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THE ULTRASTRUCTURE OF CAT FUSIMOTOR ENDINGS AND THEIR RELATIONSHIP TO FOCI OF SARCOMERE CONVERGENCE IN INTRAFUSAL FIBRES

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SUMMARY

1. Six muscle spindle poles, five from experiments in which foci of sarcomere convergence had been observed during stimulation of fusimotor axons, were serially sectioned for light and electron microscopy. Every somatic motor terminal was studied in ultrathin sections at several levels.

2. In all six poles static γ axons, or presumed static γ axons, supplying the static bag_2 fibre and/or chain fibres had no terminations on the dynamic bag_1 fibre. In five poles, the dynamic bag_1 fibre was selectively innervated by dynamic γ or β axons save in one case where a dynamic γ axon also innervated one chain fibre.

3. Seventy-seven motor endings were of four distinct ultrastructural types: 'm_a plates' lay superficially on the surface of static bag_2 or chain fibres; 'm_b plates' were deeply indented into dynamic bag_1 fibres; in 'm_c plates', found on chain fibres only, the muscle surface was thrown into projecting fingers between which the axon terminals were embedded; one type 'm_d plate' was found, fully indented into a long chain fibre. A few plates of intermediate form (m_{ab}) were variants of m_a and m_b plates.

4. The muscle membrane beneath both m_a and m_b plates was smooth, or had a few wide, shallow folds; m_c plates usually had wide, shallow subjunctional folds; numerous deep, narrow folds were characteristic of the m_d plate. The length of unmyelinated pre-terminal axon or the number of sole plate nuclei were not useful diagnostic features.

5. Obvious foci of sarcomere convergence in the capsular sleeve region of dynamic bag_1 and static bag_2 fibres coincided with the location of motor plates. Additional contraction foci were observed in the extracapsular region of dynamic bag_1 fibres where there was no motor innervation; contraction occurs principally in the outer half of these fibres. No foci of contraction or motor plates were observed in the extracapsular region of static bag_2 fibres; contraction in these fibres is typically mid-polar.

6. In some poles local contraction of chain fibres centred on the location of m_c plates. In others, very localized contraction occurred distal to the sites of m_a plates. Both m_a and m_c plates were never found on the same pole of a chain fibre.

7. Dynamic γ or β axons end in m_b plates, probably equivalent to p_2 plates. The

concept of distinctly different p_1 and p_2 plates on dynamic bag_1 fibres, supplied by dynamic β and γ axons, respectively, is not supported by ultrastructural evidence.

8. Some static γ axons end in multiple m_a plates which correspond with 'trail endings', or in single large m_a plates, on static bag_2 or chain fibres. The m_c plates are the terminations of other static γ, or occasionally dynamic γ, axons on chain fibres. Static β axons probably end in m_d plates on long chain fibres which may correspond with p_1 plates.

9. It is proposed that there are two types of static γ motoneurone, one terminating in m_a plates and the other in m_c plates, possibly directed preferentially towards static bag_2 fibres and chain fibres, respectively.

INTRODUCTION

Initially, the work described in this paper had three objectives: to discover (*a*) if the location of motor endings coincided with the positions of foci of sarcomere convergence observed when single γ or β fusimotor axons were stimulated; (*b*) whether there was any structural evidence for the proposition, which arose from observation of living muscle spindles, that dynamic bag_1 fibres are exclusively innervated by dynamic γ or β axons, and static bag_2 and nuclear chain fibres solely by static γ axons; and (*c*) whether the form of the endings bore any relation to the type of intrafusal muscle fibre on which they lay or to the type of axon innervating them.

The first experiments on the nature of the termination of γ axons of known function (Barker, Emonet-Dénand, Laporte, Proske & Stacey, 1973) showed that static γ axons terminated as trail endings on both nuclear bag and chain fibres. The trail endings were recognized by light miscroscopy in silver stained spindles which had been studied experimentally. This result was puzzling since the most cogent explanation for the actions of static and dynamic γ axons on the Ia discharge depended on the separate innervation by these axons of nuclear chain and nuclear bag fibres with their distinctly different mechanical properties (see Matthews, 1972). A new insight followed the recognition by Ovalle & Smith (1972) of two types of nuclear bag fibre (bag_1 and bag_2) in cat and monkey spindles by staining for myosin ATPase activity. Observation of the behaviour of intrafusal fibres in living spindles during stimulation of single static γ, or dynamic γ or β, axons led Boyd, Gladden, McWilliam & Ward (1975) to propose that dynamic γ axons modified Ia afferent activity by innervating one type of nuclear bag fibre only which they termed the 'dynamic nuclear bag fibre'. This fibre contracted slowly and had viscous mechanical properties (Boyd, 1976). Static γ axons operated the other type of nuclear bag fibre ('static nuclear bag fibre') whose contraction rate and mechanical properties were intermediate between the dynamic nuclear bag fibre and nuclear chain fibres, which were fast-contracting, had elastic mechanical properties and were also supplied by static γ axons (Bessou & Pagès, 1975; Boyd, Gladden, McWilliam & Ward, 1977). Examination of the experimental spindles of Boyd *et al.* (1975, 1977) by light microscopy showed that static nuclear bag fibres had more elastic fibres in the polar regions than dynamic nuclear bag fibres. Since, in sections of other spindles stained for myosin ATPase activity, elastic fibres were prominent round the bag_2 fibres of Ovalle & Smith (1972),

static nuclear bag fibres could be equated with bag_2 fibres and dynamic nuclear bag fibres with bag_1 fibres (Gladden, 1976); hence the nomenclature 'dynamic bag_1 fibres' and 'static bag_2 fibres' used in this paper.

The main conclusion of a series of experiments conducted by Brown & Butler (1973, 1975) and by Barker, Emonet-Dénand, Harker, Jami & Laporte (1976*b*, 1977), using the glycogen depletion technique of Edström & Kugelberg (1968), was that static γ axons innervated bag_1 fibres as often as bag_2 fibres although it was shown that stimulation of dynamic γ or β axons depleted bag_1 fibres almost exclusively. Emonet-Dénand, Laporte, Matthews & Petit (1977) reclassified static and dynamic γ axons into six categories ranging from purely dynamic to purely static. They suggested that the intermediate categories of response were caused by axons which innervated the bag_1 fibre as well as the bag_2 or chain fibres. Bessou & Pagès (1975) and Boyd *et al.* (1975, 1977), however, never observed contraction of dynamic bag_1 fibres during static γ stimulation. Boyd, Gladden & Ward (1979) thought that minor variations in the mechanical properties of particular types of intrafusal fibre, rather than involvement of the dynamic bag_1 fibre, could account for intermediate types of response.

It was suggested to us, however, that the large amplitude contraction of a static bag_2 fibre in response to static γ stimulation might sometimes obscure a much smaller simultaneous contraction in the dynamic bag_1 fibre if it were innervated by a branch of the same static γ axon. Hence we felt it necessary to follow up the visual observation of spindle poles with reconstruction of their motor innervation from light and electron microscopy of serial sections in order to be certain not only that all motor endings were found, but also that axons, myelinated or unmyelinated, did not branch within the spindle to supply the dynamic bag_1 fibre as well as either the static bag_2 fibre or chain fibres. Serial sectioning for electron microscopy is very laborious and could only be undertaken in a small number of spindles. It will be shown that the dynamic bag_1 fibre normally does, in fact, have its own selective innervation.

It will be shown, also, that the motor endings on all types of intrafusal fibre have the form of 'motor plates' each consisting of a number of 'axon terminals' and separated from other motor plates by at least 15 μm of intrafusal fibre free of axon terminals. 'Axon terminals' are the ultrastructural unit, comprising the terminations of the axon in contact with the sub-synaptic membrane of the muscle fibre and containing synaptic vesicles and mitochondria but no neurofilaments; they correspond with the synaptic knobs, rings or tapers of light microscopy. Four principal ultrastructural types of motor plate, and a fifth intermediate type, have been identified.

Two preliminary reports on part of the present work have been published (Arbuthnott, Boyd & Gladden, 1976; Arbuthnott, Boyd, Gladden & McWilliam, 1977).

METHODS

Correlation of positions of contraction foci with motor endings

The experimental procedures used to establish the foci of local contraction have been described previously (Boyd *et al.* 1977). The distances of contraction foci from the equator were noted during the experiments. The muscle spindles were fixed at the length they had been throughout most of

the experiment, and, after embedding, the resin block was fractured at known positions and the positions of motor endings were calculated from the number of serial sections of known thickness cut between the known position and the ending. Even so, discrepancies arose because of contraction and shrinkage during fixation and because fixation was not always at precisely the same length as when recordings were taken. This problem was solved in the following way. Serial photographs were taken of poles A, B, and F (Fig. 1) both in the living state and in the resin block. Landmarks in the living spindle were identified in both the block and in transverse sections of it (cf. Pl. 1 *A* and *B*). Thus, the precise position of foci of sarcomere convergence could be located in the resin block and correlated with the site of motor nerve terminals (cf. Pl. 1 *C* and *E; G* and *H*). In pole C the position where the motor axon bundle reached the intrafusal fibres could be identified in both the living spindle and in the sections. By summing the thickness of sections from this point individual sections could be related directly to positions in the living spindle where observations had been made.

