish neuromuscular contact with a particular fibre. This has the advantage of accounting not only for the within-spindle segregation but also the nonrandom component of between-spindle distribution, because, as we have seen, the earliest static γ axons to arrive in a developing muscle will encounter presumptive b_2 but not c fibres.

It is possible, therefore, to explain the occurrence of a degree of static γ segregation without recourse to intrinsically different motoneurons, but this does not demonstrate that the segregation is of use in motor control. Histological evidence is unlikely to provide definitive evidence here, though it can afford some insight. This has already been discussed by Banks (1994) and will not be repeated in detail, but we may note the incongruous association of a segregated static supply to b_2 and c fibres and the presence of secondary endings, even at the level of individual poles. The incongruity arises because secondary endings are predominantly situated on (Banks et al. 1982), and influenced by (Boyd, 1981) c fibres. Furthermore, if segregation is functionally important, we might expect that when sufficient static γ axons are present in a spindle (a minimum of 2) the mechanism of motor unit homogenisation that seems to operate between the dynamic and static systems (and also extrafusally) could ensure that the b_2 and c fibres were separately innervated. It is clear that this does not happen.

The contrast between the high degree of segregation of the dynamic and static systems and the lack of segregation within the static system is noteworthy and presumably reflects the functional importance of the former division. We may also note that whereas the dynamic input may largely be provided by collaterals of α motoneurons, and therefore is presumably activated in parallel with them, the static system is evidently required to be under separate control since it is almost exclusively supplied by fusimotor (γ) axons. This highlights the necessity of comparative studies of muscles involved in different motor tasks since patterns of intrafusal and extrafusal activation are unlikely to be universally applicable (see e.g. Murphy et al. 1984; Murphy & Hammond, 1993).

Ending morphology and the problem of the number of types of static γ motoneuron

Intrafusal motor endings, and the neuromuscular junctions to which they contribute, are extremely variable (Banks et al. 1985; Kucera & Walro, 1986), and much effort has gone into their description and classification (see Banks, 1994, for review). The lack of a generally accepted scheme may be due, among other reasons, to problems of sampling and to the likely presence of influential factors that are uncontrolled or unaccounted for. The latter reason in particular should be borne in mind when assessing the evidential use of junctional morphology in relation to ideas about the number of types of static γ neurons (Boyd, 1986; Arbuthnott et al. 1992).

Banks et al. (1985) concluded that the form of the motor endings was primarily determined by neuronal type, whereas postjunctional structure mainly varied according to intrafusal fibre type and to the location of the endplates in relation to the primary sensory ending. Kucera & Walro (1986) reached similar conclusions, and went on to show that among a suite of 5 postjunctional characters only one, the mean cross-sectional area of the sole-plate, showed a slight difference as between segregated and unsegregated axons, and that only for the endplates of b_2 fibres. More recently, however, Arbuthnott et al. (1992) described differences between the postjunctional structures associated with segregated and unsegregated intrafusal branches of static γ axons ending on both c and b_2 fibres.

The present statistical analysis (see Table 3) shows that some significant differences exist between the

mean values of estimates of ending size (L or PTL) and complexity (R) for endings of static γ axons according to their intrafusal distribution, whether segregated or unsegregated, and their location, whether on b_2 or *c* fibres. Combination of one or other estimate of size with that of complexity could potentially increase the discrimination of any groups that may occur, since the elements of each combination show very little correlation. In this way, by combining L and R, 3 groups emerge that can be characterized as being relatively: (1) small and simple, (2) large and simple, and (3) large and complex. They correspond respectively to endings of (1) unsegregated intrafusal branches on *c* fibres, (2) unsegregated branches on b_2 fibres together with those of segregated branches on c fibres, and (3) segregated branches on b_2 fibres. It should be noted, though, that groups (1) and (2) are incompletely separated when PTL and R are combined in that the endings on *c* fibres, irrespective of source, no longer differ significantly.

Since apparently different forms of ending occur on the same type of intrafusal fibre it could be argued that this implies the existence of intrinsically different types of static γ neuron, just as the endings of dynamic β and γ axons differ even though they both supply b_1 fibres. However, such a conclusion can only be accepted if other influential factors can be eliminated or controlled, and this has yet to be achieved in any study on intrafusal motor innervation. The potential for postjunctional factors to influence ending form is clearly indicated by the unsegregated intrafusal branches, since their endings on *c* and b_2 fibres differ in size, those on the *c* fibres being smaller. A consistent size difference also exists between the *c* and b_2 endings of the segregated branches.

Variation in size of spindles, and therefore of the component intrafusal fibres, is one obvious factor that could also influence ending form. Spindle size correlates with the number of afferent axons (Fig. 6), which in turn correlates with the number, and therefore the degree of segregation of the static γ axons. Precisely the same 3 groups of endings that are recognised above also occur in the analysis of Kucera & Walro (1986) where the differential feature is the mean distance between the motor and primary sensory endings. At least for the *c* fibres, this distance is itself correlated with an estimate of fibre size, namely polar length (Banks, 1981). Since, therefore, the endings of segregated and unsegregated intrafusal branches of static γ axons map in a biased or nonrandom fashion into fibres of different sizes, it is not possible to conclude that the above groups represent different types of neuron; a single, continuously varying population of neurons could automatically generate the groups if ending and fibre size are related.

A final speculation on motor units and development

The peripheral organisation of the motor units of the adult static γ system show some remarkable similarities with that of the skeletomotor (α) system at a stage of development typified by the neonatal rat (Jones et al. 1987*a*, *b*). These include: convergence of different motoneurons onto individual muscle fibres; clustering of component fibres; and, perhaps most significantly, heterogeneous composition. In view of the probable common origin and ontogenetic pattern of intrafusal and extrafusal fibres (Milburn, 1984; Kucera & Walro, 1990), it may be that the details of that organisation are as relevant to our concepts of neuromuscular development in general as to furthering our understanding of motor control.

The convergence seen in extrafusal development appears to be due entirely to multiple innervation at individual endplates (see review by Jansen & Fladby, 1990). A similar kind of transient convergence occurs during the equivalent developmental stage of intrafusal endplates (Kucera et al. 1988); the convergence that persists in the adult static γ system is due to the presence of several endplates in each b_2 pole, which in some cases are supplied by different axons. This may be a consequence of the lack of propagated action potentials in these fibres (as also with the b_1 fibres, but in contrast to the *c* fibres which normally have a single endplate per pole and propagate action potentials; Barker et al. 1978; Banks, 1981), but it does indicate that the reduction of multiple to single innervation of individual endplates is a locally mediated phenomenon. Temporary development of multiple innervation at single sites may be a mechanism to ensure that virtually all muscle fibres become innervated. However, even if the interaction that results in its reduction is in any sense competitive, it does not follow that the contributing axons have equal chances of winning. Indeed, the nonrandom component in the distribution of individual static γ axons suggests that this is not so, but is consistent with the possibility that the first axon to contribute to an endplate is most likely to survive there.

Persistence in the adult of heterogeneous static γ motor units suggests that the condition is functionally neutral, and that other factors, such as the need to ensure as complete an innervation as possible, are more important. Conversely, when homogeneity occurs, as in the dynamic γ system and in extrafusal motor units, we may suppose that this is a positive

adaptation arising from the functional benefits provided to overall motor performance. If so, it is not surprising that even there complete homogenisation does not seem to occur (Kucera, 1985; Walro & Kucera, 1985; Barker et al. 1992). Local, biased competitive interaction of axonal terminals coupled with a stereotyped spatiotemporal formation of the various generations of primary and secondary myotubes might set the pattern of homogenisation, with further enhancement following mutual differentiation of motoneurons and muscle fibres perhaps by withdrawal of now inappropriate connections.

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THE MOTOR INNERVATION OF MAMMALIAN MUSCLE SPINDLES

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1. INTRODUCTION

Mammals display a wide behavioural repertoire; their control of posture and locomotion is correspondingly complex and requires a variety of proprioceptive and exteroceptive sensors. The muscle spindle is one of these sensors, and it has been known and studied now for over a century. Its function as a mechanosensory proprioceptor was soon recognized by Sherrington (1894) and Ruffini (1898), since when it has principally been studied in order to elucidate its role in motor control (Matthews, 1972). More than a mere sense organ, however, the mammalian muscle spindle is a miniature neuromuscular system (Barker and Banks, 1994). As such it may be studied on several organizational levels, and from various aspects. This will, I am sure, be familiar to those for whom the spindle is their special concern, but the benefits of the spindle as a model for other neural and neuromuscular systems do not seem to be more generally appreciated, despite its long history.

The spindle informs the central nervous system (CNS) about local changes in muscle length (Windhorst *et al.*, 1989). The facility of presenting accurate and reproducible length stimuli to muscles undoubtedly contributed to the importance of the spindle, both in experiment and theory. But its importance is compounded because, uniquely among somatic proprioceptors, the spindle's response has both passive and active components; for the CNS exercises an efferent control over the response through the partially intrafusal distribution of the motor innervation. This complication makes the role of the spindle in motor control much more difficult to define, since, in the normal behavioural repertoire, the length signals provided by spindles are continuously modulated by efferent activity. Moreover, the CNS may use the length signals in a variety of contexts, related not only to the source of the signal but to the behavioural task being performed. Thus, in addition to particular muscles possessing characteristically different complements of spindles and their sensory innervation, we should expect the information they provide about muscle length to be utilized differently if, for example, a muscle were to contribute to postural adjustment as against locomotion, or to exploratory as against ballistic movements.

It is beyond the scope of the present review to

describe the part played by the intrafusal motor innervation in the exercise of motor control. The interested reader may consult the excellent reviews that are available elsewhere (Hulliger, 1984; Matthews, 1972, 1981a,b; McIntyre, 1974; Schomburg, 1990). Similarly, the internal mechanisms of the spindle, reviewed by Boyd and Smith (1984), Hulliger (1984), Hunt (1990) and Matthews (1972, 1981a,b), will be introduced only in sufficient detail to facilitate the description of the normal pattern of motor innervation. The objectives of the current review are: (i) to describe the common plan of the spindle and its innervation; (ii) to give a quantitative account of the variability of the motor innervation within the common plan, and of its relationship with the sensory innervation; (iii) to consider how the adult patterns may be produced developmentally; and (iv) to demonstrate, thereby, the relevance of these studies not only to motor control but to neural organization in general.

2. THE SPINDLE AND ITS COMPONENTS

This section briefly summarizes the basic structure and function of the spindle, necessary to understand the subsequent detailed treatment of its motor innervation. Since the subject has recently been fully reviewed by Barker and Banks (1994), references to original work are kept to a minimum.

The spindle's function as a length sensor arises essentially from its geometrical relationship with the muscle of which it forms a part. It consists of a bundle of specialized muscle fibres lying in parallel with, and hence normally surrounded by, the fascicles of the regular, force-producing fibres. Any length changes in the muscle are therefore transmitted by the intrafusal fibres to the sensory nerve terminals that occur in their mid, or equatorial, regions. The intrafusal fibres are contractile throughout their lengths except for the equatorial regions, so that each is divided into two, independently acting, polar regions. The small cross-sectional area of the intrafusal fibres in the adult means that their contraction makes an insignificant contribution to the total external force of the muscle, while being sufficient to deform the sensory terminals. It is by this means that, through the motor innervation normally supplied to both polar regions of the spindle, the CNS is able actively to modulate the sensory response.

2.1. The Sensory Innervation

It may seem perverse to begin a review of the intrafusal motor supply with a description of the sensory innervation, but this is necessary for two reasons: (i) to consider subsequently whether the presence of different sensory complements has any influence on the motor provision in individual spindles, and (ii) because the functional categories of the motor axons are defined according to their differential effects on the sensory response to muscle stretch.

The equatorial region receives the endings of one or more separate afferent axons. The maximum number is indeterminate but seems to be about eight. A detailed description of the endings has been given for the cat by Banks *et al.* (1982) and Banks (1986). The form of the terminals and their distribution among the intrafusal fibres varies according to the position of the ending in relation to the equatorial myonuclei. It is thus possible to distinguish between primary and secondary sensory endings. Primary-ending afferents appear to induce the differentiation of the intrafusal fibres during development (Zelená, 1957) and thus determine the characteristic number of spindles occurring in a particular muscle. The relative number of spindles in different muscles, as measured allometrically (Banks and Stacey, 1988), is undoubtedly an important factor in motor control, but one that has not yet received the attention it deserves.

In most spindles the sensory complement consists of a single afferent that forms a primary ending, either alone or associated with one or more afferents that usually form secondary endings but may contribute to doubly or multiply innervated primary endings (Banks and Stacey, 1988, 1990; Banks *et al.*, 1988). The frequency of occurrence of spindles with different sensory complements follows a binomial distribution, suggesting that afferents additional to those initiating intrafusal development are allocated randomly among the whole spindle population of the muscle (Banks and Stacey, 1988). In the binomial distribution the product of p, the probability of an event occurring, and n, the number of independent trials, is equal to the average occurrence of the event. In the sensory complement of most cat spindles this corresponds to the average number of secondary endings present. The precise distribution, defined by the values of the binomial parameters p and n, is again a characteristic feature of a particular muscle, and presumably, therefore, of functional significance.

2.2. Effects of Motor Stimulation on Sensory Responses to Stretch

The responses of primary and secondary endings to a variety of stretch stimuli have been analyzed so as to define the transduction properties of the spindle and to investigate its internal working. For present purposes it is only necessary to consider the ramp and hold stimulus, where a muscle is held at constant length, then stretched at constant velocity to a new length at which it is again held. There are thus two phases of static stretch separated by a dynamic phase. The responses of primary and secondary endings of passive spindles are qualitatively similar, both showing an increased firing rate when the muscle is maintained at a longer rather than a shorter length. The onset and maintenance of the dynamic phase of stretch produce complex effects in the sensory response, whose origin is still the subject of debate. There is a component that is related in some way to the rate of stretch so that the dynamic response is greater than would be expected on the basis of instantaneous length alone. This component is usually more marked in primary than secondary responses.

In general, the sensory endings are excited by stimulation of intrafusally distributed motor axons, but the effects produced are not all similar. Two main categories are recognizable according to whether or not the dynamic phase of the primary-ending response

is selectively enhanced. Excitation without dynamic enhancement may take various forms collectively known as static effects. In some cases the output of the primary ending can show entrainment at the stimulus frequency (1:1 driving) or a submultiple of it (subharmonic driving). These effects are to some extent length and frequency dependent. At high frequencies the response may show greatly increased variance in addition to an increase in its mean rate. In other cases the primary output may be biased upwards with no sign of driving at any length or frequency. When a motor axon innervates more than one spindle it almost always has the same broad effect, whether dynamic or static, on each primary ending. It is thus convenient, as well as reflecting an important functional distinction, to speak of dynamic and static axons. Only static axons commonly affect secondary endings, and those that drive primary endings tend to produce a more powerful secondary excitation than those that do not (Celichowski *et al.*, 1993).

2.3. Fusimotor (γ) and Skeletofusimotor (β) Axons

In addition to categorization by their functional effects on primary-ending responses, intrafusally distributed motor axons may be classified according to their size and overall distribution. Some axons are exclusively fusimotor. They are small, conducting in the γ range (between 15 and 55 $msec^{-1}$ in the cat), and seem to be characteristic of mammals (Barker, 1974) if not entirely confined to the class. The cost in increased complexity incurred by the provision of a separate fusimotor route is presumably met by the additional flexibility given to the control system as a whole. Nevertheless a simpler system, widespread in other tetrapod classes, continues to form a significant component of the mammalian intrafusal motor innervation. Here the intrafusal fibres form part of the motor units of axons that are otherwise distributed to extrafusal fibres and that conduct mostly in the α range. Though it is convenient to call these skeletofusimotor, or β, axons, it should be remembered that there is as yet no evidence that they are distinct from corresponding axons whose motor units are purely extrafusal.

The dynamic and static categories of fusimotor effect can be elicited by both β and γ axons, but there seems to be little or no distinction in size between dynamic and static γ axons, whereas the two types of β axon do differ in this respect (see Section 3.2.1).

2.4. The Intrafusal Muscle Fibres

The intrafusally distributed motor axons, whether γ or β, exert their influence on sensory responses through the activity of the contractile poles of the intrafusal muscle fibres. It has long been supposed that the sensory effects of motor stimulation are due to different contractile properties of the muscle fibres, so an important aim of spindle research has been to establish how many types of fibre there are and what is the nature of their contraction. Despite minor variations in details of size and some histochemical staining properties, intrafusal-fibre types are remarkably constant in a wide range of eutherian mammals including rodents, lagomorphs, carnivores and primates. Initially, large fibres containing bag-like collections of equatorial myonuclei were distinguished from smaller fibres with rows or chains of nuclei. In an important resolution of conflicting evidence relating to the intrafusal distribution of static and dynamic γ axons, the nuclear-bag-fibres were subsequently recognized as including two, metabolically quite distinct, types. Following a precedent introduced by Ovalle and Smith (1972), the different kinds of fibre are now generally referred to as bag_1 (b_1), bag_2 (b_2), and chain (c). In most spindles all three kinds are represented (b_1b_2c units), with a total complement in each spindle that is rarely less than $1b_1$, $1b_2$, and $2c$. In larger spindles the additional fibres are usually c, but some spindles may contain additional b_1 or b_2 fibres, especially in certain species or muscles. At least in the cat, smaller spindles may lack a b_1 fibre (b_2c units), when they are usually linked in tandem with a larger b_1b_2c unit by a continuous b_2 fibre.

The metabolism of intrafusal fibres, as expressed in their enzymic and immunohistochemical properties, is unusual when compared with that of their extrafusal neighbours (Rowlerson *et al.*, 1985). For example, they show marked regional variation centred on the equatorial nuclei and, thus, the primary sensory ending, whereas extrafusal fibres are noted for their homogeneity (but see Barker *et al.*, 1992). They may possess peculiar myosin isoforms as demonstrated by neonatal and tonic myosin immunoreactivity, in addition to the more normal fast- and slow-twitch varieties that also occur extrafusally. The most important differential and regional properties are summarized in the schematic diagram of Fig. 1, where the regions are defined in relation to the spindle's capsule, itself derived from the perineurium of the supplying nerves. In region A, centred on the equator, the capsule is separated from the bundle of intrafusal fibres by a periaxial space that gives rise to the eponymous shape. It is of variable extent depending on the number of sensory endings present. In region B the capsule closely surrounds the intrafusal bundle. Most of the motor innervation occurs here, but some, particularly of the b_1 fibre, is found in region C, which is that part of the intrafusal bundle extending beyond the end of the capsule.

Since the regional transitions and the changes in ATPase activity and myosin composition are not sharply defined, nor do they usually correspond precisely, the summary of the regional properties of the intrafusal fibres shown in Fig. 1 should be regarded as representative only. However, it serves to emphasize the following important points: (i) tonic, slow-twitch and fast-twitch myosins characterize the b_1, b_2 and c fibres, respectively. This is entirely consonant with the known speeds of contraction of the muscle fibres. (ii) There is a major change in the properties of the b_1 fibre at about the B/C boundary where it acquires an acid-stable ATPase activity and slow-twitch myosin, both of which occur throughout the b_2 fibre. There is an approximately corresponding transition in the ultra-structure of the sarcomeres from a characteristically tonic type in regions A and B to a twitch type in region C (Banks *et al.*, 1977). This partial switch from tonic to twitch properties may account for the large discrepancies usually found between the sites of focal

sarcomere shortening elicited by motor stimulation and the location of the endplates (Banks *et al.*, 1978). It is reasonable to envisage that activation of the b_1 fibre produces an increased stiffness in the tonic region together with some overt shortening in the distal, twitch-like region, though typical twitches are almost certainly not involved.

The extracapsular portion of the spindle, region C, may represent about half of the total length, but the chain fibres usually do not extend far into it. In some cases, however, one or more very much larger chain poles are present, when they may resemble, or even exceed, the length and diameter of the bag fibres. The fibres possessing these are often called long chain fibres, but usually only one pole of a fibre is of the long variety and the usage can lead to confusion. They have been described in detail only for the cat, but they probably occur in other species, including the rat (Kucera *et al.*, 1991).

3. THE MOTOR INNERVATION

At this point it may be useful briefly to summarize the distribution of the intrafusal motor innervation, as it is currently understood, and to set out the differing opinions concerning its interpretation, before examining the relevant evidence in detail. The summary will be based on hindlimb muscles of the cat; important variations present in other species will be considered later.

In general, dynamic axons, whether γ or β, innervate b_1 fibres alone, whereas static axons innervate b_2 and c fibres. There seem to be few exceptions to this generalization. Perhaps the most important and instructive are the axons that supply a b_1 and a c fibre in the same spindle. At least in the majority of these cases the c poles are long and the axons are probably β. More usually, long c (lc) poles are separately and uniquely innervated by static β axons. Thus, whereas the distributions of static γ and β axons are quite distinct, those of their dynamic counterparts are coincident even to the extent that both types may be supplied to a single b_1 pole.

Static γ axons supply the b_2 and c fibres of a single pole jointly or separately about equally often, though their overall distribution in all the spindles that they innervate usually includes both types. Nevertheless, they are not all equally distributed, since the segregated portion of their distribution tends to involve either b_2 or c fibres only. This differential effect may be correlated with the overall extent of their distribution and their conduction velocity, such that the larger, more widely distributed axons predominantly supply b_2 fibres.

How, then, are we to view the diversity in the intrafusal motor innervation? Because of the theoretical importance for control mechanisms of a separate fusimotor system and of dynamic and static divisions, it is very convenient, as well as reflecting the historical development of the subject, to speak of dynamic γ axons, static β axons, and so on. But the price of convenience is the implied suggestion that the functional attributes reside in the axons; that the several functional groups are all useful in specialized ways; and that hence there are several distinct types of

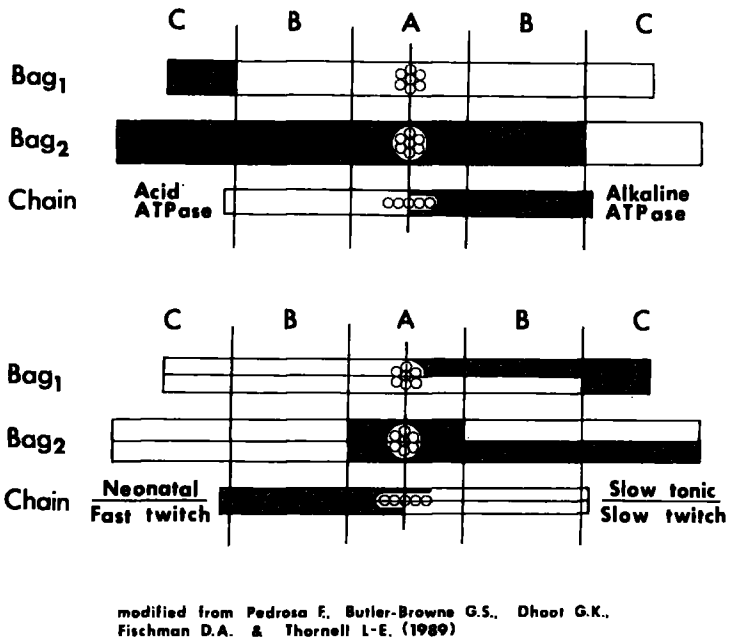


FIG. 1. Schematic diagram to show the differentiation and regional properties of the three types of intrafusal muscle fibre, as revealed by ATPase activity after acid or alkaline preincubation (top), and immunocytochemistry (bottom) using monoclonal antibodies against tonic, slow-twitch, neonatal and fast-twitch isoforms of the myosin heavy chain. High levels of reaction product are shown as black in each case. Regions A, B, and C are defined in the text. From Barker and Banks (1994), modified from Pedrosa *et al.* (1989).

TABLE 1. NUMBERS OF INTRAFUSAL MOTOR AXONAL BRANCHES HAVING DIFFERENT DISTRIBUTIONS IN TENUISSIMUS SPINDLES OF THE CAT

Distribution	Source			Totals
(a) *Serially sectioned spindles*				
	Durham	Glasgow	Boston	
b_1	7	10	66	83
b_1 (l)c	1	2	5	8
lc	0 } 5	1 } 12	23 } 60	24 } 77
c	5 }	11 }	37 }	53 }
b_2c	8	8	31	47
b_2	5	7	23	35
(b) *Silver-stained spindles*				(c) *Overall totals* ($a+b$)
	C883	Other	Totals	
b_1	45	61	106	189
b_1 lc	1	1	2	10
lc	9 } 28	12 } 44	21 } 72	45 } 149
c	19 }	32 }	51 }	104 }
b_2c	23	21	44	91
b_2	18	29	47	82
?b_1b_2		1	1	1
(d) *Distributions as proportions of relevant total* (%)				
	All serial (a)	All silver (b)	C883 (b)	Overal total (c)
b_1	33	39	39	36
$b_1$lc	3	1	1	2
lc	10 } 30	8 } 26	8 } 25	9 } 28
c	21 }	19 }	17 }	20 }
b_2c	19	16	20	17
b_2	14	17	16	16

References: (a) Durham: Banks (1981). Glasgow: Arbuthnott *et al.* (1982); Sutherland *et al.* (1985). Boston: Kucera (1985b). (b) Banks (in press).

Analyzed using (a) reconstruction from serial sections, and (b) silver-impregnated, teased spindles. Overall totals are given in (c), and proportions of the various distributional types in (d).

motoneuron. This was entirely feasible at a time when it was thought that both static and dynamic axons frequently innervated the same type of muscle fibre. Now, however, the evidence overwhelmingly supports the idea that the functional effects are consequences of the mechanical properties of the muscle fibres. In effect, we have transferred the epithet from the motor unit to the motoneuron, but this does not prove that the motoneurons themselves form correspondingly distinct groups. For the adult control system, it may be insignificant whether or not the motoneurons are inherently different at the outset of spindle development, provided the outcome of the developmental process is a high degree of segregation in their distribution. However, the question is directly relevant to our consideration of how the spindle is formed in development; how the intrafusal and extrafusal systems are related; and, of particularly current interest, whether there are more than one type of static γ motoneuron. As we shall see, the dynamic and static systems, whether γ and β, are highly segregated. This leaves little doubt that the motoneurons concerned are differentiated in the adult, having become so at the latest during peripheral reorganization of the fusimotor innervation. I shall argue that the static γ neurons, on the other hand, are not themselves differentiated into two or more types despite compelling evidence for some differential distribution among their two effectors, b_2 and c fibres.

3.1. THE INTRAFUSAL DISTRIBUTION OF MOTOR AXONS

Although the principal diagnostic features of intrafusal-fibre types remain their histochemical staining properties, subsidiary characteristics have been identified that allow fibres to be recognized in preparations suited to tracing directly the intrafusal distribution of motor axons. To the classical fibre-size and nuclear morphology (Boyd, 1962) have been added partial dissociation of b_1 from the remaining fibres in the equatorial region (Banks *et al.*, 1977), and the presence of elastic fibres most prominently associated with b_2 in the polar region (Gladden, 1976). It is thus possible to identify the fibre types in transverse sections of spindles embedded in epoxy resin. The intrafusal distribution of motor axons may be reconstructed from serial sections (Banks, 1981), with sufficient resolution to trace easily all myelinated axons and even the larger unmyelinated ones.

