

Muscle Spindles in Extraocular Muscles of Human Infants

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Key Words

Extraocular muscles, human · Muscle spindles · Infant proprioception

Abstract

The capacity of muscle spindles in adult human extraocular muscles (EOM) to provide effective proprioception was questioned on the grounds of their peculiar morphology. Their appearance could be attributable to ageing and to test this possibility examples of infant muscle spindles have been examined. Forty encapsulated structures from five extraocular muscles removed post mortem from 4 infant patients aged 6 days, 5, 23 and 30 months were examined by means of light microscopy using serial transverse sections. Seven of them were identified as false spindles. The remaining 33 structures, identified as spindles, contained a total of 175 intrafusal fibres varying from 2 to 12 (mean: 6) in each. 130 of these fibres (74.3%) were of nuclear chain type. Unequivocal evidence of bag fibres was not found. Spindles lacked or had a limited equatorial expansion, and the inner capsule was incomplete and irregularly shaped. 45 (25.7%) of the intrafusal fibres had extrafusal features with large diameters, peripherally placed nuclei, no equatorial modification and without associated sensory nerve terminals. Serial sections revealed that a majority of the nuclear chain fibres were interrupted, fragmented or terminated abruptly, and most spindles

contained at least one incomplete fibre. These observations show that the atypical features observed in adult human EOM spindles are also present in infants and are therefore not attributable to ageing.

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Introduction

Cilimbaris [1910] is generally credited with the first detailed description of muscle spindles in the extraocular muscles (EOM). He found them in ungulates and other grazing animals, and numerous attempts by his contemporaries to demonstrate them in primates failed except for a brief but convincing description of spindles in human EOM by Buzzard [1908], predating Cilimbaris' study. This work appeared to have been overlooked, and the view that human EOM lacked spindles prevailed when Cooper and Daniel [1949] and Merrillees et al. [1950] published the first full descriptions. They found the spindles to be unusually numerous, up to 71 being reported in single muscles, although it was later observed that fibre encapsulation alone is inadequate evidence of muscle spindles [Ruskell, 1989; Lukas et al., 1994], and, if used as the criterion, may lead to overestimation of their incidence.

In human EOM spindles have a number of features not found in limb muscles, including the presence of intrafusal fibres equal in size to extrafusal fibres, a thin capsule,

and little periaxial space. These characteristics were not regarded as impediments to spindle function until recently, when further examination of these and other unique features, has led to their capacity to respond appropriately to muscle activity being questioned [Ruskell, 1989]. For example, it was found that the larger intrafusal muscle fibres were continuous and unmodified throughout spindles and they lacked contact with nerves. Many of the smaller fibres were fractured and had lost continuity with the sensory locus. These anomalies were found in elderly adult specimens and the possibility that they may be the consequence of ageing has to be considered. The purpose of the present study is to determine whether or not they are present in infants. If similar anomalies are found, then they may be attributed to factors other than ageing.

During the course of the study, a report on a single human specimen was published describing an EOM spindle form in infants with essentially the same characteristics as those reported in adults [Blumer et al., 1999]. Our results confirm the similarity, but a different conclusion is reached regarding their potential function.

Material and Methods

Post-mortem tissues from the eyes of four infants were used in the study. All material was gathered by Dr. Martin Steinbach and colleagues at the Toronto Childrens Hospital. The fixed tissues were collected for further processing in London. The age of the infants, cause of death and other details are given in table 1. From a total of 14 muscles available, a single muscle from each individual and a second from one of them were selected as suitable for the present study; they showed the least autolytic changes and preparation damage. None of the infants had any recorded history of binocular vision problems or neuromuscular disease.

Muscles were dissected from the eyes and immersed in 5% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.4, for 1–12 h. Muscles were cut as close as possible to their bony attachments but the most proximal parts were missing from all of the removed pieces. Muscle size was small enough to permit preparation of them whole. Tissues were transferred from fixative to dissecting fluid (buffered sucrose) for 24 h, washed, then immersed in a 1% solution of unbuffered osmium tetroxide for 1 h. After washing they were dehydrated in graded alcohols and xylene, and then embedded in Araldite resin. Transverse sections of 0.75 μm thickness, were cut on a Reichert ultramicrotome for light microscopy. Semi-thin sections were stained with toluidine blue and were initially collected at intervals of 50 μm . When a spindle was found, uninterrupted serial sections were then taken. In some samples spindle frequency was sufficient to require serial sections throughout the whole muscle piece.