Identification of type of bag fibres

In poles A, B, C, D and F the dynamic bag_1 fibre was identified in the living spindle by the fact that it contracted when dynamic γ or β axons were stimulated. The particular fibre involved was identified in the fixed material in the following ways. In one spindle (*poles A and B*) the dynamic bag_1 fibre was marked intracellularly by ionophoretic injection of Alcian Blue dye (Gladden, 1976) 0·3 mm to the left of the primary ending (Pl. 1 *B*, *). The dye could be seen in ultrathin sections at that point. In the extracapsular region of both poles the static bag_2 fibre had more elastic fibres than the dynamic bag_1 fibre. In *pole C* the dynamic bag_1 fibre was clearly smaller in diameter than the static bag_2 fibre in the fluid space of the living spindle and from this size difference and its relationship to other landmarks it could be identified in the serial sections. The larger bag fibre of this pole also had more elastic fibres extracapsularly than the dynamic bag_1 fibre, confirming its identification as a static bag_2 fibre. In *pole D* the dynamic bag_1 fibre, from which intracellular recordings had been made during stimulation of a dynamic γ axon (experiment 026, Gladden, 1981), was deliberately damaged by injected current. Local damage was found in one bag fibre only which must have been the dynamic bag_1 fibre. The other two bag fibres both had more numerous and larger elastic fibres in the extracapsular regions than the dynamic bag_1 fibre and were thus identified as static bag_2 fibres. Identification of the dynamic bag_1 fibre in *pole F* was from its clear separation in the equatorial region from the static bag_2 and chain fibres, which all lay together in a separate compartment of inner capsule cells. Also, three secondary sensory endings in this pole all had fewer terminals on the dynamic bag_1 fibre than on the two static bag_2 and chain fibres. In *pole E* the dynamic bag_1 and static bag_2 fibres were identified solely on histological grounds from their differing elastic fibre distributions in the extracapsular region, and by the separation of the dynamic bag_1 fibre from the rest of the intrafusal bundle in the equatorial region.

Histological procedures

After completion of the experiment on a living spindle, and before it was removed from the bath, the muscle containing the spindle was tied to a glass frame to minimize length changes during staining and embedding, and the Krebs solution was replaced by 3% glutaraldehyde in Millonig phosphate buffer at pH 7·3. After one hour in aldehyde, the spindles were rinsed in phosphate buffer overnight, post-osmicated and dehydrated, except for poles A, B and F which were fixed and stained by the method of Hayat & Giaquinta (1970).

All specimens were flat embedded, still attached to the glass frame, in a 3 mm thick layer of Araldite or Epon/Araldite mixture. Batches of ultrathin transverse sections were cut at intervals varying from 0·5 to 20 μm (see below) and intervening tissue was cut in 1–4 μm thick sections for light microscopy; these thick sections could be applied with resin to the surface of a dummy block and sectioned later for electron microscopy if necessary. The intervals between the batches of ultrathin sections depended on the complexity of the motor innervation, e.g. the shortest intervals (0·5 μm) were in pole F where many axons had small endings, and the largest (20 μm) were in the extracapsular regions where no axons were present. All poles were serially sectioned transversely except for 2·1 mm of pole A (Fig. 5) which was sectioned longitudinally for both light and electron microscopy. Ultrathin sections were mounted without folds on formvar-coated copper one-hole mounts with a 0·8 mm diameter aperture (Arbuthnott, 1974) and stained with uranyl acetate and lead citrate solutions. Sections were viewed in either an AEI EM6B or in a JEOL JEM-100C electron microscope and photographed at magnifications ranging from $\times$ 1600 to $\times$ 20,000.

Analysis of motor plates

Each motor plate was studied at two or more levels, the number of levels depending upon the length of the motor plate: e.g. one large motor plate was observed in ten batches of ultrathin sections and proved to be 100 μm long. Micrographs from all levels were analysed in order to classify the motor plate. Each motor plate was analysed for the following features.

(*1*) *Protrusion of the muscle fibre at the motor plate.* The degree of protrusion of the muscle myoplasm above the myofilaments was assessed for each motor plate (e.g. Fig. 3*E*).

(*2*) *Indentation of the post-synaptic membrane.* To measure the degree to which an axon terminal was indented into the muscle surface a straight line was drawn across the terminal, joining the muscle surfaces at the two sides (see Fig. 2). The ratio of the depth below the mid point of this line to the total height of the terminal was calculated.

(*3*) *Folding of the post-synaptic membrane.* The length of the post-synaptic membrane under each axon terminal was compared with that of the presynaptic membrane. Ratios of pre: post-synaptic membrane lengths were calculated and ranged from 0·26 to 1·0, a value of 1·0 indicating no folds. The folds were also placed in categories: wide or narrow, deep or shallow, with or without branches, with or without fused central basement membranes.

(*4*) *Number of sole plate nuclei.* Some sole plates contained no nuclei, but where they were present the nuclei were followed through the ultrathin sections at each level of sectioning and the number counted.

(*5*) *Length of preterminal unmyelinated axons.* The distance between the point on the axon at which myelination ceased and the motor plate was calculated from the serial sections.

Vesicle density, vesicle shape and mitochondrial size in axon terminals were also systematically studied and rejected as being unhelpful in separating different types of ending.

Autonomic innervation

Autonomic axons were found in all the spindle poles studied. They were identified by their typical beaded appearance. The beads or varicosities contained both clear and dense cored transmitter vesicles of various dimensions. Autonomic axons in pole C (Fig. 1*C*) were described by Ballard (1978). The autonomic axons could be reliably distinguished from unmyelinated terminal branches of myelinated axons by following them through serial sections. They did not end in plates. All fusimotor axons, including those which were unmyelinated at spindle entry, ended in plates > 10 μm long on intrafusal muscle fibres, and lacked varicosities.

RESULTS

The innervation of individual intrafusal muscle fibres

Fig. 1 shows the distribution of the twenty-four axons which innervated six muscle spindle poles, together with the location and type of each of the seventy-seven motor plates they supplied. Five poles (A–E) were from the tenuissimus muscle and one (F) from the abductor digiti quinti medius muscle. Six of the seven axons supplying motor plates on dynamic bag_1 fibres were selective, i.e. supplied this fibre only; the seventh supplied one plate on one chain fibre in addition (pole D). All these axons were presumably dynamic in effect and five were known to be so.

None of the seventeen axons which supplied static bag_2 or chain fibres branched to supply dynamic bag_1 fibres in addition. This is especially remarkable in pole F in which three axons branched within the spindle to supply thirty motor plates on static bag_2 and chain fibres, and there was no capsular barrier between these fibres and the dynamic bag_1 fibre. Moreover, in spindle pole E two axons supplied eight plates on the static bag_2 and chain fibres, but there was no plate on the dynamic bag_1 fibre at all. All the axons to static bag_2 or chain fibres were presumably static in effect, and four were known to be so. Five spindle poles received one axon which supplied both static bag_2 and chain fibres while the sixth received two such non-selective axons.

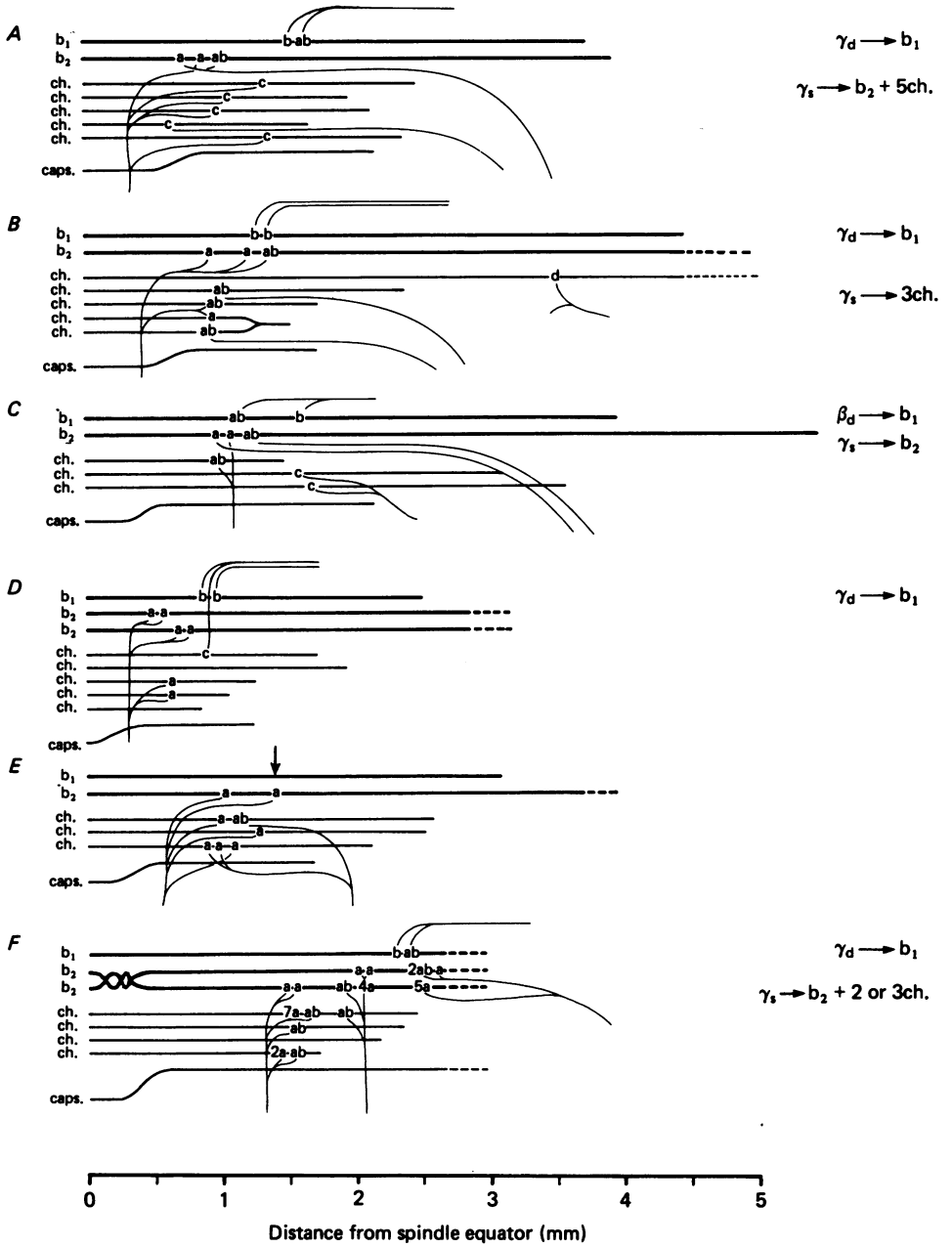

Fig. 1. Diagrammatic reconstructions of six spindle poles to show the distribution of motor axons to dynamic bag_1 fibres (b_1), static bag_2 fibres (b_2) and nuclear chain fibres (ch.), and the location and ultrastructural type of the motor plates they supplied (m_a, m_b, m_{ab}, m_c or m_d). Poles A–E from tenuissimus muscle; A and B from same spindle with orientation of A reversed for ease of comparison (see Figs. 5, 6). Pole F from abductor digiti quinti medius muscle. Poles A–D and F were studied in the living state prior to fixation; functional observations are shown on the right. Caps.: spindle capsule. ---, ends of fibres not sectioned.