The greatest number of such reconstructions has been carried out for one muscle, the tenuissimus of the cat (Arbuthnott *et al.*, 1982; Banks, 1981; Banks *et al.*, 1981; Kucera, 1982a, 1983, 1984a, b, c; Kucera and Hughes, 1983a; Kucera *et al.*, 1984; Kucera and Walro, 1987, Sutherland *et al.*, 1985). Results from three independent laboratories reveal similar distributional patterns (Table 1a), in particular an almost total segregation of the motor input to the b_1 from that to the remaining fibres. Indeed, only 55 out of 250 (22%) intrafusal motor branches were supplied to more than

one type of fibre, these examples overwhelmingly (45, 19%) involving b_2 and one or more c fibres. The b_1 was only ever coinnervated with a single chain fibre, at least one example being reported by each laboratory. The c fibre involved was always among the longest present in the pole. The histological evidence cannot rule out the possibility that in each case the motor branch concerned was part of a β axon, although Sutherland *et al.* positively identify one of their examples as a γ axon. This was presumably on the basis of coincident movement of the b_1 and c fibres on stimulation of a slowly conducting axon in the ventral root, another instance of which had been previously reported by Gladden in Arbuthnott *et al.* (1982). In addition, intrafusal branches of β axons undoubtedly make up some of the selective supply to b_1 and c fibres (Banks, 1981; Kucera, 1984a, b, 1985a, b; Kucera and Hughes, 1983; Kucera *et al.*, 1984; Sutherland *et al.*, 1985). Identification of the β input will be considered in detail below; for the moment it is sufficient to note that in the case of the c fibres, β innervation is essentially restricted to *lc* poles (including Kucera's (1980a) intermediate category), which are listed separately in Table 1. The converse that the presence of *lc* fibres implies β innervation is, however, certainly not true (Banks *et al.*, in press, a). This is particularly clear for the superficial lumbrical which has a high proportion of *lc* fibres (Decorte *et al.*, 1987), though apparently very little β innervation of them (Banks *et al.*, in press, b).

Despite the large number of spindles used in the serial-section analyses they may not represent a fair sample of the tenuissimus, some having been selected to illustrate specific features of innervation (e.g. Kucera and Hughes, 1983), whereas others were derived from correlative physiological studies which tended to utilize particular parts of the muscle. Since there may be regional and individual variations in the pattern of motor innervation as there are in the sensory innervation (Banks and Stacey, 1990), it is important that some information be obtained about complete muscles. This has now been achieved in one case, again using the cat tenuissimus in a correlative histophysiological study (Banks, 1991, and in press). In these experiments the muscles were silver-impregnated, and the spindles, together with the intramuscular nerves, were teased out and examined in whole mounts. Intrafusal fibres were identified by size, equatorial nucleation, differential presence of elastic fibres, and details of the primary sensory endings (Banks *et al.*, 1982). Intrafusal branches of motor axons were traced in camera lucida drawings using an oil-immersion, 100 × objective. Under these conditions virtually all the branches could be traced with complete confidence throughout their intrafusal distribution (Fig. 2) and in some cases well into the intramuscular nerve.

The results of this distribution analysis are summarized in Table 1b. The complete muscle is designated by its laboratory identification (C883); it will be referred to again on several occasions. The close similarity between the results of the silver and of the serial-section analyses (Table 1d) serves to increase the confidence that can be placed in the individual results obtained by either technique. Again, with one possible exception, b_1 fibres were supplied alone, or rarely, in common with a *lc* pole. The exception occurred in a spindle with three bag fibres, so some doubt remains over the identity of the b_1 in this case, particularly in view of the occasional presence in such spindles of fibres having poles of different types (Kucera, 1981a).

There can be no doubt, however, that in other muscles of other species coinnervation of b_1 with b_2 and often c fibres as well, occurs quite commonly (Arbuthnott *et al.*, 1989; Kucera, 1985c,d; Kucera *et al.*, 1991; Walro and Kucera, 1984, 1985a,b). In particular, human spindles may receive such innervation, though as yet very little information is available. Kucera (1986) has reconstructed the following distributions of 13 intrafusal branches to a single biceps brachii spindle that contained $2b_1$, $1b_2$ and $4c$ fibres: b_1, 7 axons, of which 6 supplied both b_1 fibres; b_2, 1; c, 2; b_1c, 1; and b_1b_2c, 2 axons. Intrafusal motor distributions for the lumbrical, extensor digitorum longus (EDL) and soleus muscles of the rat and for the macaque lumbrical are summarized in Table 2. The proportion of those branches that supply b_1 together with other types ranges from 10% in rat EDL to 22% in macaque lumbrical. There has been some dispute concerning the interpretation of the results obtained by the two groups who carried out the work (Walro and Kucera, 1990; Gladden, 1990), though both agree on the significant presence (4–11%) of some branches that supply all three types of fibre.

The many detailed studies referred to above demonstrate that if such branches occur at all in the cat tenuissimus they must be very rare, but in the absence of equivalent information for other muscles it is not clear to what extent the cat or its tenuissimus is unusual in this respect. It is worth recalling, however, the results of Barker and Cope (1962) who also produced some reconstructions, but based on wax sections and de Castro staining. Although this work was carried out long before the two types of nuclear-bag fibre were recognized, it is possible to infer from their reconstructions that they encountered some intrafusal motor branches distributed to all three types of fibre in spindles of cat rectus femoris and soleus.

The results of Table 2 suggest that variability in the proportion of intrafusal motor branches that supply b_1 with other fibre types is only part of a larger phenomenon that will require considerably more comparative studies before its significance can be evaluated. Thus (Table 3) there is a wide range in the proportion of intrafusal branches that have mixed distributions (18% in rat lumbrical to 46% in macaque lumbrical), and the proportions of these that involve the b_1 vary greatly, as, to a lesser extent, do those involving b_2 and c fibres. One constant feature does emerge for each muscle: of all the intrafusal branches supplying a particular type of fibre, the proportion that is exclusive to that type is greatest for b_1 and least for b_2. Indeed, in each case, the majority of branches supplying b_1 do so exclusively, whereas the majority of branches to b_2 also supply other fibres, usually c.

3.2. The Complete Distribution of Motor Axons Having Intrafusal Branches

The problem of determining the complete distributions of parent motor axons having intrafusal branches is inextricably linked with that of the distinctions between β and γ axons and their static and dynamic categories. For although Crowe and

Matthews (1964) have shown that single axons normally elicit the same effect, whether dynamic or static, in each of the spindles innervated in common, it does not necessarily follow that this is achieved by segregated inputs to different types of intrafusal fibre. Indeed, during the decade following Crowe and Matthews' work, and until the separate identities of the b_1 and b_2 fibres were recognized, an alternative possibility seemed equally likely in view of the known intrafusal distribution of the motor axons: that a single type of muscle fibre could respond with different types of contraction to static or dynamic γ activity. As we shall see, a similar kind of reasoning has more recently been applied in suggesting that there may be more than one type of static γ axon.

There are daunting technical problems involved in directly tracing the complete distributions of individual axons in muscles with intact motor innervation. To date only one such study has been published, in which Adal and Barker (1965a) examined deafferented

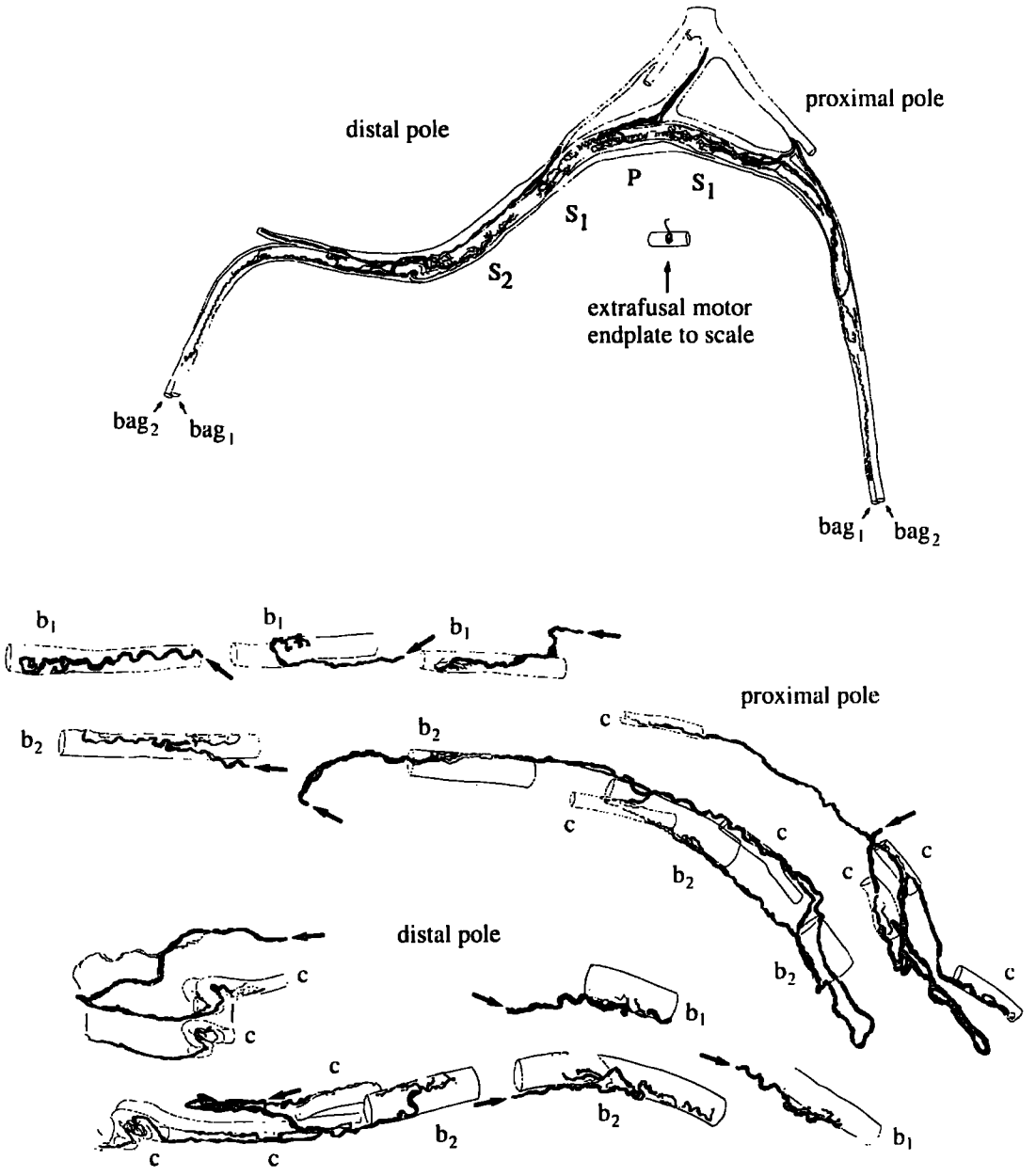


FIG. 2. Camera-lucida drawings of: (a) the complete innervation of a muscle spindle from cat tenuissimus, consisting of a primary (P) and three secondary (S) sensory endings flanked by motor endings in the distal and proximal poles; and (b) details of the motor innervation. In (b) arrows mark the incoming axon to each ending or group of endings. The two endings on the distal pole of the b_1 fibre were derived from a single intrafusal axonal branch. All the b_1 endings are morphologically of the p_1 type, indicating that their parent axons have skeletofusimotor distributions. The spindle is number eight from muscle C883: its location with respect to other spindles in its muscle is given in Fig. 11; a schematic diagram of its innervation, together with the other spindles of that muscle is given in Fig. 17.

TABLE 2. THE NUMBERS AND PROPORTIONS OF TOTALS (% IN PARENTHESES) OF INTRAFUSAL MOTOR AXONAL BRANCHES HAVING DIFFERENT DISTRIBUTIONS (a) IN LUMBRICAL (LUM), EXTENSOR DIGITORUM LONGUS (EDL) AND SOLEUS (SOL) MUSCLES OF THE RAT. AND (b) IN LUMBRICAL MUSCLE OF THE MACAQUE, AS ANALYZED BY RECONSTRUCTION FROM SERIAL SECTIONS

	Source			
(a) Rat		Kucera *et al.* (1991)		Arbuthnott *et al.* (1989)
distribution	LUM	EDL	SOL	SOL
b_1	48 (62)	45 (53)	26 (39)	9 (23)
b_1c	3 (4)	0	3 (4)	2 (5)
c	15 (19)	10 (12)	4 (6)	12 (30)
b_2c	4 (5)	15 (18)	16 (24)	8 (20)
b_2	0	7 (8)	7 (10)	6 (15)
b_1b_2	2 (3)	5 (6)	6 (9)	0
b_1b_2c	5 (6)	3 (4)	5 (7)	3 (8)

(b) Macaque	Source Kucera (1985d)
distribution	LUM
b_1	17 (31)
b_1c	2 (4)
c	9 (16)
b_2c	13 (24)
b_2	4 (7)
b_1b_2	4 (7)
b_1b_2c	6 (11)

lumbrical muscles of the cat using teased, osmiumtetroxide-stained preparations. The results showed considerable individual variation in the extent of axonal branching within a muscle (Fig. 3), and in the overall patterns of innervation displayed by each cat. But particularly important was the histological confirmation of the existence in a mammal of skelefusimotor axons, which had recently been demonstrated physiologically by Bessou *et al.* (1963).

Further progress depended on the development, in the late 60s and early 70s of combined histophysiological techniques, often very elegant. These included: reduction of the motor innervation to a single γ axon (Barker *et al.*, 1973); glycogen depletion of intrafusal fibres during prolonged γ activation (Brown and Butler, 1973); and direct observation of living spindles with intact nerve and blood supply (Bessou and Pagès, 1973; Boyd *et al.*, 1973). Common to all these, of course, was the isolation in dorsal and ventral root filaments of functionally single afferent and efferent axons (Kuffler *et al.*, 1951). We shall next consider the results that have been obtained with these techniques and their successors, and that are relevant to the complete distribution of motor axons with intrafusal branches.

3.2.1. *Dynamic and static γ and β axons*

De-efferentation of the cat tenuissimus apart from a single γ axon, followed by functional identification of the intact axon and by histological analysis of its distribution using teased, silver-impregnated preparations, allowed Barker *et al.* (1973) to establish the distributions of six static axons (Table 4). Each axon supplied from four to seven spindles. In individual

TABLE 3. A COMPARATIVE SURVEY OF THE INTRAFUSAL DISTRIBUTIONS OF MOTOR AXONAL BRANCHES BASED ON THE DATA GIVEN IN TABLES 1 AND 2

	Species/muscle				
		Rat			
	Cat tenuissimus	LUM	EDL	SOL	Macaque LUM
(a) Proportion of intrafusal branches with mixed distributions					
	19	18	28	40	46
(b) The proportion of mixed-distribution branches that involve each type of fibre					
b_1	11	71	35	44	48
b_2	90	79	100	88	92
c	99	86	78	86	84
(c) The proportion of branches to each type of fibre that are exclusive to that type					
b_1	95	83	85	65	59
b_2	47	0	23	25	15
c	59	52	36	30	30

Values are percentages.

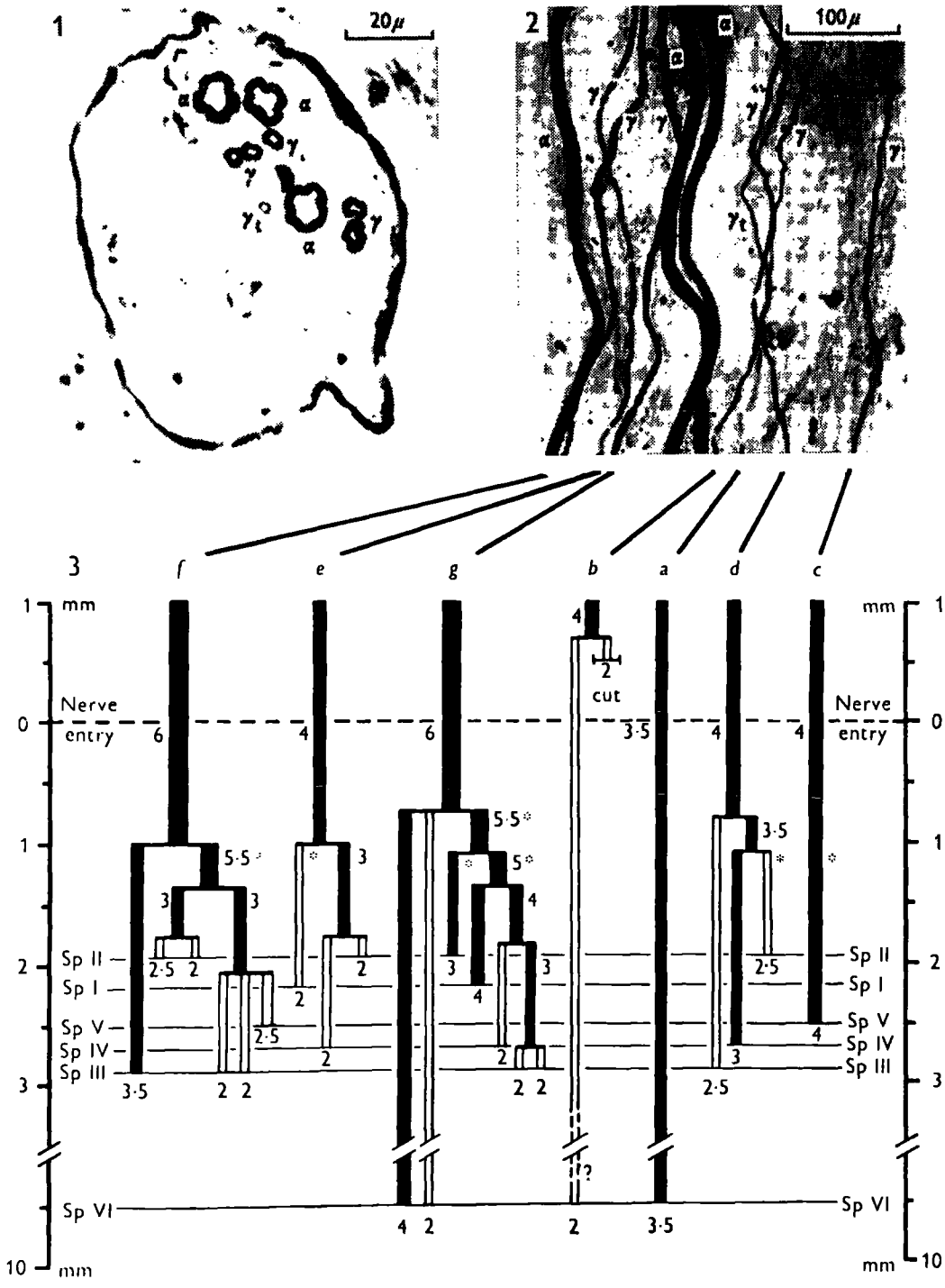


FIG. 3. (a) Transverse section of the osmium-tetroxide-stained nerve of a deafferented lumbrical muscle from cat hindlimb. (b) Whole mount of a more distal portion of the same nerve. (c) The intramuscular branching patterns of the seven γ axons revealed by teasing. All branches were myelinated; those shown white were less than 3 μm in diameter. Note that the largest stem axons tend to have the widest distributions. From Adal and Barker (1965a).

TABLE 4. THE DISTRIBUTION OF SINGLE STATIC GAMMA AXONS TO BAG AND CHAIN INTRAFUSAL FIBRES IN SIX CAT TENUISSIMUS MUSCLES, AS REVEALED BY ANALYSIS OF SILVER-IMPREGNATED, TEASED SPINDLES PREVIOUSLY DE-EFFERENTED APART FROM THE ONE γ AXON IN EACH CASE

		Pole A		Pole B	
Cat	Spindle	*b* fibres	*c* fibres	*b* fibres	*c* fibres
CT2	1				c^4c^4
	2	b^5	$c^2c^2c^3$		
	4		$c^{11}c^8c^4$	b^{13}	$c^9c^2c^7$
	7			b^5	
CT3	2			b^{13}	c^7c^4
	3				$c^{18}c^7c^1c^{12}$
	11	b^4	c^8		
CT4	1	b^4	c^{12}	b^7	$c^4c^2c^2c^2$
	2			b^7	
	3	b^{18}	$c^{12}c^4$	b^{10}	
	6	b^{12}			
CT8	1		c^8		c^{14}
	2	$b^{30}b^3$	c^5c^5		
	3			b^{22}	c^4c^{10}
	5			b^{18}	
	6	b^{14}	c^7c^3		
	7	$b^{24}b^{11}$		b^6	$c^8c^7c^3$
	8	b^{10}			
CT10	1	b^6			
	2	b^4	$c^8c^5c^5c^4$	b^5	$c^4c^6c^2c^5$
	3				$c^{26}c^8c^3$
	4		$c^2c^6c^4$		
	5		c^{13}		
	6	b^6	c^2c^2	b^3	$c^{12}c^7c^3c^{11}$
	7				$c^{10}c^5c^6c^2$
CT11	1			b^{11}	c^6c^3
	3	b^7			
	5	b^2	c^5		
	7	b^{12}			
	8				c^8

Each fibre is shown with the number of motor terminals that it received. From Barker *et al.* (1973).

spindles (30) the bag and chain fibres might be innervated separately (bag seven spindles; chain, eight spindles) or together (15 spindles); but the complete distribution of a single axon always included both types. In most spindles, bag–fibre innervation was restricted to only one of the two bag fibres usually present.

Brown and Butler (1973) used glycogen depletion to infer the distribution of both dynamic and static γ axons, again in cat tenuissimus. They found that stimulation of dynamic axons almost exclusively depleted bag fibres, often just one in each spindle, whereas static axons usually depleted both bag and chain fibres together, and often more than one bag fibre in each case.

With the third technique, observation of living spindles, Bessou and Pagès (1975) found that dynamic γ stimulation produced movements in bag fibres, that static γ stimulation produced movements equally often in chain fibres, bag fibres or both, and that dynamically activated bag fibres (11 examples) were never also activated by static axons.

The apparently conflicting results of the glycogen depletion and direct observation experiments were confirmed following the separate recognition of b_1 and b_2 fibres. Barker *et al.* (1976a) reported the glycogen depletions produced by single dynamic or static γ axons in as many as seven spindles of cat tenuissimus. The four dynamic axons always (17 examples) depleted b_1 fibres, rarely with a *lc* pole (two examples) or a b_2 fibre (one example) in addition. The eight static axons apparently depleted all three types of fibre in various combinations: b_1, 3; b_1 and *c*, 6; b_1 and b_2, 4; b_1, b_2 and *c*, 3; b_2 and *c*, 9; b_2, 1; and *c*, 6 (Fig. 4). Overall the two types of bag fibre were depleted equally often during static γ stimulation, and a similar result, though with less apparent b_1 involvement, was subsequently obtained from cat peroneus brevis by Emonet-Dénand *et al.* (1980). However, with direct observation Boyd *et al.* (1977) showed that dynamic axons (γ or β) always activated b_1 fibres, whereas static γ axons activated b_2 alone (11 axons), c alone (12 axons) or both b_2 and *c* (nine axons), but never b_1. For this they combined a tenuissimus preparation similar to that of Bessou and Pagès (1975) with Gladden's (1976) observations on elastic-fibre distributions.

Further support for the frequent innervation of b_1 fibres by static axons, this time in cat soleus, was provided by Emonet-Dénand *et al.* (1977b), who described categories of primary-ending responses to stimulation by single γ axons that were intermediate between pure static or dynamic effects and that could be mimicked by simultaneously activating the two pure effects in appropriate measure. If the intermediate effects were due to co-innervation of b_1 with b_2, *c*, or both, this would eliminate a possible reconciliation of the results obtained by glycogen depletion and direct observation: that the b_1 fibres were innervated by static γ axons, but their neuromuscular junctions were ineffective in eliciting b_1 contraction even though they could produce glycogen depletion. Again, however, direct observation with tenuissimus contradicted the joint activation of b_1 with other types of fibre in the generation of the intermediate effects (Boyd *et al.*, 1979), though the possibility of a genuine difference between muscles must remain until the motor innervation of soleus spindles has been analyzed histologically.

