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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 othe 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.

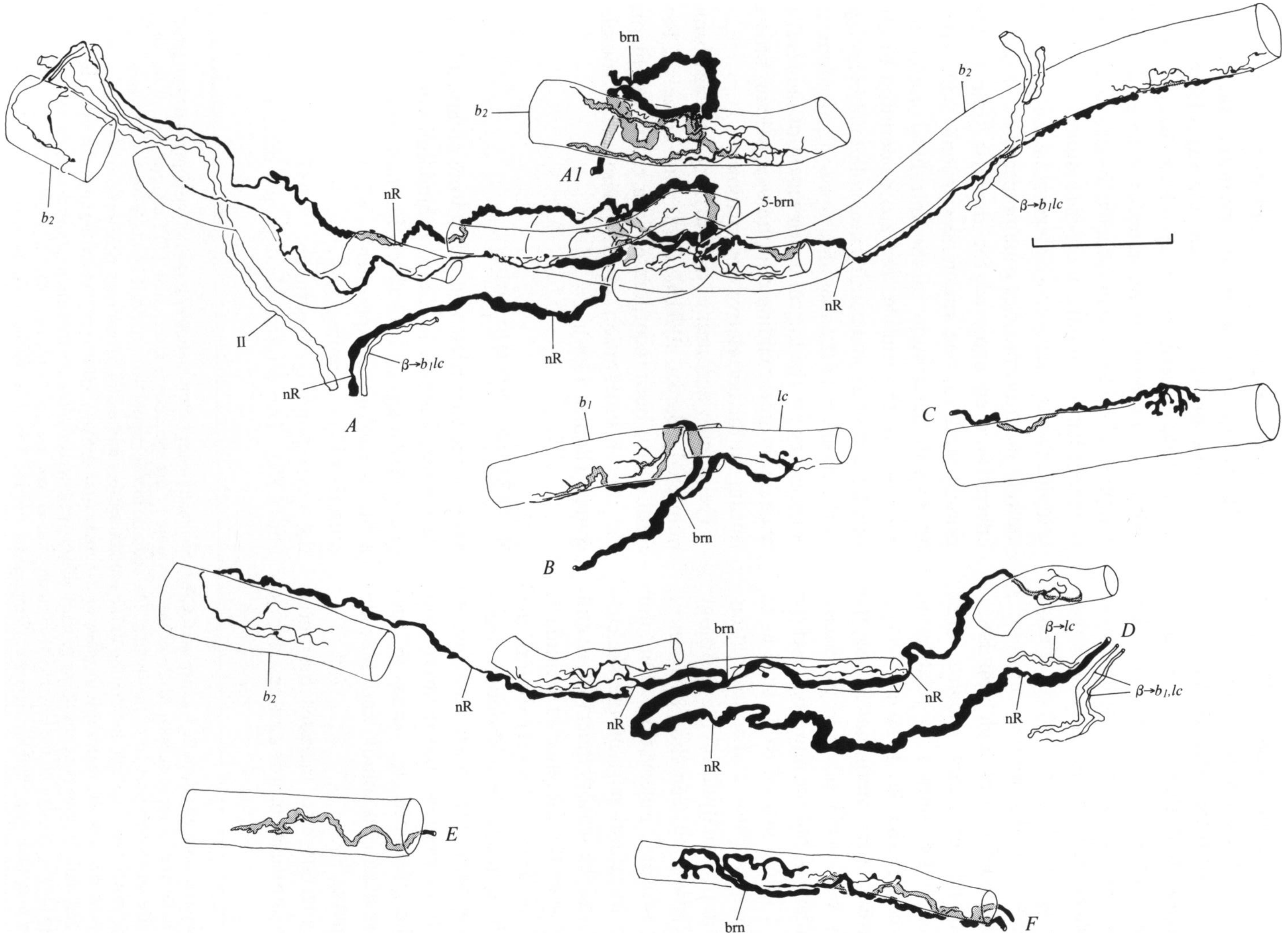


Fig. 2. Camera lucida drawings continuing the illustration of the interpretation of teased silver-impregnated material; details of all the intrafusal motor branches and their endings of spindle 7, C883. Compare with Figure 1*B*. The letters *A–F* identifying the drawings are placed next to the axonal 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 (*A*1). (*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.