The remaining ten axons to static bag_2 or chain fibres were selective in distribution, six supplying chain fibres only and four supplying static bag_2 fibres only.

Classification of motor plates in terms of ultrastructure

The motor endings could be separated into five types mainly in terms of the degree and manner of the indentation of their axon terminals into the muscle surface. The types have been labelled m_a, m_b, m_c, m_d plates, and an intermediate type m_{ab}. The following descriptions apply only to transverse sections.

Type m_a plates. Axon terminals of m_a plates lie entirely superficially on the muscle fibre and do not modify the muscle contours to any appreciable extent. Plates were assigned to this type if none of the two to fourteen axon terminals in sections cut from several regions of the plate was indented more than 25% below the muscle surface (Fig. 2*A*, 3*A*, *D* and Pl. 2*A*, *D*).

Type m_b plates. Axon terminals of m_b plates are substantially indented into the muscle surface, the degree of indentation being greater than 25% and sometimes as much as 100% (Figs. 2*B*, 3*B*, *C* and Pl. 2*B*, *C*). The curvature of indentation at the edge of the terminal is steep, sometimes almost 90°, and the muscle contour is noticeably altered by the presence of the terminal. A plate was assigned to type m_b only if *all* the axon terminals examined (9 to 18) were consistent with this description.

Type m_{ab} plates. These are intermediate between types m_a and m_b and so cannot be assigned confidently to either group. Some have some axon terminals of type m_a and others of type m_b (Fig. 2*D*). Other m_{ab} plates have terminals which are superficial at one edge and indented at the other, with a less steep angle of curvature than is present in m_b endings (Figs. 2*D* and 3*G*).

Type m_c plates. These plates consist of finger-like processes which protrude from the muscle surface between which axon terminals are inserted (Figs. 2*C*, 3*E* and Pl. 2*E*). However, any axon terminal at the edge of the plate may be deeply indented into the muscle and could be confused with an m_b terminal. Because of this, it is essential to cut sections through several levels of an m_c plate for accurate identification.

Type m_d plate. Only one example was found of a fifth type of plate, m_d, distinctly different from any of the other types (Figs. 2*E*, 3*F* and Pl. 2*F*). The plate seemed to consist of a single axon terminal which was fully indented into the intrafusal fibre and subjunctional folding was complex (see below).

Other physical characteristics of the five types of axon terminal. The majority of m_a terminals had a smooth subjunctional membrane (Pl. 2*A* and *D*) but a few had wide shallow folds. Folding in m_b plates was variable along their length: some terminals had marked wide, shallow folds (Pl. 2*C*) while others had none (Pl. 2*B*). Thus, if folding alone were used as a diagnostic feature, m_a and m_b plates would be indistinguishable. The membrane of the muscle fingers beneath m_c axon terminals usually, but not always, had wide, shallow folds; occasionally a narrow fold was seen with the basement membranes of the muscle on either side of the fold fused into a single basement membrane. The m_d plate had numerous deep, narrow folds and fused basement membranes occurred in over 50% of the folds. Thus, the nature of the folding of the subsynaptic membrane is an important feature in distinguishing m_d plates from m_a, m_{ab} and m_c plates on chain fibres.

Protrusion of the muscle fibre underlying the axon terminals was absent from m_a, m_{ab} and m_d plates, but was a constant feature of m_c plates (Fig. 3*E*). In m_b plates protrusion of the muscle fibre sometimes occurred but not directly under the axon terminals.

For the majority of plates encountered in tenuissimus spindles, the length of unmyelinated pre-terminal axon was less than 100 μm and usually the supplying axon

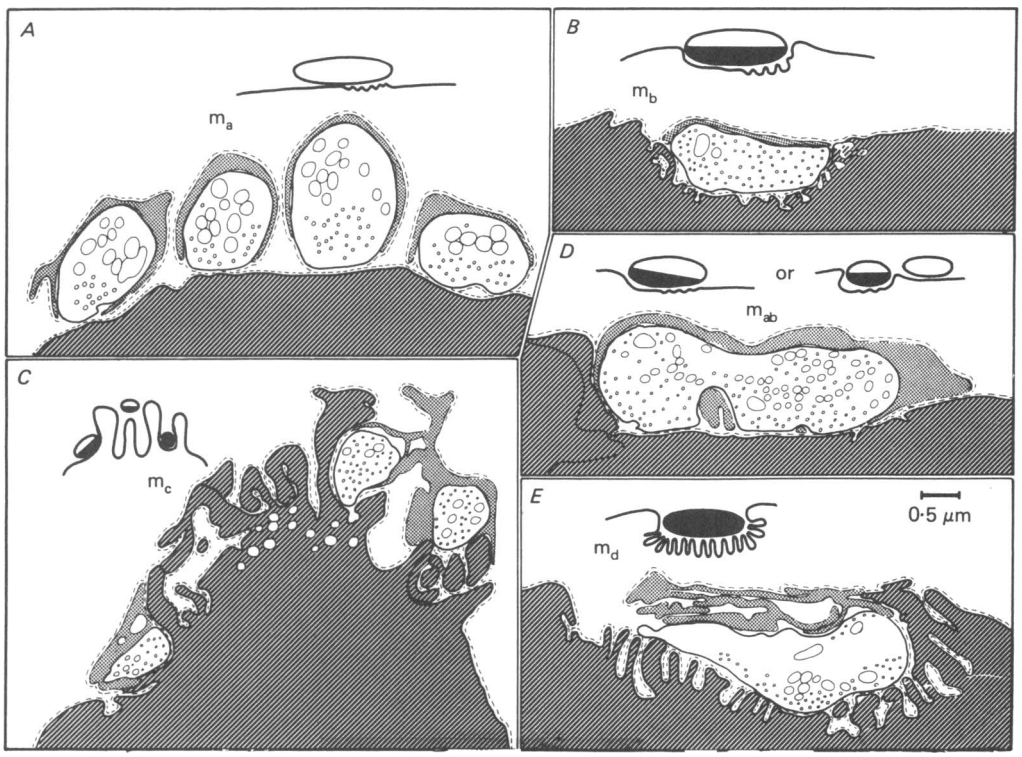

Fig. 2. Tracings of high power electron micrographs of axon terminals of each of the five ultrastructural types together with a diagrammatic representation of each type. Intrafusal fibres, hatched. Schwann cell lid, light stipple. Scale in *E* applies to all tracings. See also Fig. 3 and Pl. 2.

was myelinated to within 10 μm of the plate. When a significant length of unmyelinated pre-terminal axon was present, m_a or m_{ab} plates were involved. The axons in pole F from the abductor digiti quinti medius muscle were quite different, however. Lengths of unmyelinated pre-terminal axon greater than 100 μm supplied all the motor plates but the length of unmyelinated axon was shorter for the plates supplied by the dynamic γ axon than for the other axons (Fig. 8, thin lines). Thus, the extent of unmyelinated pre-terminal axon varies greatly for spindles in different muscles and cannot be regarded as a reliable criterion for distinguishing one type of plate from another.

The number of sole plate nuclei was generally greater in m_b plates than in all the other types of plate but seemed to be more related to plate length than to plate type. It was not a useful diagnostic feature.

In m_b plates the Schwann cells appeared to 'seal off' the axon terminals more completely than in m_a, m_{ab} or m_c plates. In cross sections of m_b terminals the Schwann cells seemed like lids over the indented terminals and often tongues of Schwann cell became inserted into the synaptic cleft (Pl. 2*B*, arrow).

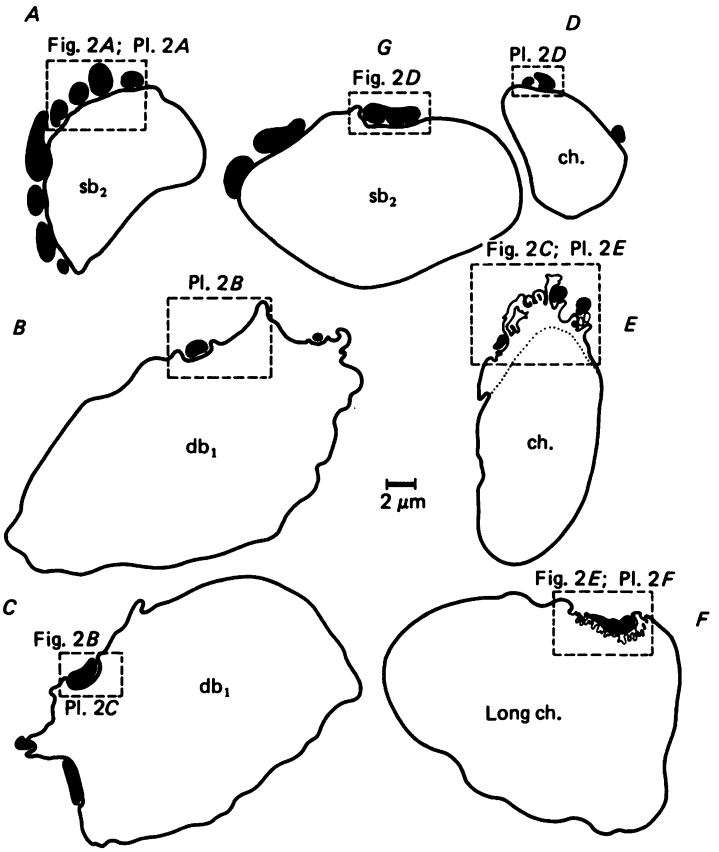

Fig. 3. Tracings of low power electron micrographs of entire static bag_2 fibres (sb_2), dynamic bag_1 fibres (db_1) or chain fibres (ch.) to show relation of the axon terminals in a single transverse section of a motor plate to the entire intrafusal fibre. Boxes indicate regions shown in Fig. 2 and Pl. 2. Scale applies to all tracings. *A*, m_a plate on static bag_2 fibre (static γ axon). *B*, m_b plate on dynamic bag_1 fibre (dynamic γ axon). *C*, m_b plate on dynamic bag_1 fibre (dynamic β axon). *D*, m_a plate on chain fibre (static γ axon). *E*, m_c plate on chain fibre cut obliquely (dynamic γ axon); dotted line shows boundary of myofibrils beneath raised plate. *F*, m_d plate on long chain fibre (? static β axon). *G*, m_{ab} plate on static bag_2 fibre (presumed static γ axon).