The results of the first histological analysis of the intrafusal motor supply following the differentiation of the two types of bag fibre (Banks, 1981) have been described above. They unequivocally demonstrated that b_1 fibres could not share their motor input with b_2 or *c* fibres anywhere nearly often enough to account for the glycogen depletion of b_1 during static γ stimulation, at least in cat tenuissimus, and implied that these depletions were largely, if not entirely, artefactual. It should be recalled, however that depletion of only b_1 was rare during static stimulation, so that the artefact seems to involve some factor, such as K^+ accumulation, arising from the activity of b_2 or *c* fibres. This was borne out in experiments by Decorte *et al.* (1984) who found that b_1 depletion resulting from static γ stimulation was only common when there was evidence that the activated intrafusal fibres had been fatigued. The existence of non-neural activation of the b_1 has also been attested in connection with its possible role in enhancing the dynamic sensitivity of

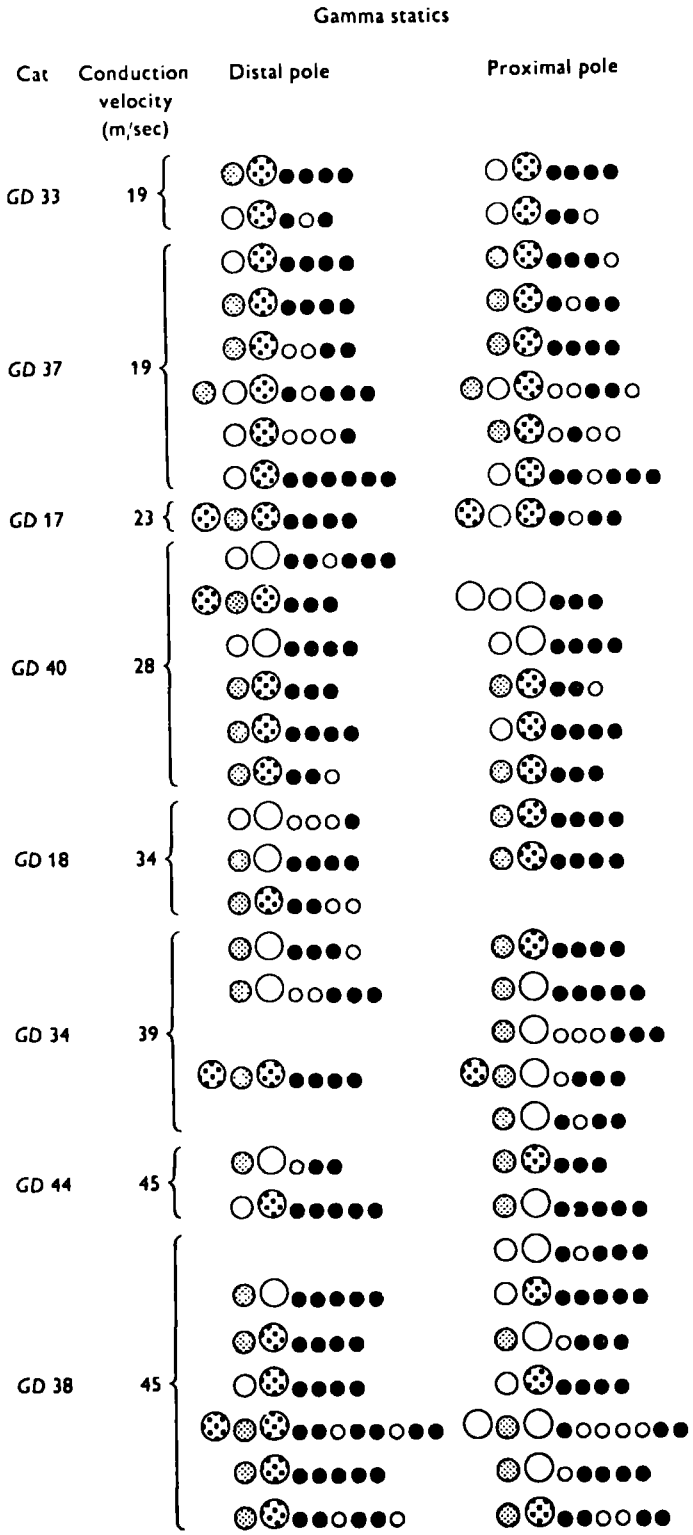


FIG. 4. Schematic diagram to show the patterns of glycogen depletion seen in cat tenuissimus spindles following prolonged static γ stimulation. Each γ axon was from a separate experiment designated by its laboratory identification (e.g. GD33). Activity in single axons depleted fibres in up to seven spindles. Filled symbols indicate undepleted fibres: large, coarse stipple, b_2; medium, fine stipple, b_1; small, black, c. Empty symbols indicate depletion. Note that every axon depleted at least one c fibre and that the b_2 was not depleted by the three slowest axons. From Barker *et al.* (1976b).

the primary ending. Decorte *et al.* (1986) showed that stretch alone could produce depletion in most (70%) of the b_1 fibres in de-efferented lumbrical muscles, but not in *c* fibres and only rarely (7%) in b_2. It follows that the results of glycogen-depletion experiments during dynamic stimulation are not invalidated, nor those relating to b_2 or *c* fibres during static stimulation.

Skeletofusimotor (β) axons were first revealed in cat lumbrical muscles by the persistence of their intrafusal effects after all extrafusal transmission had been eliminated by curare (Bessou *et al.*, 1963). They were dynamic in action with conduction velocities between 31 and 61 msec^{-1}. Emonet-Dénand *et al.* (1970) subsequently found both dynamic and static β axons in rabbit lumbrical; indeed they found only β axons in this muscle, despite looking for purely fusimotor ones. It may be noted here that the conduction velocities of the dynamic (27–70 msec^{-1}, mean 40.1 msec^{-1}, $n=8$) and static (37–50 msec^{-1}, mean 43.5 msec^{-1}, $n=4$) axons did not differ, in contrast to the situation that later became familiar from studies on the cat (see below). Despite both histological and physiological evidence for the widespread and frequent occurrence of β axons in various species as well as in several hindlimb muscles of the cat (Adal and Barker, 1965a, b; Barker *et al.*, 1970; Emonet-Dénand *et al.*, 1970; Emonet-Dénand and Laporte, 1975; Kidd, 1964; McWilliam, 1975), they seem to have been widely regarded as a vestige of the mammal's reptilian ancestry, until Emonet-Dénand *et al.* (1975) provided physiological confirmation of the histological results concerning several of the larger hindlimb muscles. The importance of skeletomotor axons, at least numerically, could no longer be denied. Studies on their distribution soon followed, as well as confirmation of a further prediction from the histology (Barker *et al.*, 1970): that they were more common than could be accounted for by the relatively slowly conducting axons so far described, and that consequently some faster-conducting axons should prove to be β also.

Glycogen depletion elicited by dynamic β stimulation in tenuissimus and peroneus brevis of the cat yielded intrafusal distributional patterns virtually identical to those of dynamic γ axons (Barker *et al.*, 1977). The b_1 was depleted in one or both poles of 18 spindles with, in only one each of these spindles, a b_2 fibre or *lc* pole in addition. Individual axons supplied up to four spindles in the portions of the muscles examined histologically. The extrafusal component of their motor units was composed of slow-oxidative fibres, equivalent to Brooke and Kaiser's (1970) type I (see also Burke and Tsairis, 1977).

Glycogen depletion was again used to demonstrate the occurrence and incidence of rapidly conducting β axons in peroneus tertius (Harker *et al.*, 1977) and tenuissimus (Jami *et al.*, 1978, 1979) of the cat. Stimulation of groups of axons conducting at 85 msec^{-1} or more resulted in the longest c fibre being depleted in one or both poles in 26 of 99 (26%) peroneus tertius and 14 of 46 (30%) tenuissimus spindles. In ten and three of these spindles respectively an additional one or, rarely, two *c* fibres were depleted, almost always the next longest fibres. When present, long *c* fibres, as originally defined by Barker *et al.* (1976b), were among those depleted. The remaining depleted fibres would presumably fall into Kucera's

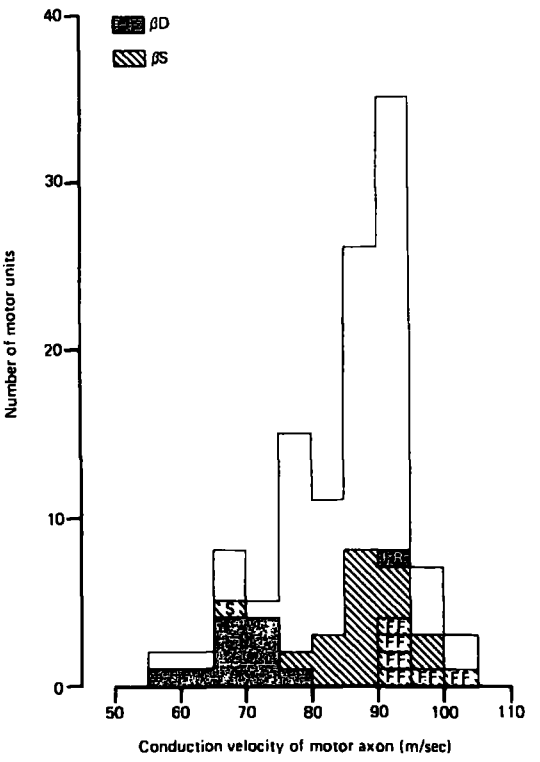


FIG. 5. Histogram of the conduction velocities of 114 skeletomotor axons in peroneus tertius of the cat. Filled blocks indicate those with intrafusal effects also: black, dynamic; hatched, static. The extrafusal motor unit types associated with the skeletofusimotor axons were slow (S), fast, fatigue-resistant (FR), or fast, fatiguable (FF) as shown. The static-associated units not individually identified were FR. Note the high proportion of skeletofusimotor effects shown by the more slowly conducting axons. From Jami *et al.* (1982a).

(1980a) intermediate category. Taking both muscles together, in three of the spindles the b_1 and in one spindle the b_2 were depleted as well as the *c* fibres. Additionally and only rarely, the b_1 and possibly b_2 (two spindles) or the b_1 (one spindle) but not *c* fibres were depleted. As expected, inclusion of axons conducting at 60 msec^{-1} or more resulted in a similar overall pattern of depletions but with greater b_1 involvement (Jami *et al.*, 1978). In this case 19 of 47 spindles (40%) contained depleted fibres, 12 of them (25%) again involving the longest *c* fibres, and nine (19%) the b_1. As estimates of the proportion of tenuissimus spindles that receive β innervation these must be considered minimum values since some β axons are known to conduct at less than 60 msec^{-1}, neither was it possible to stimulate all axons conducting at 60 msec^{-1} or more that were identified in ventral-root filaments. In practice about 90% (65/71) were included.

Jami *et al.* (1979, 1982a) demonstrated that the rapidly conducting axons usually had static effects and could excite secondary endings, as was expected on the basis of their predominantly c-fibre intrafusal distribution. In cat peroneus tertius they (Jami *et al.*, 1982a) identified as β 36 (32%) of 114 motor axons with conduction velocities between 56 and 104 msec^{-1}:

24 were classified as static and 12 as dynamic (Fig. 5). Together these activated 49% (28/57) of primary and 50% (19/38) of secondary endings. Usually only a single β axon affected each sensory ending, but two or, rarely, three might do so, generally at least one of each functional category. Since the primary endings are normally distributed to all the intrafusal fibres, their responses may be taken to reflect the balance of motor effects elicited by the isolated β axons. From a total 41 effects, 18 were dynamic. This is a greater proportion (44%) than is the number of dynamic axons compared to the total (12/36, 33%), and may indicate that on average the dynamic β axons supply more spindles than do their static counterparts, an observation that has just been confirmed by Emonet-Dénand *et al.* (1992). Adal and Barker (1965a), in their study on the intramuscular distribution of motor axons in cat lumbrical muscles, noted an approximately reciprocal relationship between the diameter and intrafusal contribution of five skeletofusimotor axons (Fig. 6). Again this may be accounted for if the slower and therefore smaller dynamic β axons are distributed among more spindles than are the static ones.

Jami *et al.* (1982a) and Emonet-Dénand *et al.* (1992) have provided the fullest descriptions so far of the extrafusal composition of β motor units. In peroneus tertius there is a clear distinction between slow and fast units and a close correlation with axonal conduction velocity (Fig. 5), the two types segregating at about 75–80 msec^{-1}. The clear distinction between dynamic/slow and static/fast β units exists also in peroneus brevis, but here correlation with axonal conduction velocity is no longer so close due to the very wide range of velocities of the dynamic axons (50–85 msec^{-1}) overlapping considerably that of the static axons (75–95 msec^{-1}). Emonet-Dénand *et al.* (1992) observed that the proportions of spindles innervated by dynamic and static skeletofusimotor axons in peroneus brevis (dynamic, 68.5%; static, 17.1%) and in peroneus tertius (dynamic, 50%; static, 40%) reflect in a general way the relative proportions of the slow and fast, fatigue-resistant extrafusal motor units in those muscles.

Although slow units form only 23% of the total in peroneus tertius (Emonet-Dénand *et al.*, 1988) they include a disproportionately large number of β units. Thus, of the axons that conducted at 75 msec^{-1} or less in the study of Jami *et al.* (1982a), 11 out of 17 (69%) were β, whereas of those that conducted at more than 80 msec^{-1} only 23 out of 82 (28%) were. Within the fast motor units, as in the complete motor-unit population, those with an intrafusal presence are predominantly of the relatively slower (fast-fatigue resistant) group at least as judged by their physiological properties (Jami *et al.*, 1982a; Emonet-Dénand *et al.*, 1992). The lower average conduction velocity of β as compared to α axons (McWilliam, 1975) is, therefore, a consequence of sampling this distributional pattern,

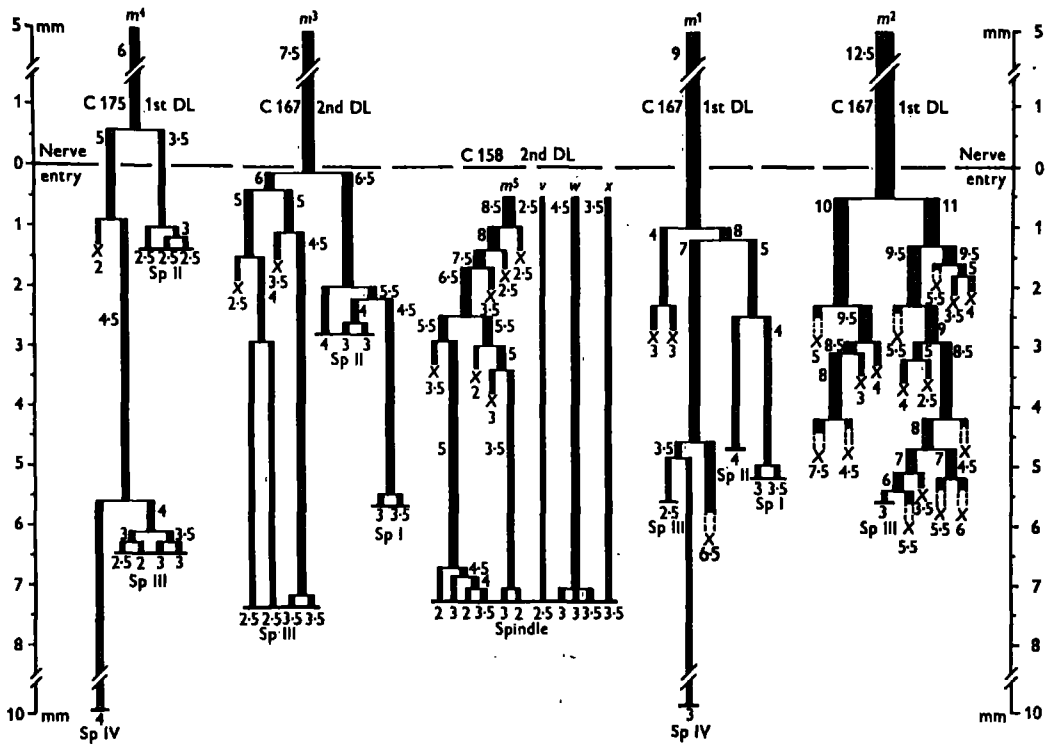


FIG. 6. Diagram of the intramuscular branching patterns of five skeletofusimotor axons (and three γ axons, *v*, *w*, *x*) from 1st or 2nd deep lumbrical (DL) muscle of cat hindlimb. Their intrafusal (Sp) and extrafusal (X) distributions are indicated. Note the approximately reciprocal relationship between stem-fibre diameter and extent of intrafusal involvement. Axons were traced by teasing deafferented muscles stained with osmium tetroxide. From Adal and Barker (1965a).

rather than arising from two distinct populations of neuron. In addition, the maximum tetanic tensions of corresponding types of α and β motor units are indistinguishable, further emphasising their essential unity (Jami *et al.*, 1982a).

Barker *et al.* (1992), using serial sections and glycogen depletion, have reconstructed the motor unit of one dynamic β axon (c.v. 72 $msec^{-1}$) and sampled the motor units of three static β axons (c.v.s. 90–95 $msec^{-1}$) from peroneus tertius. Whereas it is generally supposed that extrafusal motor units are homogeneous, they found that the extrafusal components of these units consisted of a mixture of fibre types, but predominantly type I (slow) or type IIB (fast-fatiguable) in the slow and fast units respectively.

3.2.2. *Variability of static responses and the distribution of static γ axons*

Recognition of the artefactual nature of b_1 glycogen depletion during static γ stimulation removed the major obstacle to supposing that the mechanical properties of active intrafusal muscle fibres are characteristic for different types of fibre, and are separately responsible for mediating dynamic and static fusimotor effects. The existence and distributions of dynamic and static β axons lends further support to this idea. Moreover, since b_2 and c fibres may be supplied separately or together by single intrafusal branches of static γ axons, it should be possible to provide a consistent explanation for the variety of static fusimotor responses shown by individual sensory endings and hence to infer the overall distribution of static γ axons.

Using direct observation of living cat tenuissimus spindles, Boyd and his colleagues (Boyd *et al.*, 1977, 1993a; Boyd, 1981) showed that entrainment of the primary ending at the stimulus frequency or a submultiple of it (1:1 driving, 1:2 driving, and so on), over some range of input frequencies, was due to the rapidly contracting c fibres, as others had speculated (Matthews, 1972). Driving was rarely produced by the b_2 fibre acting alone, which characteristically biased the primary response, increasing both its mean firing rate and, to some extent, its variability. Initially co-activation of b_2 and c fibres was said always to result in primary driving, usually more secure or to higher frequencies, than that produced by c fibres alone (Boyd *et al.*, 1977). Later, however, Boyd (1981, 1986) does not make clear how co-activation was recognized.

Subsequently Boyd (Boyd *et al.*, 1983b; Boyd, 1985, 1986) used these correlations, and others involving secondary endings (Boyd, 1981), to infer the distribution of single static γ axons among several spindles. He concluded that, almost without exception, each axon always supplied either b_2 or c fibres, though not always exclusively so, and that therefore there exist two kinds of static γ motoneuron which he proposed to call static bag and static chain γ motoneurons respectively (Boyd, 1986). His evidence, however, is not compelling, and is contradicted by that of others.

It is undeniable that some axons predominantly supply one or other type of fibre (Barker *et al.*, 1973, 1976a), or might appear to do so if only part of their distribution can be determined (Banks *et al.*, 1985a). But this is to be expected, to some extent, even if the intrafusal branches of individual axons are randomly distributed to b_2 or c or both. The key issue is, of course, the degree to which the overall distribution of axons is mixed, including whether single axons often innervate b_2 and c fibres separately in different spindles. The most direct evidence relevant to this problem is provided by the experiments of Barker *et al.* (1973) in which the tenuissimus was deefferented apart from a single static γ axon. Not only did each of the six axons thus isolated supply both b_2 and c fibres (in 3 to 7, average 5 spindles), but the three most widely distributed had intrafusal branches to b_2 and c fibres alone in separate spindles (Table 4). Glycogen depletion, while less direct, confirms these results (Fig. 4): in five out of six experiments (Barker *et al.*, 1976a), where depletions were produced in at least two spindles on stimulation of a single static γ axon, both b_2 and c fibres were involved. Again, three of the axons each appeared to innervate b_2 and c fibres separately in different spindles. The sixth experiment resulted in depletions in c fibres alone (ignoring b_1) in six spindles.

Boyd (1986) used the responses evoked by the activity of 37 static axons to infer the axonal distributions within and among 57 spindles. Although he reported a large number (17) that supplied either b_2 (5) or c (12) fibres alone, most of these (b_2 3, c 12) evoked positive responses from, or were tested on, only two or three spindles. A further 20 axons produced overall mixed effects (in 1–6, mostly two or three spindles), but only one of them appeared to innervate b_2 and c fibres alone in different spindles. Since the proportion of axons giving mixed effects was much greater when, for whatever reasons, more spindles were included (6/8, 75%, of axons to 4–6 spindles; 14/29, 48%, of axons to 1–3 spindles), it seems likely that many of the restricted but exclusively b_2 or c distributions were actually part of larger, mixed distributions. Nevertheless there is still a discrepancy between Boyd's (1986) observations and the histological data in that he attributed a rather small proportion (25%) of individual effects to activation of both b_2 and c fibres, and a very high proportion (47%) to c fibres acting alone.

Boyd (1986) used this discrepancy to argue that some of the intrafusal motor connexions are ineffective; thus, although the actual distributions of static γ axons might not obviously segregate into two types, what matters functionally is that their effective distributions would do so. However, not only would numerous ineffective connexions be unusually profligate, but the frequent provision of the complete polar static γ supply by single intrafusal motor branches (see Section 3.4) would imply that many intrafusal fibre poles were effectively without motor input. Since the axons and terminals are undoubtedly present, this seems to be intrinsically unlikely. In fact, both glycogen depletion (Barker *et al.*, 1976a) and observation of intrafusal fibre-movements (Bessou and Pagès, 1975; Boyd *et al.*, 1977) indicate that b_2 and c fibres are activated together in single poles about as often as the histological data suggest.

There are, of course, other possibilities that might account for the apparent paucity of joint innervation of b_2 and c fibres by single intrafusal motor branches in Boyd's (1986) observation. Length-dependency of the driving response (Banks, 1991; Bessou *et al.*, 1968;

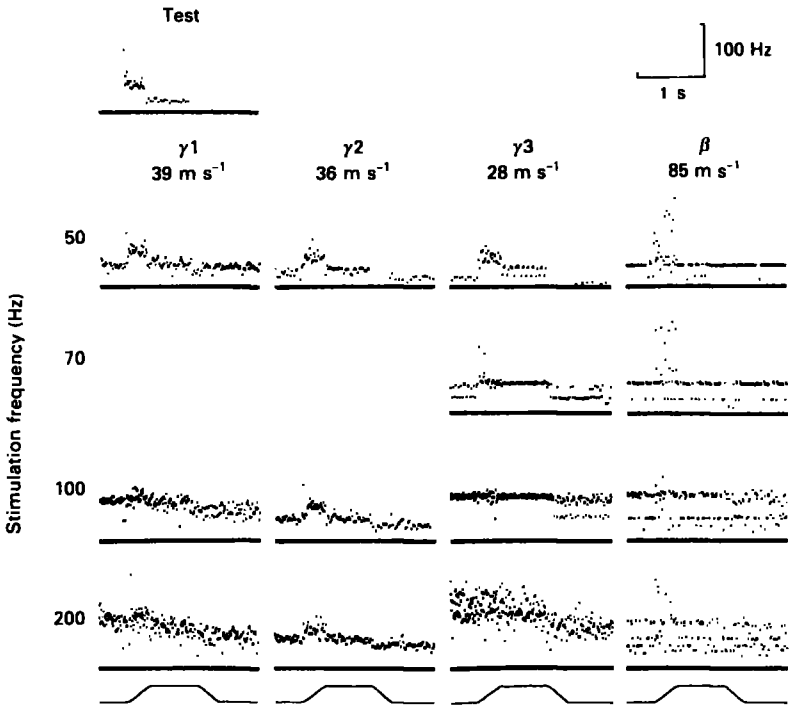


FIG. 7. Instantaneous frequency (calculated as reciprocal interspike-interval) displays showing the responses of a primary ending from cat tenuissimus to ramp-and-hold stretch in the absence (test) and the presence of motor stimulation at various frequencies. The motor axons all had static effects on the primary responses, γ1 and γ2 producing different amplitudes of biassing, γ3 and the skeletofusimotor (β) axon both driving. Note that the driving γ has the slowest conduction velocity. Each response plot includes a continuous baseline representing zero frequency. Muscle length is indicated at the bottom of each column, extension by upward displacement of the line. From Banks (1991).

Boyd *et al.*, 1985) might be a contributory factor, as could Boyd's use of secondary-ending responses alone to infer static γ distributions in some spindles. But perhaps most important could be a combination of variability in the intrafusal mechanical components with non-linearities in the mechanical transmission to the sensory endings. Thus the number of *c* fibres present and the proportion of them activated by single intrafusal motor branches can undoubtedly affect sensory responses (Jami *et al.*,1980). Non-linear mechanical coupling between intrafusal fibres has yet to be systematically studied, but it is indicated by juxta-equatorial kinking of *c* fibres at short muscle lengths and unloading of *c* fibres by b_2 contraction (Boyd, 1976; Poppele and Quick, 1985), while some tension presumably remains in the *c* fibres equatorially (Banks, 1986, 1991). Such effects could account for the instances of joint b_2c activation that Boyd (1986) acknowledges were only found after observation of intrafusal-fibre movements in the spindles concerned. Since 44% (11/25) of intrafusal branches that were identified by Boyd as jointly supplying b_2 and *c* fibres occurred in the directly observed spindles, but only 21% (12/57) of spindles were observed, his estimate of the numbers of branches to both types of fibre is most probably too low.

Finally, another and unrelated effect could have contributed to the apparent discrepancy. Boyd used the mid-portion of the tenuissimus muscle, which receives the nerve supply and contains the proximal part of the main intramuscular division of the nerve. As will be shown below (Section 3.5) it is in just this region that the greatest amount of segregated static input tends to occur.

Some of the spindles that were observed by Boyd were subsequently reconstructed from serial sections as a check on the inferred patterns of innervation (Sutherland *et al.*, 1985). This is extremely time-consuming, so that necessarily only selected spindles can be examined. To overcome this problem, Banks (1988, 1991) combined physiological mapping with silver staining, so as to allow an extensive correlation of the physiological and histological results. A series of critical correlations established the following relationships: (i) driving of primary endings at the stimulus frequency over some part of a range of fixed frequencies from about 50–100 Hz was due to *c* fibres acting alone. In one case, consistent with the observations of Jami *et al.* (1985a,b) on static β axons, driving was produced by a rapidly conducting (85 ms^{-1}) axon. The histology subsequently indicated that the effect was mediated by a single *lc* pole. (ii) Biassing of the primary response with no sign of driving at the fundamental or subharmonic stimulus frequencies was due to b_2-fibre activity, either alone or combined with *c* fibres. Examples of these correlations are shown in Figs 7–10.

Some responses could not be placed into either the

1:1 driving or biasing categories, in that they combine features of the two. For example, at high stimulus frequencies driving axons typically evoked high mean frequency, highly variable primary-ending responses, an effect that in some cases was combined with an otherwise typical biasing response. Alternatively, driving might be present but not well developed, perhaps occurring at a very restricted range of stimulus frequencies or muscle lengths, or else being exclusively subharmonic. The b_2/biasing and c/driving correlations suggested that these indeterminate responses were due to joint activation of b_2 and c fibres. This overall scheme could be applied consistently in the most extensive of the histophysiological experiments, C883, (Fig. 11), to which reference has already been made (Section 3.1).

On the basis of the biassing and indeterminate response categories and their histological correlations, Banks (1991) suggested that b_2 activity could partially or completely occlude the effects on primary endings of c-fibre contraction in the same pole. This mechanical interaction would be expected to be non-linear and length dependent. It would not be necessary to postulate ineffective neuromuscular junctions in order to account for the apparent discrepancy between the histological and physiological results. In this context it is interesting that spontaneous c activity has been observed to cease during active b_2 shortening when fusimotor neurons were recruited by stimulation in the region of the red nucleus (Dickson and Gladden, 1990) or cortex (Asgari-Khozankalaei and Gladden, 1990), though the authors took this to signify simultaneous excitation and inhibition of separate γ neurons by b_2 and c fibres respectively.

Absence of driving during combined b_2 and c activity contradicted Boyd's (1986) conclusions and may seem difficult to accept since at least with higher rates of stimulation the mean firing frequency during a biasing response is normally less than the stimulation rate and, hence, the potentially driven frequency. The phenomenon has, however, been confirmed by Dickson *et al.* (1993) using direct observation of living spindles.

Two experiments of Banks (1991) each yielded 63 combinations of primary efferents and static γ axons, of which 47 and 37% were effective (Tables 5 and 6). Although there was no evidence for the segregation of the axons into more than one kind, there was clear evidence of important correlations between the distributions of the axons and their conduction velocities. Thus, whereas individual axons could evoke biassing, indeterminate and driving responses in different spindles, faster-conducting axons tended to be more widely distributed among spindles than were more slowly conducting ones but less likely to innervate c fibres alone (as judged by driving responses). Most remarkable was that axons that produced 1:1 driving in particular spindles were likely to be the slowest- (and, when appropriate, next slowest-) conducting static axons supplied to those spindles, even though they might not have been the slowest in the muscle as a whole. Statistical tests and estimates of probability demonstrated that these relationships between distributional properties and conduction velocities were unlikely to have occurred by chance.