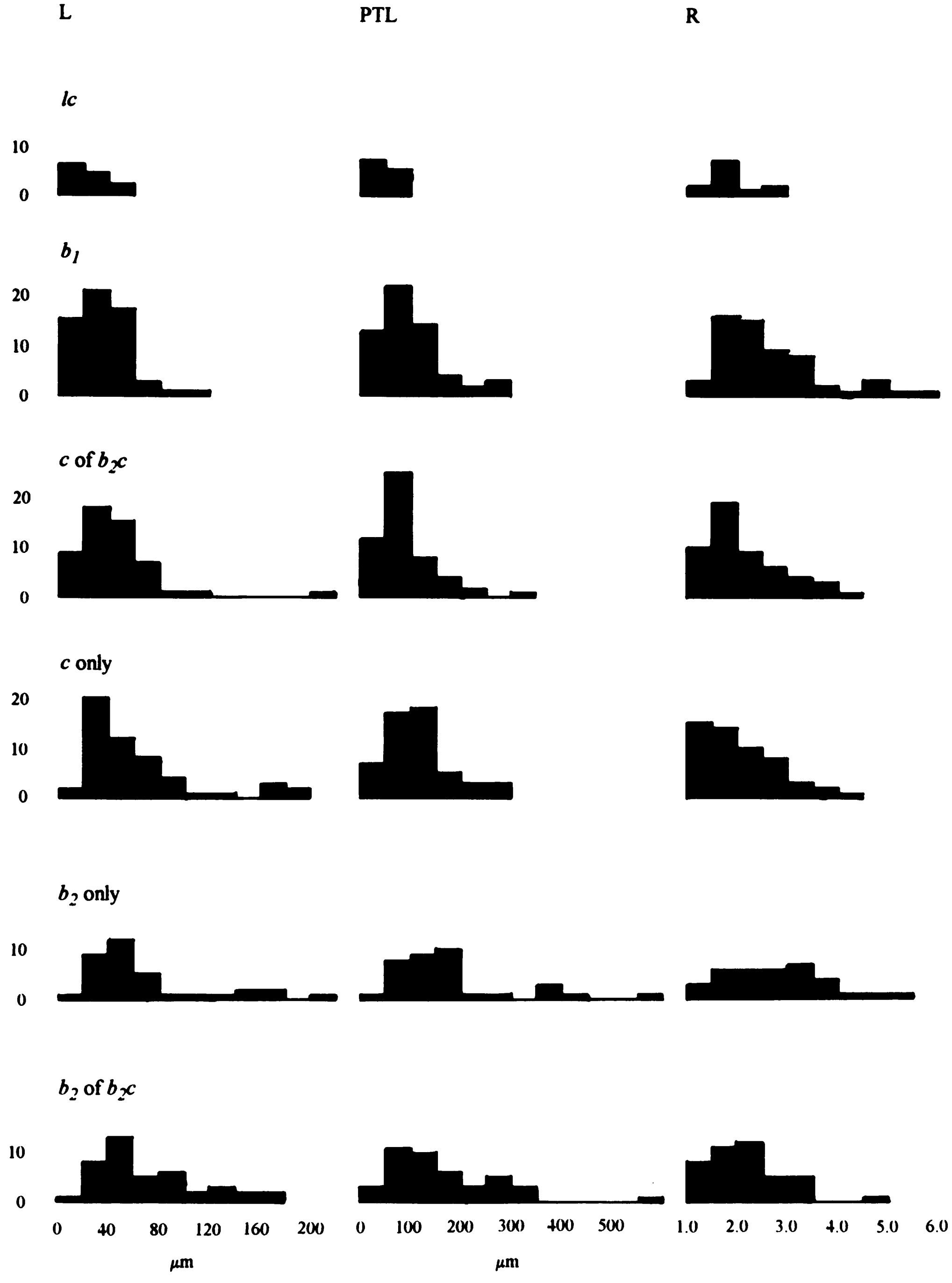


Fig. 3. Histograms showing the lengths (L), total preterminal and terminal lengths (PTL), and ratio (R) of PTL to L of motor endings on the various types of intrafusal fibre. Data relating to endings on bag$_2$ fibres (b_2) and on typical poles of chain fibres (*c*) are separately plotted according to 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.

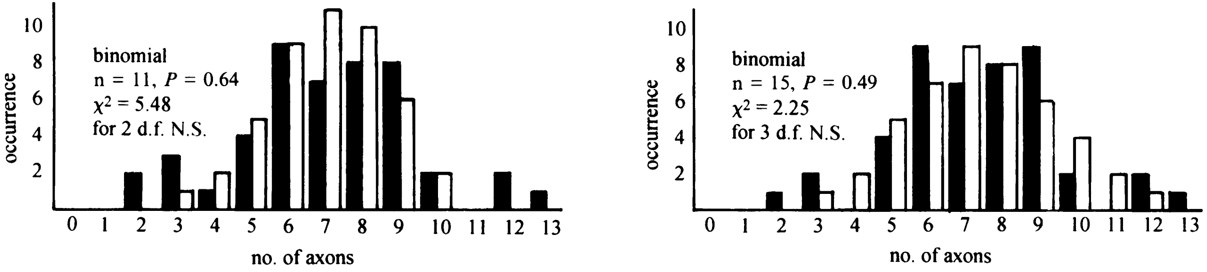
Numbers and distributions of intrafusal branches of motor axons in the complete sample of 4 muscles

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 all single units, n = 47 (a) or 38 (b and c)

b_1b_2c units only, n = 44 (d) or 35 (e and f)

