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Spiral nerve endings in human extraocular muscles terminate in motor end plates

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INTRODUCTION

Extraocular muscles are singular in possessing nerve endings that spiral around muscle fibres unrelated to muscle spindles (Daniel, 1946). The simplest form of spiral ending consists of a myelinated nerve fibre tightly applied to the muscle fibre for several turns in a helical fashion before terminating. Should the nerve divide, other forms are produced such as multiple spirals and clasp or pincer-like endings. They have been noted in cats and monkeys (Cooper & Fillenz, 1955), but illustrated and described adequately only in man (Daniel, 1946; Sas & Appletauer, 1963) until recently when details of their fine structure in monkeys have been presented (Ruskell & Wilson, 1983). A variety of spiral ending, distinguished from the others by the presence of a thin capsule, is considered to represent an atypical muscle spindle (Sas & Appeltauer, 1963).

The sensory nature of spiral endings was proposed by Daniel (1946), who argued that their form "... is designed to record the cross-sectional expansion which follows the contraction of the encircled muscle fibres". A contrary view has been expressed by Ruskell & Wilson (1983), who note the morphological and histochemical similarity of spiral endings and motor end plates in monkey extraocular muscles. The present study was undertaken to see whether or not the structure of spiral endings in human extraocular muscles share the motor features of monkey spirals.

MATERIALS AND METHODS

Muscle samples were taken from the orbits of five patients after enucleation of an eye, either for malignant melanoma (two cases) or because they were blind and painful. The patients were aged 25, 30, 42, 70 and 74 years and they had no record of impairment of eye movements or of neurological or muscular disease. Five inferior oblique muscles and three medial, two superior and two inferior recti muscles were sampled; each piece was of full width and included the motor end plate band. Muscle samples were immersed without further cutting and with little delay, in cold 5% glutaraldehyde buffered with 0·1 M sodium cacodylate, pH 7·4, and stored in the fixative at about 4 °C. Most muscles were cut into slender pieces, suitable for electron microscopy, up to 6 mm in length along the muscle axis. Others were cut into full transverse slices about 2 mm thick. Tissues were transferred from fixative, after washing, to a 1% unbuffered solution of osmium tetroxide for one hour and subsequently passed through graded ethanols and xylene, and then embedded in Araldite. Sample semithin sections were cut at different positions along each muscle to give transverse or longitudinal sections of the fibres, and full or interrupted serial

sections were cut from appropriate blocks, mainly from the motor end plate region. The sections were stained with toluidine blue. Some of the full transverse pieces were divided into smaller pieces after initial histological examination. Thin sections, suitable for electron microscopy, were taken at intervals from the serially sectional blocks, mounted on copper grids, immersed in a saturated solution of uranyl acetate in 30 or 70% ethanol for about 20 minutes and then, after washing, in 0·4% lead citrate in 0·1 N sodium hydroxide for 10 minutes.

One of the inferior oblique muscle samples was fixed in 10% buffered formalin and a small piece removed, teased and stained for acetylcholinesterase using a copper thiosulphate method (Karnovsky & Roots, 1964). The fibres were then teased again and sometimes exposed briefly to osmium tetroxide vapour to obtain a light staining of myelin before mounting on slides.

The collection of silver-stained slides of human extraocular muscles, used by Daniel (1946) and by Cooper, Daniel & Whitteridge (1955) in their pioneering studies of nerve endings, was kindly given to the author by Professor Whitteridge on a recent visit to his laboratory so that they might be compared with the preparations of this study. They have been fully re-examined and it was felt unnecessary to prepare any further specimens stained with silver.

RESULTS

Silver preparations

Examination of the thick sections stained with silver confirmed Daniel's (1946) original description of human muscle spiral endings as summarised in the Introduction. Certain features not described by Daniel, but pertinent to the present study, were noted. Most of the spiral and other complex nerve endings identified by Daniel gave off several fine, mostly unmyelinated, branches which terminated on the muscle fibres within the length of the spiral. The branches usually terminated in small, fine rings as shown in Figure 1 and, significantly, structures of similar form and size were observed in many neuromuscular junctions identified as typical motor end plates. The rings were arranged in several well separated clusters disposed on different aspects of the muscle fibre and spread over a length usually greater than that of conventional motor end plates, up to a maximum of 220 μm.