Evidence for a relationship between the likelihood of driving or c-fibre innervation by a static γ axon and its conduction velocity can also be found in several studies on tenuissimus (Barker *et al.*, 1976a; Boyd *et al.*, 1977), tibialis anterior (Brown *et al.*, 1965) and peroneus brevis (Dickson *et al.*, 1993). In one case (Barker *et al.*, 1976a) the results can be shown to be statistically significant (χ^2 test). Such a relationship allows the possibility of a limited amount of separate central or reflex control of b_2 and c fibres (as has been reported by Gladden and McWilliam, 1977a,b, and Wand and Schwartz, 1985), or of sequential recruitment according to the size principle

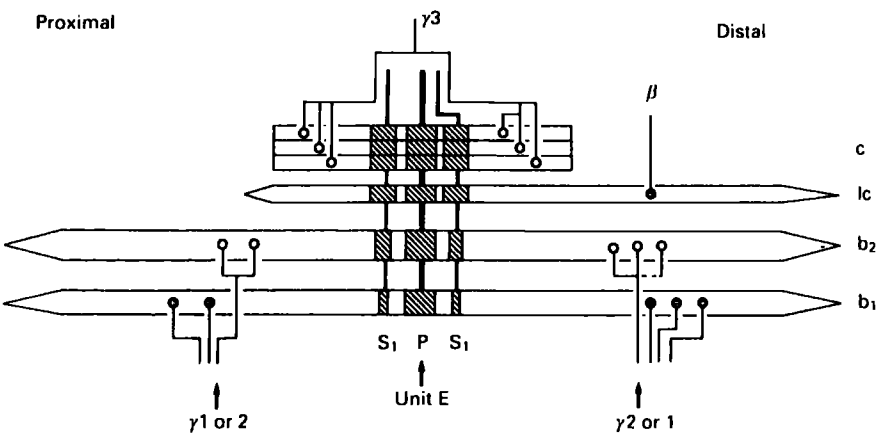


FIG. 8. Schematic diagram of the innervation of the spindle that contained the primary ending whose responses are shown in Fig. 7. There were probably four static motor axons present in the spindle: the connection shown by the dashed line is uncertain. Since the skeletofusimotor (β) axon drove the primary and innervates a chain (specifically *Ic*) fibre, it may be assumed that the only other axon to supply c fibres was the driving γ (γ3). This leaves the biassing γ axons (γ1 and γ2) to supply both poles of the b_2 fibre, at least one of which must do so. Filled symbols indicate p_1 motor endings characteristic of skeletofusimotor axons (see Section 3.3.1). Based on a teased, silver-impregnated preparation. From Banks (1991).

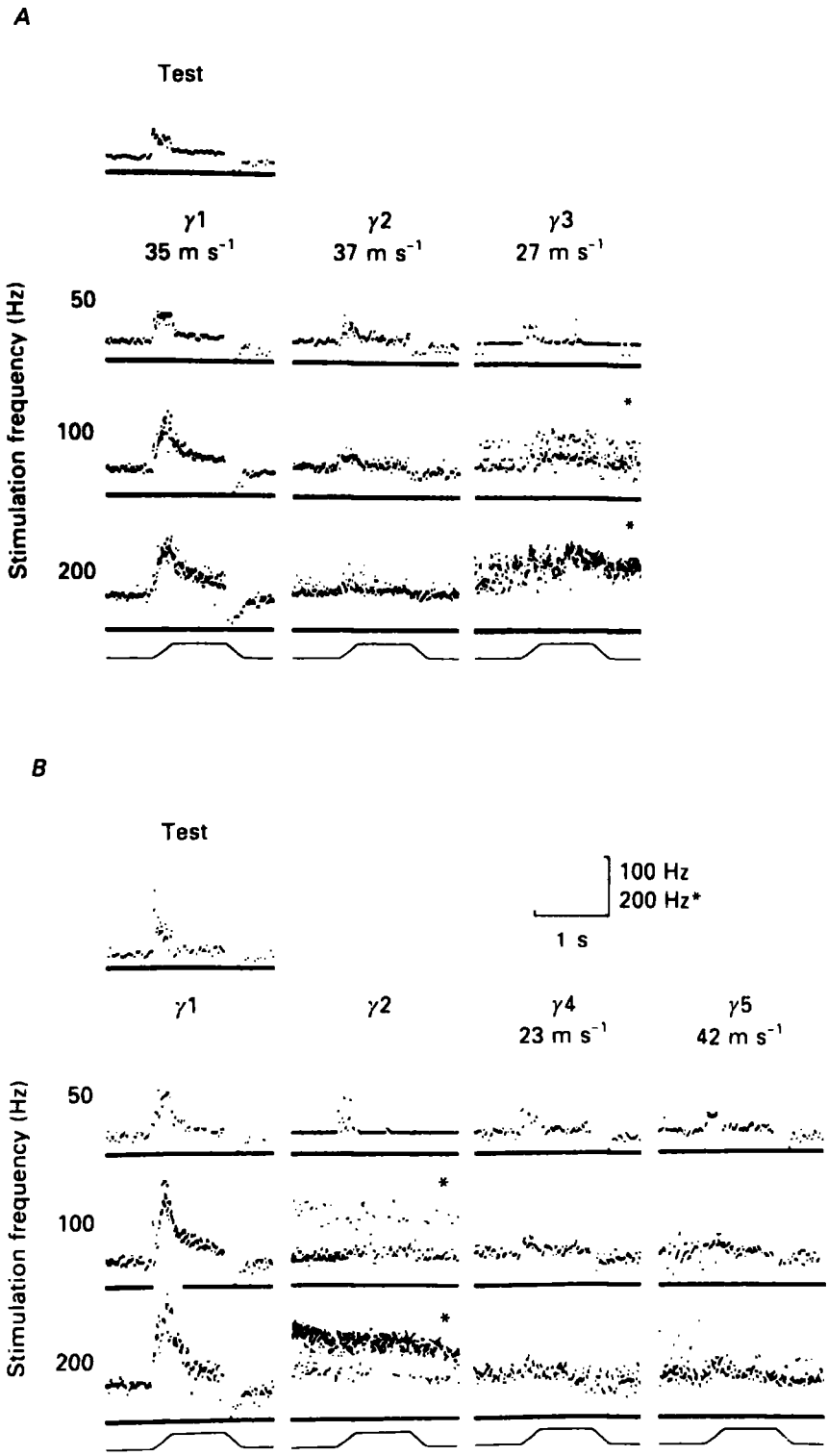


FIG. 9. Instantaneous frequency displays showing the responses to ramp-and-hold stretch of two primary endings in adjacent spindles of cat tenuissimus, in the absence and the presence of fusimotor stimulation. Arrangement as in Fig. 7. (A) The effects of activation of three γ axons on one primary (afferent unit D of muscle C689): γ1 is dynamic; γ2, biasing static, but with some sign of subharmonic driving (1:2) during the hold phase of the stretch at 200 Hz stimulation; γ3, driving static. A secondary ending (afferent unit E) present in the same spindle was only excited by γ2. (B) The effects of activation of four γ axons on the second primary (afferent unit F): γ1 and γ2 are the same as in (A); γ1 is again dynamic, but γ2 now drives the primary; γ3 had no effect, but two new axons, γ4 and γ5, had similar biasing static actions. From Banks (1991).

(Henneman, 1981) for which there is also some evidence (Murphy, 1981). However, the segregation, as shown by the actual or effective distributions of the static γ axons, is so imprecise that it cannot be taken as evidence for more than one type of neuron. An example of this imprecision in a muscle other than tenuissimus is provided by Emonet-Dénand *et al.* (1977b) who found that in peroneus brevis 1:1 driving, which occurred in 10 of 76 static effects on primary responses, was produced by 8 of the 25 γ axons involved, each of which also evoked other effects in different spindles (Fig. 12). Although Dickson *et al.* (1993) describe some static γ axons supplying the peroneal or tenuissimus muscles of the cat as non-driving in that they produce only a biasing effect in all of their target spindles, there is nothing to indicate that they form a distinctly separate group from the remaining γ axons that evoke mixed or driving effects.

Celichowski *et al.* (1993), using peroneus tertius, found that 57% of 42 static γ axons, each one distributed to 3–6 spindles, elicited effects attributable to both b_2 and c fibres, acting separately or jointly in at least two spindles. The remaining 43% were equally divided between those that always appeared to innervate either b_2 or c fibres. Celichowski *et al.* call these "specific slow" and "specific fast" respectively. It should be noted that "slow" and "fast" refer here to the apparent speed of the intrafusal contractile events elicited by axonal activity, and not to the conduction velocities of the axons themselves. Moreover, the authors included an axon as specific even when b_2 and c fibres seemed to be co-innervated in one of the spindles supplied by it. In a full treatment of their results, including a statistical analysis, Celichowski *et al.* (1994) conclude that the apparently specific axons can be accounted for on a probabilistic basis assuming only one type of static γ neuron with the ability to innervate either b_2 or c fibres.

Dickson *et al.* (1993) appear to have used a stricter criterion in describing static axons either as "non-driving" or "driving" (otherwise equivalent to "specific slow" and "specific fast" respectively), in that the same effect had to be produced in each affected spindle. However, this is probably offset to some extent by including in each category those axons acting on only two spindles. The greater mean number of effects per axon seen among the group showing mixed actions (3.24) than either the non-driving (2.96) or driving (2.36) groups (data derived from Table 1 of Dickson *et al.*, 1993), indicates that categorization is as much a sampling as a real phenomenon, as Banks *et al.* (1985a) argued was to be expected.

The "non-driving" effect of Dickson *et al.* (1993) seems to be directly equivalent to that called "biasing" by Banks (1991). However, any significantly detectable entrainment of the primary response at fundamental or sub-harmonic stimulus frequencies is called "driving" by Dickson *et al.*, whereas Banks restricted use of the term to the fundamental frequency, and allocated subharmonic responses to his "indeterminate" effect. A low probability of entrainment is perhaps best detected with post-stimulus-time histograms (Emonet-Dénand *et al.*, 1977a; Hulliger, 1979) as used by Dickson *et al.* (1993), but not by

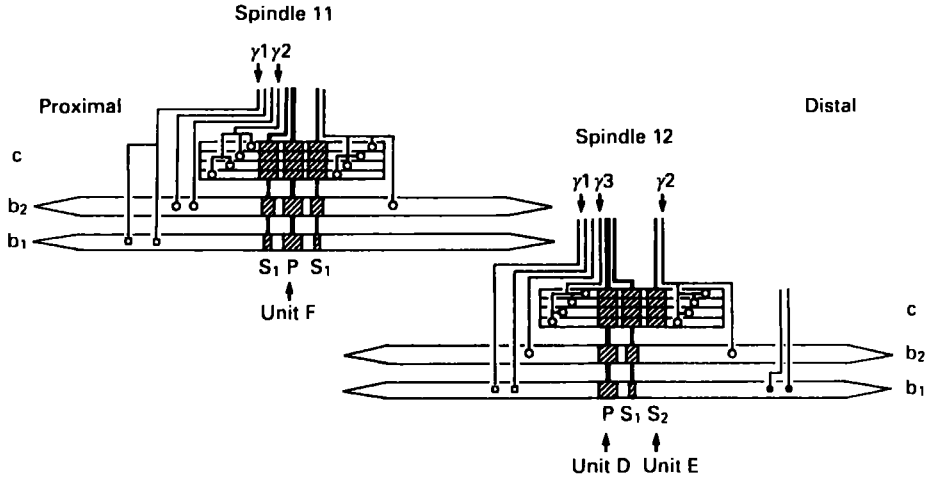


FIG. 10. Schematic diagram of the innervation of the spindles that contained the primary endings whose responses are shown in Fig. 9 (spindles 11 and 12, muscle C689). The distal pole of spindle 11 overlapped with the proximal pole of spindle 12. Note that: the dynamic γ (1) innervated only one pole of the b_1 fibre in each spindle (though in spindle 12 it might be represented by either or both of two axons); the asymmetrical arrangement of the secondary endings in spindle 12 allows the static γ (2) with an indeterminate effect on unit D to be identified as supplying b_2 and c fibres, since γ2 excited the secondary ending, unit E (identified as S_2 because of its slow conduction velocity, 30 m/sec); the axon that drove unit D (γ3) and that did not activate the secondary ending is therefore located in the proximal pole and by comparison with the results shown in Figs 7 and 8 may be identified with the branch to c fibres only; in spindle 11, γ2 probably supplied c fibres only in the proximal pole; γ4 and γ5 are not individually identified, but the similarity of their effects on unit F, with no sign of driving, suggest that they may be represented by the two branches to the proximal pole of the b_2 spindle in 11. Square symbols indicate p_2 motor endings characteristic of dynamic γ axons (see Section 3.3.1). Based on a teased, silver-impregnated preparation. From Banks (1991).

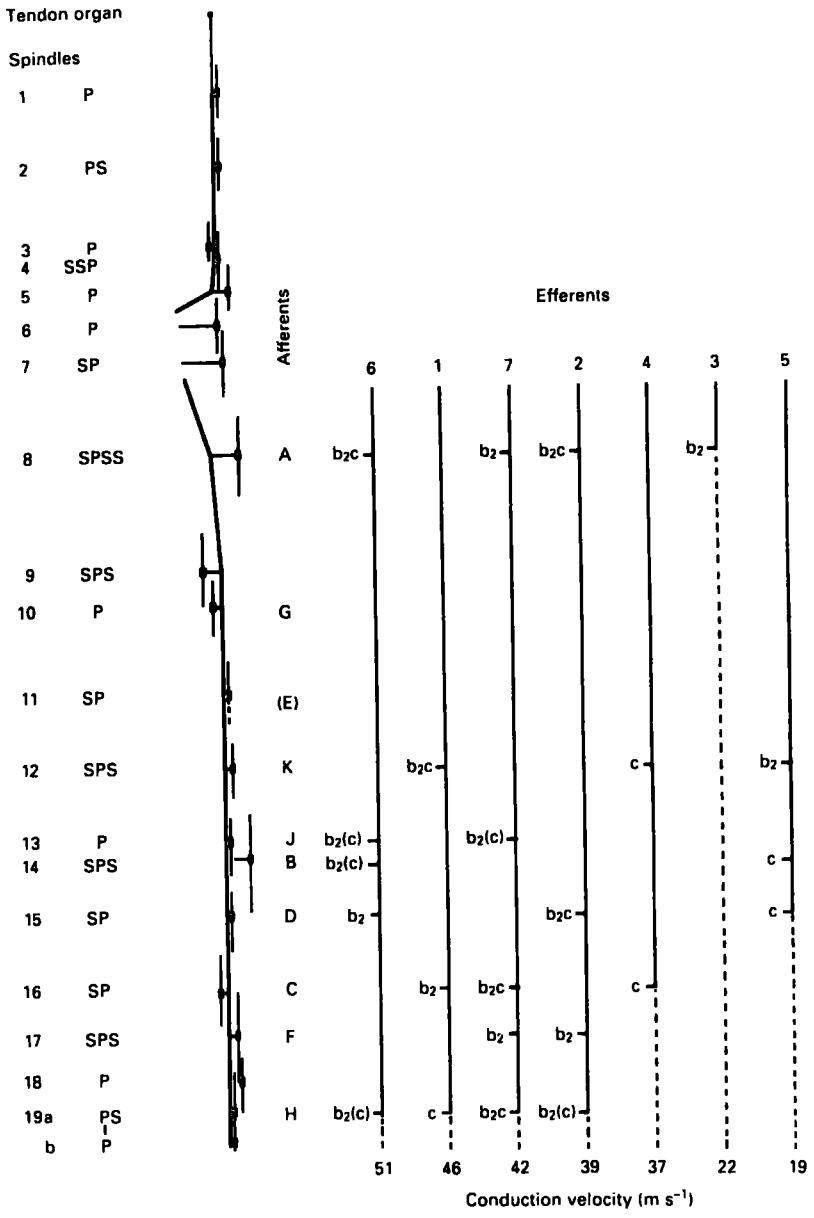


FIG. 11. Graphic representation of the distribution of γ axons among the spindles supplied by the distal nerve branch in a cat tenuissimus muscle (C883), together with a schematic diagram of the arrangement of the spindles and their nerve supply in the whole muscle. From the left: the spindles are numbered in their proximal-to-distal sequence within the muscle; their sensory complements are listed; their relative lengths (short vertical lines) and location with respect to the intramuscular branches of the tenuissimus nerve are indicated in the schema; functionally single afferent axons isolated in dorsal root filaments, and identified alphabetically according to the order of isolation, are shown next to the spindles whose primary endings they supplied; and the distributions of seven γ axons among and within those spindles are tabulated. The γ axons were all static: their distributions were inferred by the effects of their stimulation on the primary-ending responses and on subsequent histological analysis as in Figs 7–10. Each is numbered according to its order of isolation in the ventral root, and is positioned according to its conduction velocity, fastest to the left. Afferent E died before any motor effects could be sought, but all the remaining afferents, including G, were tested against all seven γ axons. Spindles 3 and 19b were b_2c units, 19b being linked in tandem to 19a by a continuous b_2 fibre. In the efferent distribution table, $b_2(c)$ signifies that the b_2 was activated, possibly together with one or more c fibres. For the complete innervation of the b_1b_2c units see Fig. 17, and for the classification of the primary-ending responses to γ stimulation see Table 6.

Banks. Nevertheless the results obtained with tenuissimus are remarkably similar in that the proportions of total static effects recognized as non-driving (54%, derived from Table 1 of Dickson *et al.*) or biasing (52%, derived from Tables 5 and 6 of the present work) are virtually identical. The values

TABLE 5. SYMBOLIC REPRESENTATION OF THE EFFECTS OF STIMULATING γ AXONS ON THE RESPONSES OF PRIMARY ENDINGS IN A CAT TENUISSIMUS MUSCLE (C870)

Efferents by c.v.	Afferents: proximal → distal D	E	A	J	B	H	C	No. of spindles 7
41	●	●	●	●	○	—	○	6
40	—	—	●	●	●	●	●	5
33	●	○	●	—	○	▲	▲	6
31	—	—	○	○	—	—	—	2
30	—	▲	—	—	—	—	—	1
28	—	—	—	●	—	—	—	1
27	▲	—	—	—	—	▲	—	2
27	▲	—	●	—	○	—	—	3
24	—	—	—	●	—	—	—	1
23	□	□	□	□	□	—	—	5
No. static	4	3	5	5	4	3	3	
No. dynamic	1	1	1	1	1	0	0	
Total	5	4	6	6	5	3	3	

Each row shows the effects of a single efferent on the several primaries activated by it. —, no effect; ●, biasing; ○, indeterminate; □, dynamic; ▲, driving. The afferents are arranged in the proximal to distal sequence of their corresponding spindles and are identified alphabetically according to the order in which they were isolated. Efferents are arranged by conduction velocity (c.v. in m sec^{-1}) as shown in the column at the left; the total number of spindles supplied by each one is given in the column at the right. The numbers of efferents supplying each spindle are summarized at the bottom of the table in the corresponding column.

From Banks (1991).

provide further evidence that not all of these effects can be attributed to activity of b_2 alone, as Banks (1991) first argued, since a much smaller proportion of intrafusal branches of known or presumed static γ axons has been found to supply b_2 exclusively (see Table 1c, where, assuming all c input is γ and all lc input is β, the b_2 proportion is 82/277, or 30%).

3.3. ON THE CLASSIFICATION OF INTRAFUSAL MOTOR ENDINGS AND THE STRUCTURE OF THE NEUROMUSCULAR JUNCTION

The pattern of motor innervation of skeletomotor twitch fibres, in which each fibre receives a single motor ending close to its mid-point, is familiar in the mammalian extrafusal system but has only limited applicability intrafusally. If each pole is regarded as being functionally independent, the c fibres, including

TABLE 6. SYMBOLIC REPRESENTATION OF THE EFFECTS OF STIMULATING γ AXONS ON THE RESPONSES OF PRIMARY ENDINGS IN A CAT TENUISSIMUS MUSCLE (C883). DETAILS AS IN TABLE 5

Efferents by c.v.	Afferents: proximal → distal A	G	K	J	B	D	C	F	H	No. of spindles 9
51	○	—	—	●	●	●	—	—	●	5
46	—	—	○	—	—	—	●	—	▲	3
42	●	—	—	●	—	—	○	●	○	5
39	○	—	—	—	—	○	—	●	●	4
37	—	—	▲	—	—	—	▲	—	—	2
22	●	—	—	—	—	—	—	—	—	1
19	—	—	●	—	▲	▲	—	—	—	3
Total	4	0	3	2	2	3	3	2	4	

See also Figs 11 and 17. From Banks (1991).

lc poles, most closely approach the extrafusal condition. Each bag–fibre pole, however, typically receives multiple endings that may be supplied by one or more axons. In a sample of 71 endings from three cat tenuissimus spindles Banks (1981) found the following mean numbers per pole: b_1, 2.4 (range 2–4); b_2, 2.4 (range 2–3); c, 1.2 (range 0–3). The corresponding values obtained from 42 tenuissimus muscles by Kucera (1982c), with a very much larger sample of almost 2000 discrete endplates marked by cholinesterase activity, were: b_1, 1.7 (range 0–5); b_2, 1.9 (range 0–6); c, not given precisely but evidently close to 1.0. Recently, Banks (in press) has examined all the single b_1b_2c units ($n = 16$) of one complete tenuissimus muscle (C883) using silver impregnation. There were on average 1.8 endings (range 0–3) per b_1 pole, 2.4 (range 0–5) per b_2 pole, and 1.2 (range 1–2) per lc pole. The number of c poles other than the long type could not be accurately determined, but it was clear that most possessed only one ending.

Intrafusal motor neuromuscular junctions are much more variable in form and structure than their extrafusal counterparts. This became evident in the 1960s at a time when the static and dynamic subdivisions of γ axons, and the existence of skeletofusimotor axons, were first recognized. It was inevitable, therefore, that attempts would be made to partition the range of variability and to equate the histological classes, thus defined, with the physiological groups.

3.3.1. *Motor endings and neuromuscular junctions of the cat*

It is convenient to begin this section with the seminal paper by Barker *et al.* (1970), which provided descriptions of three types of motor ending—trail, p_2 plates and p_1 plates— based on their appearance in teased, silver-impregnated spindles from various hindlimb muscles of the cat. The details used to define each category need not detain us, but because they do not appear to be exclusive it is clear that in some cases a certain amount of subjective judgement was necessary to assign individual endings to particular categories. Barker *et al.* (1970) argued that trail and p_2 plates represented the endings of static and dynamic γ axons respectively. Although the argument was based in part on the premiss of bag–fibre homogeneity, the correlation of trail endings with static axons was soon conclusively demonstrated by reducing the motor innervation of the tenuissimus to a single γ axon (Barker *et al.*, 1973). The six axons isolated in this way all proved to be static. Their intrafusal distributions have been described above (Section 3.2.1).

Barker *et al.* (1970) had direct evidence concerning the nature of the p_1 plate, for not only did it closely resemble endplates on the surrounding extrafusal muscle fibres, but three examples were found of axons that branched to supply both p_1 and nearby extrafusal endplates. Furthermore, following nerve section, p_1 plates degenerated at the same rate as extrafusal endplates and more rapidly than the trail or p_2 plates, indicating that all p_1 plates were supplied by fast-conducting axons. Conversely, after selective degeneration of γ axons (Barker *et al.*, 1980) only p_1 plates remained as the intrafusal endings of skeletofusimotor axons. That the axons must indeed

be skeletofusimotor rather than exclusively fusimotor α axons had been shown conclusively by Ellaway *et al.* (1972).

The differentiation of the bag fibres into b_1 and b_2 types and the recognition of their largely segregated motor supplies led Barker and Stacey (1983) and Banks *et al.* (1985a,b) to reexamine the classification of intrafusal motor endings. They made a quantitative and statistical comparison of endings on the different intrafusal fibres in normal spindles and those present after all large (presumably α and β) axons had degenerated or after only specific axons, β or static γ, remained. Thus there was no subjectivity required to allocate individual endings in particular groups. It was found that: (i) the population of normal endings on b_1 fibres was indistinguishable from that of a combination of β and presumed dynamic γ axons; (ii) static γ axons had endings indistinguishable as a group from those on normal b_2 and *c* fibres; (iii) β endings on b_1 were similar to the presumed β endings on long or intermediate *c* poles, but both these groups were significantly smaller on average than either known static or presumed dynamic γ endings; and (iv) compared with presumed γ endings on b_1 fibres, static γ endings, though of similar mean length, were significantly less complex as measured by the total length of unmyelinated preterminal and terminal branches. These comparisons allowed three categories of ending to be distinguished: (i) those of static γ axons on b_2 or *c* fibres; (ii) those of presumed dynamic γ axons on b_1 fibres; and (iii) those of β axons, whether presumed dynamic or static, on b_1 or long and intermediate *c* poles. The similarity of these to the earlier (Barker *et al.*, 1970) scheme encouraged Banks *et al.* (1985a,b) to retain the original names, except that it was proposed to call the endings of static γ axons *trail plates* in recognition of the discrete nature of their contributions to individual muscle fibres (Banks, 1981). It is important, however, to emphasize that this classification, though similar to that of Barker *et al.* (1970), is logically and formally independent of it.

Whereas whole, teased spindles impregnated with silver emphasize the pre-terminal and pre-junctional axonal branches, in sectioned material the neuromuscular junctional relationships and postjunctional structures are more evident. Barker *et al.* (1970) attempted to identify the neuromuscular junctions formed by trail, p_2 and p_1 plates mainly on the basis of their relative locations, trail endings being more equatorially located than plates. They proposed a simple correlation between type of ending and ultrastructural appearance, especially the degree of secondary folding of the post-junctional membrane. Thus trail endings were associated with smooth post-junctional membranes, and plates with shallow (p_2) or deep (p_1) folding. The scheme was successfully applied by Arbuthnott *et al.* (1976) to the presumed trail and p_2 endings of static and dynamic γ axons in a spindle from abductor digiti quinti medius, and was initially accepted by Kucera in interpreting the results of cholinesterase staining of serial frozen sections of tenuissimus (Kucera, 1980b,c, 1981b, 1982b,c). However in preliminary descriptions (Barker *et al.*, 1976b) of a series of histophysiological studies subsequently described in full (Barker *et al.*, 1978; Banks *et al.*, 1978; Banks, 1981), it was soon abandoned by its authors who instead suggested that postjunctional features, including sole-plate development and secondary junctional folding, were primarily related to muscle-fibre type and distance of the motor ending from the equatorial (primary) sensory ending.