Length of intrafusal motor plates

The lengths of the five types of motor plate are shown separately in Fig. 4. With the exception of two very long m_a and m_{ab} plates in longitudinal sections of one static bag_2 fibre (Fig. 5; Pl. 1*E*) all the plates on all three types of intrafusal fibre were less than 100 μm in length. There were proportionately more smaller plates on static

bag_2 fibres and chain fibres than on dynamic bag_1 fibres but, out of the total of thirty-eight m_a and m_{ab} plates less than 25 μm in length, twenty-nine were situated in the same spindle from the abductor digiti quinti medius muscle (Fig. 8). The mean length of the twenty-eight m_a and m_{ab} endings in the five tenuissimus spindle poles was 53 μm, almost the same as the mean for m_b endings (54 μm). Thus, there was no reason to designate motor endings on static bag_2 fibres and chain fibres in terms of length differently from those on the dynamic bag_1 fibre and they are all called 'motor plates' in this paper.

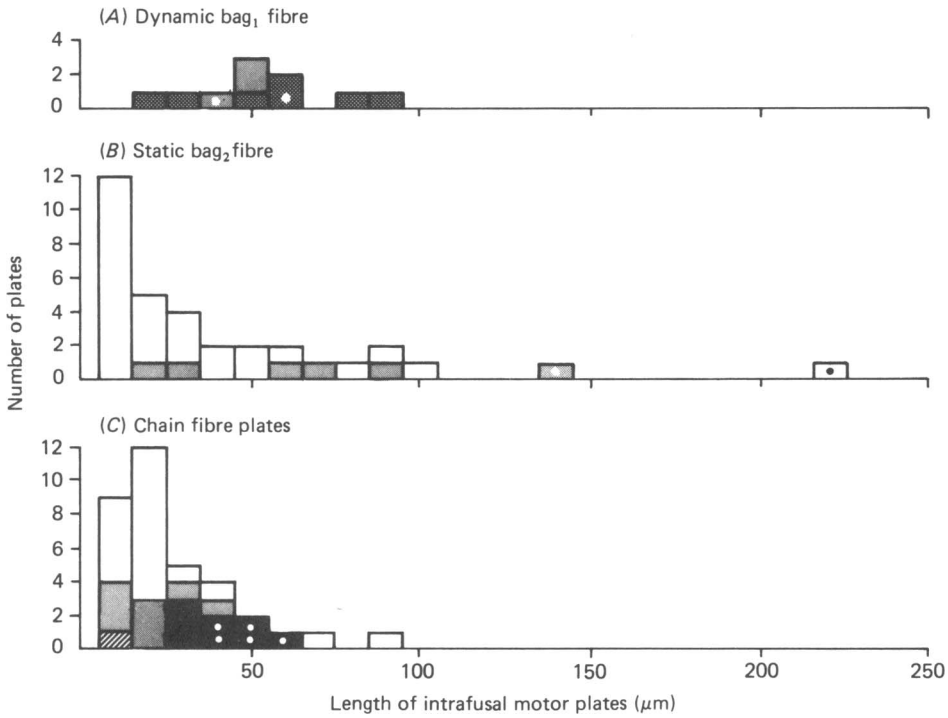

Fig. 4. Length of seventy-seven motor plates on dynamic bag_1 fibres (*A*), static bag_2 fibres (*B*) and chain fibres (*C*). Spotted values from serial longitudinal sections, the remainder from transverse sections. m_a plates, no shading, 10–220 μm (mean 28 μm). m_{ab} plates, light stipple, 10–135 μm (mean 46 μm). m_b plates, heavy stipple, 15–86 μm (mean 54 μm). m_c plates, black shading, 25–65 μm (mean 41 μm). m_d plate, cross hatched shading, 10 μm.

Type and number of motor plates related to type of intrafusal fibre

Most of the plates on the dynamic bag_1 fibre were m_b plates though some fell in intermediate type m_{ab} (Table 1). There were no m_a, m_c or m_d plates on dynamic bag_1 fibres. The dynamic bag_1 fibres in five poles received two plates (Fig. 1) but in two of the poles the two plates were very close together and supplied by the same axon (Fig. 5, 50 μm apart; Fig. 8, 35 μm apart).

The great majority of plates on the static bag_2 fibre were m_a plates, though again a few fell into type m_{ab}. There were no m_b, m_c or m_d plates on static bag_2 fibres. In the five tenuissimus spindle poles, the static bag_2 fibres received either two or three

plates supplied by from one to three axons. In the spindle pole from abductor digiti quinti medius, the two static bag_2 fibres were each innervated by a considerable number of plates (Fig. 8).

In the six poles studied about half the plates on nuclear chain fibres were m_a plates, about one quarter were m_{ab} plates and one quarter were m_c plates. No individual chain fibre at any pole received m_c innervation as well as either m_a or m_{ab} innervation. In the five tenuissimus spindle poles, however, m_a, m_{ab} and m_c plates on chain fibres occurred with about equal frequency and by far the most common arrangement was for each chain fibre to receive a single motor plate.

TABLE 1. The distribution of seventy-seven plates of five ultrastructural types on the three types of intrafusal muscle fibre. The functional type of axon innervating each type of plate, where known, is shown in parentheses

Type of motor plate	Type of intrafusal fibre innervated		
	Dynamic bag_1 fibre	Static bag_2 fibre	Chain fibres
m_a	0	26 (12 on γ_s)	17 (9 on γ_s)
m_{ab}	3 (1 on γ_d, 1 on β_d)	6 (1 on γ_s)	9 (5 on γ_s)
m_b	7 (4 on γ_d, 1 on β_d)	0	0
m_c	0	0	8 (5 on γ_s, 1 on β_d)
m_d	0	0	1 (on long chain)
Total	10	32	35

The one m_d plate lay far out on the only long chain fibre encountered (Fig. 6). This fibre had no other innervation at this pole, while at the opposite pole it was about the same length as the other chain fibres and was innervated by an m_c plate (Fig. 5). Long chain fibres (Barker, Banks, Harker, Milburn & Stacey, 1976*a*) occur in about 13% of tenuissimus spindle poles (Kucera, 1980*b*).

With the exception of one m_a plate and one m_c plate which lay near to the end of the fluid space in pole A, and the extracapsular m_d ending in pole B, all the motor plates lay in the capsular sleeve region of all three types of intrafusal fibre.

Position of motor plates relative to intrafusal fibre behaviour

The dynamic bag_1 fibre. In three spindle poles in the present study maximal activation of the dynamic bag_1 fibre resulted in a shift of the fibre in the fluid space region of about 20 μm towards the pole, and in extension of the primary sensory spiral of 6%. In two of these poles (Figs. 5 and 8) an obvious focus of sarcomere convergence was observed in the capsular sleeve region precisely at the point where two motor plates, supplied by the same axon, were situated. In the third pole (Fig. 7) the capsular sleeve region, where two motor plates lay some distance apart, was obscured by connective tissue and intramuscular nerves so that any focus of contraction would not have been seen. In a fourth pole (Fig. 6) contraction of the dynamic bag_1 fibre was very weak judging by the small displacement of the fibre, and no contraction focus was observed in the sleeve region where two motor plates lay. In this last case

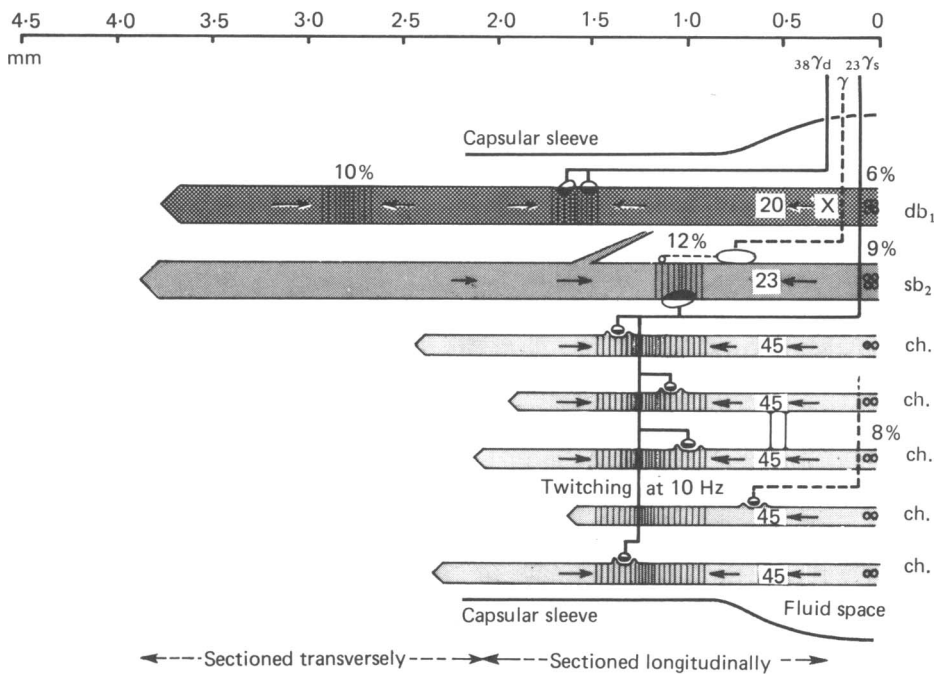

Fig. 5. This and Figs. 6–8 are diagrammatic reconstructions of four spindle poles from serial thin and ultrathin sections. Transverse scale about eight times the longitudinal scale which indicates distance from spindle equator. Variation in diameter of fibres along their length is not shown. The precise location and actual length of all the somatic motor endings is indicated but sensory and autonomic innervation is omitted. Axons enter at about the correct position but their path in the spindle is diagrammatic. Continuous lines, axons activated in ventral roots and identified histologically (d, dynamic; s, static; conduction velocity in m/s). Broken lines, axons found in the spindle but action not determined. Thin lines, unmyelinated preterminal parts of axons. Vertical lines connecting chain fibres indicate sites of *zonulae adhaerentes*. Foci of sarcomere convergence are shown by vertical lines closest together at the point of maximum sarcomere shortening; percentage shortening of twenty sarcomeres given whenever measured, as is extension of the corresponding primary sensory spiral. Arrows, points of observation of longitudinal displacement of intrafusal fibres during maximal activation (values in μm).