New material

Arcing of myelinated nerve fibres around muscle fibres was observed in single transverse semithin sections, especially in the region of the motor end plate band. Inspection of serial sections showed that the arcs were often segments of spirals (arbitrarily defined here as endings completing at least one full turn). Most of the spirals made one to three turns around muscle fibres in a helical fashion before terminating. In some cases, two or more fibres contributed to the spiral and in others, the nerve fibre divided to form a spiral in each direction along the muscle fibre. Serpiginous and clasp-like terminating fibres were also present.

At the commencement of a spiral the nerve fibre was often still within its perineural sheath, emerging from the sheath during the course of the spiral. The perineural sheath and, subsequently, the nerve fibre were applied to the muscle surface so tightly that they indented the sarcoplasm, which bulged on each side. The spacing of the turns was often almost regular and the associated helical furrows imprinted on the muscle fibre surface suggested a pattern like the thread of a bolt pin.

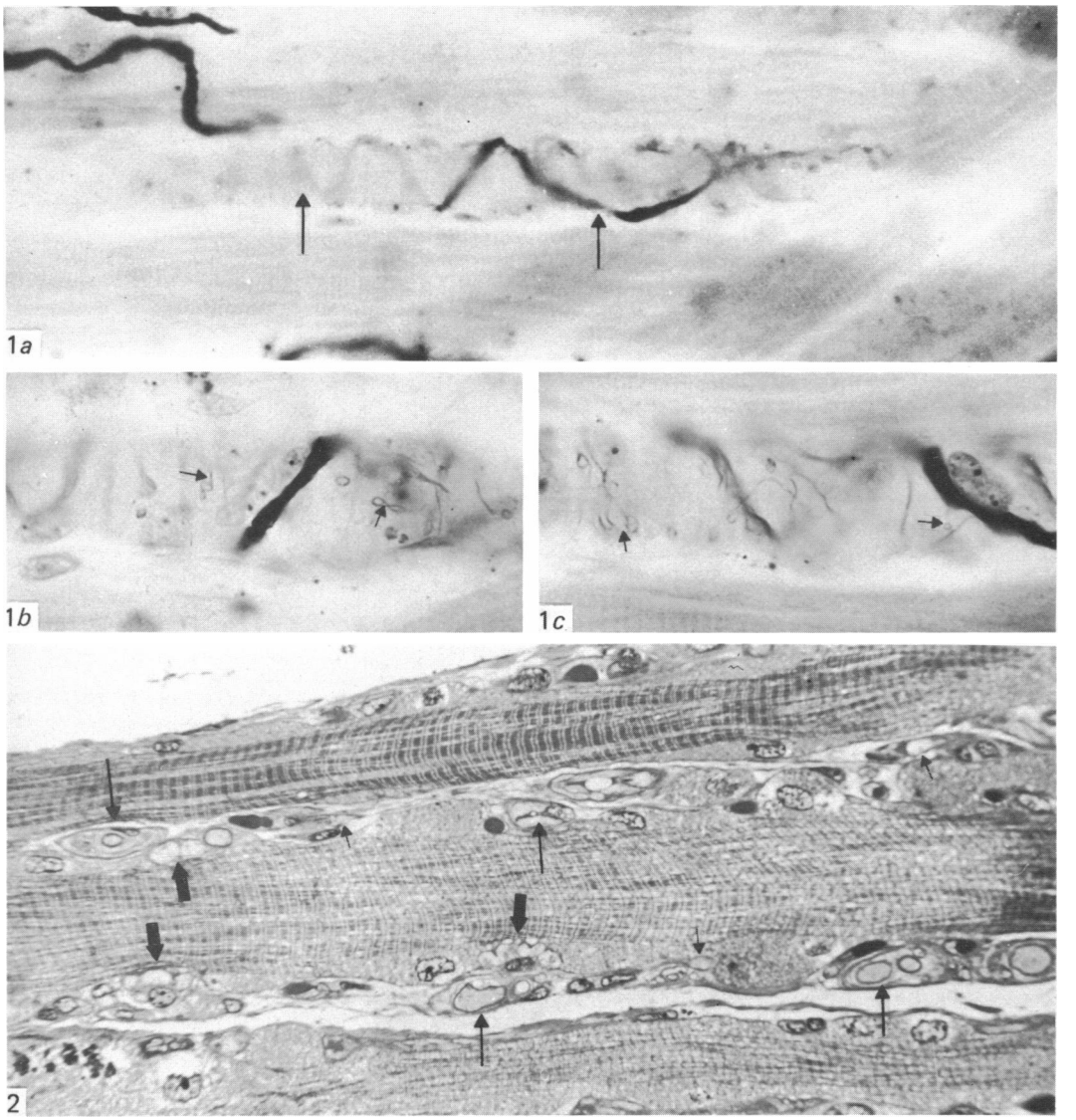
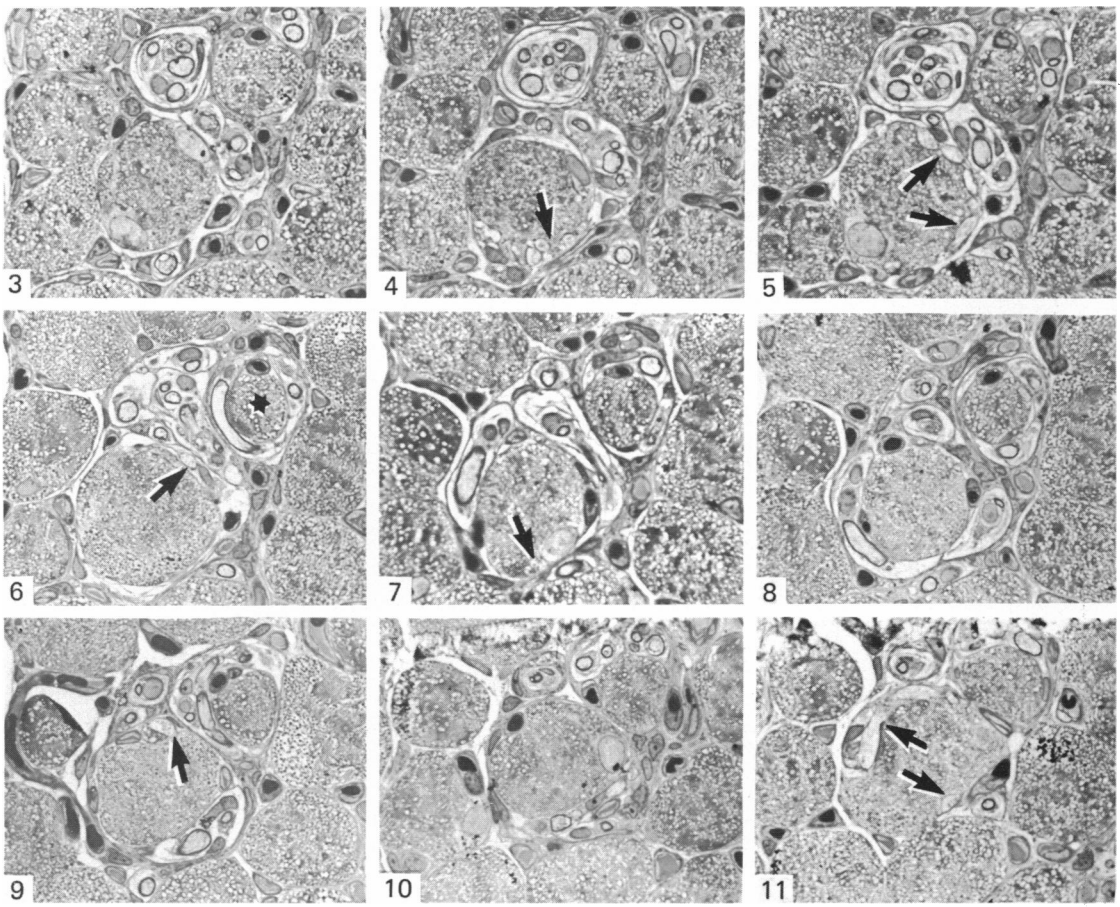


Fig. 1 (*a–c*). Complete muscle spiral with three loops. The portion lying between the arrows in (*a*) is represented in (*b*) and (*c*) with higher magnification, focussed at the top and deep surfaces to show the fine branches from the spiral and some of their numerous circular and oval terminals (small arrows). Lateral rectus muscle, Gros–Bielschowsky technique. From a preparation originally used by P. Daniel and given to the author by D. Whitteridge. × 560 and × 920.