The evidence was that neuromuscular junctions of b_2 or *c* fibres, in some cases individually identified as activated by static γ axons, might have smooth or folded post-junctional membranes with correspondingly no or little sole-plate development. Junctions on *c* fibres tended to be more complex than those on b_2 fibres even when supplied by the same axon. Bag_1 fibres, however, usually had neuromuscular junctions without secondary folds, whether innervated by dynamic γ axons, or, in one case, a dynamic β axon. A single, distally located ending, also suspected to be that of a β axon, had a well developed sole-plate with secondary folding (Banks, 1981).

In one of these studies, Banks (1981) found that *c* fibres usually had a single, plate-like, neuromuscular junction in each pole, even though the preterminal axon might be much branched and, therefore, trail-like. He pointed out that this precluded the simple cholinesterase rim deposits of Kucera (1980b,c) from marking the post-junctional component of trail, or even somatic, motor endings, since *c*-fibre poles almost always had a single plate-like structure, with intense cholinesterase activity, which surely marked the trail junction. This was immediately accepted by Kucera (1982d) who now agreed that the structure of neuromuscular junctions might be influenced mainly by intrafusal-fibre, rather than axonal, type.

Using the 1 μm serial-section technique together with skip-serial ultrathin sections of neuromuscular junctions, Kucera went on to show that the post-junctional membrane associated with skeletofusimotor endings on *lc* poles was characteristically highly folded (Kucera and Hughes, 1983a,b; Kucera, 1984a). In this it resembled the post-junctional membrane of p_1 plates as originally described by Barker *et al.* (1970). However, on b_1 fibres, as Banks (1981) had found, the equivalent membrane was usually smooth (Kucera, 1984b; Kucera *et al.*, 1984, 1985a), closely resembling that associated with dynamic γ endings, but differing from the folded post-junctional membranes of extrafusal fibres that were coinnervated with b_1 by the presumed dynamic β axons (Kucera, 1985a) (Fig. 13). Moreover, since the same relationships obtain even in the rare instances of b_1 and *lc* coinnervation (Kucera, 1984c), the evidence compels us to conclude that post-junctional structures have properties primarily determined by muscle-fibre type.

I have been careful to maintain a distinction between (motor) endings, implying axonal structures exclusively, and neuromuscular junctions or endplates, which include components of the muscle fibres. This is important because it does not necessarily follow, as Kucera supposed (e.g. Kucera, 1984a), that endplates with different post-junctional structures are precluded from exhibiting a single morphological type of ending, such as the p_1 plate (Banks *et al.*, 1985a). However, it must be admitted that the continued use of "p_1 plate", "p_2 plate", and "trail plate" is misleading since these terms are now defined only by pre-junctional criteria. Their original derivation, of course, was from "endplate" at a time when it was felt

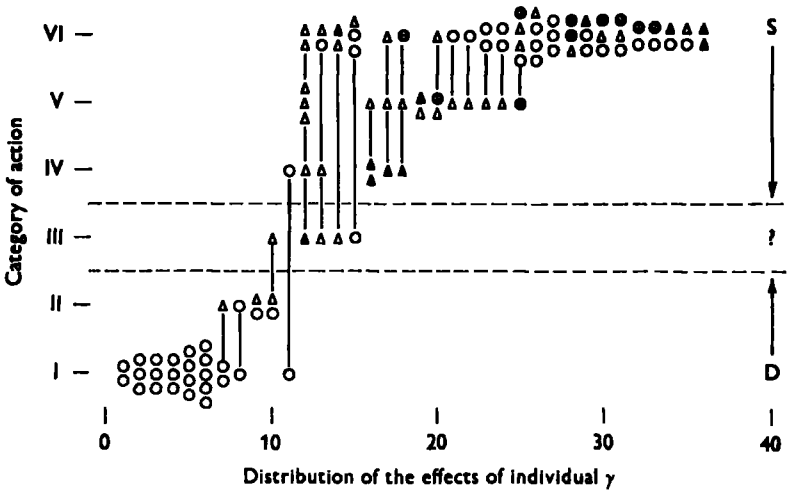


FIG. 12.Classification of the effects of stimulating each of 36 γ axons on 2–7 primary endings in cat soleus, from Emonet-Dénand *et al.* (1977b). The authors categorized the responses according to the purity (I and VI) or apparent degree of admixture (II to V) of dynamic (D) and static (S) effects. Different symbols relate to the regularity of afferent firing: open circles, regular firing; open triangles, moderate irregularity; filled triangles, considerable irregularity; filled circles, 1:1 driving. Comparison with the results shown in Figs 7–11 and 17 suggest that individual static axons (12–36) might supply b_2 or *c* fibres, or both, in different spindles.

that pre- and post-junctional structures were closely correlated (Barker *et al.*, 1970). I therefore propose that in future they be called p_1, p_2, and trail *endings* to avoid any confusion. In the case of the trail ending this involves the restriction of an earlier usage in which the expression described the entire ramification and terminations of single intrafusal axonal branches, often extensively distributed to several muscle fibres and forming discrete neuromuscular junctions on each.

As we have seen, there is clear evidence that different post-junctional structures may indeed be associated with a single morphological type of ending. Nevertheless, if it could be demonstrated that distinctly different types of endplate could occur on a single type of muscle fibre, this would presumably be strong evidence for innervation by more than one kind of axon. Boyd and his colleagues have argued that this is the case for the *c* fibres (Arbuthnott *et al.*, 1982, 1985; Sutherland *et al.* 1985). They described two types of neuromuscular junction one of which, their m_a plate, was similar to the neuromuscular junction of the b_2 fibre and was often coinnervated with it. The other, their m_c plate, was characterized by the presence of finger-like sarcoplasmic projections between the motor terminals, and was usually associated with intrafusal motor branches distributed exclusively to *c* fibres. Boyd (1986) proposed that m_a and m_c plates represent the neuromuscular junctions formed by his static bag and static chain γ-motoneurons respectively. There are, however, some inconsistencies in this scheme. For example, according to Boyd the intrafusal axonal branches of static bag γ-motoneurons virtually always includes b_2 (see Section 3.2.2) so exclusively *c* branches terminating at m_a plates should be rare, yet in Sutherland *et al.* (1985) three such branches occurred in a total of 11 that were distributed only to *c* fibres. Conversely an axon inferred by Boyd to be of static chain type (his γ_{46}, experiment 83/1, Table 3D; Boyd, 1986) proved to terminate in at least one spindle in m_a plates on several *c* fibres and the b_2 fibre (the spindle is identifiable as that reconstructed in Fig. 6 of Boyd *et al.*, 1985b). It is worth noting that the response evoked by stimulation of this axon in the primary ending of the reconstructed spindle was weak, that is mostly sub-harmonic, driving. This would probably have been categorized by Banks (1991) as indeterminate, suggesting both b_2 and *c* fibre involvement, whereas Boyd interpreted it as typically and purely *c* until the spindle was isolated and the intrafusal-fibre movements were observed (Boyd, 1986).

The evident difficulties with Boyd's scheme may have prompted Gladden and Sutherland (1989) to suggest not two but three types of static γ motoneuron in cats, in effect subdividing Boyd's static chain type into those predominantly supplying *c* fibres and terminating at m_a plates, and the remainder that supply *c* fibres exclusively and terminate at m_c plates. The evidence seems to be based on individual intrafusal branches rather than overall distributions of complete axons. If so, Gladden and Sutherland's criteria for individual branches cannot be applied consistently to the complete distributions of static γ axons given in Barker *et al.* (1973) (see Table 4).

A more serious doubt, however, concerns the reality of the distinction between m_a and m_c plates. There is undoubtedly considerable variability in the complexity of form and structure of endplates on *c* fibres, but this is probably matched by comparable variability in such features as the length and diameter of the fibres themselves. Following the initial suggestion by Barker *et al.* (1976b) that some variable features of neuromuscular junctions might be related to location with respect to the primary sensory ending, as well as to muscle fibre type, Banks (1983; Banks *et al.*, 1985b) demonstrated that this is indeed the case for the degree

to which the motor terminals are indented into the post-junctional surface of the muscle fibres (Fig. 14). A similar result was obtained independently by Kucera and Walro (1985, 1986), who also made numerous other measurements of endplate structure from carefully sampled electron micrographs. Drawing on data from 184 intrafusal and 30 extrafusal endplates, by far the largest sample up to that date, Kucera and Walro (1986) were able to make comparisons between the endplates of the different types of muscle fibre and between those on a single type of fibre but associated with selective or non-selective intrafusal branches. Essentially these confirmed the primary association of endplate structure and fibre type. Even when differences in structure existed between groups of endplates on the same type of fibre, such as the degree of indentation of presumed γ or β axon terminals in b_1 endplates, mean distances of the endplates from the primary endings also differed significantly so that distance was a complicating factor that could not be removed.

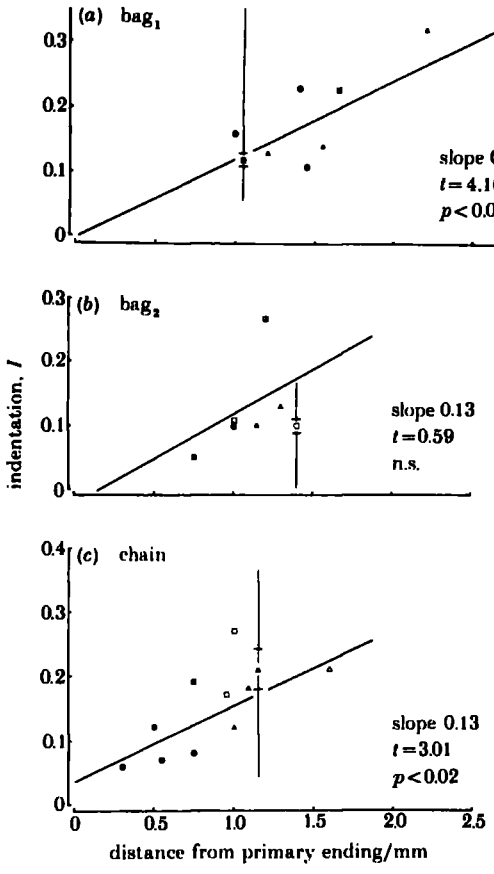


FIG. 14. Scatter plots of mean values of motor terminal indentation (I) into muscle-fibre surface, measured as the proportion of terminal thickness below the local surface of the fibre, against distance between the motor and primary sensory endings, together with regression of indentation on distance, for 27 motor endings. Circles, squares, and triangles represent the separate origins of the data from three tenuissimus muscle spindles each from a different cat. For each fibre type, endings supplied by different axons in the same spindle are indicated by different versions (filled, unfilled, half-filled) of the same symbol. Measurements of I were made on every terminal profile from serial, 1 μm transverse sections. For one ending in each graph a vertical line shows the range of mean values of I for the individual terminals of the ending. Horizontal bars on the line indicate the standard error of the mean value for the whole ending. The extent of the variability within individual endings indicates that a large amount of data is necessary for the relationship between I and distance to emerge. From Banks *et al.* (1985b).

On c fibres presumed to be innervated by static γ axons Kucera and Walro (1986) found that the mean location of endplates of selective (c only) intrafusal branches was significantly further from the equator than that of endplates of non-selective (b_2 and c) branches. This is important since, on the evidence of Sutherland *et al.* (1985), the former group would be expected to be dominated by m_c plates and the latter by m_a plates. Arbuthnott *et al.* (1985), however, found no difference in the location of m_a and m_c plates. Although Kucera and Walro (1986) do not mention finger-like processes of the sole-plate surface, they did measure the extent of its profile and found no difference in this respect, either as absolute length or proportion of total fibre profile, between the two groups of endplate.

In a recent quantitative study Banks (Section 3.4 and in press) also divided motor endings of known or presumed static γ axons on c fibres into those of intrafusal branches supplied either exclusively to c fibres or jointly to b_2 and c fibres, and found that on average the former were significantly longer than the latter. Using the results of Barker *et al.* (1973), who denervated tenuissimus muscles except for single (static) axons (Table 4), it can be shown that on average, c-fibre endplates received more motor terminals when supplied by an intrafusal branch distributed only to c fibres (mean 8.15) than when supplied by one that also innervated a b_2 fibre (mean 5.57). This is especially interesting because it demonstrates the necessity to take account of variability between individual animals. Thus, although the probability that the overall means differ is not quite significant (t-test for unequal variances after log transformation, $t=2.079$, 66d.f., $0.1 \gg p > 0.05$), the difference between the mean values for c only and b_2c branches of each axon was always positive (excluding CT4 which had no c-only branches) and the mean difference (3.06, variance 1.54; $n=5$) itself differed highly significantly from 0 ($t=5.508$, 4d.f., $p<0.01$).

It can also be inferred that the mean length of endplates identified by Arbuthnott *et al.* (1982, 1985) as m_a plates on c fibres (subsuming the m_{ab} plates of 1982) is significantly less than that of their m_c plates. (If endplates in their Fig. 4C, 1982, are assigned the central value of the corresponding histogram bin, then after logarithmic transformation and a modified t-test for unequal variances $t=-4.7$, $p<0.05$). In a recent study in which they compared the post-junctional structure of c endplates that were supplied by γ axonal branches distributed either exclusively to c or jointly to b_2 and c fibres, Arbuthnott *et al.* (1992) found the former group to have significantly greater and more variable post-junctional folding than the latter. However, there was no obvious relationship with

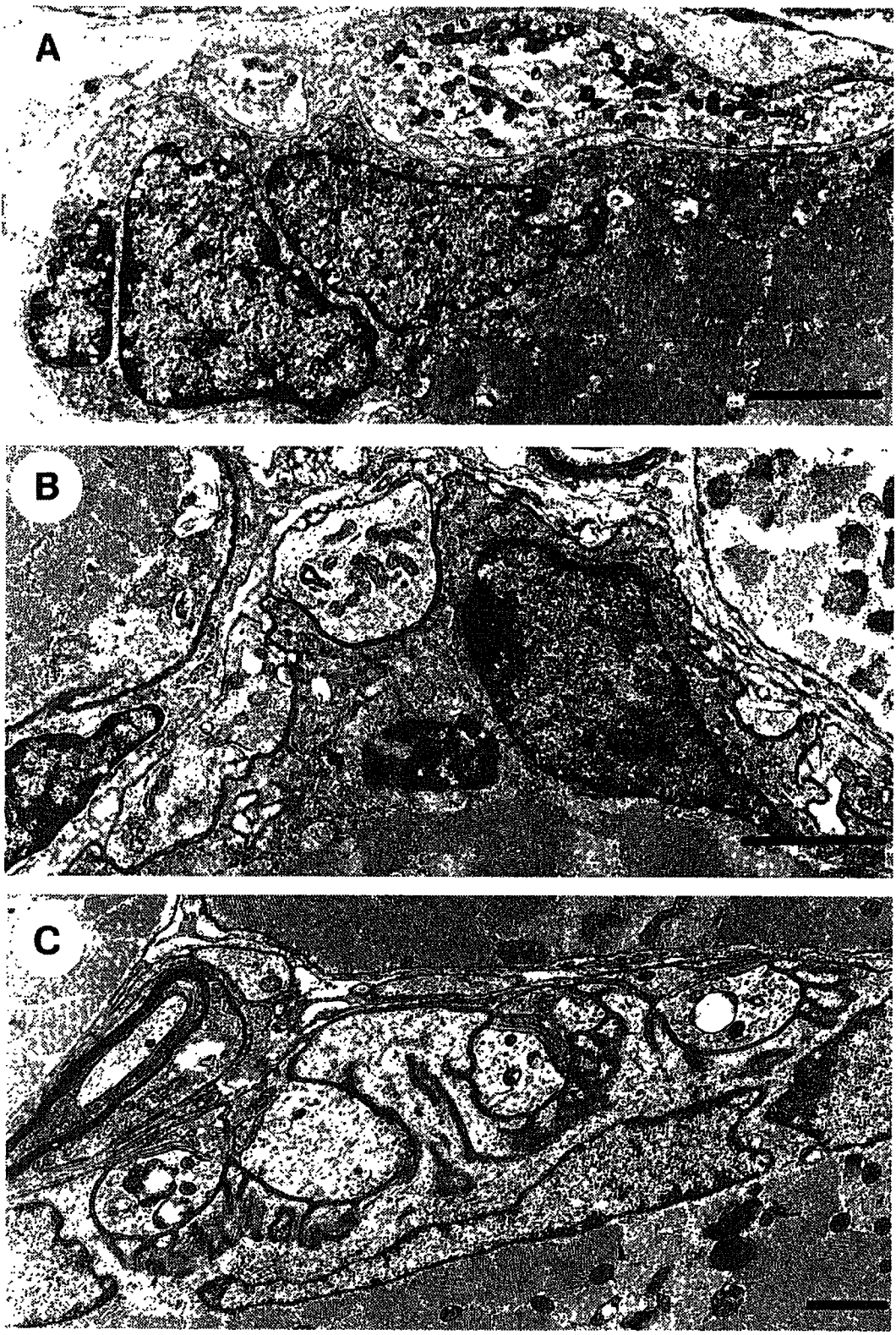


FIG. 13. Electron micrographs of transverse sections through intracapsular (A) and extracapsular (B) endplates supplied by two skeletofusimotor axons to the same b_1 fibre, and of an extrafusal endplate (C) co-innervated with the b_1 endplate of (B). Note the relative amounts of post-junctional folding. From Kucera *et al.* (1984).

endplate size although the mean number of sample sections per endplate in the more complex group was 30% greater than in the simpler group. It must be recalled that, whereas these intrafusal branches may have been derived from axons whose local distribution in other spindles was similar to that analysed in detail (Arbuthnott *et al.*, 1992), the overall distribution of the axons would most probably have included both *c* and b_2 fibres (Barker *et al.*, 1973, 1975b; Emonet-Dénand *et al.*, 1980).

Since intrafusal branches of individual fusimotor axons may supply *c* fibres exclusively in some spindles and b_2 and *c* fibres jointly in others, it is important to realize that none of this implies the existence of more than one type of static γ neuron with different types of endplate. Instead a picture emerges in which endplate size is correlated with complexity of pre- and post-junctional structure and a tendency for the longer endplates to be supplied by intrafusal γ-branches distributed exclusively to *c* fibres, though at present, as we have seen, some results are inconsistent or even contradictory. If the endings of exclusively *c*-intrafusal branches also tend to be located further from the primary sensory terminals, this would imply that they innervate correspondingly larger *c* poles (Banks, 1981), and suggests that a matching process occurs between fibre size and endplate size and complexity.

3.3.2. *Motor endings and neuromuscular junctions in mammals other than the cat*

Several descriptive light and electron microscopical studies, mostly conducted before the general recognition of b_1 and b_2 fibre-types, show that a similar range of neuromuscular junctional size, form and structure occurs in various mammals. These include both eutherian (rat, rabbit, guinea pig, baboon, and man, as well as cat) and metatherian (opossum, *Trichosurus*) species (Banks and James, 1973; Corvaja *et al.*, 1969; von During and Andres, 1969; Gladden, 1969; Gladden *et al.*, 1985; Goglia, 1970; Greer, 1985; Hennig, 1969; Jones, 1966; Kucera, 1988; Mayr, 1969; Ovalle, 1972; Swash and Fox, 1972). Many of the results were interpreted using the trail, p_2 and p_1 classification of Barker *et al.* (1970), and seem to have been consistent with it, at least as it was understood at that time.

Quantitative serial-section analyses of rat extensor digitorum longus, soleus, and lumbrical spindles and of *Macaca* lumbrical spindles by Kucera and his colleagues (Kucera 1985c,d,e; Kucera *et al.*, 1991; Walro and Kucera, 1985b) confirmed the essential similarity of their neuromuscular junctions and those of the cat. This was especially true of the junctions of γ axons on b_2 and *c* fibres and of β axons on both b_1 and *lc*, which were most frequently encountered in the lumbrical muscles of both species. Unusual features, at least by comparison with the familiar picture derived from cat spindles, included: the presence of β endings on b_2 fibres in *Macaca*; the relatively large size of the presumed p_1 endings in that species; multiple, often grouped, small junctions associated with innervation of bag fibres, also seen in human spindles (Gladden *et al.*, 1985; Kucera, 1986); and, in the case of the rat b_1 fibre, a tendency for those junctions to be located juxta-equatorially, in marked contrast to the cat where endings on b_1 fibres are typically the most distal (Fig. 15).

This last result was foreshadowed by Mayr (1969) who described diffuse, multiterminal endings similarly located in rat lumbrical spindles on light (i.e. pale staining) fibres, that he equated with bag fibres. Mayr used a cholinesterase technique with teased, whole spindles, and Kucera *et al.* (1978), also using cholinesterase but with serial sections, subsequently found equivalent diffuse structures to occur mainly on b_1 fibres in rat soleus. There can be little doubt that these represent neuromuscular junctions, unlike the superficially similar rim deposits that Kucera (e.g. 1980c) found mostly on *c* fibres in the cat (see the previous section).

The diffuse γ endings of the rat b_1 fibre seem to be supplied by intrafusal branches that may be distributed exclusively to the b_1 or jointly with the b_2, or *c* fibres, or both (Walro and Kucera, 1985b; Kucera *et al.* 1991). Some, at least, could be derived from [illegible] parent axons whose overall distribution would allow them to be classified as dynamic, but the physiological observations of Andrew *et al.* (1978) leave this question open, since only single primary endings were tested. It is remarkable that dynamic effects were only commonly found when several γ axons that activated a previously isolated primary afferent were tested, and doubly remarkable that it should be the slowest axon in each case ($n=8$). This is reminiscent of the occurrence of driving effects by static γ axons of the cat (see Section 3.2.2). Until more comparative studies are made, the relationships of the diffuse γ endings on rat b_1 fibres to the p_2 ending of the cat must therefore remain questionable.

Quantitative morphological studies on ultrathin sections of intrafusal endplates from *Macaca* have been carried out by Subramani *et al.* (1986) and Sahgal *et al.* (1990). Endplates were classified only as belonging to b_1, b_2 or *c* fibres. In some cases there were significant differences between the classes, and, as might be expected, there was some evidence of significant variability between different muscles. Evidence on the effects of location was equivocal.

3.4. On the Relationship Between the Numbers of Motor Axonal Branches and the Numbers of Afferent Axons Supplied to Spindles

There is surprisingly little comparative data on the numbers of motor axonal branches entering spindles. Most of what there is is summarized in Table 7, as mean numbers of branches per spindle pole. The twofold range seen in the total numbers for various muscles of the cat suggests that there may be real differences between muscles, whereas homologous muscles of different species have rather similar numbers. Since some intrafusal branches divide to supply both poles of a spindle, the total numbers per spindle will usually be slightly less than twice the corresponding polar values.

Banks (in press) has collected data on 333 intrafusal branches of motor axons that supplied 47 tenuissimus spindles from four muscles. The intrafusal fibre distributions of most of the branches have been given in Table 1. Only 10 were found to innervate more than one pole, the majority in a single muscle, C883,

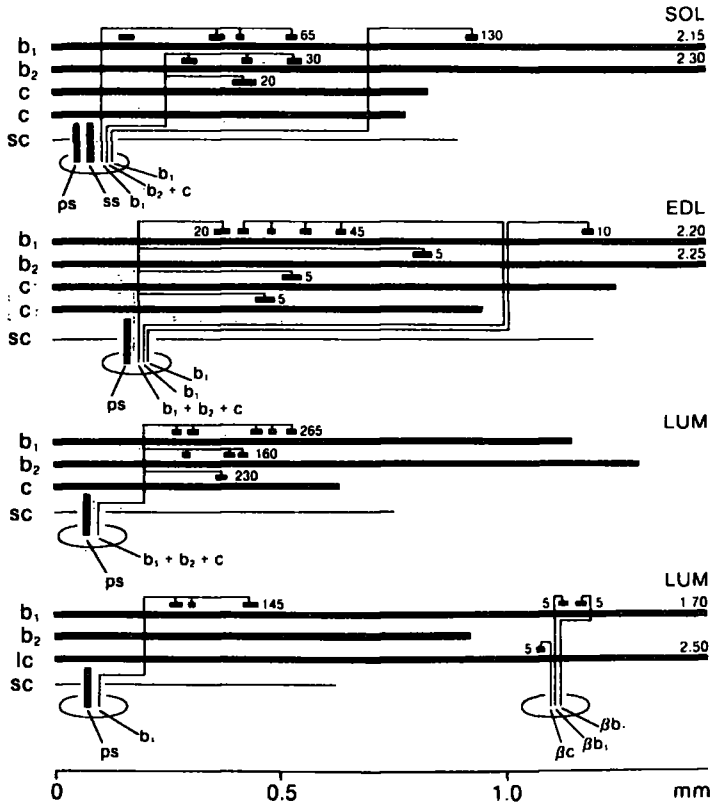


FIG. 15. The innervation of four representative spindle poles from rat soleus (SOL), extensor digitorum longus (EDL) and lumbrical (LUM), reconstructed from serial transverse sections. The primary (ps) and the secondary (ss) sensory endings are indicated only by their incoming axons. The authors assumed that the motor innervation was all γ apart from three axons supplying the b_1 and a *lc* fibre in the lowermost LUM pole. Values next to motor endings (short black bars) are the lengths (μm) of the unmyelinated preterminal segment of the axon, whereas those at the extreme right of some of the intrafusal fibres are the total lengths (mm) of the fibres in the reconstructed poles. The extent of the capsule (sc) is also indicated in each case. From Kucera *et al.* (1991). American Journal of Anatomy © 1991 Wiley–Liss. Reprinted by permission of Wiley–Liss, a division of John Wiley and Sons, Inc.

which provided the most complete data. Each spindle received 2–13 branches, average 7.09. This is probably fewer than any cat muscle listed in Table 7, but is considerably more than the average of 3.3 axons per spindle (2.6 γ, 0.7 β) in the lumbrical muscles studied by Adal and Barker (1965a).

The frequency of occurrence of spindles that received different numbers of motor branches appears

TABLE 7. THE NUMBERS OF INTRAFUSAL MOTOR BRANCHES IDENTIFIED AS OF FUSIMOTOR (γ) OR SKELETOFUSIMOTOR (β) ORIGIN BY THE ORIGINAL AUTHORS

		No. of intrafusal branches			
Species	Muscle	γ	β	Total	Source
Cat	Peroneus longus	3.8	0.25	4.1	a
	Peroneus brevis	5.1	0.9	6.0	a
	Peroneus tertius	3.6	1.0	4.6	a
	Flexor hallucis longus	5.3	2.1	7.4	a
	Soleus	4.7	0.8	5.5	a
	Unnamed tail muscle (Extensor caudae medialis?)	5.7	2.5 (1–5)	8.2	b
Rat	Soleus	3.7±0.3	0	3.7±0.3	c
	Extensor digitorum longus	3.9±0.3	0.2±0.1	4.0±0.3	c
	Lumbrical	1.4±0.2	1.0±0.2	2.4±0.2	c
Macaque	Lumbrical	1.6 (0–3)	0.8 (0–8)	2.3 (0–9)	d

Key to source material: a, Barker *et al.* (1970); b, Hunt *et al.* (1972); c, Kucera *et al.* (1991); d, Kucera (1985d,e). Criteria used to identify γ and β branches differ according to source.

