Structure, distribution and innervation of muscle spindles in avian fast and slow skeletal muscle

WILLIAM K. OVALLE, PIERRE R. DOW AND PATRICK C. NAHIRNEY

Department of Anatomy, Faculty of Medicine, University of British Columbia, Vancouver, Canada

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ABSTRACT

Muscle spindles in 2 synergistic avian skeletal muscles, the anterior (ALD) and posterior (PLD) latissimus dorsi, were studied by light and electron microscopy to determine whether morphological or quantitative differences existed between these sensory receptors. Differences were found in the density, distribution and location of muscle spindles in the 2 muscles. They also differed with respect to the morphology of their capsules and intracapsular components. The slow ALD possessed muscle spindles which were evenly distributed throughout the muscle, whereas in the fast PLD they were mainly concentrated around the single nerve entry point into the muscle. The muscle spindle index (number of spindles per gram wet muscle weight) in the ALD was more than double that of its fast-twitch PLD counterpart (130.5 ± 2.0 vs 55.4 ± 2.0 respectively, $n = 6$). The number of intrafusal fibres per spindle ranged from 1 to 8 in the ALD and 2 to 9 in the PLD, and their diameters varied from 5.0 to 16.0 μm and 4.5 to 18.5 μm, respectively. Large diameter intrafusal fibres were more frequently encountered in spindles of the PLD. Unique to the ALD was the presence of monofibre muscle spindles (12.7% of total spindles observed in ALD) which contained a solitary intrafusal fibre. In muscle spindles of both the ALD and PLD, sensory nerve endings terminated in a spiral fashion on the intrafusal fibres in their equatorial regions. Motor innervation was restricted to either juxtaequatorial or polar regions of the intrafusal fibres. Outer capsule components were extensive in polar and juxtaequatorial regions of ALD spindles, whereas inner capsule cells of PLD spindles were more numerous in juxtaequatorial and equatorial regions. Overall, muscle spindles of the PLD exhibited greater complexity with respect to the number of intrafusal fibres per spindle, range of intrafusal fibre diameters and development of their inner capsules. It is postulated that the differences in muscle spindle density and structure observed in this study reflect the function of the muscles in which they reside.

Key words: Intrafusal muscle fibres; avian skeletal muscle; muscle spindles.

INTRODUCTION

The majority of studies on muscle spindle morphology, distribution and function have been conducted on mammalian skeletal muscles (Matthews, 1972; Barker, 1974; Boyd & Smith, 1984). Comparatively little information exists on avian muscle spindles and the degree to which they resemble or differ from those of other vertebrates (Barker, 1974; Adal & Chew Cheng, 1980; Maier, 1992; 1997). Avian muscle spindle structure has been found to be basically similar to mammalian examples (DeAnda & Rebollo, 1967; Rebollo & DeAnda, 1967; Maier & Eldred, 1971; James & Meek, 1973; Ovalle, 1976; Maier, 1992), although some morphological characteristics have been reported as being more reminiscent of those of amphibian muscle spindles (Barker & Cope, 1962; Maier & Eldred, 1971).

Studies which have examined muscle spindles in synergistic or antagonistic avian muscles are relatively few in number (Veggetti & Palmieri, 1971; Adal & Chew Cheng, 1980; Maier, 1981; Bout & Dubbeldam, 1991). Each muscle is thought to contain its own characteristic sensory and motor supply which lends credence to its overall functioning. Spindle density has typically been employed to designate the sensory

Correspondence to Dr W. K. Ovalle, Department of Anatomy, Faculty of Medicine, University of British Columbia, Vancouver, Canada V6T 1Z3.

feedback capacity of a muscle (Maier, 1992). Some researchers have reported that muscle spindle indices are higher in slow-twitch than in fast-twitch mammalian muscles (Swett & Eldred, 1960; Richmond & Abrahams, 1975*b*). Apart from Maier's study (1981) on the pigeon gastrocnemius, no other comparable data on muscles with differing histochemical and contractile properties exist for avian muscles, but an ideal experimental model exists in the avian latissimus dorsi muscle. In the chicken it is composed of 2 separate and distinct portions: the slow (or tonic) anterior latissimus dorsi (ALD) and a fast-twitch (or phasic) posterior latissimus dorsi (PLD). These synergistic muscles differ morphologically, histochemically, physiologically, biochemically and developmentally (Ginsborg, 1960*a*, *b*; Hess, 1970; Shear & Goldspink, 1971; Atsumi, 1977) and together represent a unique paradigm of a true slow and fast avian skeletal muscle.

The aim of the present study, therefore, is to examine and compare the density, distribution and morphology of the muscle spindles in these 2 synergistic avian muscles, the ALD and PLD, and to ascertain some structural and ultrastructural features of the intrafusal fibres and innervation patterns found in these receptors. An account of the fine structure of the avian muscle spindle capsule in the same species has been reported elsewhere (Ovalle, 1976).

MATERIALS AND METHODS

Male and female white leghorn chickens, aged 7 wk, were killed with an overdose of chloroform. Body weights ranged from 550 to 600 g. The anterior (ALD) and posterior (PLD) latissimus dorsi muscles chosen for this study form the most superficial layer of dorsal musculature of the back. Each arises from cervical and thoracic segments of the vertebral column, respectively, and they insert by separate tendons onto the humerus. They are distinctly innervated by 2 branches of the median nerve and remain separate throughout their course to their insertions (Ginsborg, 1960*b*). Each muscle was quickly excised, trimmed of superficial fascia and tendon, and their weights were subsequently recorded. Muscles were then pinned to a cork board in a moderately stretched position and moistened with physiological saline.

Muscle spindle quantitation

For muscle spindle quantitation, whole ALD and PLD muscles were quick-frozen in isopentane cooled to -150 °C in liquid nitrogen. Muscles were then transferred to a cryostat set at -20 °C, adhered to a mounting chuck, and serially sectioned from origin to insertion at a thickness of 10 μm. Every tenth section was mounted on a glass slide and stained with haematoxylin and eosin (H & E) or with a modified Gomori trichrome stain.

Muscle spindles from 6 ALD-PLD pairs were located by brightfield microscopy at a magnification of $\times 400$ and numbered. Capsules and intrafusal fibres were then indicated on a scale drawing of each muscle cross section using the micrometer scale readings on the mechanical stage of the microscope. This procedure was repeated at 100 μm intervals and the length of each receptor was approximated by counting the number of sections in which intrafusal fibres were seen and then multiplying by a factor of 10. The total number of spindles per muscle was counted and a muscle spindle index was obtained by dividing the number of spindles by the gram weight of muscle. Quantitative data obtained from these measurements were analysed for significance by using an unpaired Student's t test ($P < 0.001$).

A longitudinal reconstruction of a single ALD-PLD pair was made from serial transverse tracings using a Leitz camera lucida drawing attachment on the microscope. In addition, a transverse plane representation of the same 2 muscles was made from 2 mm-spaced serial tracings in order to ascertain muscle spindle positions relative to ventral and dorsal surfaces. The number of intrafusal fibres was counted in 100 ALD and 100 PLD muscle spindles, and the diameters of 250 ALD and 250 PLD intrafusal fibres were measured in juxtaequatorial regions of these muscle spindles. Size histograms of intrafusal fibres from muscle spindles in the ALD and PLD were then constructed from these data.