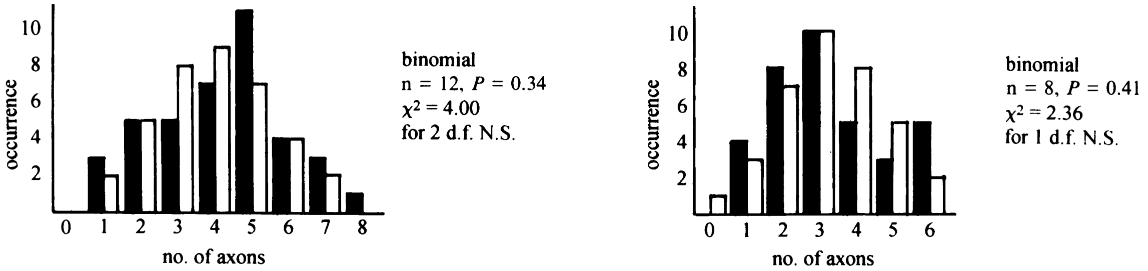
(a) all axons

(d) all axons



(b) possible or definite γ axons to b_1, b_2 and c fibres

(e) all axons to b_1 and lc fibres



(c) definite γ axons to b_2 and c fibres

(f) probable or definite β axons to b_1 and lc fibres

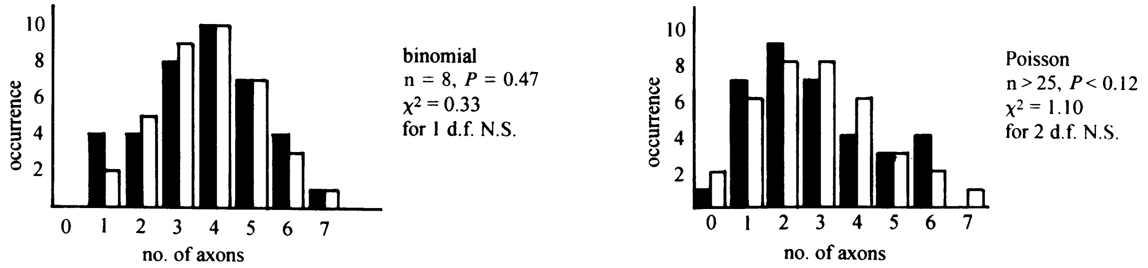


Fig. 4. Observed (filled bars) and best-fitted calculated (open bars) probability distributions of the numbers of motor axons that supplied each spindle, for various more or less inclusive samples. In no case does the observed distribution differ significantly from the theoretical one. The ordinate (occurrence) 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