Fig. 2. Longitudinal section through a Fibrillenstruktur muscle fibre displaying well separated groups of motor end plate boutons (thick arrows). The preterminal myelinated nerve fibre profiles indenting the sarcoplasm (long arrows) belong to a single spiral ending. Several unmyelinated branches (short arrows) are present. Inferior rectus muscle. Toluidine blue. × 535.

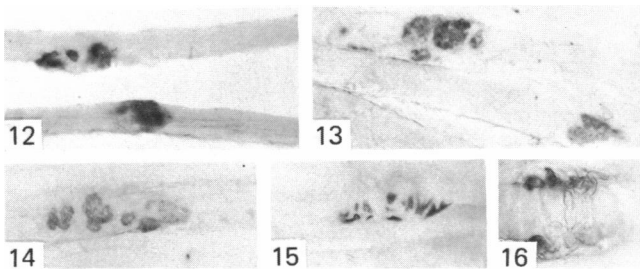


Figs. 3–11. Serial transverse sections through a spiral nerve ending; a selection presented in sequence. Figs. 4, 5, 6, 7, 9 and 11 display end plate boutons (arrows). A sheath encloses the muscle fibre in Figs. 7, 8 and 9. Evidence of spiralling of both the principal myelinated axon and unmyelinated branches is discernible in several of the Figures. A second, small, sheathed muscle fibre with a spiral nerve ending is marked with a star in Fig. 6. The distance separating Figs. 3–11 is 120 μm. Inferior oblique muscle. Toluidine blue. × 516.

Spiral endings were quite frequent but they represented a small proportion of all the terminals in the end plate band. Surveys of 100 to 320 terminals in each of six muscles sectioned transversely (four inferior oblique and two superior recti muscles) showed that 2–11 % of the endings were spirals with a mean of 5 %.

Nearly all spiral endings gave off several unmyelinated branches; five or six primary or secondary branches were common and as many as twelve were present. Longer branches often followed the angle of the spiral to their termination but otherwise the directions taken appeared random.

Spiral ending branches terminated in neuromuscular junctions consisting of clusters of boutons characteristic of motor end plates. However, unlike conventional motor end plates, several discrete bouton clusters, often well separated, were applied to several aspects of single muscle fibres. The bouton clusters therefore corresponded to the clusters of rings found in the silver-stained preparations.



Figs. 12–16. Teased muscle fibres stained for acetylcholinesterase. Figs. 12 and 13 each show two motor end plates, one of them continuous, the other discontinuous or dapple. Figs. 14–16 show discontinuous end plates of different forms, and additional light staining with osmium tetroxide vapour reveals the myelinated spiral nerve ending in Fig. 16. Inferior oblique muscle. Magnification varies from ×130 to ×220.

Separate clusters were readily apparent in semithin longitudinal (Fig. 2), and in transverse (Figs. 3–11) sections, in which up to nine separate groups of boutons were recognised and five or six groups were common. The acetylcholinesterase technique also revealed satisfactorily the discontinuity of end plates (Figs. 12–16), but few examples of more than five stained loci were seen, although eleven were present on one muscle fibre. Disparity in the number of bouton groups seen in semithin sections and acetylcholinesterase-stained material may have been attributable to the limited amount of tissue used for the histochemical method.

A few spiral endings were associated with a single cluster of boutons in the conventional manner of motor end plates. Such spirals were unbranched except for the short terminal branches. They were mostly located near the periphery of muscles and some were well removed from the motor end plate band.

The size of motor end plates varied considerably. Those of spiral nerve endings with a continuous end plate were 10–40 μm wide, which was approximately the size range of non-spiralling motor endings. The bouton clusters of discontinuous end plates were often small, a majority having a greatest width of 6–12 μm, but the total number of boutons, or the area stained for acetylcholinesterase, was usually larger, often much larger, than in conventional, continuous end plates. The distance from the first to the last cluster varied from about 40 μm (with two clusters), to 360 μm (with nine clusters), but one exceptionally long group, 600 μm in length, was found.