Silver impregnation

To demonstrate spindle innervation patterns, 3 whole ALD-PLD pairs were fixed in moderately stretched positions in 10% neutral buffered formalin for 24 h. After dehydration and paraffin embedding, serial longitudinal sections (15 μm) of each muscle were cut on a rotary microtome, mounted on albuminised glass slides, and stained by a modification of the Bielschowsky (1909) silver impregnation technique. Slides were initially incubated in a 20% silver nitrate solution in the dark at 37 °C for 24–48 h, with subsequent reduction in 20% neutral buffered formalin for 1–2 min. Further development was under-

taken in a 20 % ammonical-silver nitrate solution for 5 min followed by toning in 1 % gold chloride for 1 h. Sections were then placed in 20 % thiosulphate fixing solution for an additional 1 h, rinsed in distilled water, dehydrated, cleared in xylene, mounted in Permount and coverslipped.

Electron microscopy

Four chickens were anaesthetised with an intraperitoneal injection of sodium pentobarbital, followed by left ventricular perfusion of Karnovsky's (1965) fixative diluted to 50 % with 0.1 M sodium cacodylate buffer at pH 7.3 for 15–20 min. ALD and PLD muscles were exposed and rapidly excised in toto, placed in moderate stretch in stainless steel clamps, and immersed for 1 h in fresh fixative. Muscles were then cut into small pieces, reimmersed in fresh fixative for an additional 3 h and washed in repeated changes of buffer. The minced tissue specimens were postfixed in 1 % aqueous osmium tetroxide for 1 h, stained en bloc in a saturated aqueous solution of uranyl acetate, dehydrated in a graded series of ethanols and propylene oxide, and embedded in a 1:1 mixture of Epon/Araldite.

To localise muscle spindles embedded in each muscle, transverse sections (1 μm) of each block were cut with glass knives. Sections were stained with 1.0 % aqueous toluidine blue for 15 s at 60 °C and examined by light microscopy. Thin transverse sections through polar, juxtaequatorial and equatorial regions of each muscle spindle were then cut with a diamond knife, collected on copper grids, stained briefly with Reynolds (1963) lead citrate , and examined in a Philips EM 300 transmission electron microscope operated at 60 kV.

RESULTS

Range and density of muscle spindles

Comparative data from quantitative analysis of muscle spindles and whole muscle wet weights in 6 ALD-PLD pairs are shown in the Table. The mean weight of the ALD was 0.28 ± 0.06 g which was consistently less than that of the PLD with a mean weight of 0.37 ± 0.07 g. A total of 315 muscle spindles was located and examined; 212 in the ALD and 103 in the PLD. The number of muscle spindles ranged from 32 to 40 per muscle in the ALD with a mean of 35.3 ± 0.8, and 16 to 20 in the PLD with a mean of 17.2 ± 0.5. Muscle spindle indices (the number of muscle spindles per gram of muscle weight) were significantly higher in the ALD (130.5 ± 2.0) than in the PLD (55.4 ± 2.0).

Distribution of muscle spindles

The ALD had a relatively uniform distribution of muscle spindle units throughout the muscle with a slight decrease towards the origin on the vertebral column (Fig. 1). A small increase in their density was seen in the region close to the entry of each of the 3 branches of the median nerve into the muscle. A more uneven distribution was found in the PLD (Fig. 1) with the highest density in the distal third of the muscle near the region of the entry point of the single branch of the median nerve. Three of the 6 PLD muscles in this sample contained only 1 muscle spindle in the proximal third of the muscle belly near its origin.

Figure 2 shows the density of muscle spindles per transverse section at 2 mm intervals. Several PLD spindles were present in 2 adjacent sections, thus indicating a length of more than 2 mm. Muscle spindles were more frequently located ventrally in each muscle where the nerve supply enters. Their lengths, including extracapsular regions, were shorter in the ALD with a range of 0.8–3.7 mm (mean = 1.9 mm) compared with a range of 1.1–4.8 mm (mean = 2.3 mm) in the PLD.

In both muscles, the majority of intrafusal fibres extended beyond the capsule and terminated a short distance from each other in the adjacent endomysial tissue. Some fibres extended more than 1 mm extracapsularly at each pole; these occured most commonly in the PLD. This situation is reminiscent of some bag

Table. *Number and density of muscle spindles in a total of 6 ALD-PLD pairs*

	ALD	PLD	ALD:PLD ratio
Weight (g)	0.28 ± 0.06	0.37 ± 0.07	1.0:1.2
Muscle spindle content	35.3 ± 0.8	17.2 ± 0.5*	2.1:1.0
Muscle spindle index	130.5 ± 2.0	55.4 ± 2.0*	2.4:1.0

The muscle spindle index is calculated by dividing the number of spindles by the wet weight (in g) of each muscle. Asterisks indicate a significant difference between the ALD and PLD at $P < 0.001$ using an unpaired Student's t test.

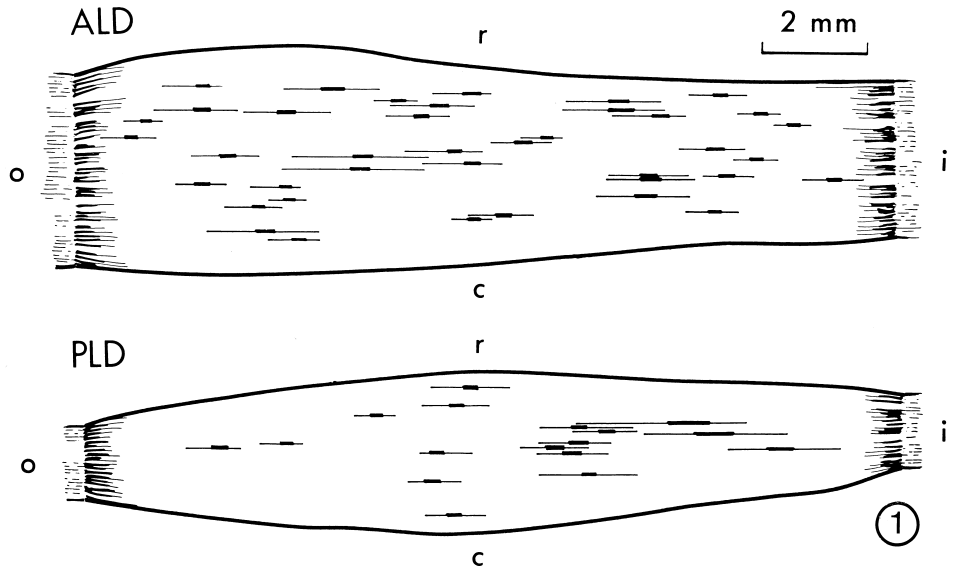


Fig. 1. Longitudinal reconstruction of an ALD and a PLD showing the distribution and lengths of muscle spindles in each muscle. Lengths of equatorial regions are indicated by thickened lines. r, rostral; c, caudal; o, origin; i, insertion.