This Figure shows the left-hand pole of tenuissimus spindle T12 (Boyd *et al.* 1977), pole A of Fig. 1. Montages of living spindle (Pl. 1 *A*) and of fixed spindle (Pl. 1 *B*) matched as described in Methods. X, point of dye injection. A dynamic γ axon (38 m/s) produced a modest contraction of dynamic bag_1 fibre only. Note coincidence of m_a and m_{ab} motor plates with contraction focus 1·6 mm from equator (Pl. 1 *G*). Second distal focus of contraction at 2·8 mm had no associated motor plate. Five chain fibres were activated powerfully by a static γ axon (23 m/s) which also produced a relatively weak contraction of the static bag_2 fibre (Pl. 1 *C* and *D*) precisely at the site of an m_{ab} motor plate (Pl. 1 *E*). Chain fibre m_c plates coincided with site of more widespread contraction, maximal at 1·2 mm. Axon to m_a plate on static bag_2 fibre not studied functionally.

the two plates were supplied by different axons only one of which could have been responsible for the contraction.

In the present study (Figs. 5 and 6), as in earlier studies, at the end of the capsular sleeve the dynamic bag_1 fibre was observed to move *towards the pole*, and in one instance there was an obvious focus of sarcomere convergence in the extracapsular

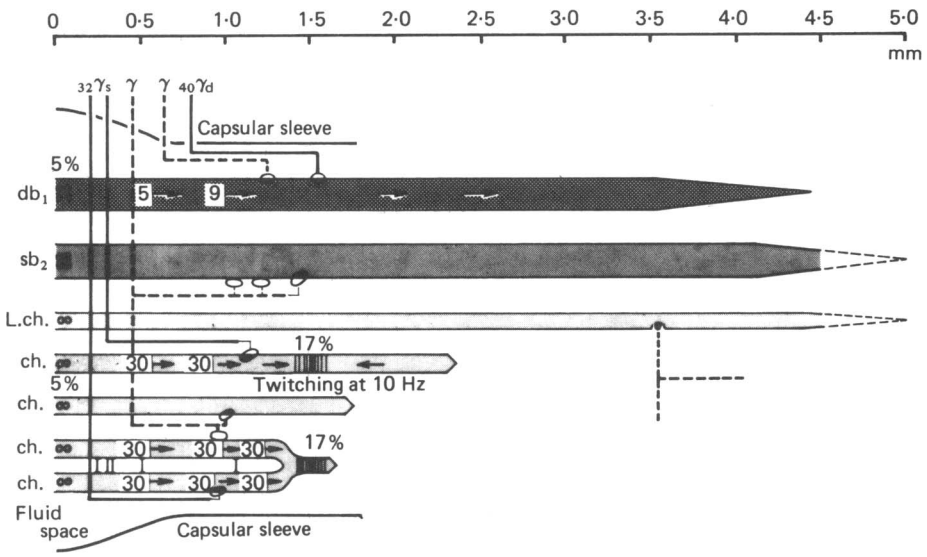

Fig. 6. Right hand pole of tenuissimus spindle T12 (Boyd *et al.* 1977), pole B of Fig. 1. A dynamic γ axon (40 m/s) caused weak contraction of the dynamic bag_1 fibre alone. No focus of sarcomere convergence was observed in the capsular sleeve region where two m_b plates lay at 1·3 and 1·6 mm. The dynamic bag_1 fibre was still moving outwards 2·5 mm from the equator beyond which the intrafusal fibres could not be seen. Modest rapid contraction of three chain fibres only, probably produced by branches of the same selective static γ axon (32 m/s), unloaded and kinked the long chain fibre; note that contraction foci at 1·5 mm were several hundred micrometres distal to the m_{ab} motor plates responsible. Non-selective axon to m_a and m_{ab} plates on static bag_2 fibre and two chain fibres, and another axon to an m_d plate on a long chain fibre, were not studied functionally.

region (Fig. 5). There were definitely no motor plates in this region, however, in the five poles which were serially sectioned throughout (poles A–E).

The static bag_2 fibre. Maximal activation of the static bag_2 fibre usually results in a powerful contraction, displacing the fibre in the fluid space markedly towards the pole (Fig. 7), extending its primary sensory spiral by up to 25%, and often unloading and kinking the dynamic bag_1 fibre and chain fibres. In the two poles in the present study in which foci of sarcomere convergence were observed in the capsular sleeve region, motor plates were located at the same position (Figs. 5 and 7). No motor plates or foci of convergence occurred in the extracapsular region of the static bag_2 fibre in any of the spindle poles studied, and the fibre at the end of the capsular sleeve always moved *towards the equator* (Figs. 5, 7 and 8).

The chain fibres. Two types of behaviour occurred in chain fibres. Marked local contraction, centred upon a point midway along the capsular sleeve, was associated with innervation by m_c plates in this region (Fig. 5). Stimulation of the appropriate static γ axon at 10 Hz caused pronounced twitching, and 1:1 driving of the Ia discharge occurred at frequencies up to 75 Hz. Mid-polar m_c plates were found in a second pole (Fig. 7), and the behaviour described above is typical of many tenuissimus spindles (Boyd, 1976). In two other poles the entire chain fibre innervation consisted of m_a plates. In one such case (Fig. 6) foci of sarcomere convergence were definitely

located more than 300 μm distal to the plates; relatively weak contraction caused local twitching at low stimulation frequencies and 1:2 driving of the Ia discharge at higher frequencies. In the second case (Fig. 8) both the localized contraction and the motor plates were located near to the ends of the chain fibres.

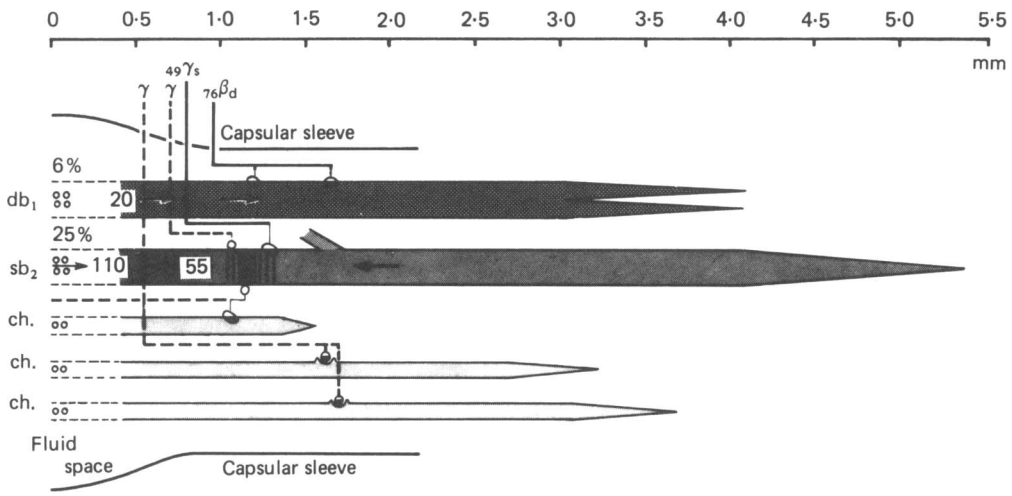

Fig. 7. Right hand pole of tenuissimus spindle T2 (Boyd *et al.* 1977), pole C of Fig. 1. Spindle possibly fixed at length slightly different from that when contraction was recorded. A dynamic β axon (76 m/s) produced modest contraction of the dynamic bag_1 fibre which was obscured beyond 1·2 mm where it still moved outwards. The β axon terminated in m_{ab} and m_b plates at 1·2 mm and 1·65 mm. A static γ axon (49 m/s) produced pronounced contraction of the static bag_2 fibre alone which unloaded and kinked both the dynamic bag_1 fibre and the chain fibres. Sarcomere convergence at 1·2 mm was close to an m_{ab} plate showing signs of repetitive activity. Additional axons not studied functionally were a selective axon to an m_a plate at 1·1 mm on the static bag_2 fibre, a selective axon to two m_c plates on chain fibres at 1·6 mm, and a non-selective axon to an m_a plate on the static bag_2 fibre and an m_{ab} plate on one chain fibre.

Type of motor plate relative to functional type of axon

It can be seen from Figs. 1 and 5–8 and Table 1 that m_a plates are the terminations of static γ axons, whereas m_b plates are the terminations of either dynamic γ or β axons. The intermediate m_{ab} plates may be the termination of dynamic γ or β axons if they lie on the dynamic bag_1 fibre, or the termination of static γ axons if they lie on the static bag_2 fibre. Type m_c plates are the terminations of static γ axons, though one such plate was supplied by a dynamic axon which also supplied the dynamic bag_1 fibre. The type of axon innervating the m_d plate was not known.

DISCUSSION

For the purposes of this Discussion, m_{ab} plates on static bag_2 fibres or chain fibres will be included in the m_a category, both types being quite different from the m_c plates or m_d plates on chain fibres. The m_{ab} plates on static bag_2 fibres appear to be variants

of m_a plates since all contain some m_a axon terminals and some contain partially indented, but no fully indented (m_b) terminals; m_{ab} plates on dynamic bag_1 fibres are variants of the usual m_b type on these fibres since all their axon terminals are either indented or partially indented.