Values are means per spindle pole with ±s.e. or (range) when given.

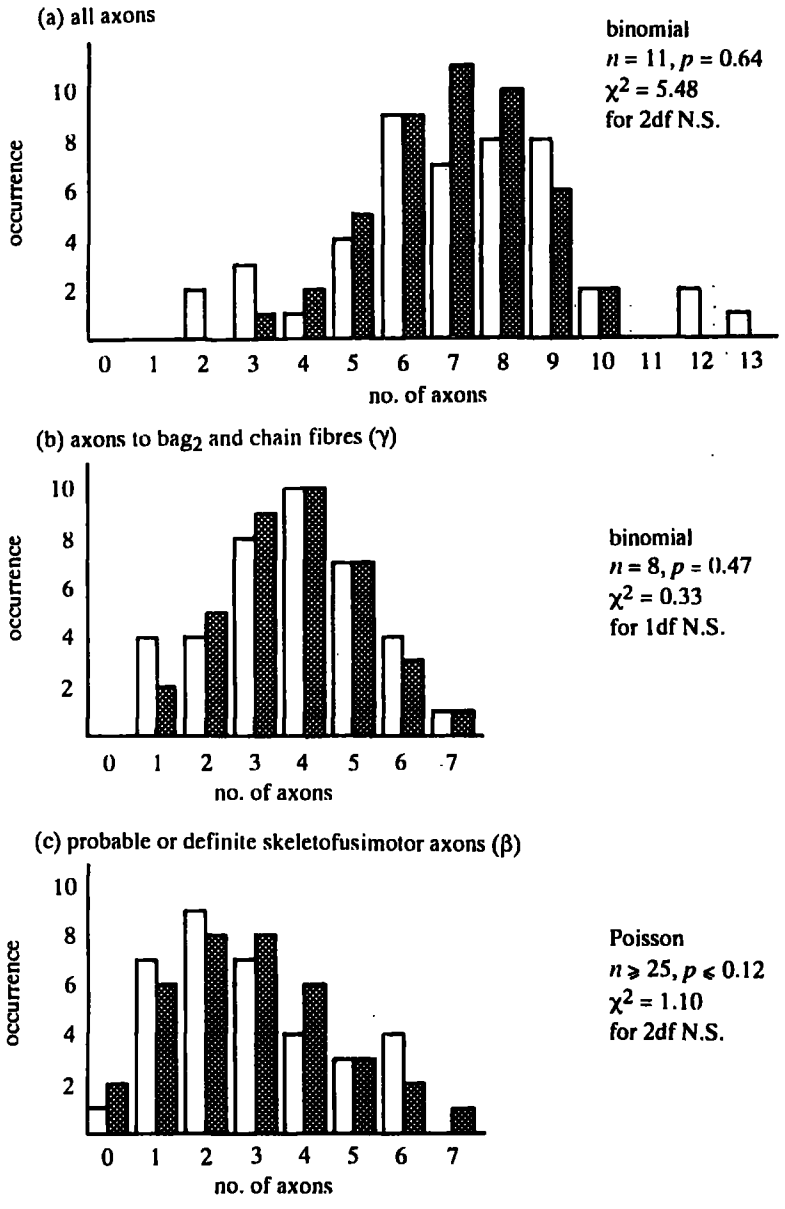


FIG. 16. Distributions of the frequency of occurrence of single-unit spindles supplied by different numbers of motor axonal branches (unfilled bars) compared with theoretical binomial distributions (shaded bars) calculated from the corresponding observed means. The histograms show the distributions for skeletofusimotor (β), and purely fusimotor (γ) axons separately, as well as combined. In each case the observed distribution does not differ significantly from the corresponding binomial distribution.

to follow binomial form (Fig. 16), indicating a random association of branches with spindles. The same is true for the number of branches that supplied b_2 and c fibres (1–7, mean 3.74), representing a virtually pure sample of γ axons, and for those intrafusal branches to b_1 and lc poles that were probably of β origin (0–6, mean 2.89). However, as Banks and Stacey (1988, 1990) have shown, spindles having different sensory complements also follow a binomial frequency of occurrence. This raises the possibility that the numbers of afferent axons and efferent branches entering each spindle could be correlated, while being randomly determined separately with respect to the spindle.

The results for only a single muscle, C883, are sufficiently complete to test this possibility, but a few results are also available from studies on tandem spindles in cat tenuissimus (Kucera and Walro, 1987) and dorsal neck muscles (Richmond *et al.*, 1986), and in *Macaca* lumbrical (Kucera, 1985a), that are consistent with the conclusions to follow. The analysis is complicated by uncertainty as to the origin, whether β or γ, of some of the motor branches. Banks (in press) concludes that the innervation of the b_2 and c poles in C883 was entirely γ apart from a single b_2 branch, whereas that of the b_1 and lc poles was entirely β. The evidence for the origin of the b_1 and lc endings was principally that their size conformed very

closely to that of known β endings (p_1 plates) in the study of Banks *et al.* (1985b). For example, the mean length of b_1 endings in C883 was 35.9 μm ($n=58$) as against 36.1 μm ($n=23$) for the known β endings. Furthermore, many of the intrafusal branches supplying these endings entered their spindles from nerves otherwise distributed only to extrafusal fibres, or had collateral branches that entered such nerves. These arrangements are typical of β axons (Barker *et al.*, 1980; Kucera *et al.*, 1984). There were, however, a few endings of b_1 fibres whose size exceeded the maximum range of the p_1 plates of Banks *et al.* (1985b) and could have represented p_2 plates of a dynamic γ axon.

The complete patterns of innervation of all single-unit, b_1b_2c spindles are shown schematically in Fig. 17. A single-unit b_2c spindle and a tandem-unit b_1b_2c–b_2c spindle were also included in the analysis but are not shown in the figure. Both regression (Fig. 18) and χ^2 analyses demonstrated significant correlations between the numbers of afferent axons and γ branches, but not β branches. This was true irrespective of whether the few doubtful endings on b_1 fibres were regarded as derived from γ or β axons. It may be accounted for if β axons are merely α axons that happen to have encountered spindles during a critical stage of development, since unlike γ axons they would not then require to be guided to the developing spindle.

3.5. On the Degree of Segregation of the Static γ Input and its Relationship to the Secondary Sensory Innervation

Although most static γ axons include both b_2 and c fibres in their overall distribution, individual intrafusal branches supply either or both types of fibre (Section 3.2). Each complete spindle pole might then receive an entirely segregated (S) or an entirely mixed (M) static γ input, or one showing an intermediate degree of segregation (I). In a sample of 66 tenuissimus spindle poles, again drawing mainly on C883, there were 25S, 22M and 19I. The various combinations that were found in complete spindles are shown in Table 8. A comparison of the numbers observed in the several groups (SS, MM, SM, etc.) with their corresponding expected values, assuming that the polar types actually occur about equally often and are randomly associated, showed no significant difference between the two sets.

However, this apparent randomness in the association of poles, irrespective of their degree of static-γ segregation, might conceal a relationship with the afferent supply, which, as we have seen, is itself subject to random variations. The possibility was tested using a sample of four silver-impregnated muscles, predominantly C883, augmented with serially sectioned spindles published by Banks *et al.* (1981, GS5, 6, 9 and 12) and by Kucera *et al.* (Kucera and Hughes, 1983a, Figs 3 and 4; Kucera *et al.*, 1984, Fig. 1; Kucera, 1984b, Fig. 1). Individual poles of single-unit, b_1b_2c spindles were scored according to their degree of segregation: M = 0, I = 1; S = 2. Poles containing at least one secondary ending (38/74) had a mean motor-supply score (1.37) more than twice that of poles without secondary endings (0.64). Mean total scores for complete spindles with primary endings only (0.75), with secondary endings in one pole (2.14), and with secondary endings in both poles (3.00) show that the static γ supply is increasingly segregated as first one and then both poles receive secondary endings. The actual total scores (7, 45, 24 respectively) were significantly different from the corresponding values (16, 43, 16) that would be expected if no such relationship were to exist ($\chi^2=10.34$; for 1 d.f., $p<0.01$). However the relationship is not, as might be supposed, simply a progressive segregation due to the number of static γ branches to a spindle increasing in step with the number of afferent axons (see previous section), since there was a tendency for the segregated poles ($n=26$) to be supplied by fewer branches (mean 2.2) than the partially segregated poles ($n=23$, mean 2.5 branches). Unsegregated poles ($n=25$) were almost always supplied by single branches (mean 1.04).

Although afferent axons, and consequently intrafusal γ branches, might be allocated at random to individual spindles, regional differences within a muscle can still occur. Thus it may be supposed that, in development, newly formed spindles closest to the muscle's nerve input would have a greater chance of receiving multiple afferents than those further away, due to a progressively declining supply of afferents available for allocation. The favourable geometry of tenuissimus has allowed this proposition to be tested and confirmed by Banks and Stacey (1990), who found that spindles supplied by the proximal half of the main, distally directed, intramuscular nerve (see Fig. 11) had on average 2.8 afferents whereas those supplied by the distal half had significantly fewer with only 2.3. The consequences for the numbers of intrafusal γ branches and the amount of segregated input to b_2 and c fibres might partially account for the high proportion of such segregated input reported by Boyd (1986) (see Section 3.2.2) since he used that portion of the muscle where, on the present argument, the greatest amount of segregation would be expected.

There is an additional, and somewhat surprising aspect to the relationship between static-γ segregation and secondary endings: in spindles with secondary endings in only one pole, the static γ innervation tends to be more segregated in that pole than the other. This was true in 11 of 15 spindles that had secondary endings in only one pole, and whose static γ innervation was differentially segregated in the two poles. If there were no correlation, the more segregated innervation would be equally likely to be associated with either pole. Then the probability that the observed numbers occurred by chance would be $^{15}C_{11}(0.5)^{11}(0.5)^4=0.042$, which is sufficiently low to confirm the relationship (Banks, in press).

Despite its impressive complexity any functional basis for this organization is elusive since the secondary endings, although often having some terminals on the b_2 fibre, are predominantly distributed to c fibres (Boyd, 1962; Banks *et al.*, 1982). Moreover in spindles without secondary endings the static γ input is typically unsegregated despite there being extensive primary terminals on both b_2 and c fibres. Such spindles frequently receive two static γ branches, yet it is normally the poles rather than the b_2 and c fibres that are innervated separately.

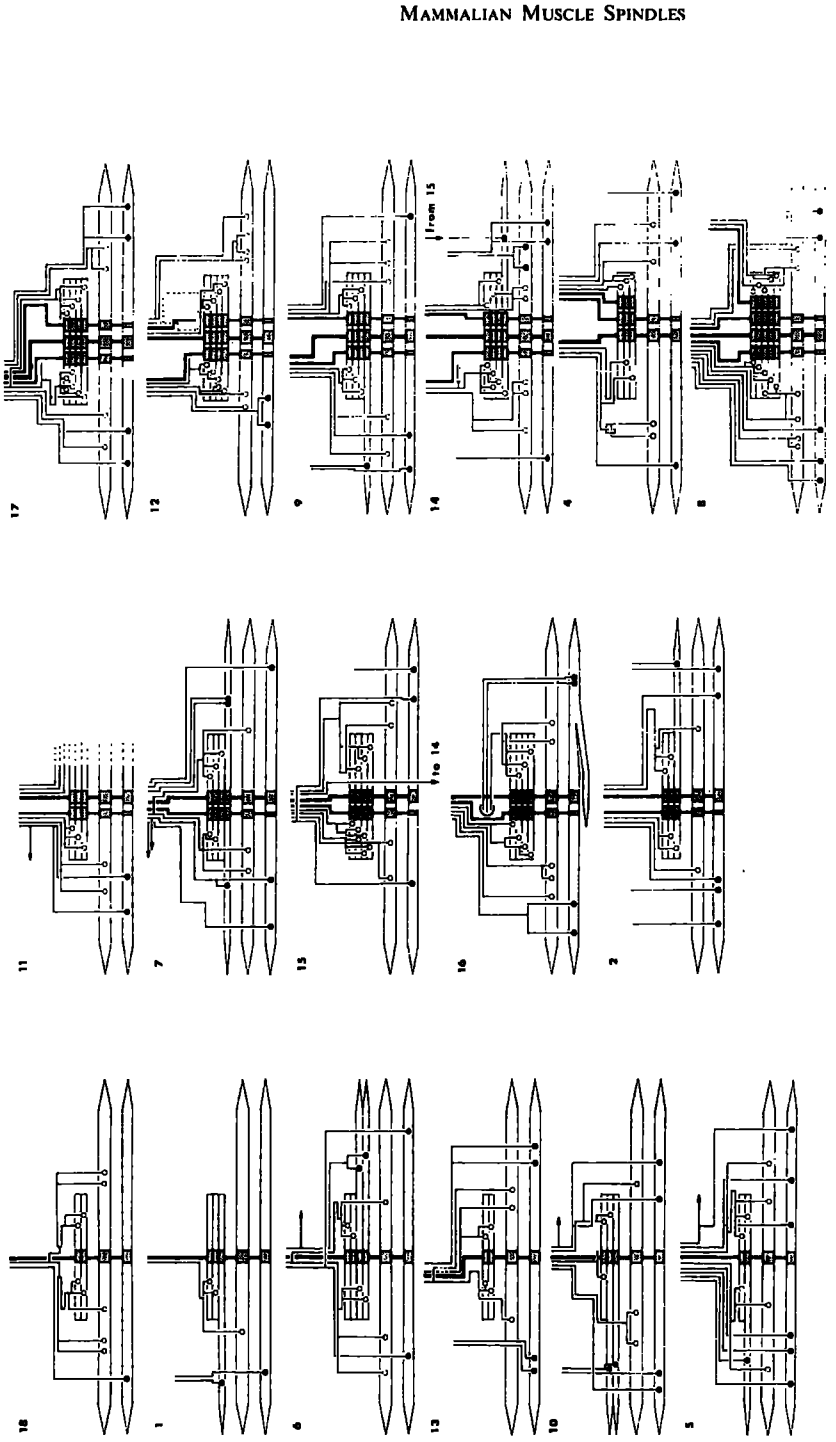


FIG. 17. Schematic diagrams of the innervation of all b_1b_2c single-unit spindles from a cat tenuissimus muscle, C883. The spindles are numbered sequentially corresponding to their proximal to distal locations in the muscle (see Fig. 11), but are arranged in columns, according to the number of afferent axons (1, 2, and 3 or 4) they received. Within each column the spindles are ordered according to the numbers of motor axonal branches entering them. The arrangement provides a visual impression of a correlation between the numbers of afferent axons and efferent branches, that was confirmed for the motor branches identified as γ in origin (whose endings are shown as open circles), but not for those identified as intrafusal branches of skeletofusimotor axons (endings shown as filled symbols). Representation of the intrafusal fibres and their sensory and motor innervation uses the same conventions as in Figs 8 and 10. Collateral branches of motor axons shown with horizontal arrowheads entered nerves having an apparently pure extrafusal distribution. Whereas the great majority, at least, of the b_1 motor innervation appeared to be skeletofusimotor in origin, some endings (filled diamonds) were larger than the known γ_1 plates of Banks *et al.* (1985b). They may therefore represent a possible γ input in some cases (spindles 2, 4, 6, 9, 14 and 17), but of seven axons isolated and tested for effects of their stimulation on most of the spindles 8–18 (and 19, not shown), none was dynamic (see Fig. 11 and Table 6). From Banks (in press).

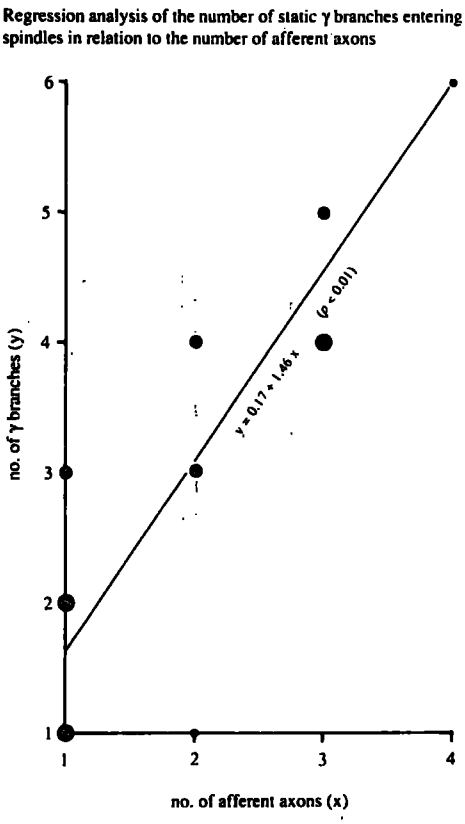


FIG. 18. The relationship between the number of γ branches and afferent axons entering the b_1b_2c single unit spindles of C883 (see Fig. 17, spindle 11 omitted). Two or three coincident observations are shown by the medium and large symbols respectively.

But perhaps we should not concentrate too closely on individual spindles in this context. It may be that for the animal the most important functional requirement is an integrated motor control system in which there are several, exclusively fusimotor, static inputs distributed among the spindle complement of a muscle. It may be that the observed relationship of segregation and secondary endings is an inevitable consequence of a development programme involving contact guidance by afferents and the sequential appearance of the c fibres after the b_2 (see Section 4) but one that is of little functional significance.

3.6. RÉSUMÉ

In the preceding sections, I have attempted to give a critical and comprehensive review of the growth of knowledge about the organization of the adult fusimotor innervation. Inevitably this means that important conclusions are scattered among, and perhaps obscured by, the detailed arguments employed. It may be helpful, therefore, to draw these conclusions together to form an overall picture that can be readily grasped, before moving on to consider fusimotor development. The feline spindle, especially that of the tenuissimus, will serve as the paradigm; important variations must be sought in the relevant sections above. The theme is one of a system constructed by an interplay of deterministic and random factors where, as in all things created through biological evolution, efficiency but not perfection is the watchword.

Skeletofusimotor (β) innervation is now known to be of common occurrence in mammalian spindles. It is provided by collateral branches of α motoneurons that are otherwise indistinguishable from those without such connexions. Though the presence of β innervation in a particular spindle is a matter of chance, the intrafusal branches are precisely distributed to b_1 and c fibres, especially $1c$, almost always separately. They thus form separate dynamic and static systems, the extrafusal components of whose motor units consist of slow (S) and fast, mostly fatigue-resistant (FR), fibres respectively. Although the absolute number of static axons may be greater than that of the dynamic, the latter form a greater proportion of extrafusally homotypic motor units than do the former.

The β-innervated poles of $1c$ fibres never seem to be supplied also by γ axons, though such axons are present on other c fibres in the same spindle poles, and even on the same c fibres in the opposite poles. The two sources of innervation, β and γ, therefore provide

TABLE 8. THE INTRAFUSAL DISTRIBUTIONS OF STATIC γ AXONS IN 32 MUSCLE SPINDLES OF CAT TENUISSIMUS, GROUPED ACCORDING TO THEIR DEGREE OF SEGREGATION

	Distribution		Number			
Segregation	Pole 1 : Pole 2	Obs	Total	Exp		$(obs-exp)^2/exp$
SS	b_2 c : b_2 c	4	4	4		
MM	b_2c : b_2c	4	4	4		
II	b_2 b_2c : b_2c	1	13	11		0.333
	b_2c c : b_2c c	1				
	b_2 b_2c : b_2 b_2c c	1				
	b_2c c : b_2 b_2c c	1				
	b_2 b_2c c : b_2 b_2c c	1	5	4		
SM	b_2 c : b_2c c	10	10		7	1.286
SI	b_2 c : b_2 b_2c	2	6		7	0.143
	b_2 c : b_2c c	3				
	b_2 c : b_2 b_2c c	1				
MI	b_2c : b_2 b_2c	1	3		7	2.286
	b_2c : b_2c c	2				
						$\chi^2 = 4.048$ for 2 df: NS

From Banks (in press).

alternative inputs for some *c* fibres. With b_1 fibres, conversely, the two sources may converge onto individual poles.

The pre-junctional components of both dynamic and static β endplates are morphologically similar. These p_1 endings are small versions of their extrafusal counterparts. Post-junctionally, muscle-fibre membranes range from smooth in intracapsular b_1 endplates to markedly folded in extracapsular *lc* endplates, though they are always less folded than the coinnervated extrafusal plates.

Exclusively fusimotor (γ) innervation is also segregated into dynamic and static types with correspondingly precise distributions to b_1 fibres and to b_2 and *c* fibres respectively. To judge by the tenuissimus, dynamic γ axons are not invariably present, whereas static γ axons, which are always more numerous, never seem to be absent. The number of γ axons that innervate a particular spindle is closely correlated with the number of afferent axons, which itself seems to be randomly determined.

The static γ supply to the b_2 and *c* fibres of a single pole may be mixed, segregated, or partially segregated, depending on the number of intrafusal motor axonal branches present. A mixed input is almost invariably provided by a single branch, whereas a segregated input is most commonly provided by two branches and partial segregation by more than two. Remarkably, when the two poles of a spindle show different degrees of segregation and when secondary sensory endings occur in only one pole, it is most likely the same as that which has the greater motor segregation.

Individual static γ axons almost always include both b_2 and *c* fibres in their motor units though they do show some differential distribution. Thus faster-conducting axons tend to be more widely distributed and are less likely to innervate *c* fibres alone in any spindle than are more slowly conducting ones. When intrafusal branches supply *c* fibres alone, their parent axons tend to have the slowest conduction velocity of all the axons innervating the same spindles, even though they may not be the slowest in the muscle as a whole.

The pre-junctional components of dynamic and static γ endplates are morphologically dissimilar to each other and to the p_1 endings of β axons. They are described as *p_2 endings* and *trail endings* respectively, though the typical p_2 ending may not be recognizable in some species. Post-junctionally, as with the β innervation, muscle-fibre membranes range from smooth (intracapsular b_1) to folded (extracapsular *c*). There is some evidence that static γ endplates in segregated poles tend to be larger and more complex than those in unsegregated poles. It is disputed whether this may be related to endplate location and muscle-fibre size.

4. THE MOTOR INNERVATION IN DEVELOPMENT

The pattern of formation of the intrafusal muscle fibres is essentially similar to that of the extrafusal fibres, both intrafusal and extrafusal types probably being derived from the same groups of myoblasts (Milburn, 1984; Barker and Banks, 1994; Kucera and Walro, 1990). The first myoblasts to fuse form discrete primary myotubes each of which acts as the centre of a growing cluster of subsequent generations of secondary myotubes. Younger myotubes arise by the fusion of myoblasts that align themselves on the older, especially primary, myotubes. As they mature into muscle fibres the myotubes progressively separate from the cluster, the oldest of the secondary myotubes doing so first. Each cluster eventually forms a part or the whole of a fascicle of extrafusal muscle fibres, or the complete intrafusal bundle of a muscle spindle.

Primary myotubes appear in the absence of any innervation. They apparently remain undifferentiated until some are contacted, presumably at random, either by α motoneurons or ingrowing sensory axons, sensory contact initiating their ultimate conversion into b_2 intrafusal fibres (Kucera *et al.*, 1989). Only after this contact, which perhaps evokes the change in heavy meromyosin expression that occurs at about the same time (Pedrosa and Thornell, 1990), do the secondary myotubes begin to form. The oldest of these develop into b_1 fibres and subsequent ones into *c* fibres whose size at maturity may reflect their sequence of development. In particular, when present, *lc* fibres are probably the oldest of the *c* fibres, appearing just after the incipient b_1 fibres.

The sensory innervation is necessary and seemingly sufficient for the initiation and maintenance of intrafusal fibres that are recognizably of b_2, b_1 and *c* types (Zelená and Soukup, 1974; Soukup and Zelená, 1985; Kucera and Walro, 1988). In contrast, motor innervation seems to have relatively little effect on the properties of the intrafusal fibres, though it perhaps influences the ultimate size of the poles. So far as the expression of heavy meromyosin is concerned, neonatal motor denervation of rat spindles only has significant effects on bag fibres, resulting in b_1 and b_2 exhibiting greater similarity than in normal spindles (te Kronnie *et al.*, 1982; Soukup *et al.*, 1990). Perhaps the most important change is that the tonic myosin, which is normally restricted to the intracapsular portions of b_2, is present throughout the fibre after motor denervation, as it is in both the normal and de-efferented b_1.

The earliest motor innervation occurs on the primary myotubes, including some of the incipient b_2 fibres, at the same time as the formation of the sensory neuromuscular contacts (Kucera *et al.*, 1989). It is presumably provided by α motoneurons and, at least in the rat soleus where these observations were made, that on the b_2 fibres is transiently present only on the 18th day of gestation. The developing spindles are thus without motor innervation until two days later when the b_2 again receives motor innervation, this time presumably by γ motoneurons. By now the presumptive b_1 myotube is also present, but it possesses only sensory innervation, motor endings appearing some two days later still. An essentially similar pattern of development, described in terms of primary and secondary myotubes, had previously been obtained for the extensor digitorum longus of the mouse by Kozeka and Ontell (1981), who noted that the first appearance of the (presumably definitive γ) motor innervation usually occurred in one pole of the primary intrafusal myotube (b_2).