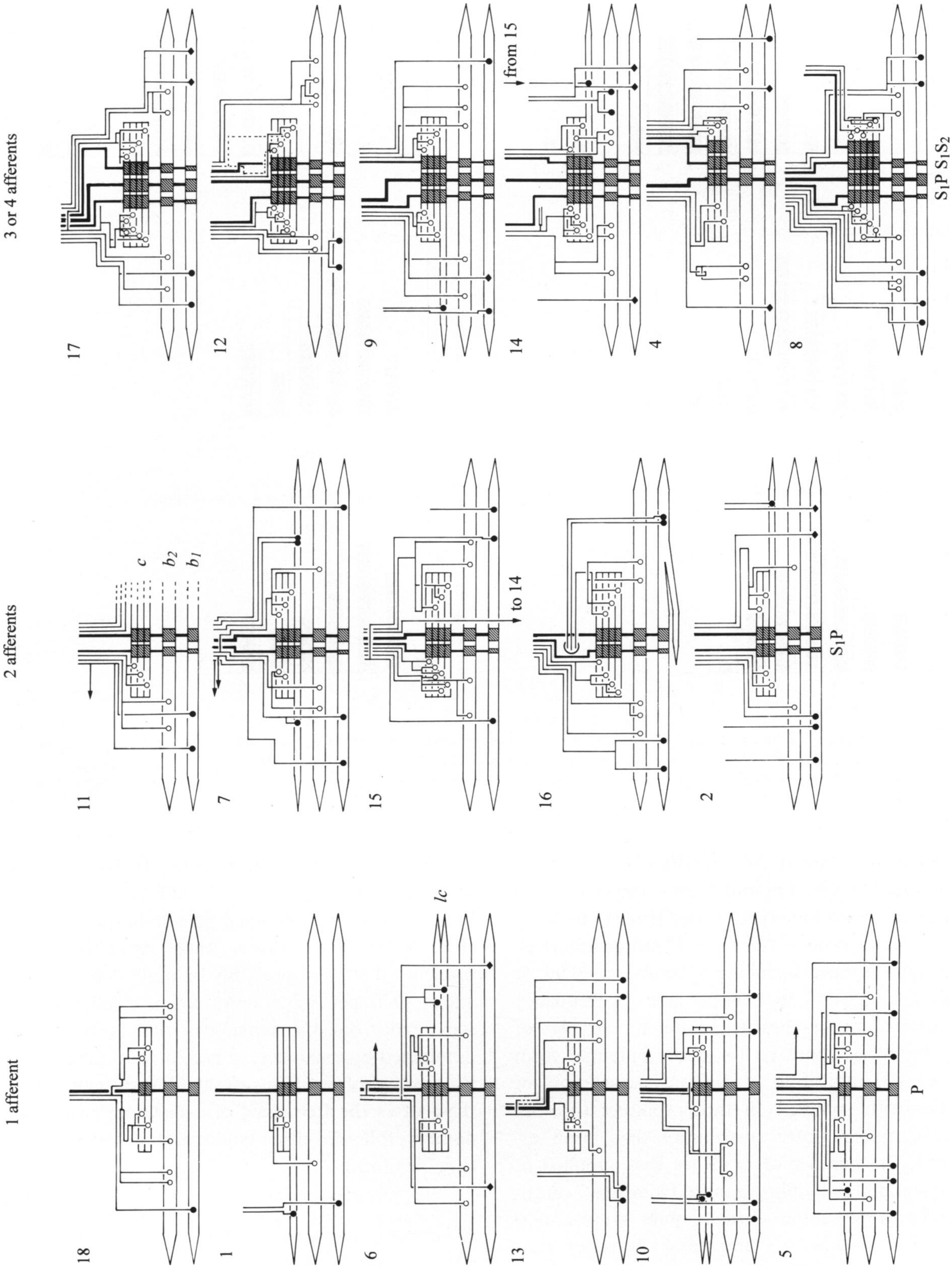


Fig. 5. For legend see opposite.

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		
		b_1	lc	b_1lc	Total	b_1	b_2	γ
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 *c*, chain fibres (shown with typical poles in spindle 11, column 2); other abbreviations as in Figure 1.