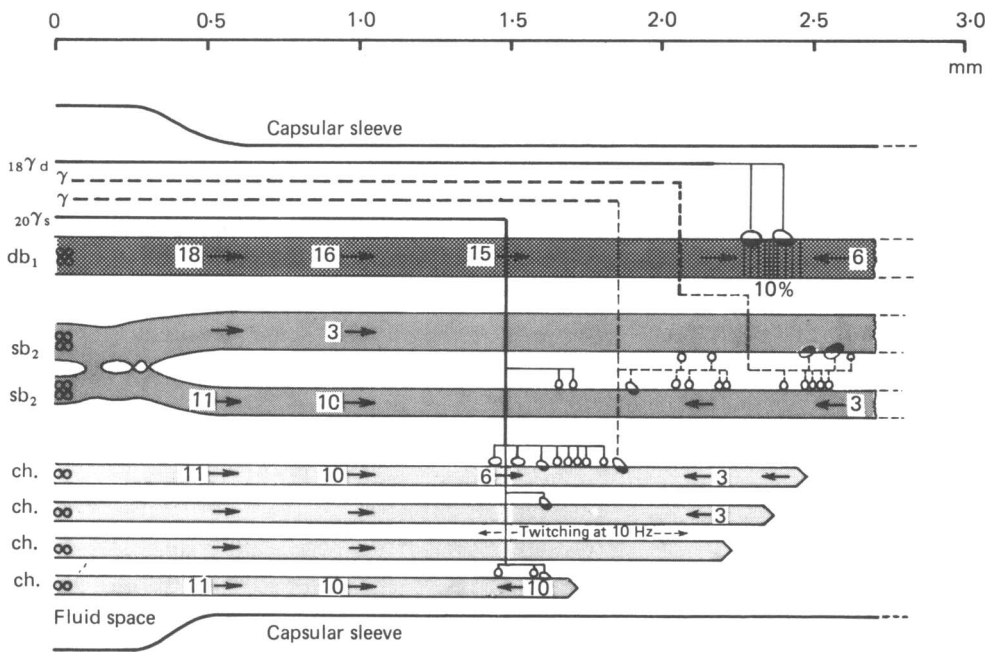

Fig. 8. Right hand pole of abductor digiti quinti medius spindle A2 (Boyd *et al.* 1977), pole F of Fig. 1. A dynamic γ axon (18 m/s) produced a modest contraction of the dynamic bag_1 fibre alone. The contraction focus at 2·4 mm coincided with the location of m_b and m_{ab} plates on the same axon. A static γ axon (20 m/s) caused local contraction in several chain fibres 1·4–2·1 mm from the equator where twelve m_a and m_{ab} plates lay, and a very small contraction in the static bag_2 fibre near to two m_a plates. Two axons ending in numerous m_a and m_{ab} plates, all but one on the static bag_2 fibres, were not studied functionally.

Selective innervation of dynamic bag_1 fibre

In the present study eighteen static γ axons, or axons presumed to be static, which innervated the static bag_2 fibre and/or chain fibres, definitely did not have any terminations on the dynamic bag_1 fibre in six spindle poles. The dynamic bag_1 fibre was innervated by dynamic γ, or dynamic β, axons which supplied this fibre only, save for one instance in which a dynamic axon also innervated one chain fibre. Since the poles were extensively studied by serial electron microscopy it was possible to trace unmyelinated axons, or parts of axons, as well as myelinated axons, to their terminations. Likewise, in a light microscopy study of 1 μm thick serial sections of seven spindle poles from the tenuissimus muscle Banks, Barker & Stacey (1981) and Banks (1981) found that out of a total of eighteen axons which innervated the static bag_2 and/or chain fibres none branched to supply the dynamic bag_1 fibre. Further,

stimulation of static γ axons produces depolarization of static bag_2 or chain fibres but not of dynamic bag_1 fibres (Barker, Bessou, Jankowska, Pagès & Stacey, 1978; Gladden, 1981).

Thus, innervation of the dynamic bag_1 fibre by static γ axons must be very rare, in the tenuissimus and abductor digiti quinti medius muscles at least. It follows that depletion of glycogen in the dynamic bag_1 fibre during stimulation of static γ axons, which is as common as depletion in the static bag_2 fibres (Barker *et al.* 1976*b*), cannot have been due, in most instances, to innervation of the dynamic bag_1 fibre by static γ axons. Barker & Stacey (1981) suggested that the dynamic bag_1 fibre depletion might have accompanied depolarization produced by raised K^+ levels consequent on static bag_2 or chain fibre contraction, though they still maintained that innervation of the dynamic bag_1 fibre by a static γ axon occurred in 8% of tenuissimus spindle poles and 17% of those in the peroneus brevis muscle.

Another explanation of the glycogen depletion anomaly is that prolonged intermittent activation of static bag_2 or chain fibres under ischaemic conditions leads to leakage of acetylcholine from the motor plates on these fibres. This overflow of transmitter may depolarize the dynamic bag_1 fibre, particularly in regions where it is not isolated in a separate compartment of the inner capsule. Of all the intrafusal fibres the dynamic bag_1 fibre is the most sensitive to acetylcholine (Gladden, 1976). This hypothesis is supported by the finding that, in pole E of the present study, the dynamic bag_1 fibre had no motor innervation, but serial electron microscopy revealed a 'sole plate' consisting of an accumulation of mitochondria and nuclei at the point indicated by an arrow in Fig. 1*E*, adjacent to a motor plate on the static bag_2 fibre. There was a break in the inner capsule between the two bag fibres at this point.

Banks (1981) encountered one axon, similar to the one in the present study, which innervated one chain fibre as well as the dynamic bag_1 fibre. In both cases the chain fibre lay close to the dynamic bag_1 fibre and, in the present study, the dynamic bag_1 fibre and the chain fibre were the sole occupants of a separate capsular compartment. Further, one of us (M.G.) has observed one instance of the contraction of a single chain fibre along with the dynamic bag_1 fibre when a known dynamic γ axon was stimulated, so all such axons are almost certainly dynamic in function. Whether such occasional chain fibres have a special function is not known. In any event, it is certain that the vast majority of cat muscle spindles contain a discrete dynamic intrafusal system consisting of the dynamic bag_1 fibre and its dynamic γ, or sometimes β, axons.

Correlation of contraction foci and motor plates

In our experience, when an obvious focus of sarcomere convergence is observed in the capsular sleeve region of either dynamic bag_1, or static bag_2 fibres it coincides precisely with the location of a motor plate. Banks, Barker, Bessou, Pagès & Stacey (1978) obtained reasonably good correlation between foci of convergence and motor endings on static bag_2 fibres, but coincidence of plate and contraction focus only occurred in two out of twelve dynamic bag_1 fibre poles. Better correlation is certainly attained by matching photographs of the living spindle with those of the fixed spindle in the histological block (see Methods), than by calculating the position of a motor plate from the number of transverse sections from a fixed point, since section thickness and the degree of shrinkage during fixation is known approximately only.

Further, the maximum shortening of sarcomeres in a dynamic bag_1 fibre is 10% or less (Fig. 8; Boyd, 1976). Foci of convergence may easily be missed in the capsular sleeve region which is often obscured by connective tissue or intramuscular nerves. Finally, sarcomere convergence in this region may not be apparent if two motor plates, supplied by the same axon, lie some distance apart (e.g. Fig. 7) or if the spindles are very extended, as seems to have been the case in the study of Banks *et al.* (1978).

Foci of sarcomere convergence in the extracapsular region of the dynamic bag_1 fibre, where there is definitely no motor innervation, were observed not only in the present study but also in that of Banks *et al.* (1978). It seems highly probable that in all dynamic bag_1 fibres with no extracapsular innervation (the great majority) contraction is induced in this region. This explains why, at the end of the capsular sleeve, the dynamic bag_1 fibre is always seen to move towards the pole when activated. Such contraction is not the result of action potential propagation since only local potentials can be recorded intracellularly from dynamic bag_1 fibres when they are impaled at intervals (Gladden, 1981). It is known that the ultrastructure of the dynamic bag_1 fibre changes along its length (Barker *et al.* 1976*a*) and it may be that electrotonic spread of depolarization from the motor plate in the capsular sleeve region results in contraction in the extracapsular region where the membrane may have properties different from those near to the motor plate. Whatever the mechanism, it is clear that contraction of the dynamic bag_1 fibre is concentrated in the outer half of one, or sometimes both, poles of the fibre.

In contrast, the static bag_2 fibre behaves as expected, namely, contraction occurs in the capsular sleeve region where the motor plates lie and probably little, if any, tension is developed in the extracapsular region of the fibre which is readily extensible. Thus, at the end of the capsular sleeve the static bag_2 fibre always moves towards the equator when activated. These differences between the dynamic bag_1 and static bag_2 fibre account, in large measure, for the fact that activity in the dynamic bag_1 fibre increases the sensitivity of the primary sensory ending to length changes whereas activity in the static bag_2 fibre reduces it (Boyd, 1981).

It has been shown that diffuse contraction in the polar half of nuclear chain fibres, centred upon a point midway along the capsular sleeve, is associated with the presence of m_c plate innervation at this point (Fig. 5). Such behaviour is suggestive of action potential propagation in the sleeve region. Both Barker *et al.* (1978) and Gladden (1981) were able to record propagated potentials in a few chain fibres. Alternatively, weaker contraction may occur near to the end of chain fibres more than 300 μm distal to the sites of m_a plates (Fig. 6). If the m_a plates lie far out on chain fibres in spindles that have several secondary sensory endings (Fig. 8, three secondary endings) than local contraction occurs in the neighbourhood of the plates. It may be that local depolarization occurs at m_a plates on chain fibres as it does at m_a plates on static bag_2 fibres. No pole of a chain fibre is ever innervated by both m_c and m_a plates, though a single spindle may have chain fibres with m_c plates at one pole and m_a plates at the other (Figs. 5 and 6). Further work will be necessary to establish whether two types of chain fibre activity occur in every spindle. This might be important in determining whether or not changes in the length sensitivity of secondary sensory endings were produced by activity in static γ axons.

Correlation of present electron microscope classification with earlier light microscopy

We have shown that the most reliable criterion for the ultrastructural classification of motor endings is the degree of indentation of the axon terminals into the muscle fibre membrane, provided that many axon terminals in each ending are examined. Further, subjunctional folding is an important criterion for distinguishing between m_a plates, m_c plates and m_d plates on chain fibres. Barker, Stacey & Adal (1970) thought that the nature of subjunctional folding could be used to distinguish the p_1 plates, p_2 plates and trail endings observed in light microscopy of silver stained material, but Barker *et al.* (1976*a*) concluded subsequently that the presence or absence of subjunctional folding was not a reliable criterion for distinguishing between p_2 plate and trail terminals. We, likewise, have found that m_b plates on dynamic bag_1 fibres and m_a plates on static bag_2 fibres cannot be distinguished in this way.