The fine structural features of spiral nerve endings were fully consistent with those of motor end plates. Boutons contained large numbers of agranular vesicles, about 50 nm in diameter, more or less aggregated towards the side opposite the muscle fibre (Figs. 17, 18). Small tightly packed groups of vesicles were applied to the synaptic membrane at foci of increased density (Fig. 18). Some sections displayed boutons packed throughout with vesicles, while others possessed large areas free of vesicles. These areas often contained mitochondria, which were the second most prominent organelle.

A basal lamina occupied the synaptic cleft of all junctions, and post-junctional folds were regularly present, but they were usually shallow and irregular in form (Figs. 17, 18).

Muscle fibres were thickened at spiral motor end plates by large accumulations of subsarcolemmal mitochondria, which were displaced to accommodate the indenting

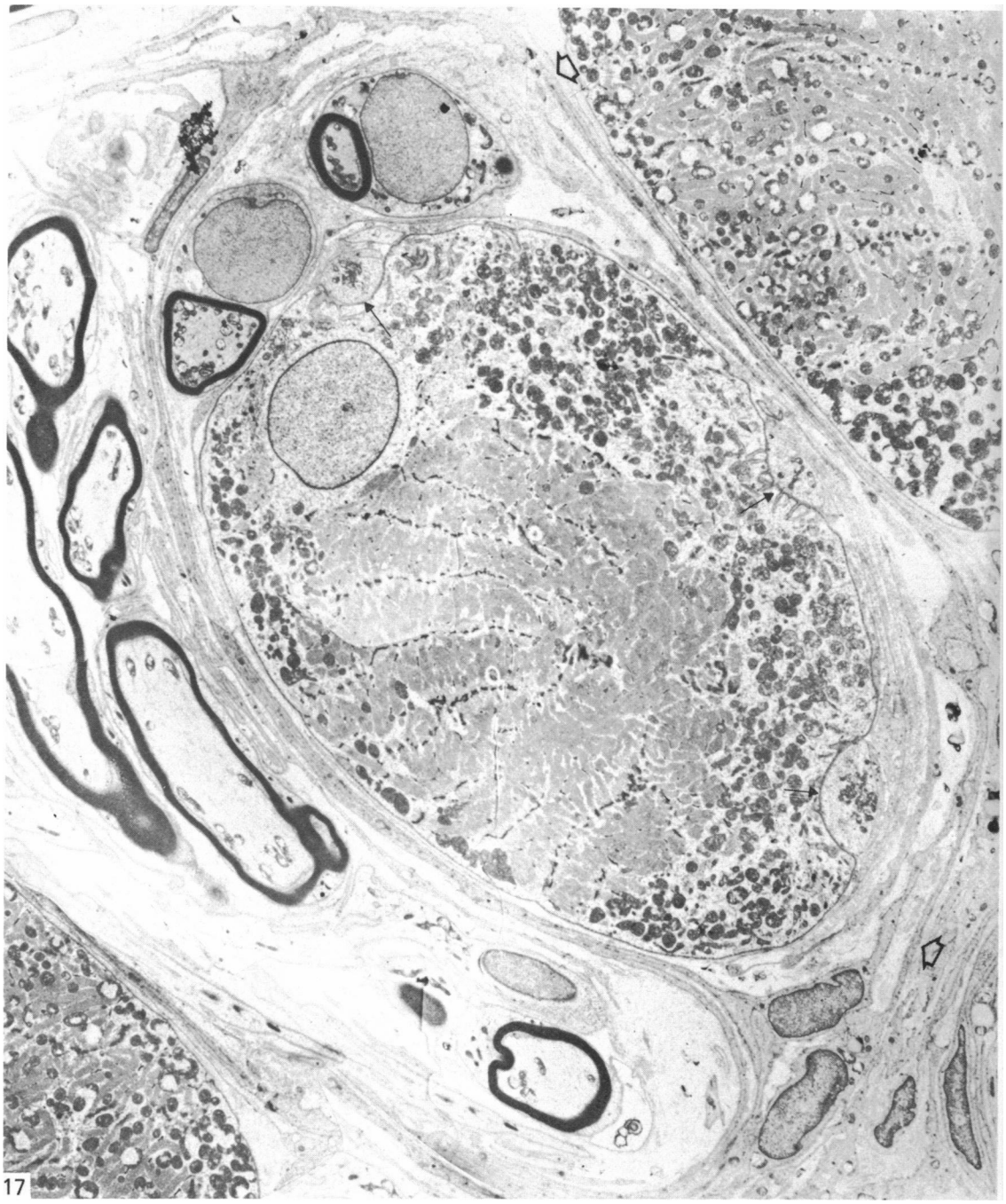


Fig. 17. Montage of a transverse section of a spiral nerve ending. The perineural epithelial layers of the spiralling nerve have split to form a sheath (arrowheads) around the muscle fibre and some nerve fibres lie within the sheath. Three motor end plate boutons (small arrows) form neuromuscular junctions that have postsynaptic folds. Inferior oblique muscle. × 3730.

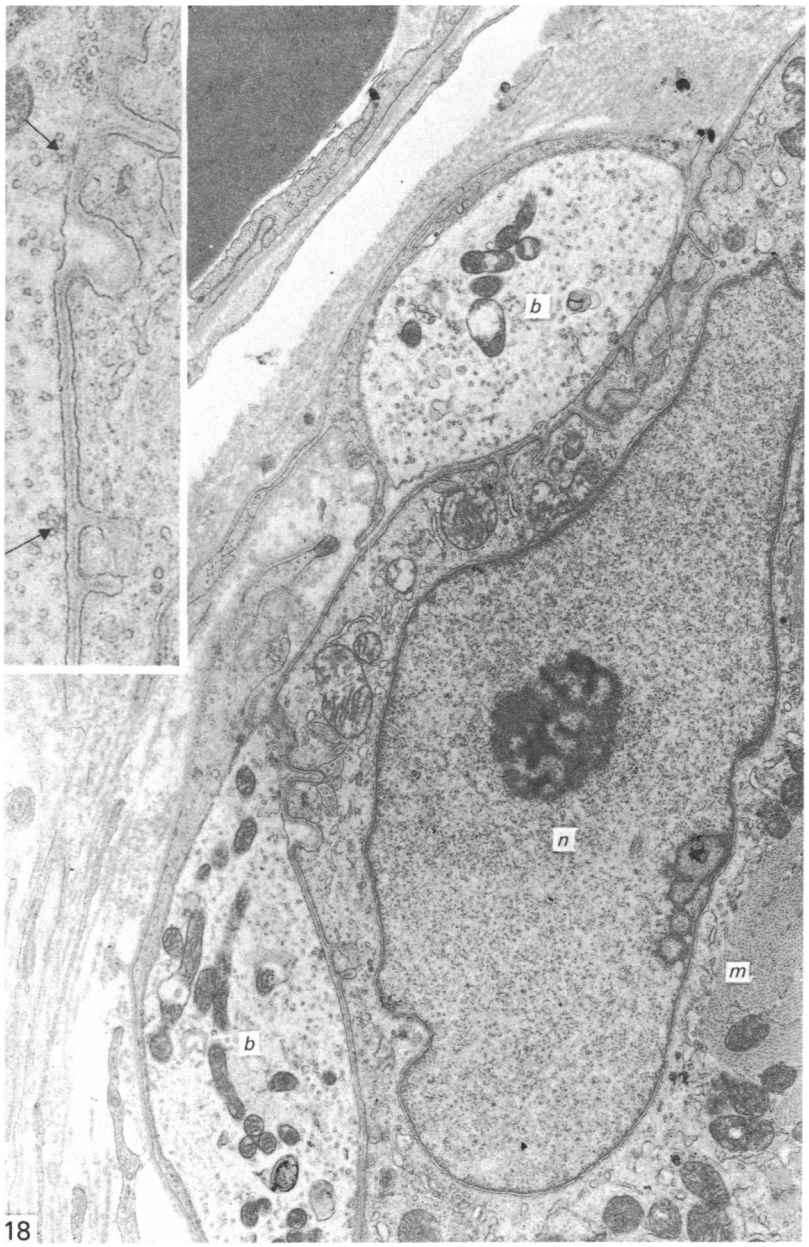


Fig. 18. Two motor end plate boutons (*b*) of a spiral ending showing their content of vesicles, a basal lamina in the synaptic cleft, and shallow postsynaptic folds, irregular in form. The inset enlargement shows small clusters of vesicles adjacent to the axon membrane at zones of increased density (arrows). *m*, myofilaments; *n*, nucleus. Inferior oblique muscle. × 12000 (inset × 27300).