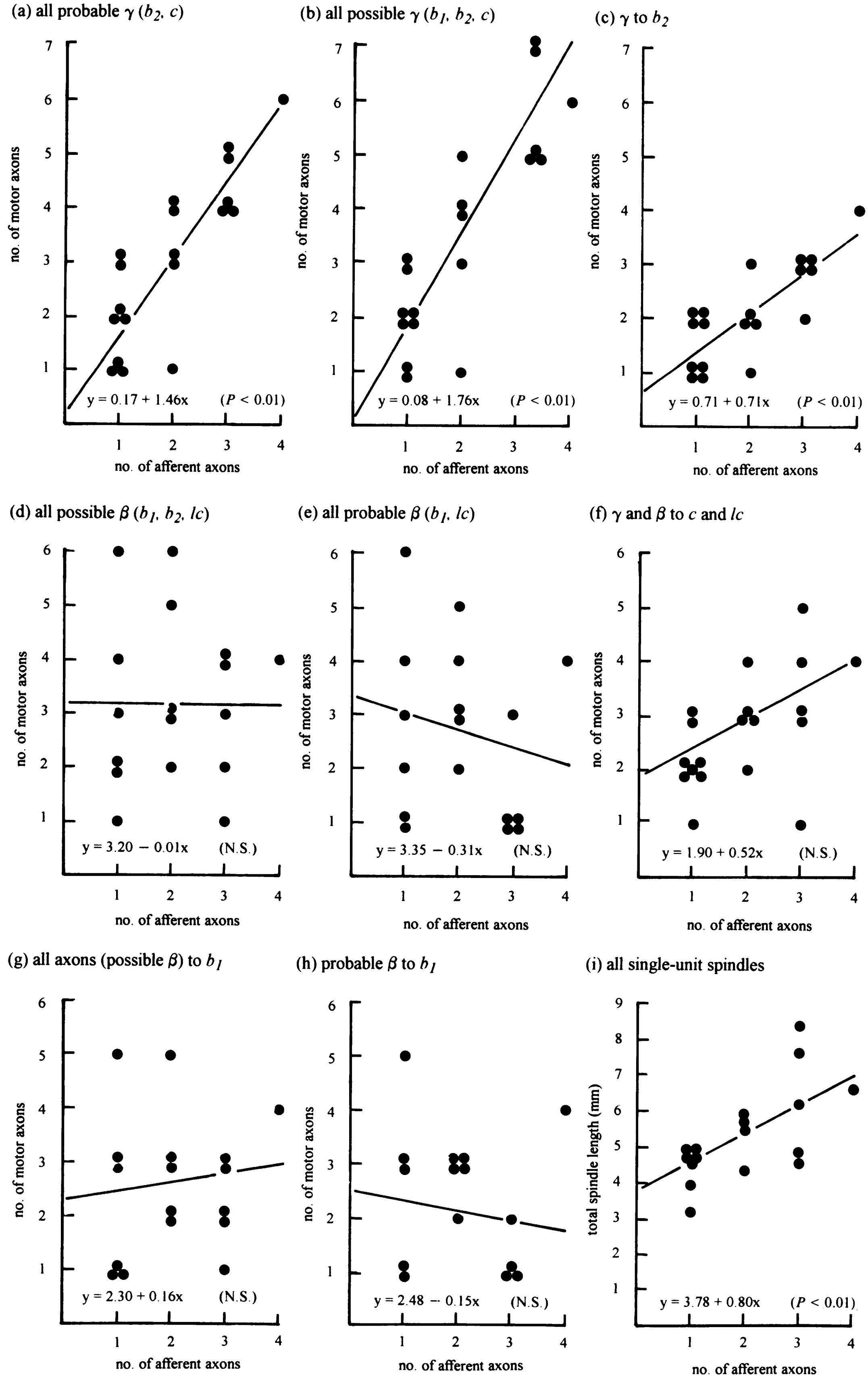


Fig. 6. Scatter plots showing the relationships between numbers of motor and afferent axons for various samples (a–h). The calculated regression of ordinate (y) against abscissa (x) is plotted and given in each case; all those, and only those, involving probable or possible γ axons alone had slopes that 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*

<table>
<tr><th>Segregation</th><th>Distribution
pole 1 : pole 2</th><th>No.
observed</th><th colspan="2">Total</th><th colspan="2">Expected</th><th>$\frac{(\text{obs}-\text{exp})^2}{\text{exp}}$</th></tr>
<tr><td>SS</td><td>$b_2\quad c:b_2\quad c$</td><td>4</td><td>4</td><td rowspan="7">13</td><td>4</td><td rowspan="7">11</td><td rowspan="7">0.333</td></tr>
<tr><td>MM</td><td>$b_2c : b_2c$</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>II</td><td>$b_2\ b_2c : b_2c\ c$</td><td>1</td><td rowspan="5">5</td><td rowspan="5">4</td></tr>
<tr><td></td><td>$b_2c\ c: b_2c\ c$</td><td>1</td></tr>
<tr><td></td><td>$b_2\ b_2c :b_2\ b_2c\ c$</td><td>1</td></tr>
<tr><td></td><td>$b_2c\ c:b_2\ b_2c\ c$</td><td>1</td></tr>
<tr><td></td><td>$b_2\ b_2c\ c:b_2\ b_2c\ c$</td><td>1</td></tr>
<tr><td>SM</td><td>$b_2\quad c: b_2c$</td><td></td><td colspan="2">10</td><td colspan="2">7</td><td>1.286</td></tr>