The progressive elaboration of the fusimotor innervation has been studied in rat soleus by Kucera

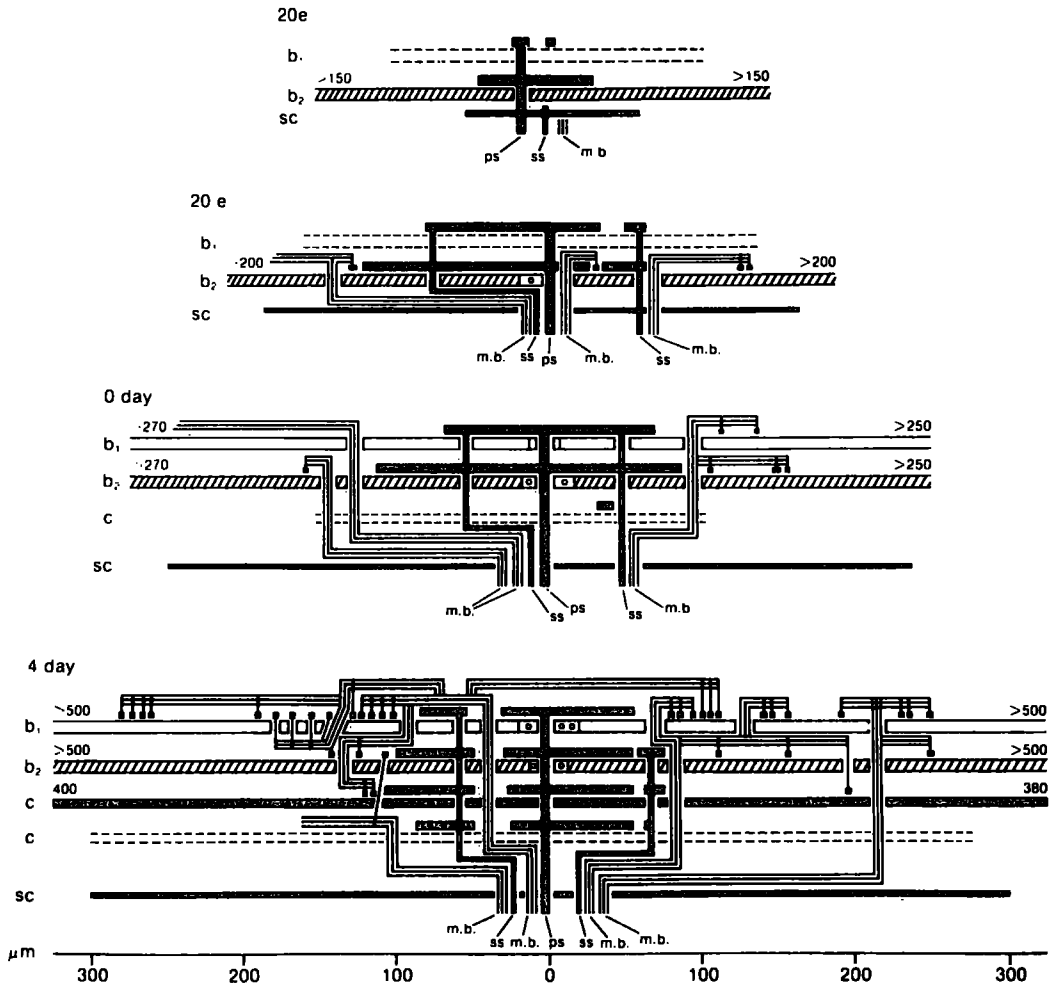


FIG. 19. The elaboration of the innervation of the muscle spindle in development as illustrated by schematic diagrams of representative spindles from 20 day foetal, neonatal and 4 day post-partum rat soleus, reconstructed from semiserial ultrathin transverse sections. Note the sequential appearance of the intrafusal fibres, initially as myotubes (dashed outlines) and polyaxonal nature of the motor innervation throughout this period of development, indicated conventionally as three axons in each motor bundle (m.b.) Compare with Fig. 15, which shows adult rat spindles. From Kucera *et al.* (1988b). American Journal of Anatomy © 1988 Wiley–Liss. Reprinted by permission of Wiley–Liss, a division of John Wiley and Sons, Inc.

et al. (1988a,b). From the 20th day of gestation, when terminals are present on most b_2 fibres only, there is a gradual approach to the adult condition. In terms of the relative numbers and locations of the motor endings, and of the arrangement of the routes taken by the axons, the process is essentially complete by the 4th post-natal day, though the innervation still has to spread to the youngest *c* fibres which are present as myotubes (Fig. 19). An important difference between the neonatal and the adult fusimotor innervation, however, is that individual endings are multiply innervated in the neonatal spindle, just as occurs in the extrafusal motor innervation at this time. This is an important result for our understanding of the development of motor innervation in general since it implies a common ontogenetic purpose for the formation and subsequent elimination of multiply innervated endings, both intrafusally and extrafusally, yet the outcome of the developmental process is different in each case. It may therefore restrict some of the possibilities for that purpose.

Extrafusally, the period of multiple innervation coincides with the existence of mixed motor units that share fibres in common, are frequently clumped, and that are larger relative to the whole muscle, but not more numerous, than in the adult (Jones and Ridge, 1987; Jones *et al.*, 1987a; Jansen and Fladby, 1990). The reduction to singly innervated endings corresponds to the formation of spatially overlapping, though otherwise distinctly separate, motor units of different types, each essentially homogeneous in fibre composition. Despite the well-known plasticity of adult extrafusal muscle-fibre types, this does not seem to come about by conversion of fibres through the imposition of properties determined by the remaining motoneuron of each unit (Jones *et al.*, 1987b). If that is so it would seem to follow that either there is precise matching of neuronal and fibre types, or the neurons

differentiate only after initial contacts with developing fibres and subsequently withdraw any inappropriate contacts. Multiple innervation may be seen as an expression of neuronal competition in either case, but only in the latter should we expect to see even the temporary formation of mixed motor units. Moreover, the trophic nature of the interaction, which has both ontogenetic and phylogenetic advantages (Purves, 1988), would be preserved.

Intrafusally, the temporary existence of multiply innervated motor endings demonstrates that the reduction to single innervation is a local phenomenon, since not only does each fibre retain innervation in both poles, usually involving different axons, but individual poles, particularly of the bag fibres, frequently retain more than one ending, (see Section 3.3). The reduction may lead to some differentiation of motor units, though there is no direct evidence of this, indeed the high proportion (42%) of intrafusal branches supplied to b_1 and either or both b_2 and c fibres in neonatal cat tenuissimus (Gladden *et al.*, 1989), apparently after the loss of multiply innervated endings, indicates that the processes of reduction and differentiation are causally independent. Furthermore, though many of these connexions with the b_1 fibre are presumably withdrawn, mixed b_2 and c motor units persist into the adult. In other species even the b_1 fibre may continue to form part of mixed units (see Sections 3.1 and 3.2).

Neither intrafusally nor extrafusally, therefore, can the developmental purpose of multiple innervation be related to the formation of motor-unit types. Perhaps, through neuronal competition, it is necessary to ensure both spatial divergence of individual units and local convergence of different units, leading to effective integration both of skeletomotor and fusimotor activities.

5. CONCLUDING REMARKS

The muscle spindle is so obviously a length transducer, and what might be called the engineering, or linear systems, approach to its study has been so successful, that it is tempting to suppose that every detail of its adult organization has a discrete role to play in motor control. But one must not forget that this same adult organization has both developmental and evolutionary histories which have their own necessities and whose traces may persist, provided they are not detrimental to the spindle's main function. We cannot merely assume, therefore, that because we find some feature to be reliably present, it must be attributable to a motor-control requirement. In these concluding remarks I shall speculate on the possible contribution of developmental and evolutionary factors to the organization of the spindle's motor supply, functional considerations already having been dealt with as they arose.

Let us first examine the so-called β innervation. The motor input of intrafusal fibres by collateral branches of skeletomotor neurons was well known from non-mammalian tetrapods long before it was discovered in mammals (see review by Barker, 1974). It is undoubtedly phylogenetically old, and presumably older than the γ system. Provision of a separate intrafusal input may be more advanced in the sense of increased flexibility in motor control, but it is also more expensive and would have required selection pressure in response to actual need to bring about. There is no reason to suppose that, despite their behavioural complexity, mammals need a completely separate intrafusal input, desirable though this may seem to an engineer. From the evolutionary perspective, therefore, the retention of skeletofusimotor connections is readily understood.

It is unnecessary to postulate a specific functional role for the neurons providing those connexions; that is to say, one that could not be fulfilled by α and γ neurons separately. Indeed, the evidence is consistent with β neurons being nothing more than α neurons that happen to have encountered differentiating intrafusal fibres at a suitable stage of development. The absence of a distinct group of β neurons is attested not only peripherally in the evidence described in Section 3.2.1, but also centrally in a study of the nuclear and synaptic morphology of peroneal motoneurons (Destombes *et al.*, 1992). The higher incidence of fusimotor connexions among the neurons of slow rather than fast motor units can be seen to reflect the greater chance of intrafusal contact at a time in development when only the primary and earliest secondary myotubes are present.

If correct, these ideas have several important implications: (i) any α neuron could potentially make intrafusal connexions; (ii) it is a matter of chance which ones do; (iii) it is therefore preferable not to refer to skeletofusimotor neurons as though they were intrinsically different from α neurons, by calling them β neurons; and (iv) the total muscle-fibre composition of all motor units is actually or potentially mixed. This last implication may seem surprising when we are so used to the concept of homogeneous motor units, but the homogeneity is a feature only of the normal adult extrafusal component, indicating that it has been positively selected presumably as providing definite functional advantages.

The fusimotor connexions made by α motoneurons are not, of course, entirely random since they are virtually restricted to b_1 and lc poles, at least in the majority of examples reported so far. This is highly significant developmentally: as we have seen above (section 3.6) the b_1 and lc fibres appear sequentially as the earliest and subsequent secondary intrafusal myotubes. The absence of α input to b_2 fibres is therefore especially noteworthy. It suggests that there exists a mechanism, presumably initiated as a result of afferent contact with a primary myotube, which serves to exclude such connections. The temporary presence of motor terminals at a very early stage of spindle development in the rat soleus, when only primary myotubes have been formed, is particularly interesting in this respect.

Long c poles form only a small part of the static equipment of spindles, most of which, indeed, lack them altogether. Conversely, b_1 fibres are regularly present except in some small, tandem-linked, units. The accumulated evidence indicates that their motor input is often substantially, and sometimes exclusively, provided by α collaterals. It therefore seems to be more important for the intrafusal static input to be separate from the α output than it is for the dynamic input.

Consonant with this observation are the results of Murphy *et al.* (1984) who have provided evidence that during locomotion in premammillary cats even the dynamic γ activity is phasically modulated in parallel with the e.m.g., whereas static γ axons are tonically active.

Chance encounter with a developing spindle is presumably too fallible a mechanism for the purely fusimotor γ neurons to rely on in order to establish their peripheral connexions. Rather, the axonal growth cones might be expected to follow paths leading directly to the incipient spindles. If the afferent axons provide the guiding stimulus or recognition signal, a possible basis for the correlation between the numbers of afferent and efferent axons supplied to individual spindles is apparent.

Despite this difference between α and γ neurons, the underlying unity in the pattern of extrafusal and intrafusal fibre development, and the existence of skeletofusimotor connections, indicate that we should seek a common theory to account for the establishment of their motor units. If so, then the static γ system is particularly instructive.

As has been demonstrated above (Section 3.2.2), the motor unit of each static γ neuron is typically mixed in composition, but the b_1 fibre is notably excluded. The exclusion of the b_1 fibre occurs at a relatively late stage of development (Gladden *et al.*, 1989), apparently after the period when endplates are multiply innervated. This is consistent with the possibility that the γ neurons, although already distinct from α neurons, are relatively undifferentiated when they first encounter the developing spindle. Initially they may attempt to establish contact with as many intrafusal fibres as possible, but it may be recalled that when the first permanent motor innervation arrives in the spindle only the b_2 fibres are sufficiently mature to receive them and *c* fibres are altogether absent. Whether through intrinsic properties, or as a result of this contact, the neurons then differentiate and are able to recognize functionally inappropriate connexions and withdraw them. That this is not a competitive process at multiply innervated endings is further suggested by those instances in which b_1 poles are left without any motor input.

A similar mechanism could operate extrafusally. For example, the earliest α axons to arrive in the muscle primordium would encounter only primary myotubes, and might differentiate accordingly. The primary myotubes will themselves subsequently differentiate into type I extrafusal fibres, and though the motoneurons also innervate secondary myotubes as they appear, virtually all extrafusal contacts apart from those on the type I fibres are withdrawn. Intrafusally, the α motoneurons are similarly promiscuous at first, as indicated by the evidence of glycogen-depleted single motor units in neonatal rat lumbrical muscle (Jones *et al.*, 1987a, Fig. 1), which undoubtedly include several b_2 as well as other intrafusal fibres. These α collaterals may persist into the adult but only the b_1 and *lc* fibres continue to receive them (Kucera *et al.*, 1991).

The proposed mechanism of motoneuronal differentiation after initial myotubal contact depends crucially on the sequential appearance of myotubes having different potentials for development. The necessary intrinsic properties of the myotubes certainly seem to be present intrafusally, or at least are not dependent on the presence of the motor innervation. They may also be present extrafusally (Jones *et al.*, 1987b). Reinnervation following nerve injury would therefore be expected to be abnormal since the different muscle-fibre types are simultaneously present, but adult motoneurons are well known to be able to modify extrafusal fibre properties, and this could mitigate some of the effects of misconnexions.

Peroneus tertius motoneurons, whose axons had regenerated to give skeletofusimotor distributions after nerve section, have revealed unusual connexions in glycogen depletion experiments (Barker, Scott and Stacey, in press). Although the sample of seven axons is small, the intrafusal connexions clearly tended to be much more random than normal. Thus, among 14 spindles, depletions were found in nine b_1 poles, eight b_2 poles, and 17 *c* poles, of which only two were long. The frequent occurrence of depletions in b_2 fibres is particularly noteworthy. Moreover, despite an apparent distinction between dynamic and static axons such that, as normal, b_1 fibres were rarely co-innervated with other types of fibre, the motor units usually exhibited abnormal associations of intrafusal and extrafusal fibre types. The extrafusal components ranged from exclusively slow (type I) to predominantly fast-fatiguable (IIB with an admixture of I), with some suggestion of a correlation with axonal conduction velocity. However, in a reversal of the normal pattern, the slowest-conducting axons had static effects, thus linking b_2 and *c* fibres intrafusally with mostly type I fibres extrafusally.

Competition between motoneurons undeniably occurs and may also, of course, contribute to the determination of motor-unit composition. However, as suggested in Section 3.6, it may be more important for the production of overlapping motor units, thus providing efficient spatial integration both in the mechanical output (extrafusal fibres) and in the proprioceptive feedback (intrafusal fibres). Support for this idea comes from the observation that in spindles receiving two static γ axons it is usually the poles rather than the b_2 and *c* fibres that are separately innervated. It would be very interesting to know whether, during development of such spindles, the two axons had previously supplied both poles at multiply innervated endings. (In C883 four spindles each received a single static γ axon, of these three did indeed supply both poles of their spindles.)

When a spindle receives three static γ axons, such that two of them supply a single pole, the b_2 and *c* fibres in that pole are then usually separately innervated. This may be readily interpreted as due to competition in which a later-arriving axon is able to displace from the newly formed *c* fibres an already present axon that has established contact with the b_2. It is obvious that a similar process could operate in the homogenization of extrafusal motor units. Intrafusally, however, it is probably of little functional significance within the static system, as has been argued above (Section 3.5), other than creating further spatial integration. This

seems to be confirmed by the observation that continued increase in the number of static γ axons to a spindle results in a reduction in their overall degree of segregation among the two types of muscle fibre.

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Paper 50.

R. W. Banks, M. Hulliger, K. A. Scheepstra & E. Otten (in press)
Pacemaker competition and the role of preterminal-branch tree architecture: a combined morphological, physiological and modelling study.
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PACEMAKER COMPETITION AND THE ROLE OF PRETERMINAL-BRANCH TREE ARCHITECTURE: A COMBINED MORPHOLOGICAL, PHYSIOLOGICAL AND MODELLING STUDY

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INTRODUCTION

Peripheral sensory endings often consist of groups of unmyelinated nerve terminals, specialized for transduction, that ultimately converge on the single afferent axon through a system of myelinated preterminal branches. At least in the mammalian muscle spindle the sensory terminals are in continuity only through the preterminal branches (Banks, 1986), so each may be supposed to have an associated spike-initiation (encoding) site, potentially able to act as a separate pacemaker. Interaction in such a system is expected to be highly competitive (Eagles & Purple,1974) so that the final output of the afferent is in general a non-linear function of the activities of the separate encoders.

The primary sensory ending of the mammalian muscle spindle is a favourable example in which to study pacemaker interactions because it is frequently derived from two first-order branches that separately supply terminals on different types of intrafusal muscle fibre: the bag_1 (b_1) fibre and bag_2 and chain (b_2 and c) fibres together (Banks 1986). The greatly expanded axonal terminals, where mechanosensory transduction may be presumed to occur, are derived from short, unmyelinated preterminal branches. These in turn arise almost exclusively from the heminodes of the ultimate myelinated preterminal branches which may be of first to fourth order (Banks, 1986). The geometry of this arrangement indicates that the heminodes act as sites of spike initiation, a hypothesis that is supported by histochemical evidence from the cat showing that primary-ending heminodes share staining properties with known spike-initiation

sites such as motor axonal initial segments (Quick et al., 1980). The high degree of segregation between the dynamic and static fusimotor systems (reviewed by Banks, 1994) enables selective activation of the b_1 and b_2-c terminals respectively, by eliciting contractile activity in the appropriate muscle fibres.

Physiological studies have repeatedly shown that at least two pacemakers exist in primary endings of the cat and that they may be separately excited by dynamic and static fusimotor stimulation. Although competitive interactions predominate, a variable amount of summation may also occur (Hulliger & Noth, 1979; and other references therein). The possibilty that the topology of the preterminal-branch tree architecture might influence, in part, the amount and pattern of summation has been supported by an unpublished modelling study (Otten, Hulliger & Schaafsma). Here we extend those observations by determining, in the same spindle, both the nature of pacemaker interactions and the tree architecture of individual primary endings, and comparing the results with model simulations.

METHODS

The preparation was the tenuissimus of the anaesthetized cat. This facilitated precise location of the primary endings, an essential prerequisite for accurate histophysiological correlation. Details of the preparation, and of the data acquisition and control of experimental parameters, are given in Banks (1991) and Baumann & Hulliger (1991) respectively. Briefly, the left tenuissimus muscle was exposed by removal of biceps femoris, after extensive denervation of the sciatic distribution to the limb, sparing only the tenuissimus nerve. The muscle was freed from surrounding connective tissue for the greater part of its length, so as to allow effective transmission of stretch applied by an electromagnetic puller at its distal end. An efficient blood supply for the distal part of the muscle was maintained by creating a pedicle at the level of the popliteal fossa. Control signals for muscle stretch and fusimotor stimulation were taken from a hybrid signal generator that permitted reproducible synchronization of electrical and mechanical stimulation patterns. Spikes obtained from single afferents were converted to TTL pulses, and stored on-line using a LS11/73 computer. Routinely, fusimotor actions were tested using both constant and triangularly modulated stimulation rates. Constant stimulation rates ranged from 25 to 150Hz. During trapezoidal stretches, both static and dynamic axons were typically stimulated at 100Hz. Triangularly modulated stimulation was applied with the muscles held at constant length. Maximal slope of the modulations was 50Hz/s, with a peak stimulation rate normally not exceeding 150Hz. This particularly facilitated dignosis of the static effectors, using criteria established in a previous histophysiological study (Banks, 1991).

The proximo-distal locations of individual spindles were marked by epimysial stitches. At the end of the physiological part of each experiment the muscle was removed and fixed under light tension for about 7 hours in a Karnowsky fixative. In most cases, located spindles and their nerve supplies were teased out before or during secondary fixation in OsO_4, enabling the equatorial regions containing the primary endings to be identified and separately embedded in epoxy resin. Serial, 1µm sections were prepared, usually in the longitudinal plane, stained with toluidine blue, and used to reconstruct the afferent tree architecture.

Simulations were carried out using a model based on likely intrafusal-fibre mechanical properties to produce a receptor potential associated with each type of fibre (Schaafsma et al., 1991). An ionic model of the action potential, based on modified Frankenhaeuser-Huxley kinetics (Otten, Hulliger & Scheepstra, in press), generated spikes at the heminodes of individual trees and propagated the spikes through the tree. The firing rate of each heminodal

pacemaker might be influenced by the receptor potential of its own terminal, electrotonic spread from other terminals via the preterminal branches, and antidromic invasion by upstream nodal spiking.

A coefficient of interaction index (C_i, see Figure 1) was calculated both for the real and simulated data, to provide a quantitative estimate of the amount of competition or summation that occurred between the dynamic and static pacemakers, and to allow correlations with parameters of tree structure to be estimated. The coefficient was defined as the difference between the response to combined dynamic and static fusimotor stimulation (R_C) and the higher of the responses to separate stimulation (R_S or R_D), normalized with respect to the lower of the separate responses; formally

$$C_i = \begin{cases} (R_C - R_S)/R_D & R_S \geq R_D \\ (R_C - R_D)/R_S & R_D > R_S \end{cases}$$

C_i might be expected to vary between 0 (high competition, or complete occlusion of the momentarily slower pacemaker by the faster one) and 1 (complete summation, in which the overall output is the algebraic sum of the separate pacemakers).

If all heminodes are potential pacemakers, there are usually in a single tree more than one possible pathway linking dynamic and static pacemakers that could be active. We have selected the minimum path length in each case, estimating the path length quantitatively by the number of complete nodes (nMPL) contained in it.

RESULTS

Responses to fusimotor stimulation were obtained both during trapezoidal stretch and with the muscle at constant length. Here we give a preliminary account of the results obtained using trapezoidal stretch, from eighteen different combinations of pairs of dynamic and static fusimotor action on 17 primary endings of 9 adult cats. C_i was calculated using data collected in 200ms bins. It usually varied according to the phase of the stretch, for example it often fell during the phase of muscle lengthening when the dynamic response greatly exceeded the static. A falling value of C_i indicates increasing competition. The effect proved to be a function of the difference in firing rate between the separate pacemakers, as shown by plotting the C_i against the difference between the separate dynamic and static responses for individual bins. The resulting scatter plot showed a clear tendency for C_i to reach maximum values (greatest summation) when the difference between the separate dynamic and static firing rates was 0.

Mean C_i, averaged from 40 bins, normally ranged from 0.07 indicating very strong competition, to 0.33 in which summation was evident but the response was still dominated by competition. Exceptionally, one primary showed very high summation ($C_i = 0.66$). The results are here tabulated in full (Table 1) according to decreasing order of mean C_i., together with the number of nodes in the minimum path length between the inferred dynamic (b_1) and static (b_2 or c) effectors. Routinely, fusimotor actions were tested using both constant and triangularly modulated stimulation rates. This particularly facilitated diagnosis of the static effectors, using criteria established in a previous histophysiological study (Banks, 1991). However, in most cases (13/17) there was no difference between the minimum path lengths for b_2 or c, and in no case was the difference greater than 1.

In most primary endings the Ia afferent branched dichotomously into first-order branches that supplied b_1 and b_2-c fibres separately. The preterminal trees of these endings

possessed a minimum of 3 to 9 nodes between the probable dynamic and static pacemakers. There were two exceptional endings: 258/11 in which there were 5 first-order branches derived from a single node; and 266/2 in which the b_1 fibre was supplied only by a second-order branch, whose complementary division supplied several *c* fibres. In the latter case it was possible to be virtually certain that the *c* fibres were activated by the static γ axon that had been stimulated, since histologically there appeared to be only a single static γ axon present, which supplied all (6) *c* fibres, as well as b_2, usually in both poles. The minimum path length was therefore 1.

Simulations were carried out using trees having the same topology as the second-order b_1-*c* part of 266/2 and the first-order b_1-*c* part of 266/5, since these were closely adjacent spindles in the same muscle and they exhibited virtually the most extreme values of mean C_i and minimum path length: 266/2, $C_i = 0.66$, MPL = 1; 266/5, $C_i = 0.11$, MPL = 8. Both showed considerably greater competition than the real endings, but whereas it was nearly complete in the 266/5 simulation, there was a much greater degree of summation in the 266/2 simulation.

DISCUSSION

Our results confirm previous reports (see Hulliger & Noth, 1979) in which it was concluded that mammalian primary endings contain multiple pacemakers, and that these pacemakers interact in a predominantly competitive manner. Detailed temporal analysis of the simulations showed that competition arises as predicted on theoretical grounds (Eagles & Purple, 1974) because of antidromic invasion of the momentarily less active pacemaker by spikes generated by the more active one.

A variable amount of summation can, however, be shown even by individual endings. There are several conceivable reasons for this variabilty, but we have now been able to demonstrate that in primary endings under comparable conditions of stretch and motor stimulation, a component is attributable to preterminal-branch structure. So far we have only considered topological features of the tree architecture and not the absolute dimensions of the trees. Those that we considered as potentially influential included the number of terminals, the proportion of branching nodes, and the number of nodes in the maximum path length. However, a multivariate analysis revealed that the only significant structural component was related to the number of nodes in the minimum path length. Moreover, the influence of this factor seems to decline very rapidly as minimum path length increases, as would be expected if the effect is mediated by the electrotonic spread of, say, receptor potentials. We may note that Hunt et al. (1978) were able to record a compound receptor potential in the parent Ia axon after blocking Na+ spikes with TTX.

The multivariate analysis also revealed that two other factors contributed to the variable amount of summation shown by individual endings: the overall firing rate, and the difference in rates when the dynamic and static pacemakers were separately activated. Summation increases with overall firing rate, a surprising observation and one not predicted by the simulations. However, it serves to remind us that competition normally predominates even, indeed especially, at low rates. This is perhaps explicable by supposing that the firing rate of an individual pacemaker is determined by its relative refractoriness, and that this can be counteracted by increasing the receptor potential. The increased summation seen at higher rates is more difficult to explain, but may be due to a shift in the effective pacemaker site to the next most proximal node if its threshold is exceeded by the electrotonically spread receptor potential. This would, of course, have the effect of reducing the minimum path length, and

would therefore increase summation due to the structural factor. The histochemical study that showed heminodes to be potential pacemaker sites (Quick et al., 1980) also showed that at least some of the preterminal nodes shared the same staining properties, indicating a similar excitability.

The general similarity between the real and simulated data is encouraging, particularly in regard to the preponderance of competition and the effect of tree topology on summation. Nevertheless, it must be noted that overall the simulated results show very much greater competition than is exhibited by the real primary endings. The reasons for the discrepancy remain to be investigated, but presumably involve oversimplifications in the assumptions that form the basis of the model.

Finally we reflect on that feature of the primary ending with which we began: the dichotomous first-order division into b_1 and b_2-c parts. Despite, or perhaps because of, its regular occurrence in cat spindles, it seems to have been taken for granted when perhaps we should have been asking whether it must be so. Certainly it is one of the few quite constant features of the preterminal and terminal branch system of the primary, and the first-order branches often lie alongside each other for what seem to be extravagantly long distances (Banks, 1986). Could it be that the division is an adaptation to ensure that the interaction between separate dynamic and static pacemakers is highly competitve? We need to know more about the use made by the CNS of such a multiplexed signal in motor control, and whether summation would compromise that control. If so, an apparently unrelated fact of spindle development, which again has rather been taken for granted, becomes explicable: that the oldest of the secondary myotubes differentiates as the b_1 fibre (Milburn, 1984). The overall pattern of myotubal development is common to both intrafusal and extrafusal fibres (Kozeka & Ontell, 1981). Thus, secondary myotubes separate from the growing cluster, centred on the primary myotube, in the order of their formation. This sequence will enhance the likelihood of a preterminal division of the Ia containing segregated dynamic and static pacemakers.

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Figure 1. A: Population density plot showing all the responses to trapezoidal stretch, averaged in 200ms bins, during static (R_S), dynamic (R_D), and combined static and dynamic (R_C) fusimotor stimulation. Average stimulation rates were approximately 100Hz in each case. B: Schematic representation of the calculation of the coefficient of interaction (C_i), as formally defined in the text, for the three representative bins (5, 15 and 35) shown in A.