It is obvious that the three axons to the static bag_2 fibre and chain fibres in pole F (Fig. 8) end in typical trail endings; the individual axon terminals had m_a type ultrastructure i.e. they lay superficially as did the trail terminals of Barker *et al.* (1970). However, in many instances in the present work the m_a endings were large, single plates supplied by a separate axon myelinated almost to its termination (Figs. 5 and 7). Clearly such endings must have been included in the group of γ_1 end-plates found on nuclear bag fibres by Boyd (1962). Thus, m_a axon terminals on static bag_2 and chain fibres correspond with trail terminals, but only a proportion of them in some muscles are, in fact, the terminals of true trail ramifications. The other m_a endings are motor plates in every sense (Table 2).

Our m_c plates on chain fibres may have formed part of trail ramifications. Alternatively, m_c plates, which formed about one quarter of the total plate population on chain fibres, may correspond with the p_1 plates on chain fibres of light microscopy which formed a quarter of the total p_1 plate population of spindles (Barker *et al.* 1970). The protrusion of the fingers of muscle under the m_c plate would give rise to the Doyère eminence characteristic of p_1 plates in light microscopy. However, the wide, shallow subjunctional folds of m_c terminals resemble those of the plate on a chain fibre thought to be a p_2 plate by Barker *et al.* (1970, Fig. 59) and are quite unlike the deep, narrow folds of what were thought to be p_1 plates on chain fibres (Barker *et al.* 1970, Figs. 73 and 75). The latter clearly correspond with our m_d plate on a long chain fibre (Table 2) not only because they had numerous deep, narrow subjunctional folds but also because the axon terminals were fully indented into the muscle fibre membrane.

While there are certainly three ultrastructural types of motor ending on chain fibres, we cannot provide any ultrastructural evidence for the presence of two distinctly different types of plate on dynamic bag_1 fibres which might correspond with p_1 and p_2 plates. According to Barker *et al.* (1970, 1978) both p_1 plates and p_2 plates are clearly indented into dynamic bag_1 fibres, as are our m_b plates. The p_1 plate is said to have deep, narrow, branched subjunctional folds (Barker *et al.* 1970). This description was derived from electron micrographs of presumed p_1 plates on *chain* fibres, however, and no plate with such folding has been encountered on any nuclear bag fibre by Barker *et al.* (1970), Banks (1981) or by ourselves.

TABLE 2. Correlation of present ultrastructural classification of fusimotor endings with the γ_1/γ_2 nomenclature of Boyd (1962), the p_1 plate, p_2 plate, trail nomenclature of Barker *et al.* (1970) and with the function of their supplying axons. The corresponding density of plate deposits of cholinesterase (ChE) is also shown (Kucera, 1980*a*, *b*, *c*). m_{ab} plates are not shown as a separate group (see text)

Action of supplying axon	Type of intrafusal fibre			
	Dynamic bag_1 fibre	Static bag_2 fibre	Chain fibres	Long chain fibre*
Static γ →	—	m_a plate: trail ending; γ_1 plate; ChE 'medium' plate	m_a plate: trail ending; γ_2 ending; ChE 'dense' plate m_c plate: ?; γ_2 ending; ChE 'dense' plate	— —
Static β →	—	—	—	m_d plate: p_1 plate; ChE 'very dense' plate
Dynamic γ →	m_b plate: p_2 plate; γ_1 plate; ChE 'pale' plate	—	m_c plate (occasional): ?; γ_2 ending; ChE 'dense' plate	—
Dynamic β →	m_b plate: p_1 plate; γ_1 plate; ChE 'pale' plate	—	—	—

* Present in 13% of tenuissimus spindles (Kucera, 1980*b*).

Further, p_1 plates are said to be shorter than p_2 plates. Individual m_b plates vary in length from 15 to 85 μm, a range which covers p_1 and p_2 plates. The pairs of m_a and m_{ab} plates close together on the dynamic bag_1 fibre in poles A (Fig. 5) and F (Fig. 8) resemble the diagrams of pairs of p_1 plates shown by Barker, Emonet-Dénand, Laporte & Stacey (1980). However, p_2 plates can be less than 50 μm in length (Barker *et al.* 1978). Also, we have been unable to distinguish different types of plate on the dynamic bag_1 fibre in terms of the number of sole plate nuclei or the presence or absence of protrusion of the muscle under the plate which presumably corresponds with a Doyère eminence; p_1 plates are said to have a well developed sole plate and to lie on a Doyère eminence (Barker *et al.* 1970).

Finally, p_1 plates have tapering axon terminals while p_2 plate terminals are mostly knob-like. In this case one might expect p_1 terminals in electron micrographs to be smaller and more uniform in size than p_2 terminals. However, the great majority of m_b terminals are less than 1 μm^2 in cross-sectional area, as are m_c terminals on chain fibres. It is m_a terminals which tend to be larger and less uniform, about half of them being 1–2·5 μm^2 in area (Fig. 3).

Kucera (1980*a*, *b*, *c*, 1982) has found that there is a gradation in intensity of staining of acetylcholinesterase deposits from 'pale' on dynamic bag_1 fibres to 'medium' on static bag_2 fibres, 'dense' on chain fibres, and 'very dense' on long chain fibres (Table 2). If cholinesterase density were related to the presence of subjunctional folding, and hence to area of synaptic contact, then one might expect the density to be greater in m_c plates on chain fibres than in m_a plates on static bag_2 fibres, and greatest in m_d plates on long chain fibres. But the density in m_b plates on dynamic bag_1 fibres should be the same as that in m_a plates on static bag_2 fibres since subjunctional folding is minimal in both types. The appearance of tongues of Schwann cell in the synaptic gap of m_b terminals and the 'lids' of Schwann cells apparently sealing off the synaptic gap (Pl. 2*B* and *C*) suggest, however, that in the case of the dynamic bag_1 fibre acetylcholine may be removed mainly by uptake into Schwann cells and axon terminals, as in nerve terminals on slow tonic extrafusal fibres in the frog (Burke, 1956). For this reason the cholinesterase content of m_b terminals may be lower than that of m_a terminals.

Correlation of type of fusimotor ending with the function of its axon

Barker *et al.* (1973) established that static γ axons terminate in trail endings. We have confirmed that some static γ axons end in m_a plates on static bag_2 or chain fibres which may be grouped in the form of trail endings, but which may also be single, large, m_a plates on static bag_2 fibres (Table 2). Other static γ axons end in m_c plates on chain fibres. Not all m_c plates on chain fibres are the terminations of static γ axons, however, since the occasional dynamic γ axon, which innervates one chain fibre as well as the dynamic bag_1 fibre, ends in an m_c plate on the chain fibre (Fig. 1*D*, Pl. 2*E*). We have little doubt that m_d plates on long chain fibres are the terminations of static β axons, stimulation of which is known to deplete long chain fibres of glycogen near the end of the capsular sleeve (Jami, Lan-Couton, Malmgren & Petit, 1979).

Barker *et al.* (1976*a*) established that at least some dynamic γ axons end in p_2 plates on dynamic bag_1 fibres. Barker *et al.* (1980) later concluded that p_1 plates on dynamic bag_1 fibres were the terminations of dynamic β axons. We have shown, however, that both dynamic γ axons and dynamic β axons terminate in the capsular sleeve in m_b plates on the dynamic bag_1 fibre, or in the intermediate m_{ab} form. The m_b and m_{ab} plates cannot be the terminals of dynamic γ and β axons, respectively, since a single

axon, β or γ, may terminate in both an m_b and an m_{ab} plate (Figs. 5, 7 and 8). Further, the motor plates on dynamic γ and β axons cannot be distinguished in terms of subjunctional folding since in both cases folding varies along the length of the plate from wide, shallow folds to none at all. Banks (1981) likewise noted no obvious difference in the ultrastructure of the terminations of dynamic γ and dynamic β axons on the dynamic bag_1 fibre. Further, in ascribing a motor ending to the p_1 plate, p_2 plate or trail category he appears to have relied on the function of the supplying axon, where known, rather than on any feature of his three dimensional reconstructions. We wonder whether the p_1 plate on dynamic bag_1 fibres is not simply a variant of the p_2 plate, whereas the p_1 plate on chain fibres is a quite different entity as described above. Some confusion may have arisen because Barker *et al.* (1970) appear to have assumed, quite naturally, that the endings of a particular motor axon on different types of intrafusal fibre were structurally the same, which is not always the case.

Factors determining the form of intrafusal motor plates

The presence of the primary sensory innervation is essential for the development of muscle spindles and the differentiation of intrafusal fibres into their adult types (Zelena & Hnik, 1963; Zelena, 1964; Zelena & Soukup, 1973). The motor innervation is not essential, however, since the three types of intrafusal fibre in the rat develop their typical equatorial nucleation, ultrastructure and histochemistry despite neonatal de-efferentation (Zelena & Soukup, 1974). Further, the regional variations in ATPase activity typical of adult cat intrafusal fibres are found in the occasional poles of all three types of intrafusal fibre which are without motor innervation in adult spindles (Kucera, 1981), such as the dynamic bag_1 fibre in pole E, and one of the chain fibres in pole F, of the present study.

Is the form of motor plates determined by the type of intrafusal fibre on which they lie, or by the type of fusimotor neurone forming the synaptic contacts? Findings in the present study which suggest that the intrafusal fibre determines the nature of its motor plates are the following. Plates of the m_b type are found only on the dynamic bag_1 fibre (Table 1) either as terminations of dynamic γ axons, or of dynamic β axons which also terminate on extrafusal fibres in end-plates which are quite different from m_b plates. Plates on the static bag_2 fibre are always of the m_a type. Plates of the m_c type are found only on nuclear chain fibres. Further, the occasional non-selective dynamic axon which innervates the dynamic bag_1 fibre and one chain fibre ends in an m_c plate on the chain fibre whereas the plate on the dynamic bag_1 fibre is of the usual m_b type. Finally, a non-selective static γ axon terminating in m_c plates on chain fibres ends in the usual m_a plate on the static bag_2 fibre.