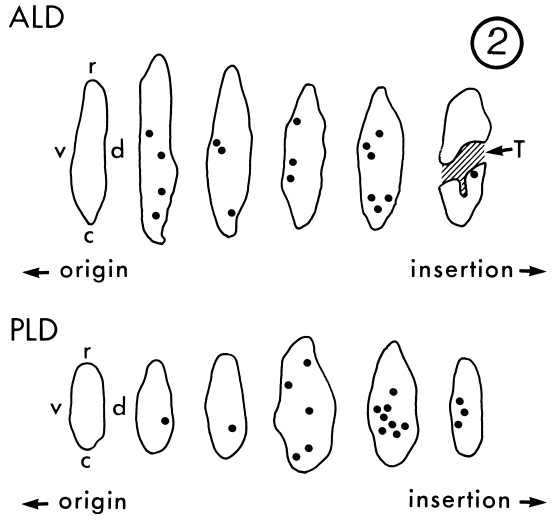


Fig. 2. Transverse reconstruction showing the distribution of muscle spindles in one ALD-PLD pair from origin to insertion. Each dot indicates a spindle. Intervals between traced transverse sections are 2 mm. r, rostral; c, caudal; v, ventral; d, dorsal; t, tendon.

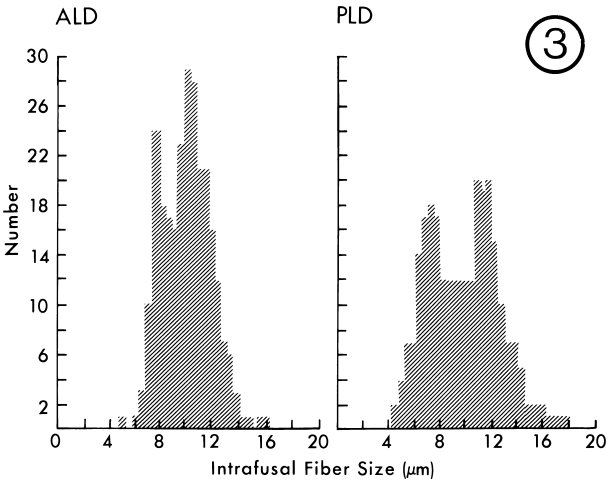


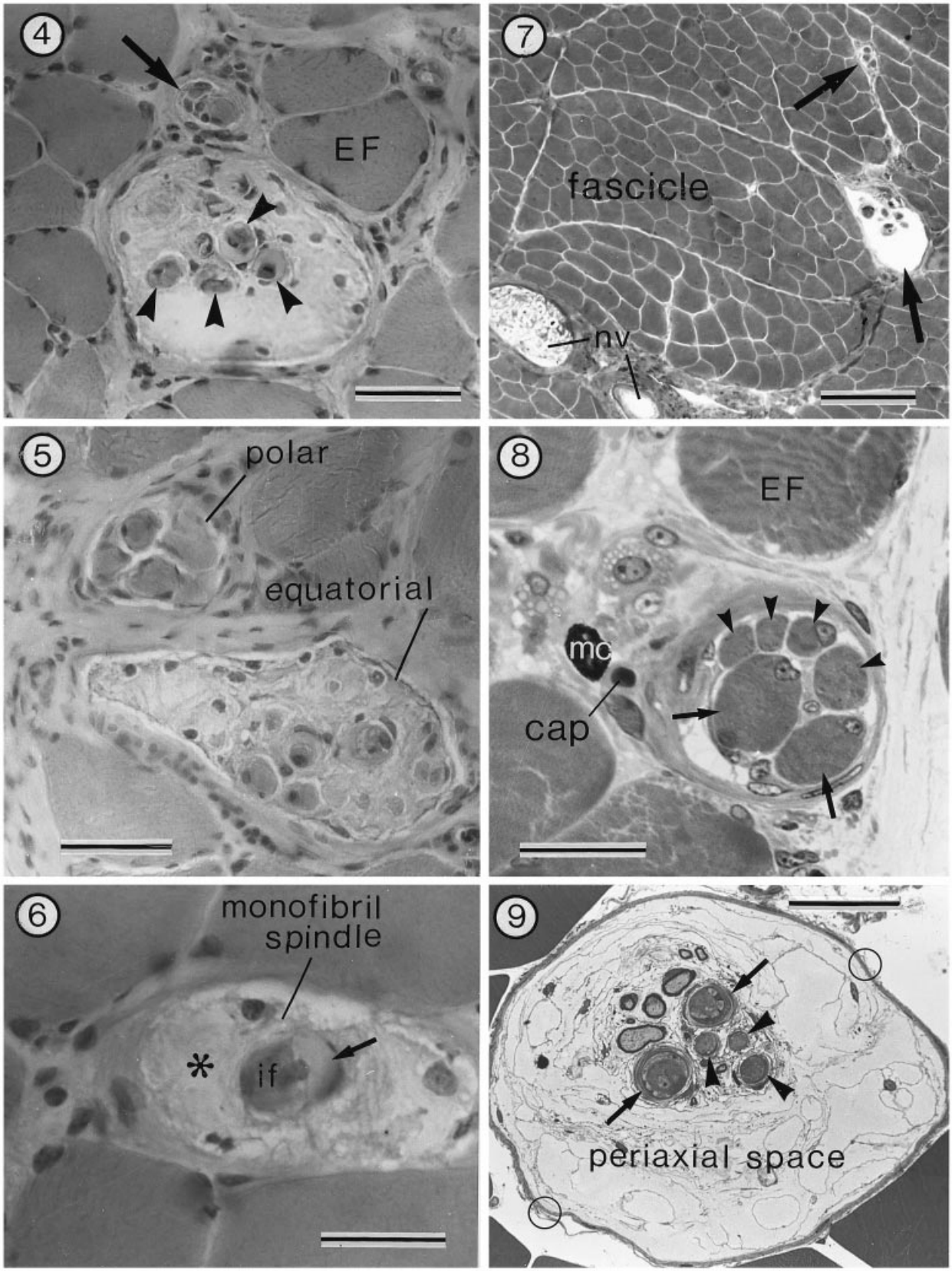
Fig. 3. Size histograms of intrafusal fibre diameters from selected spindles in the ALD and PLD. A total of 250 intrafusal fibres from each muscle was measured and the data were collected from 6 ALD-PLD pairs. Note the apparent bimodal distribution of cross-sectional diameters in the 2 muscles.

fibres in mammalian muscle spindles (Swett & Eldred, 1960). Within the ALD, monofibre muscle spindles which contained a solitary intrafusal fibre were rarely longer than 1 mm.

Light microscopic appearances

The light microscopic appearance of muscle spindles observed in the chicken ALD and PLD was similar in many respects to that described for other avian muscles (DeAnda & Rebollo, 1967; Maier & Eldred, 1971; Hikida, 1985). Our observations, however, reveal several differences between the muscle spindles of the ALD and PLD including their location, relationship with adjacent structures, intracapsular components and intrafusal fibre characteristics.

Muscle spindles in the ALD were usually located on the periphery of muscle fascicles and at their junctures. They were surrounded by prominent perimysial connective tissue (Figs 4, 5) and showed a comparatively thicker outer capsule in equatorial regions than those in the PLD. Extracapsular regions of ALD intrafusal fibres were typically embedded in a dense perimysium. Paired muscle spindle complexes, having some form of mechanical contact without sharing



Figs 4–9. Light microscopic views of muscle spindles in the ALD on the left (Figs 4–6) and the PLD on the right (Figs 7–9).