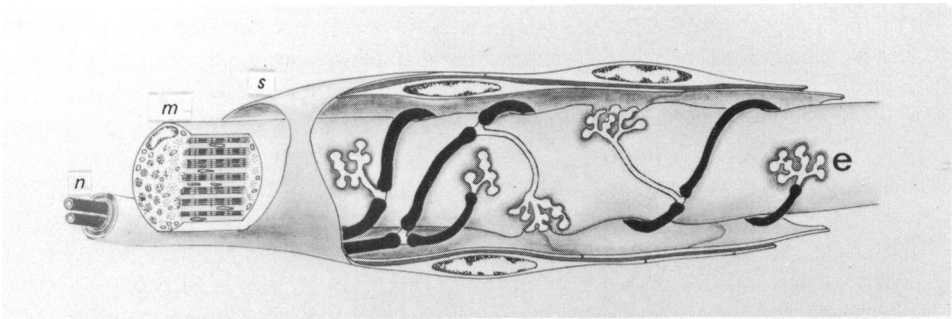


Fig. 19. Schematic drawing of a sheathed spiral nerve ending with a dapple motor end plate. Part of the sheath is removed to reveal the clusters of boutons and the spiralling axon. e, end plate bouton clusters; *m*, muscle fibre; *n*, nerve; *s*, sheath.

nerve fibre coils, and by sole plate nuclei. All fibres receiving spiral nerve endings were of the Fibrillenstruktur type with discrete myofibrils and a well developed sarcoplasmic reticulum. A few of the fibres displayed Ringbinden opposite neuromuscular junctions and elsewhere, with the peripheral myofibrils orientated circularly or spirally around the normally disposed central myofibrils. The unusual disposition of the myofibrils of Figure 17 heralded the commencement of a Ringbinden. A predisposition for Ringbinden in fibres with spiral endings is unlikely, as they appeared to be equally common among other muscle fibres, but their high frequency in muscles at the age of 74 years was consistent with evidence that they are a feature of ageing in extraocular muscles (Muhlendyck & Ali, 1978).

Approximately two thirds of spiral endings were fully or partly enclosed by sheaths formed from the perineurium of the terminating nerve (Figs. 3–11, 17). The outermost layers of the perineural epithelium cells extended to enclose the muscle fibre in a compartment separate from the nerve fibre or fibres. The dividing septum then terminated to permit access of the nerve to the muscle fibre. Sheath length varied from about 20–260 μm. Sheaths were bound to muscle fibres here and there in small patches, but otherwise they were not in contact and the sheaths were open ended. In other details, the sheaths conformed to recent descriptions (Ruskell, 1984).

DISCUSSION

Attribution of a sensory function to extraocular muscle spirals rests insecurely on their potential capacity, suggested by their form, to record increases in cross sectional area of contracting muscle fibres (Daniel, 1946; Sas & Appeltauer, 1963). A parallel, helpful to this view, may be drawn with the spiral forms encountered in known sensory receptors, as in the muscle spindle. Yet spiral endings in monkey extraocular muscles possess most of the structural characteristics of motor terminals and none of sensory terminals (Ruskell & Wilson, 1983), and the same has now been found in man. In common with monkeys, the neuromuscular junctions of human spiral endings display (*a*) boutons filled with vesicles, with small numbers aggregated at the synaptic membrane at foci of increased density, (*b*) a basal lamina in the synaptic cleft, (*c*) post-junctional folds and (*d*) sole plate nuclei in the sarcoplasm underlying the synaptic area. These features are characteristic of motor end plates. The spiral terminals differ from other end plates in being discontinuous, larger and spread over a greater length: the peculiar spiral form may be explained as a device for distributing discrete groups of boutons to several aspects of a muscle fibre.