<tr><td>SI</td><td>$b_2\quad c:b_2\ b_2c$</td><td>2</td><td colspan="2" rowspan="3">6</td><td colspan="2" rowspan="3">7</td><td rowspan="3">0.143</td></tr>
<tr><td></td><td>$b_2\quad c: b_2c\ c$</td><td>3</td></tr>
<tr><td></td><td>$b_2\quad c:b_2\ b_2c\ c$</td><td>1</td></tr>
<tr><td>MI</td><td>$b_2c :b_2\ b_2c$</td><td>1</td><td colspan="2" rowspan="2">3</td><td colspan="2" rowspan="2">7</td><td rowspan="2">2.286</td></tr>
<tr><td></td><td>$b_2c : b_2c\ c$</td><td>2</td></tr>
<tr><td></td><td></td><td></td><td colspan="2"></td><td colspan="2"></td><td>$\chi^2 = 4.048$</td></tr>
</table>

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*

<table>
<tr><th rowspan="2">Secondary endings</th><th colspan="3">Static γ innervation</th></tr>
<tr><th colspan="2">More segregated in one pole</th><th>Neither pole more segregated</th></tr>
<tr><td>Present in both poles or in neither</td><td colspan="2">8 (5)</td><td>7 (5)</td></tr>
<tr><td>Present in one pole</td><td colspan="2">15 (5)</td><td>5 (2)</td></tr>
<tr><td></td><td>same pole as secondary
11 (5)</td><td>opposite pole to secondary
4 (0)</td><td></td></tr>
</table>

* 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 be 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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