Fig. 4. Transverse frozen section of the ALD showing 2 spindles arranged side-by-side and forming a paired complex. Their outer capsules are fused but their inner contents remain separate and distinct. The spindle above is a monofibre unit (arrow), whereas the equatorial region of the subjacent spindle contains 4 intrafusal fibres (arrowheads). H & E. ×950. Bar, 20 μm.

intrafusal fibres, were found in the ALD in the regions of high overall muscle spindle density (Figs 4, 5). In addition, monofibre muscle spindles (Fig. 6) were also commonly found in areas of the ALD containing a high muscle spindle density. These single intrafusal fibre units typically contained a dense connective tissue capsule along their lengths.

At low magnification, the PLD was characterised by tightly-packed extrafusal fibres grouped into pleomorphic fascicles with relatively little perimysial connective tissue (Fig. 7). In the PLD, spindles were typically located within fascicles and were, subsequently, in close contact with surrounding extrafusal fibres along their entire lengths. Only one paired muscle spindle complex was observed in the distal third of a PLD. Occasional mast cells were encountered near the muscle spindles (Fig. 8) and numerous capillaries in close apposition to the spindle capsule were also present. Equatorial regions of muscle spindles in the PLD exhibited a large periaxial space which contained a delicate array of extensions from inner and outer capsule cells (Fig. 9). A thin outer capsule was present at the equator and was in close contact with neighbouring extrafusal fibres. In juxtaequatorial regions, the outer and inner capsule cells became indistinguishable and a thin multilayered perineurial capsule invested the intrafusal fibres. Comparatively less noncellular connective tissue (e.g. collagen) was associated with the capsule of the PLD than with that of the ALD.

Intrafusal fibres

Attempts to classify avian intrafusal fibres according to size have been inconclusive at best (De Anda & Rebollo, 1967; Maier & Eldred, 1971; Adal, 1973); however, in our samples, differences were found between ALD and PLD intrafusal fibre sizes based on measurement of juxtaequatorial regions of 500 fibres (250 from ALD and 250 from PLD muscle spindles, excluding monofibre units).

The range of intrafusal fibres per ALD muscle spindle was 1–8 (mean = 3.5) and 2–9 (mean = 4.9) in the PLD. A total of 27 monofibre units was found in the ALD samples with a range of 3–8 per muscle and accounted for 12.7% of the ALD total. No monofibre units were observed in the PLD.

ALD intrafusal fibre diameters ranged in size from 5.0 to 16.0 μm (mean = 10.1 μm) compared with 4.5–18.5 μm (mean = 10.7 μm) in the PLD. There was little variation in fibre diameters in monofibre units, with a mean diameter of 9.3 μm. Histogram distribution data (Fig. 3) of intrafusal fibre diameters were suggestive of a trend towards bimodality in intrafusal fibres in both the ALD and PLD. Larger diameter intrafusal fibres were more frequently encountered in the PLD. There was no marked increase of equatorial nucleation in either ALD or PLD intrafusal fibres. One to 3 eccentrically located nuclei were typically seen on cross-section. These findings confirm the results of other avian muscle spindle studies (Maier & Eldred, 1971; Adal, 1973).

Ultrastructure of the intrafusal fibres

From the morphometric measurements, intrafusal fibres of muscle spindles in both the ALD and PLD were classified into either type 1 (small diameter) or type 2 (large diameter) fibres. Type 1 fibres were the most numerous (3–5) per muscle spindle, and they were significantly smaller in cross-sectional size with a mean fibre diameter in polar regions of 7.4 ± 2.5 μm. Myofibrils in small-diameter fibres were relatively large and tightly packed (Fig. 12). They existed as poorly defined myofilament bundles which exhibited a 'Felderstruktur' appearance (Kruger & Gunther,

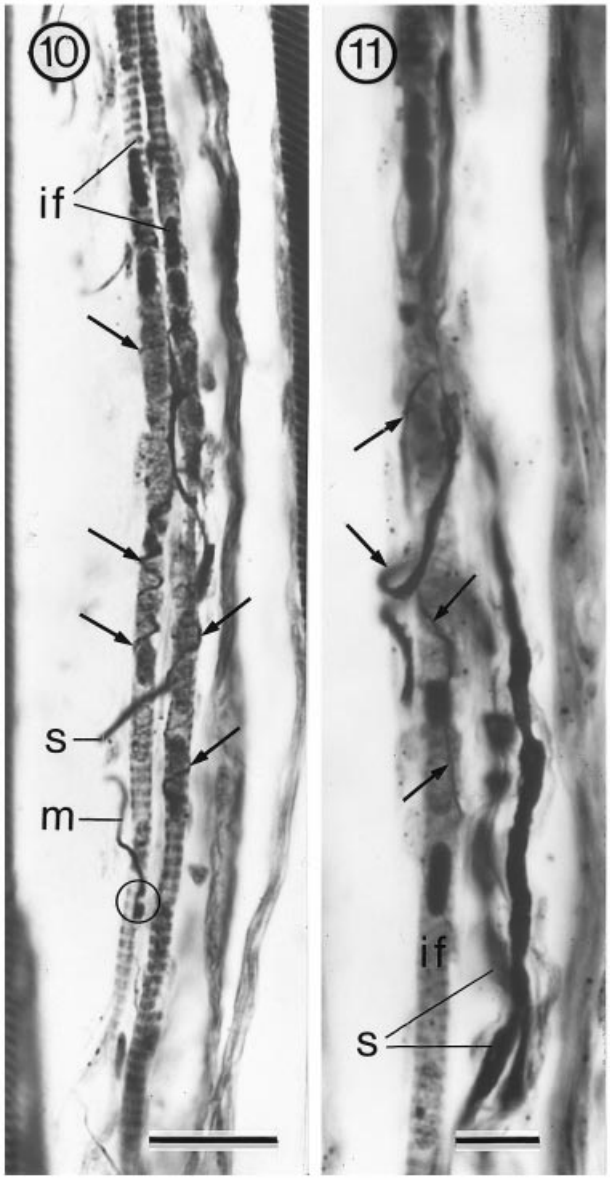
Fig. 5. Transverse section of the ALD stained with H & E. Two closely adhering spindles sectioned through polar and equatorial regions occupy the field. ×1000. Bar, 20 μm.

Fig. 6. High magnification view of the equator of a monofibre spindle. A crescent-shaped collagenous cap (arrow) abuts the sensory region of the intrafusal fibre (if). An expanded periaxial space (*) contains processes of inner capsule cells. Frozen section stained with H & E. ×1150. Bar, 25 μm.

Fig. 7. Transverse frozen section of the PLD stained with Gomori trichrome. Extrafusal fibres are grouped into pleomorphic fascicles and 2 spindles are located in the perimysium. The polar region of one spindle (upper arrow), the equatorial region of another spindle (lower arrow), and a neurovascular unit (nv) are indicated. ×175. Bar, 100 μm.