The histological preparations, made nearly 40 years ago and used by Daniel (1946) to describe human extraocular muscle spiral endings, were in excellent condition. The observations that had led Daniel to propose that spiral endings are sensory are confirmed but, benefiting from the experience of other findings noted in this study, it is possible to identify additional features pointing to their motor character. The fine ring terminals at the end of unmyelinated branches of the myelinated spiral fibre are the silver-stained facsimiles of the structures identified as motor boutons by other methods. The number of rings in a cluster, the size range of the rings, and the cluster separation and spread are consistent with the appearance of spiral endings seen in semithin sections and with the electron microscope. Moreover, non-spiralling nerve fibres, terminating in the conventional cluster of motor end plate boutons, also display rings.

Practically all spiral endings in silver-stained sections possess fine, unmyelinated branches, but many lack ring terminals. Bearing in mind the limitations of silver techniques, it is more likely that the difference is attributable to incomplete staining rather than to a different type of ending. The presence of many conventional motor end plates without stained boutons supports this conclusion.

The form of the neuromuscular junction of spiral nerve endings displays greater variation in man than in monkeys. In man, the endings vary from the simple terminal with a single cluster of boutons to the most complex spiral ending with numerous bouton clusters, whereas in monkeys discontinuous end plates form a discrete group seldom containing less than four clusters (Ruskell & Wilson, 1983). The size of bouton clusters also displays greater variation in man, with the smallest composed of a few slightly separated boutons and others as large and as close-packed as in continuous end plates. Despite the diversity of form, the word 'dapple', used to describe discontinuous end plates in monkeys, is also appropriate in man.

In various skeletal muscles, the fast twitch fibres are served by the largest motor end plates (Padykula & Gauthier, 1970; Gertler & Robbins, 1978; Robbins, 1980). Accepting this relationship and assuming its general application, fibres in receipt of dapple end plates are potentially the fastest in extraocular muscles, as other end plates are smaller and often much smaller. The singularity of extraocular muscles in possessing dapple end plates may be related to the fact that they are the fastest contracting muscles in the body (Cooper & Eccles, 1930), capable of producing exceptionally high velocity eye movements (Westheimer, 1954).

Unlike Sas & Appeltauer (1963), Daniel (1946) does not refer to encapsulation or sheathing of spiral endings. Re-examination of his material has shown that connective tissue in general is poorly represented and sheaths are not visible. There can be little doubt that with a suitable staining technique, sheaths would have been revealed. The proposal that sheathing provides additional evidence of the sensory nature of spiral endings, and that the sheathed spirals represent modified muscle spindles (Sas & Appeltauer, 1963) has been challenged. Sheathing of motor end plates is common in human extraocular muscles (Ruskell, 1984) and the present results show that the spiral form is simply a particular example of it.

The sheaths of spiral endings have a similar composition to the capsules of muscle spindles and Golgi tendon organs; all three are extensions of perineural epithelial cells. However, the capsules of muscle spindles are probably sealed at each pole (Shanta, Golarz & Bourne, 1968), as are those of Golgi tendon organs (Schoultz & Swett, 1972), whereas spiral ending sheaths appear to be open ended. For this reason, and in order to avoid confusion with sensory end organs, the word *sheath* rather than

capsule has been used to describe the perineural extensions associated with spiral endings. Another difference is worth noting. Capsules are a definitive part of the end organs they serve whereas sheaths are not, as shown by the invariable encapsulation of sensory endings on muscle fibres in contrast to spiral endings which are not always sheathed. Spiral endings are thought to lack sheaths at birth (Ruskell, 1984), unlike sensory endings of muscle, which are encapsulated at, or shortly after birth (Landon, 1972; Zelená & Soukup, 1977).

SUMMARY

The long held view that spiral nerve endings in extraocular muscles are sensory, recently shown to be incorrect for monkeys, was tested in man. Muscle samples were taken from orbits of five patients after eye enucleation and prepared for light and electron microscopy. Most spiral endings terminated in the motor end plate band in several well separated clusters of boutons applied to various aspects of individual fibres, in contrast to the single group of boutons of other endings. They displayed a dapple appearance using the acetylcholinesterase technique and possessed fine structural features characteristic of motor end plates. Approximately 5 % of motor end plates had spiral endings and most were sheathed by extensions from perineural epithelial cells.

Hence, the spiralling of nerve fibre endings in man, as in monkeys, is a device for conveying boutons to dapple motor end plates rather than for monitoring the contraction of muscle fibres. It is suggested that muscle fibres with dapple motor end plates may be responsible for the exceptional speed of contraction of extraocular muscles.

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