On the other hand, if the type of intrafusal fibre determines the form of its own plates, why are all the plates on chain fibres not of the m_c type? Some static γ axons end in m_a plates on chain fibres. If two types of ending indicate that chain fibres are of two distinct types, then it is necessary to postulate that the two poles of the same chain fibre can be of different types since a single chain fibre may receive an m_c plate at one pole and an m_a plate at the other pole. It seems more likely that there are two types of static γ motor neurone as has already been suggested by Boyd *et al.* (1977), Gladden & McWilliam (1977) and Gladden (1981), one type ending in m_c plates and the other in m_a plates. Finally, a single chain fibre may receive an m_c plate at

one pole and an m_d plate at the other, if it is a long chain fibre at this latter pole, which suggests that it is the static β motoneurone which determines the form of the m_d plate which closely resembles an extrafusal plate.

We suggest that both the type of fusimotor neurone and the type of intrafusal fibre are involved in the determination of the structure of motor plates and that there is an optimum match between the two. When optimum matching occurs the fibre may reject earlier connexions with other fusimotor neurones. If this hypothesis is correct then the rejection of imperfect matching must be more complete in the case of the dynamic bag_1 fibre, on which static γ axon connexions are rarely found, and in the case of chain fibres, since m_a and m_c plates are never found on the same pole of one fibre, than it is in the case of the static bag_2 fibre. Selective and non-selective connexions to the static bag_2 fibre quite often co-exist. Not all non-selective connexions are necessarily functionally perfect, however, and evidence for this is provided by the finding in pole A of the present study (Fig. 5) that the non-selective, static γ connexion to the static bag_2 fibre produced a relatively weak contraction and the axon terminals of the plate itself contained a large number of lysosomes and membrane fragments suggesting that it was in the process of degenerating. This plate may have been in the process of rejection because the static bag_2 fibre had formed an optimum match with a static γ motoneurone innervating it selectively.

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EXPLANATION OF PLATES

PLATE 1

Correlation of foci of sarcomere convergence and site of motor plates in the left hand pole (Fig. 1, pole A) of spindle T12 of Boyd *et al.* (1977).

A, montage of 2 mm of the living spindle. Scale indicates distance from point of termination of group Ia axon with primary sensory ending. fs, fluid space; caps, capsule; small box, contraction site in static bag_2 (sb_2) fibre enlarged in *C* and *D*; large box, inner contraction site in dynamic bag_1 (db_1) fibre enlarge in *G*. Vertical arrows mark fragments of extrafusal fibres. Sloping arrows mark fat cells.

E, montage of same region of fixed spindle in resin block at same magnification as *A*. *, injected dye mark in dynamic bag_1 fibre. Arrows mark corresponding structures in *A* and *B*.

C, region of sarcomere convergence in static bag_2 fibre. Triangles indicate site of m_{ab} motor plate on static bag_2 fibre at rest. Pairs of arrows mark twenty sarcomeres of static bag_2 fibre at motor plate and 150 μm to left of plate.

D, same region as *C* during maximal contraction of static bag_2 fibre when static γ axon stimulated at 150 Hz (see Fig. 5). Shortening of twenty sarcomeres, 12% at motor plate, 5% 150 μm to left of motor plate.

E, same region as *C* and *D* in resin block showing static γ axon (γ_s) and actual motor plate on static bag_2 fibre and some nuclei. Arrows indicate capsular sleeve.

F, diagram of same region of fixed spindle as in *E* showing extent of the static bag_2 fibre motor plate. Arrowheads indicate width of static bag_2 fibre, dynamic bag_1 fibre, and one chain fibre. Scale applies to *C*, *D*, *E* and *F*.

G, region of dynamic bag_1 fibre where sarcomere convergence occurred during stimulation of a dynamic γ axon. Fibre at rest. Triangles indicate situation of two motor plates on a single axon to dynamic bag_1 fibre (see Fig. 5).

H, corresponding region to *G* in resin block. Dynamic γ axon (γ_d) is visible. Arrows indicate capsular sleeve and red blood cells in capillary.

I, diagram of same region of fixed spindle as in *H* showing extent of the two dynamic bag_1 fibre motor plates; γ_d axon visible at right is obscured as it crosses the intrafusal bundle. Arrowheads indicate width of intrafusal fibres. Scale applies to *G*, *H* and *I*.


A
B
C
D
E
F
G
H
I
fs
Ia
caps
0
0·5
1·0
1·5
γ
caps
caps
50 μm
γs axon
sb2
db1
ch.
caps
rbc
caps
γd axon
sb2
db1
ch.
50 μm

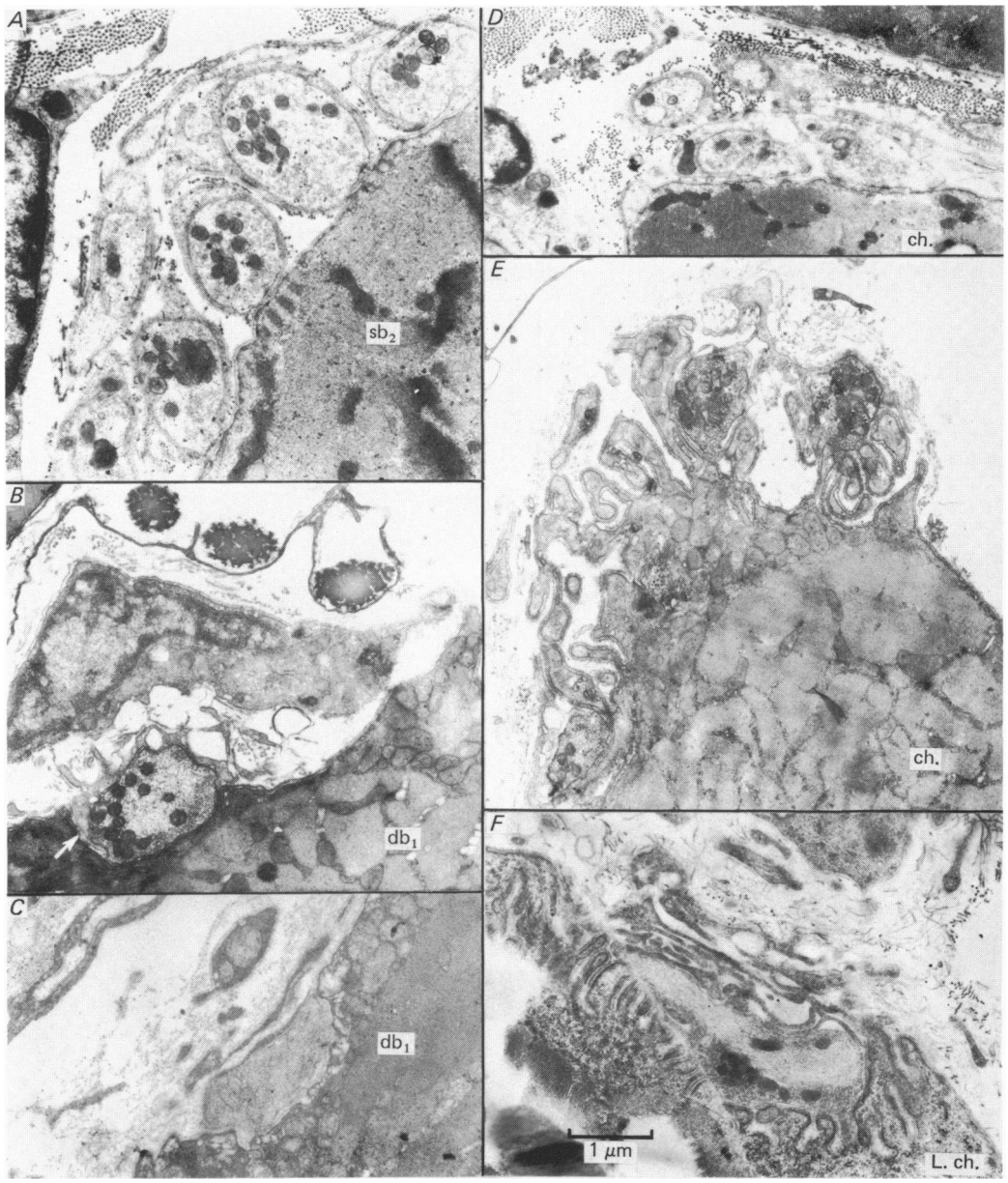
A
sb2
B
db1
C
db1
D
ch.
E
ch.
F
1 μm
L. ch.

PLATE 2

Electron micrographs of transverse sections of axon terminals of intrafusal motor plates. Scale applies to all micrographs.

A, four axon terminals of a type m_a motor plate on a static bag_2 fibre (see also Figs. 2*A* and 3*A*). Termination of a known static γ axon. Terminals entirely superficial with no folding of subjunctional membrane. From pole F (Fig. 8).

B, axon terminal of a type m_b motor plate on a dynamic bag_1 fibre (see also Fig. 3*B*). Termination of a known dynamic γ axon. Terminal deeply indented into fibre with no subjunctional folding. At other levels folding as in *C* was present. Arrow indicates tongue of Schwann cell lid. From pole D (Fig. 1).

C, axon terminal of a type m_b motor plate on a dynamic bag_1 fibre (see also Figs. 2*B* and 3*C*). Terminations of a known dynamic β axon. Terminal deeply indented into fibre with some wide, shallow subjunctional folds. At other levels folding was absent as in *B*. From pole C (Fig. 7).

D, two axon terminals of a type m_a motor plate on a nuclear chain fibre (see also Fig. 3*D*). Terminations of a known static γ axon. Terminals superficial with no subjunctional folding. From pole F (Fig. 8).

E, type m_c motor plate on a nuclear chain fibre (see also Figs. 2*C* and 3*E*). Three axon terminals indented into protruding fingers of muscle. Termination of a known dynamic γ axon. From pole D (Fig. 1).

F, axon terminal of a type m_d motor plate on a long chain fibre (see also Figs. 2*E* and 3*F*). Possible termination of a static β axon. Terminal fully indented and with numerous deep, narrow subjunctional folds. From pole B (Fig. 6).