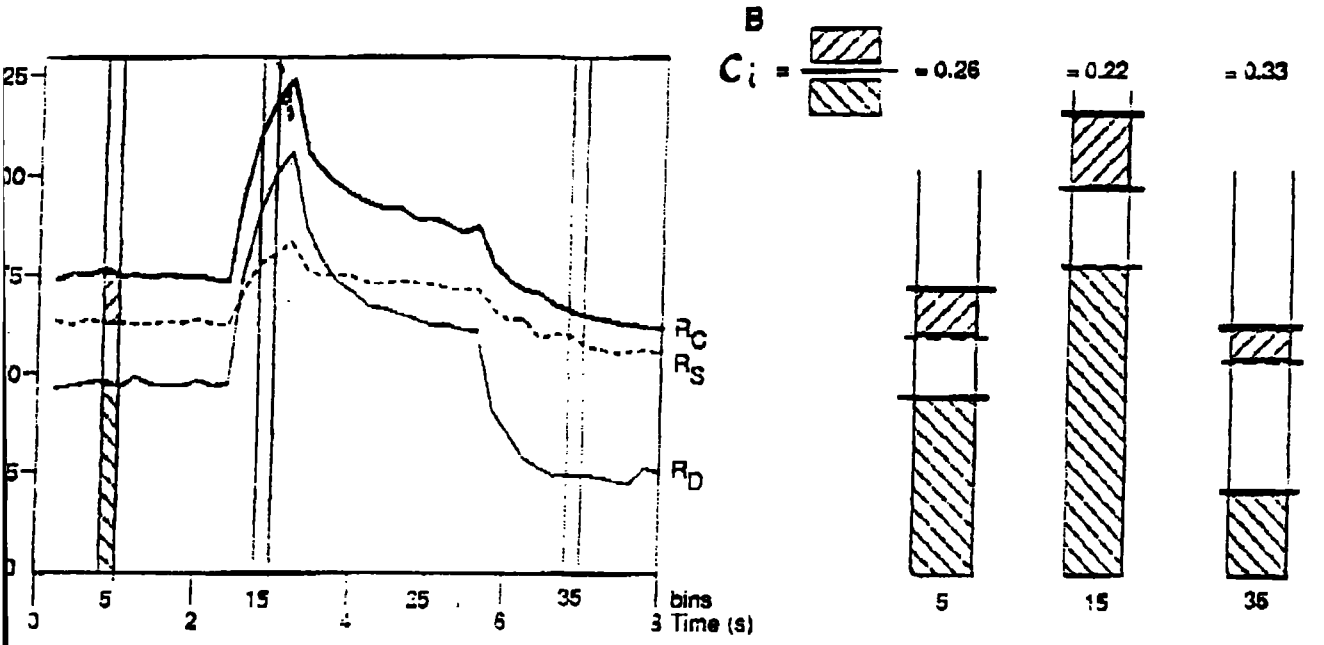


Table 1. Mean coefficient of interaction ..... (C_i) and the number of nodes in the minimum path lengths (nMPL) for dynamic and static pacemaker interactions in cat tenuissimus primary endings.

experiment/spindle	C_i	nMPL	experiment/spindle	C_i	nMPL
266/2	0.66	1	258/3	0.17	5
262/4	0.33	5	269/9	0.17	8
270/11	0.31	3	269/4	0.17	9
258/2	0.30	3	261/1	0.16	6
270/4	0.24	5	258/12	0.15	4
264/9	0.22	6	264/3	0.14	9
258/11	0.20	2	269/11	0.11	9
262/10	0.19	6	266/5	0.11	8
269/11	0.17	9	270/1	0.07	6

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Paper 51.

R. W. Banks, L. Decorte, F. Emonet-Dénand, M. H. Gladden & F. Sutherland (in press)
The exceptional structure of muscle spindles in superficial lumbrical muscles of the cat hind limb.
In A. Taylor & M. H. Gladden (Eds.) Alpha and Gamma Motor Systems Plenum Press, New York.

THE EXCEPTIONAL STRUCTURE OF MUSCLE SPINDLES IN SUPERFICIAL LUMBRICAL MUSCLES OF THE CAT HIND LIMB

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INTRODUCTION

Histophysiological experiments using the superficial lumbrical muscle of the cat have provided the opportunity to analyse the structure of a large number of spindles (Decorte *et al.*, 1986). This muscle was initially chosen because of its small size, spindle content and known motor-unit properties (Emonet-Dénand *et al.*, 1988).

Compared with the classical picture of the spindle, derived principally from tenuissimus, superficial lumbrical spindles show several important differences. In particular, a high proportion of lumbrical spindles possess long chain fibres (71% of poles, Decorte *et al.*, 1987) and greater individual variability in the number of bag fibres present (Decorte *et al.*, 1990).

Here we present further original features of superficial lumbrical (SL) spindles, emphasizing the importance of the bag_1 (b_1) fibre, which is known to be innervated by both small (γ range) and large (α range) motor axons.

METHODS

The observations are based on serial cryostat sections, stained to demonstrate ATPase activity for fibre typing. Details of the method are given in Decorte *et al.*.(1987).

RESULTS

The longest intrafusal fibre present was almost invariably a b_1 (26/30). The difference in length between b_1 (mean 3388μm) and bag$_2$ (b_2; mean 2320μm) fibres is especially notable. In only one case was a b_2 longer than a b_1, whilst in two cases the longest fibre was a (long) chain. Mean diameters of each fibre type were measured at regular intervals. The b_2 fibre was slightly smaller than the b_1 fibre at the equator, and chain (c) fibres were significantly smaller than both. All types progressively decreased in diameter from region A through B; b_2 and most c fibres continued to decline through region C, whereas the mean diameter of long c fibres did not decrease further until beyond 2000μm from the equator. In complete contrast, the mean diameter of b_1 fibres increased markedly at the start of region C, and then again declined steadily. The b_1 fibres therefore were normally not only the longest, but considerably the largest, intrafusal fibres in the extracapsular poles.

In a few spindles there was only a single bag fibre, which was more often b_1 (2/4 complete spindles, or 7/11 poles) than is the case for similar spindles in tenuissimus. Perhaps related to this is the finding that in tandem-linked spindles continuous bag fibres were more often b_1 than b_2, which is again at variance to the familiar picture from tenuissimus. Some spindles appear to consist of two intrafusal bundles more or less conjoined so that the equator, and often one pole in addition are common to both.

CONCLUSION

Our observations add to the growing evidence that different muscles exhibit characteristic features of the structure and innervation of their proprioceptors. These are often of a statistical or quantitative nature, nevertheless they may be supposed to reflect adaptations to local control requirements.

ACKNOWLEDGEMENTS

We are very grateful to Dr. D. W. Harker, who participated in the initial histological analysis. RWB thanks the Wellcome Trust for Financial support.

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THE INNERVATION OF MUSCLE SPINDLES IN AN INTRINSIC MUSCLE OF THE HIND FOOT: THE SUPERFICIAL LUMBRICAL OF THE CAT.

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An important, if largely unrecognized, feature of the design of the mammalian skeletomotor proprioceptive system is the characteristic variation from muscle to muscle in the provision of its components. This applies not only to the relative abundance of muscle spindles, but also to quantitative differences in their sensory-ending complement (Banks and Stacey, 1988). Furthermore, Banks (in press) has shown that there is a linear correlation between the numbers of static γ and afferent axons supplied to each spindle, at least in the tenuissimus. Considerably more comparative information of this type is needed to contribute to a fuller understanding of the contribution that proprioception makes to particular motor tasks. Here we describe some preliminary observations on the innervation of the superficial lumbrical muscle of the cat's hind foot.

SENSORY INNERVATION

A sample of 53 teased, silver-impregnated spindles was analysed. The results from both 1st and 2nd superficial lumbrical muscles were combined, since there appeared to be no differences between the two parts. The spindles contained 55 primary (P) endings, two of which occurred singly in small, tandem-linked units that probably consisted only of bag1 (b_1) and chain (c) fibres. All of the remaining units

had the full complement of intrafusal-fibre types, and almost all comprised single spindles, though a few were conjugated in parallel. In addition to a primary ending, each possessed from 0 to 4 secondary (S) endings with the following frequency distribution: P 23%; PS 55%; P2S 17%; P3S 4%; P4S 2%. Of the the 57 S endings 49 were in the S_1 position (next to P) and 8 in S_2 (next to S_1).

It is clear that a single Ia afferent is both necessary and sufficient to maintain (and prbably to initiate) intrafusal-fibre differentiation. Any additional afferent axons are usually of group II and supply secondary endings, as is the case in the superficial lumbrical described here. The mean number per spindle of these additional afferent axons in a particular muscle seems to be an important controlled variable of mammalian sensorimotor systems, since it has a characteristic value for that muscle while varying between different muscles. The value for the superficial lumbrical is lower (1.08) than that of any of a sample of 7 hind limb muscles of the cat (range 1.22, peroneus brevis, to 2.72, popliteus) studied by Banks & Stacey (1988). Precisely which spindles receive secondary endings appears to be a matter of chance, since the frequency of occurrence of spindles with different complements of afferent axons can be fitted by probabilistic statistics. The mean number of additional afferents per spindle is used to estimate the statistical parameters (Banks & Stacey, 1988). In most cases the two parameters (n, p) of the binomial distribution are necessary to describe adequately the observed distributions, but when n is large and p is small the distribution approximates to Poisson. For the superficial lumbrical spindles that we have examined, binomial distributions with parameters $n = 2$, $p = 0.54$, or $n = 3$, $p = 0.36$ most closely approximate to the observed data. There are, however, insufficient degrees of freedom remaining after parameter estimation to test whether the theoretical distribution corresponding to either set of parameters differs significantly from the data.

MOTOR INNERVATION

The distributions of 131 intrafusal branches of motor axons within 29 poles of 16 teased spindles (5 P, 8 PS, 3 P2S) have been determined as follows: to bag_2 (b_2) or c or both, 56; to b_1, 75. Despite the presence of a high proportion of long-chain (lc) fibres in superficial lumbrical spindles (Decorte et al., 1987) only 2 axons to lc fibres showed characteristics of skeletofusimotor (β) innervation, a much lower incidence (0.13/spindle) than in tenuissimus (0.59/spindle; Banks, in press). Conversely the incidence of b_1 innervation (4.7/spindle) that appeared to be dominated by p1 plates and thus, potentially, β innervation, was much greater than in tenuissimus (1.9/spindle). The β nature of some of this innervation was demonstrated by Barker et al. (1980). We have studied this innervation using serial, cryostat sections of combined cholinesterase and silver-gold impregnation (Pestronk and Drachman, 1978), interspersed with alkaline- and acid-preincubated ATPase for fibre-typing. Our observations confirm that it almost invariably supplies the b_1 fibre.

The large majority (54/56) of the axons innervating b_2 and c fibres (separately or jointly) may be safely identified as purely fusimotor (γ). Mean values of the number of these axons per spindle, for each of the different sensory complements, were: P, 2.9; PS, 4.0; P2S, 4.4. Corresponding values for tenuissimus are 1.7, 3.0, and 4.4 respectively. A similar correlation between the numbers of afferent and γ axons may therefore exist in both muscles, though the constant of proportionality may differ.

CONCLUSION

We conclude that the pattern of innervation of superficial lumbrical spindles, although qualitatively similar to that of tenuissimus, differs quantitatively in several important respects. It seems possible, even likely, that such characteristics vary independently between different muscles.

ACKNOWLEDGEMENT

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Paper 53.

R. W. Banks, M. H. Gladden, F. I. Sutherland & A. Yoshimura (in press)
The sensory and motor innervation of the abductor digiti quinti medius muscle of the cat.
In A. Taylor & M. H. Gladden (Eds.) Alpha and Gamma Motor Systems Plenum Press, New York.

THE SENSORY AND MOTOR INNERVATION OF THE ABDUCTOR DIGITI QUINTI MEDIUS MUSCLE OF THE CAT

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INTRODUCTION

The abductor digiti quinti medius (adqm) is an intrinsic muscle of the foot which abducts the fifth digit. Despite its small size (about 30mg) it contains all the elements of a complete skeletomotor and fusimotor system. It may therefore provide a practical model for studying integrated fusimotor and skeletomotor activity under a variety of conditions. Morphological investigation of the innervation of the adqm muscle of the cat hind limb was carried out as an adjunct to electrophysiological studies.

METHODS

Five adqm muscles from 3 cats were prepared for silver impregnation and teasing according to the method of Barker and Ip (1963).

One muscle was frozen in isopentane cooled with liquid-N_2, cut transversely at 10μm and reacted with antibodies raised against anti-tonic and anti-neonatal myosin (Rowlerson *et al.*, 1985).

One muscle was fixed with glutaraldehyde, embedded in Araldite resin and serially sectioned transversely at 1 and 5μm. Sections were stained with toluidine blue for 30s on a hotplate.

All specimens were observed using light microscopy. Whenever possible, the numbers and identification of intrafusal fibres were noted, as were the number and position of sensory endings and the distribution of motor axons.

RESULTS

Bag fibres were distinguished from chain fibres by their greater diameters. The two types of bag fibre were distinguished histochemically by the marked presence of tonic myosin combined with a virtually total lack of reaction against neonatal myosin in the bag_1 fibre, as has been previously found in triceps (Rowlerson *et al.*, 1985). In the resin sections the bag_2 fibre had a more granular appearance than the bag_1, had more elastic fibres surrounding its poles (Gladden, 1976). Also it often shared a capsular compartment with chain fibres. In the silver material the bag fibres were distinguished by the form of the sensory terminals and by the distribution of elastic fibres.

Each muscle contained 3 to 6 spindles and 3 to 7 tendon organs. Additionally, the silver material contained 4 paciniform corpuscles.

Table 1. Numbers of spindles and tendon organs observed using the various techniques

Method	Number of spindles	Number of tendon organs
immunohistochemistry	4	7
toluidine blue	3	6
silver	17	26
total	23	37

Spindles contained up to 2 bag_1 fibres, 4 bag_2 fibres and 7 chain fibres, the highest numbers occurring in one spindle from the histochemical series that appeared to consist of two intrafusal bundles conjugated in parallel within the same capsule. This is a feature that has also been noted in the superficial lumbrical muscle (Banks *et al.*, these proceedings). Only one spindle appeared to be a b_2c unit. All 4 spindles studied by immunohistochemistry, 2 from the resin-embedded material, and at least 2 from the silver were linked in series with tendon organs. The overall sensory complements of the latter two associations were:

$S_3S_2S_1PS_1S_2S_3$-TO and TO-$S_2S_1PS_1S_2$-TO

In the overall sample the sensory complements of the b_1b_2c spindle units were:

P 2; PS 0; P2S 7; P3S 3; P4S 6; P5S 0; P6S 1.

The mean number of secondary endings per spindle is 2.90. This is an unusually high average for a hind limb muscle, where mean values have previously been found to range from 1.08 in

superficial lumbrical (Banks et al., these proceedings) to 2.72 in popliteus (Banks and Stacey, 1988).

In a preliminary physiological experiment we have separately recruited 4 Ia afferents and 2 paciniform corpuscles, recording spikes at two sites on the nerve supply to adqm so as to establish conduction velocities.

Motor endings as seen structurally in the resin-embedded material, and as indicated by acetylcholinesterase activity in the immunohistochemical material were located in reconstructions. The great majority (about 94%) were intracapsular, although dynamic β innervation is known to occur regularly in this muscle (F. Emonet-Dénand, personal communication). Motor axons are predominantly unmyelinated at spindle entry.

CONCLUSION

Although this preliminary structural analysis demonstrates that the muscle contains all the sensory complement expected in a hind limb muscle, there are certainly some unusual features, notably the high incidence of secondary endings, and the in-series arrangement of many spindles and tendon organs.

ACKNOWLEDGEMENT

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Paper 54.

R. W. Banks, F. Emonet-Dénand, M. H. Gladden & F. I. Sutherland (in press)
Evidence for γ_S innervation of long chain fibres.
In A. Taylor & M. H. Gladden (Eds.) Alpha and Gamma Motor Systems Plenum Press, New York.

EVIDENCE FOR γ_S INNERVATION OF LONG CHAIN FIBRES

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INTRODUCTION

Nuclear chain fibres are classically regarded as being much shorter and thinner than bag fibres either bag_1 or bag_2. However, Barker *et al.* (1976) noticed that some spindles possessed one or more chain fibres with unusually long poles, often of similar length to the bag fibres. As an estimate of the incidence of these 'long chain' fibres they assessed the proportion of spindles containing at least one chain-fibre pole that extended for more than 1mm beyond the end of the capsule. Kucera (1980) formalised this practical criterion as a definition of a class of long chain fibres each of which possessed at least one long pole. Since that time long chain fibres have often been treated as though they formed a distinct type of intrafusal fibre with a specific static beta β_S innervation (Kucera, 1982, 1984; Barker & Banks, 1986). Here we give evidence in detail of both static β and static γ innervation of long chain fibres.

METHODS

Two methods were used to examine the motor innervation of tenuissimus muscle spindles which were obtained from previous work (Banks, 1981, 1991; Arbuthnott *et al.*

1982; Sutherland *et al.* 1985; Boyd, 1986). these were silver staining and teasing of muscle spindles and serial sectioning of resin-embedded muscles.

Tenuissimus muscles were silver stained and teased, according to the method of Barker & Ip (1963) and the motor innervation traced. In addition, seven adult cat tenuissimus muscles were glutaraldehyde fixed, resin embedded and serially sectioned in the transverse plane throughout at 1μm. Ultrathin sections were either taken every 10μm throughout the sleeve region (Arbuthnott *et al.*, 1982) when photographs were taken with the Zeiss 109 electron microscope at x8,300 and x20,000 enlarged x2.5, or were taken when a batch of 1μm sections ended in a motor ending. Motor innervation of the spindles was in all cases reconstructed. In several instances of each method the function of the motor axons had been determined. Fusimotor axons were classified as static or dynamic from the response of the primary sensory afferent to muscle ramp and hold stretches and ramp stimulation between 0 and 150 Hz.

RESULTS

The intrafusal distributions of all motor axons that supplied at least one long pole of a chain fibre are given in Table 1, which also includes 2 instances of long chain poles that received no motor innervation. The most frequent distribution (6/16, 38%) was to long chain poles only, but long chain poles could also be supplied in common with bag_1, bag_2 or other chain fibres. The common innervation with bag1 fibres is particularly noteworthy.

Specific examples in which physiological properties of the motor innervation were known are considered in more detail below.

Table 1. Long chain fibre pole innervation. γ_S, β_S were physiologically characterised static fusimotor axons.

No innervation	lch	lch + ch	lch + b_2	lch + b_1	total
2	6 ($1\beta_S$) ($2\gamma_S$)	2	3 ($1\gamma_S$)	3	16

Long chain fibre pole innervated by known static γ axons

In the spindle innervated by a known β axon, one chain fibre pole was long. The motor axon terminating on this long chain fibre pole had a conduction velocity of 85m/s and the primary sensory ending was driven, which is characteristic of static β axons (Jami *et al.*, 1985). The two endings on this long chain fibre pole were p_1 plates 15μm apart and positioned inside the B region of the capsule.

Long chain fibre poles innervated by known static γ axons

In the specific example described here in which the spindle was reconstructed from serial sections there was a chain fibre both poles of which were long. The motor innervation of these poles was totally contained within the spindle capsule sleeve. One static fusimotor axon (γ_{20}, where 20 refers to the conduction velocity of the axon in m/s) terminated only

on the proximal long chain fibre pole. On stimulation, this axon drove the primary sensory ending. A second static motor axon (γ_{18}) had an ending on the distal long chain fibre pole but also terminated on the bag_2 fibre in this pole. This axon showed bag_2 effects combined with some chain involvement (Boyd & Ward, 1982).

The form of the motor endings on each long chain pole was different. One was of the simple type and one of the complex type, as determined by Arbuthnott *et al.* (1992). The motor endings of the axon terminating on both bag_2 and long chain fibre poles were similar and of a simple type, i.e. axon terminals lying on a smooth post-synaptic muscle membrane. The motor ending on the proximal long chain fibre pole was complex, i.e. the post-synaptic muscle membrane was protruded into 'fingers', the axon terminals lying on or between these protrusions.

CONCLUSIONS

A long chain pole can be innervated by gamma or beta motor axons, and the incidence of long chain poles cannot be used to estimate the incidence of static beta innervation.

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Paper 55.

K. A. Scheepstra, E. Otten, M. Hulliger & R. W. Banks (in press)
Modelling of chaotic and regular Ia afferent discharge during fusimotor stimulation.
In A. Taylor & M. H. Gladden (Eds.) Alpha and Gamma Motor Systems Plenum Press, New York.

MODELLING OF CHAOTIC AND REGULAR Ia AFFERENT DISCHARGE DURING FUSIMOTOR STIMULATION

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Introduction

Muscle spindle Ia afferents often show 'driving', the firing of action potentials time-locked to the stimulus pulse, during static fusimotor stimulation. Driving is thought to be caused by rapid unfused contractions of nuclear chain fibres.

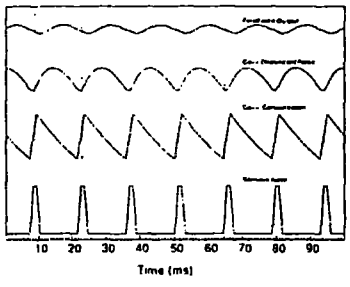
In order to give a more quantitive account of the mechanisms involved in driving, a model study was conducted based on detailed mathematical discriptions of both mechanical events and ionic events .

References

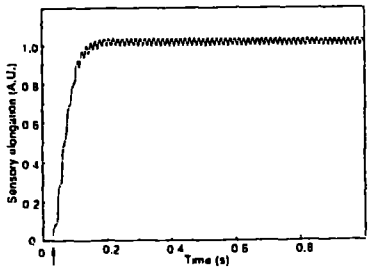
Bessou et al, 1968. J. Physiol. Lond. 196:47-62
Boyd et al, 1985, The muscle spindle. pp. 195-199.
Otten,1987, J. Neurosci. Methods 21: 287-302
Schaafsma et al, 1991, J. Neurophysiol. 65: 1297-1312.

Methods

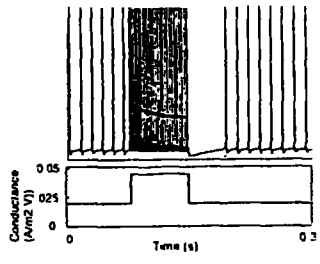
The mechanical model simulated the process of force production, using equations describing this process for type II extrafusal muscle fibre (Otten, 1987).



Stimulation pulses at 70 Hz. cause an unfused tetanus. The final force fluctuates at the stimulation frequency with an amplitude of 5% of the total force level.

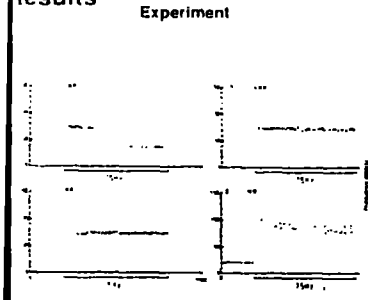


The sensory ending is stretched by the contracting fibre. The sensory elongation is tranduced into a depolarising conductance, proportional to the amount of sensory elongation. Note the remaining 5% oscillation.

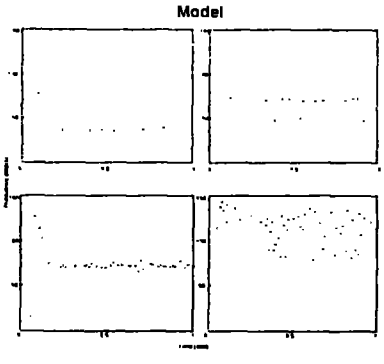


The conductance is used as an input for the ionic model, which generates action potentials. The ionic model is based on t Frankenhaeuser-Huxley equations. A sl K-channel (τ = 70 ms) was added to enal low frequency repititive firing. It also causes spike frequency adaptation after step change.

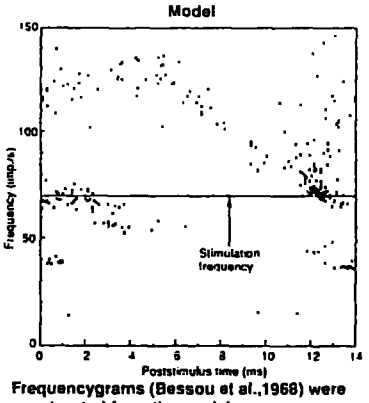
Results



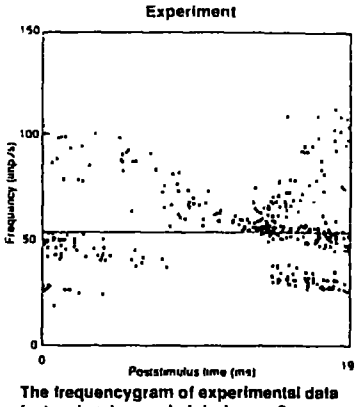
Experimental data (cat, peroneus tertius) from Boyd et al. (1985) shows that the initial muscle length can alter the driving pattern. 1:2 (top left), 1:1 (top right, bottom left) and irregular firing occur at resp. minimal, intermediate and maximal physiological muscle lengths.



All driving patterns could be reproduced with the model by adjusting the level of input conductance. The irregular firing occurs when the average afferent firing rate exceeds the stimulation frequency.



Frequencygrams (Bessou et al.,1968) were constructed from the model responses. Irregular firing (above stimulation frequency) forms a pattern in the frequencygram which resembles the intrafusal contraction. Irregular firing due to random noise will show no pattern.

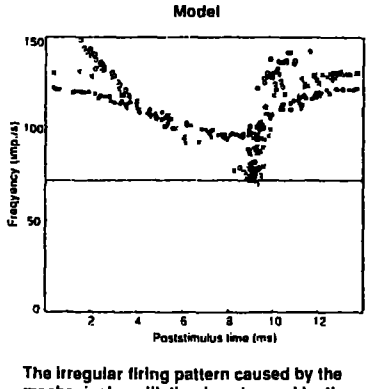


The frequencygram of experimental data (cat, soleus) recorded during an 8 mm ramp and hold stretch with static fusimotor stimulation is comparable to that of the model.

The hypothesis that the frequencygram reflects the contraction dynamics of the chain fibre was tested with the model (crosses).

When the amplitude of the mechanical oscillation is doubled, the modulation in the frequencygram increases (open circles).

Removing the slow K-channels reduces the modulation (closed circles). Therefore the frequencygram also reflects ionic mechanisms.



The irregular firing pattern caused by the mechanical oscillation is enhanced by the slow potassium conductance. In other words, the process of spike initiation itself is sensitive to dynamic changes in receptor potential.

Discussion

Small oscillations of the sensory elongation (5%) can cause different patterns of driving.

The type of driving is determined by the average afferent firing rate, which in turn is determined by the average amount of sensory elongation.

The observation (Boyd et al, 1985) that stable 1:1 driving can occur at all muscle lengths, can only be explained by the present model by assuming that the change in sensory elongation is small.

Possibly a transducer process sensitive to dynamic changes is needed to account for this phenomenon.

Conclusions

The irregular firing pattern observed during chain fibre stimulation is a chaotic form of driving which is expressed when the average afferent firing rate is higher than the stimulus frequency.

Driving is enhanced by the presence of a slow K-channel at the encoder site.