Fig. 8. Transverse Epon section of the polar region of a spindle in the PLD. Two large (arrows) and 4 small (arrowheads) intrafusal fibres are invested by a prominent capsule. A mast cell (mc) and a capillary (cap) closely abut the outer capsule. Neighbouring extrafusal fibres are indicated (EF). Toluidine blue. ×1150. Bar, 20 μm.

Fig. 9. Transverse Epon section of the equatorial region of a spindle in the PLD. Two large (arrows) and 3 small (arrrowheads) intrafusal fibres occupy a central axial compartment. An outer capsule (circles) invests an expanded periaxial space which contains processes of inner capsule cells and myelinated nerve fibres. Toluidine blue. ×1000. Bar, 20 μm.



Figs 10–11. The sensory innervation patterns of a multifibre (Fig. 10) and a monofibre (Fig. 11) spindle in the ALD are seen in longitudinal section treated with the Bielschowsky silver stain.

Fig. 10. A sensory axon (s) terminates in the equatorial zone of 2 intrafusal fibres (if) in an annulospiral fashion (arrows). A small motor axon (m) is also seen terminating in the juxtaequatorial region (circle). ×840. Bar, 20 μm.

Fig. 11. In this monofibre spindle, 2 sensory axons (s) form complex spiral endings (arrows) on the surface of a solitary intrafusal fibre. ×1100. Bar, 10 μm.

1955) due to their irregular shapes in transverse section. The mean myofibril cross-sectional area was 1.1 ± 0.2 μm². Mitochondria within these fibres were small and sparse, and they usually occurred as either isolated or paired organelles in the intermyofibrillar spaces.

The type 2 intrafusal fibres were considerably larger in size with their mean fibre diameter measuring 11.7 ± 1.6 μm. Myofibres in this fibre type were small, rounded, well demarcated bundles which exhibited a 'Fibrillerstruktur' appearance (Kruger & Gunther, 1955) in cross-section (Fig. 12). The mean myofibre area was 0.6 ± 0.2 μm². Mitochondria were larger and more abundant than in type 1 fibres, and often occurred in long, closely-packed rows in the intermyofibrillar spaces. Peripherally placed, ovoid nuclear profiles were present in the majority of the intrafusal fibres in polar and juxtaequatorial regions, whereas equatorial regions contained slightly eccentric, spherical nuclei especially in the large diameter fibres. Intermediate-sized intrafusal fibres (approximately 8–10 μm in diameter) that were commonly encountered in the PLD shared morphological features of both the large and small diameter fibres.

Satellite cells were observed in equatorial and juxtaequatorial regions of both types of intrafusal fibres (Figs 12–14). These mononucleated cells were situated on the surface of the intrafusal fibres. They were invested by the intrafusal fibre basal (external) lamina and were less electron dense than the adjacent intrafusal fibres containing an array of ribosomes, rough endoplasmic reticulum, mitochondria and glycogen. The majority of these cells were associated with sensory terminals (Fig. 14), although isolated profiles of satellite cells were present in juxtaequatorial and polar regions of the intrafusal fibres (Figs 12, 13).

At high magnification, electron-dense plaques resembling macula adherens junctions were present between the adjacent borders of the satellite cell and intrafusal fibre (Fig. 12 inset).

Sensory and motor nerve terminals

Sensory nerve terminals were seen in equatorial and juxtaequatorial regions of all types of intrafusal fibres. At the light microscopic level, silver-stained longitudinal sections of muscle spindles revealed large-diameter axons which encircled the intrafusal fibres in an annulospiral fashion (Figs 10, 11) resembling to a degree the primary endings seen in mammalian spindles. By electron microscopy, sensory nerve terminals were in intimate contact with the intrafusal fibre and shared the same basal lamina (Fig. 14). They were filled with small tubular mitochondria, clear and dense-cored vesicles, neurofilaments and occasional lysosomes (Fig. 14). On the surface of the intrafusal fibre and adjacent to the sensory nerve terminals were small satellite cells (Fig. 14). They typically intervened between the 2 crescent-shaped sensory nerve profiles and were covered on their outer surface by a common basal lamina.

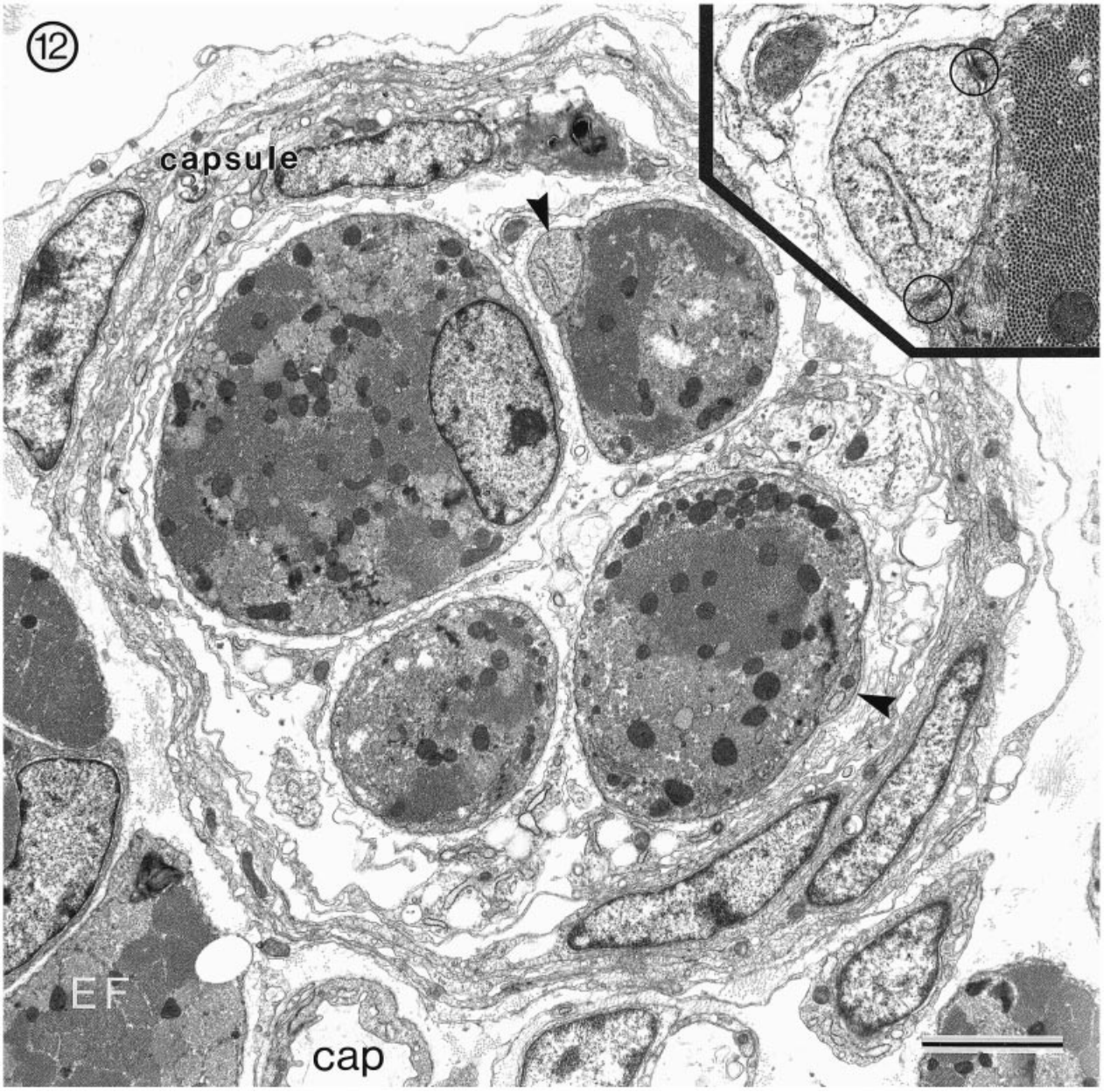


Fig. 12. Juxtaequatorial region of a spindle in the ALD viewed by electron microscopy. Four intrafusal fibres are surrounded by several layers of flattened outer capsule cells that possess discrete epithelial extensions separating each intrafusal fibre. Portions of 2 satellite cells are seen on the surface of the intrafusal fibres. An extrafusal fibre (EF) and a capillary (cap) lie adjacent to the outer capsule. Inset: higher magnification of a portion of a satellite cell seen on one of the intrafusal fibres. Adhesion junctions resembling that of the macula adherens type are present at its lateral borders (circles). ×4500. Bar, 5 μm. Inset ×13,000.

Between the sensory nerve terminal and intrafusal fibre a small gap measuring 20 nm in width and lacking an intervening basal lamina was present. Both surfaces were highly corrugated and maintained a close apposition throughout their contact regions. The sarcoplasm of intrafusal fibres at myosensory junctions consisted of a conspicuous array of smooth tubular aggregates with an overall lack of myofibrillar structures. In addition, large accumulations of mitochondria were often seen in parajunctional regions of the sarcoplasm and typically surrounded the eccentrically located euchromatic nucleus (Fig. 14).

A collagenous perifibre sheath, or collagenous crescent-shaped cap (Barker, 1974; Hikida, 1985), partially encircled the intrafusal fibres in equatorial regions (Fig. 14). It consisted of tightly packed collagen fibres which formed a crescent on one side of the sensory terminal and contained processes of inner capsule cells intermingled within the collagen fibre bundles (Fig. 14). The sheath was thicker in equatorial

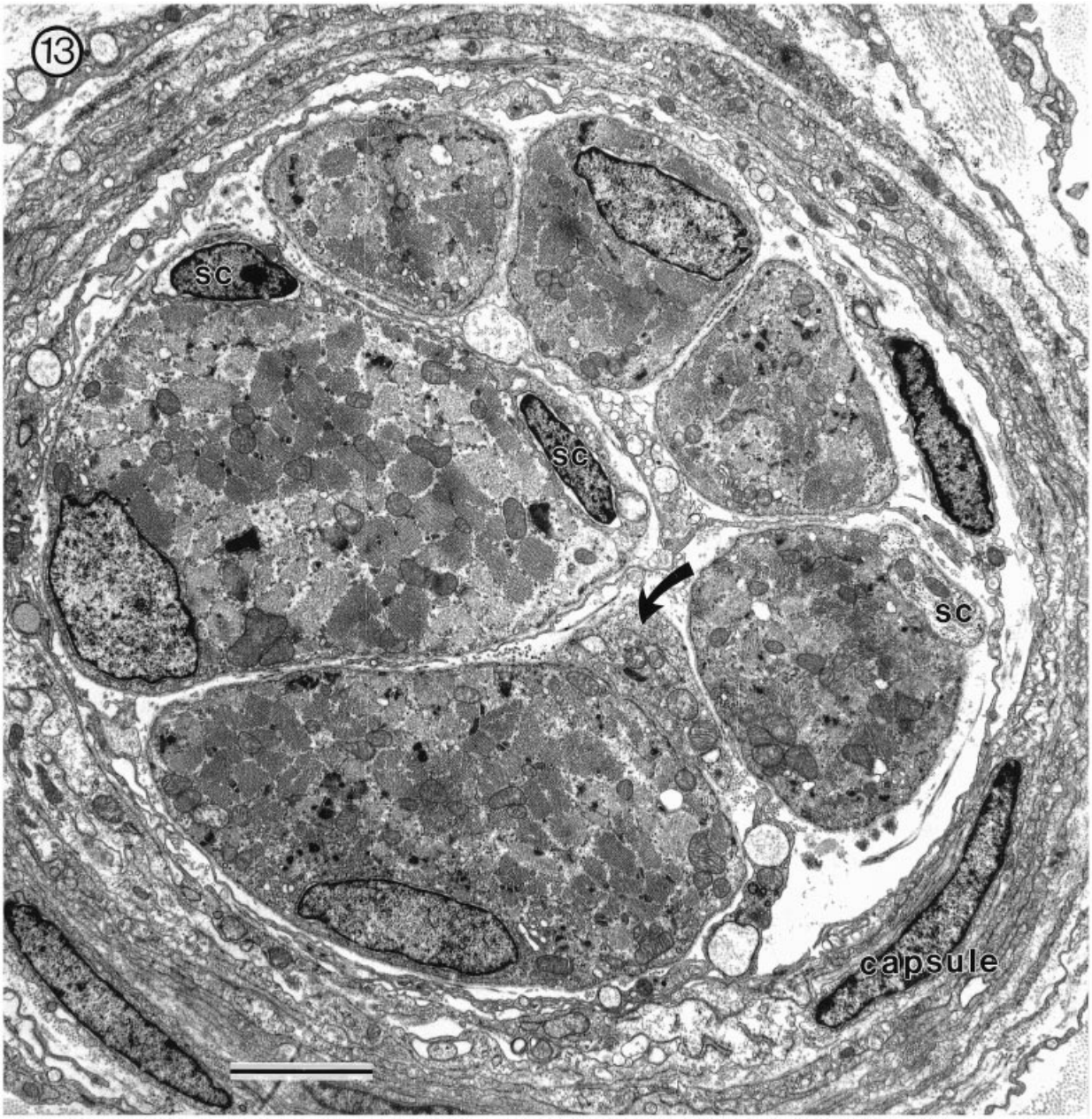


Fig. 13. Juxtaequatorial region of the same PLD spindle seen in Fig. 8 viewed by electron microscopy. A multilayered outer capsule envelops 6 intrafusal fibres. The 2 large-diameter fibres contain small and well delineated myofibrils which exhibit a punctate pattern and are interspersed with prominent mitochondria. The 4 small-diameter fibres contain irregular and poorly defined myofibrils with a 'felderstruktur' appearance. Peripherally-placed nuclear profiles are seen on some of the intrafusal fibres. A motor nerve terminal (curved arrow) and satellite cells (sc) are indicated. ×4500. Bar, 5 μm.

regions adjacent to sensory terminals and gradually disappeared in juxtaequatorial regions. The precise function of the sheath is presently unknown, although it has been suggested that this collagen-rich structure protects sensory terminals from undue distortion (Hikida, 1985).

Motor nerve terminals were present in both juxtaequatorial and polar regions of the intrafusal fibres (Figs 10, 15, 16). Both plate and trail-like endings were revealed by silver-staining methods (Fig. 10). Ultrastructurally, their outer surfaces were covered by thin Schwann-cell processes which appeared to seal the edges of the synaptic cleft by abutting with the intrafusal fibre (Fig. 16). The synaptic cleft ranged in width from 80 to 120 nm and contained a fused or thickened intervening basal lamina (Fig. 15). Within the motor nerve terminal, numerous uniformly-sized vesicles (measuring 40 nm in diameter) and large mitochondria were present. The postjunctional surface of the intrafusal fibre contained discrete sarco-

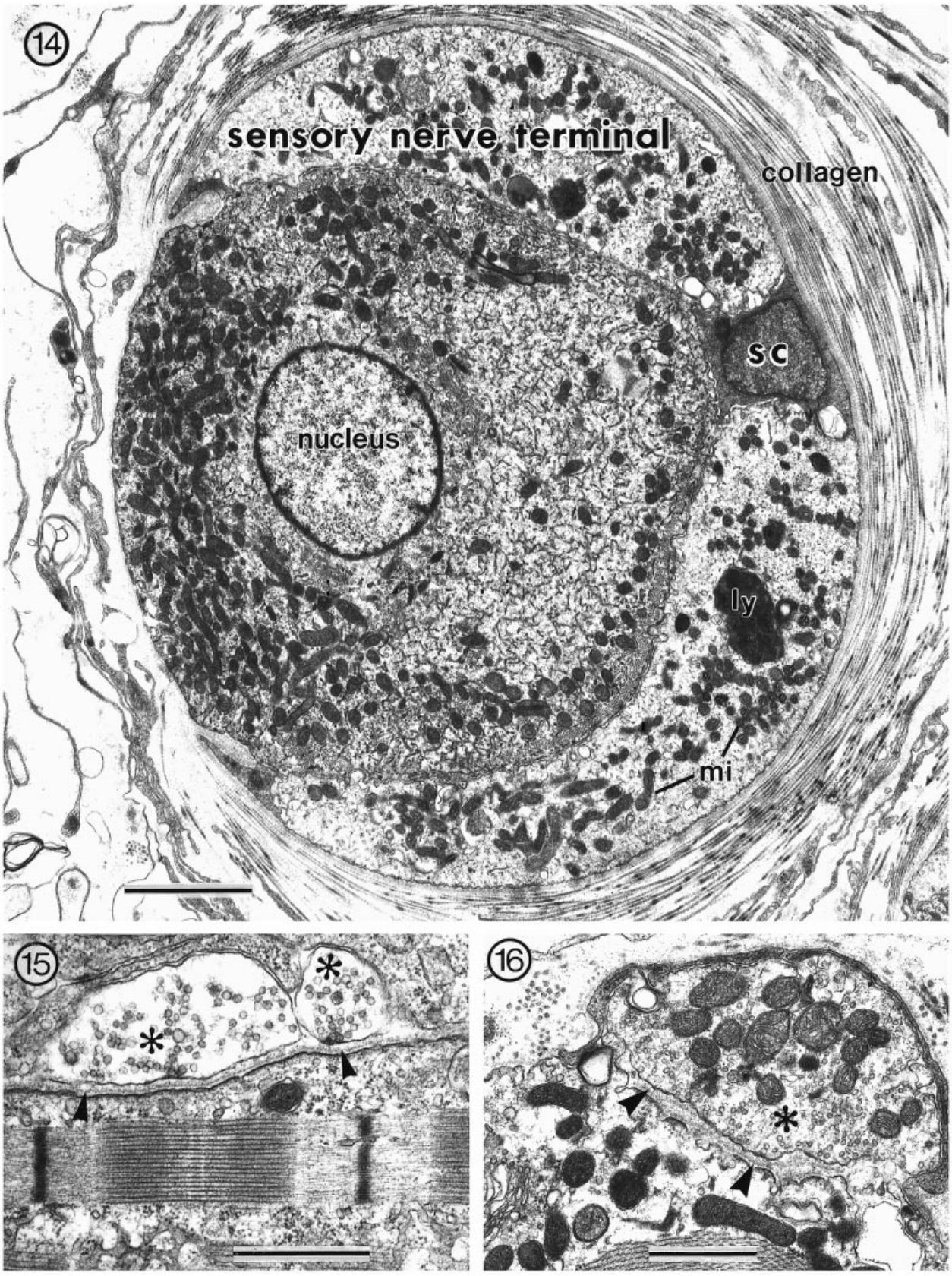


Fig. 14. Electron micrograph. Transverse section of the equatorial region of a monofibre spindle in the ALD. A sensory nerve terminal wraps around the external surface of the single intrafusal fibre, sharing its basal lamina. A satellite cell (sc) intervenes between the 2 crescent-shaped sensory nerve profiles. The intrafusal fibre exhibits an eccentric nucleus and its sarcoplasm contains an abundance of mitochondria and a prominent network of smooth-surfaced tubules. A plethora of mitochondria (mi), lysosomes (ly), and neurofilaments occupy the sensory nerve terminal. Tightly packed collagen fibres form a cap on one side of the sensory terminal and processes of inner capsule cells are seen on the left. $\times 10600$. Bar, 2 μm.

lemmal infoldings and intermittent electron-dense plaque regions along the membrane, whereas the underlying sarcoplasm of the intrafusal fibre was typified by numerous mitochondria, distended cisternae of the sarcoplasmic reticulum, and occasional Golgi complexes (Fig. 16). This, however, was not common for all motor nerve terminals on the intrafusal fibres (see Fig. 15). No attempt was made to distinguish putative subtypes of motor axons or their terminations on the intrafusal fibres.

DISCUSSION

The results of the present study demonstrate that muscle spindles in the ALD are different from those in the PLD quantitatively, morphologically and in their relationship with surrounding extrafusal muscle fibres. Statistical data from our study indicate that the ALD contains, on average, 52% more muscle spindles than the PLD and has a muscle spindle index of even greater value. The large disparity in density of muscle spindle units in the 2 muscles may reflect their different functions, probably in a manner similar to that which is thought to occur in slow and fast-twitch mammalian muscles. For example, the slow-twitch soleus muscle of the cat was reported by Swett & Eldred (1960) to have a significantly higher muscle spindle index than the adjacent fast-twitch medial gastrocnemius. Small postural muscles of the cat neck have been shown to contain up to 500 muscle spindles per gram muscle weight (Richmond & Abrahams, 1979), and large muscles maintaining overall head position not only have high muscle spindle densities (46–610 per gram) but contain the majority of spindles in regions of predominantly slow-twitch extrafusal fibres (Richmond & Abrahams, 1975*b*).

The principal action of the avian ALD and PLD muscles would appear to be adduction of the humerus; however, the ALD also supports the wing and prevents it from drooping (Vrbova et al. 1978). The PLD, on the other hand, demonstrates maximum isometric tension with the wing folded next to the body (Shear, 1978). The ALD contracts slowly, attains relatively low tension, but can maintain maximum tension for long periods of time. The PLD, in contrast, can change length rapidly and attain high tension, but is unable to sustain prolonged contractions (Ginsborg, 1960*a*, *b*). It is postulated that the anatomically separate avian ALD and PLD muscles work in a similar manner to achieve and maintain optimum wing position, i.e. the PLD acts as the prime mover in adduction of the wing while the ALD stabilises the wing in the appropriate position attained by its synergist. It is in this way that slow-contracting fibres are thought to maintain the position developed by fast-twitch fibres so that they work as functional synergists (Vrbova et al. 1978).

In comparing 2 synergistic muscles, it is possible that spindle densities per gram may not reflect actual function since the spindles and the extrafusal fibres in which they are surrounded need not be a functional unit. It has been suggested that muscle spindles may be 'dislocated' in, for example, a synergist muscle which acts in parallel across a joint, as is the case with the ALD and PLD. Smaller, spindle-rich muscles such as the ALD would perform a guiding action which controls the larger parallel muscle, thus acting as 'kinesiological monitors' (Peck et al. 1984). It would be of interest to compare the size of the motor units between these 2 muscles, correlate this with the spindle density of each muscle, and perform physiological studies on possible parallel reflex arcs in either muscle.

The muscle spindle distribution tended to follow the overall pattern of innervation, particularly in the PLD where 50% of the total number of spindles was found in the distal third of the muscle in the region where the median nerve enters and immediately divides. The ALD, as might be expected, had a relatively even distribution from origin to insertion, a feature which may reflect its function in monitoring small changes in muscle fibre length over a wide range of limb motion. Moreover, ALD muscle spindles were usually located between, rather than within, muscle fascicles and were only found in thick planes of connective tissue which isolated these receptors from surrounding structures. In contrast, a more intimate association of muscle spindles with extrafusal fibres

Figs 15, 16. Electron microscopic views along different regions of an intrafusal fibre in an ALD monofibre spindle at sites of contact with motor nerve terminals. In both sections, a basal lamina (arrowheads) occupies the synaptic cleft between nerve and muscle.

Fig. 15. Longitudinal section in the polar zone showing 2 axonal profiles (asterisks) filled with synaptic vesicles and resting on the surface of the muscle fibre. ×23000. Bar, 1 μm.

Fig. 16. Transverse section in the juxtaequator showing an axonal profile (asterisk) in contact with a slightly-infolded sarcolemmal membrane. Mitochondria and synaptic vesicles occupy the axon terminal. The sole-plate region of the intrafusal fibre contains many closely-packed organelles including a Golgi complex on the left. ×18500. Bar, 1 μm.

was seen in the PLD where spindles were found either at the periphery or enclosed within groups of extrafusal fibres. It is likely that the ALD, which was divided by large planes of connective tissue, may function as a group of interrelated subunits which are supplied by independent branches of the median nerve. This has been noted to be reminiscent of some slow-twitch postural muscles of the cat neck (Richmond & Abrahams, 1975*a*). In contrast, no evidence of dense connective tissue inscriptions was observed in the PLD.

The outer capsule of ALD muscle spindles was generally thicker than that of spindles in the PLD, even though at the light microscopic level it was sometimes difficult to distinguish the capsule from the surrounding interfascicular perimysium. It can be surmised that the contents of ALD spindles are more isolated from the surrounding extracellular space than those of the PLD, particularly in the polar region where the capsule is well developed (Ovalle, 1976). Bridgeman & Eldred (1964) have suggested that the outer capsule might function as a pressure sense-organ. In view of the more intimate relationship between PLD muscle spindles and surrounding extrafusal muscle fibres, it had been postulated (Ovalle, 1976) that muscle contraction would produce a greater amount of lateral pressure on the contents of its spindles than those of the ALD.

Classification of avian intrafusal fibres by size has been attempted by many researchers (Rebollo & DeAnda, 1967; Maier & Eldred, 1971; Adal, 1973; Maier, 1977) although variations in the data still exist. One to 4 types of intrafusal fibres have been described in birds (Maier, 1977, 1983; Ovalle, 1978; Uehara, 1978; Toutant et al. 1981; Hikida, 1985). Earlier work in our laboratory has revealed 2 types of intrafusal fibres, types 1 and 2, which are distinguishable by myosin adenosine triphosphatase (ATPase) histochemical staining characteristics (Ovalle, 1978). Morphometric data from our study suggested 2 intrafusal fibre populations in muscle spindles of the ALD and PLD, with smaller diameter fibres being more prevalent in the ALD. More recently, immunocytochemical studies have shown that avian intrafusal fibres can be subdivided into 3 types based on differences in their myosin heavy chain composition (Maier & Zak, 1990; Maier, 1991, 1992) and motor innervation (Maier, 1991). A review by Maier (1992) contains a compiled list of results for avian intrafusal fibre classifications.

Ultrastructurally, sensory and motor innervation of the intrafusal fibres did not differ between the 2 muscles and showed similar morphology to that reported in other studies on avian spindles (Adal, 1973; James & Meek, 1973; Adal & Chew Cheng, 1980; Hikida, 1985). Sensory endings were encountered throughout the equatorial and portions of the juxtaequatorial regions and consisted exclusively of primary-type sensory endings; no secondary type of sensory endings, such as flower-spray endings reported in mammalian spindles, were present. Motor terminals, on the other hand, were located in juxtaequatorial and polar regions and were characterised by little or no postsynaptic infolding.

In addition to sensory and motor endings, satellite cells which lie in close apposition to the sensory nerve terminals and the intrafusal fibres were observed. Dense plaques resembling macula adherens junctions were particularly evident between intrafusal fibres and the adjacent borders of satellite cells. It has been postulated that these cells consist of 2 types, one which is associated with sensory nerve terminals, known as the sensory satellite cell, and one which is associated with the intrafusal fibre, referred to as the myosatellite cell (Hikida, 1985). The former cells are believed to be necessary for the process of regeneration of intrafusal fibres (Maier, 1997) whereas the latter may be a modified form of Schwann cell which preferentially associates at the intrafusal fibre surface with the sensory nerve terminals (Hikida, 1985). If this is the case, the sensory satellite cell represents a different structural organisation at the myosensory junction from that seen on mammalian intrafusal fibres where Schwann cells are normally absent.

Exclusive to the ALD was the presence of monofibre muscle spindles reminiscent of those found in the snake and lizard (Pallot & Ridge, 1971; Proske, 1973). Two functional kinds of monofibre spindles have been identified in the snake (Fukami, 1970); however, on morphological grounds, only one type was found in our ALD samples. Since monofibre spindles did not appear in any PLD muscles examined, it is surmised that the influence of sensory innervation patterns during development in the ALD differs with respect to the number of sensory nerve contact sites with myotubes. While the functional significance of avian monofibre spindles remains obscure, they appear to be morphologically similar in many respects to multifibre spindles observed in this study and published previously by other investigators (Hikida, 1985; Maier, 1992).

In summary, the results of this study provide evidence that the muscle spindle density in the slow ALD is more than double that of its fast PLD counterpart. It is postulated that the large number of spindles which are spread throughout the ALD are

necessary to provide precise information on contractile activity and to monitor changes in muscle length over a wide range of motion. It is hoped that a more complete understanding of the distribution and morphology of these neuromuscular receptors will contribute to a better understanding of their complex functions.

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