Measurements of the intrafusal fibres, the capsule and surrounding extrafusal fibres were made using a calibrated microscope eyepiece graticule.

Table 1. Details of specimens

Reference number, age of specimen	Type of muscle	Cause of death	Time of fixation after death, h	Sex
HC 2 5 months	MRM IOM	died following cardiac surgery	7	male
HC 4 6 days	MRM	died following cardiac surgery	10	male
HC 5 23 months	IOM	died following motor vehicle accident	7	male
HC 6 30 months	IOM	accidental strangulation	1	male

MRM = Medial rectus muscle; IOM = inferior oblique muscle.

Results

Spindles were identified in the majority of muscle samples and, with few exceptions, they were traced from pole to pole. Forty encapsulated structures, from 4 to 17 in each muscle, were assumed to be spindles. At a later stage, on completion of the analysis, 7 of them were found to lack the principal characteristics of spindles and were identified as false spindles [Ruskell, 1989; Lukas et al., 1994]. Muscle fibres of the false spindles did not show any modification in diameter or nuclear distribution within or beyond the encapsulated length and they had no characteristics to distinguish them from the extracapsular fibres. The capsule invested the fibres tightly and was composed of an extension from the perineurium of a neighbouring nerve. Most of these structures were found close to the motor endplate region. Nerve fibres seen running alongside these structures never entered the muscle compartment of the perineurium. Single encapsulated fibres were also present, but these structures were mostly associated with motor endings encapsulated by the perineural sheath.

The following observations refer to the 33 structures identified as true spindles. The spindles varied in length from 47 to 683 μm (mean 323 μm) and diameters varied from 10 to 110 with a mean of 50 μm . There was a tendency towards a linear relationship between capsule length and equatorial diameter (fig. 1). The number of intrafusal muscle fibres ranged from 2 to 12 (mean 6) and their number was unrelated to spindle length (correlation factor = 0.077). The shortest spindles were found among

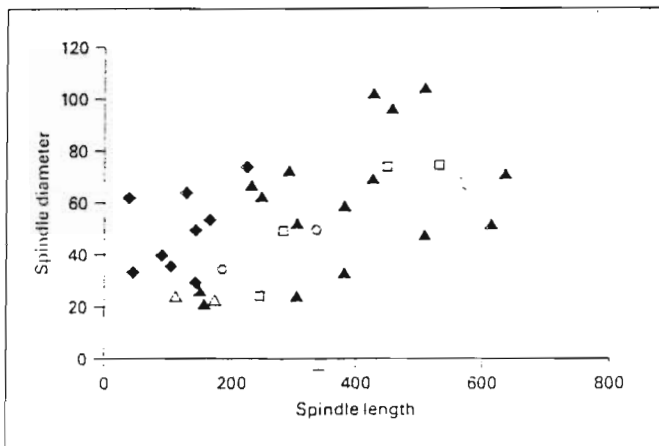


Fig. 1. Scatter chart showing the relationship between spindle length and diameter of spindles obtained from 4 infant patients aged 6 days, 5, 23 and 30 months (Indicated by diamonds, triangles, squares and circles, respectively). Grey symbols indicate inferior oblique muscles while solid black symbols indicate medial rectus muscles. Correlation factor $r = 0.55$.

those of the youngest subject, but a clear age related size variation was not found. A discrete periaxial space was not always present and there was little space around the individual fibres. Strands of connective tissue invested the fibres individually and were loosely interconnected. In a few spindles, the strands displayed a limited organisation forming an incomplete, irregular inner capsule (fig. 2). Some spindles were close to cylindrical in shape with equatorial diameters matching those of the polar regions. In these spindles the term 'equatorial' is used to describe the region of centrally placed nuclei, rather than the region of maximum diameter.

On approaching the equatorial region, the expected accumulation of centrally placed nuclei, usually confined to this region of the spindle in other mammals, was less discretely organised. Most centrally placed nuclei were located close to the equatorial region of the spindle, but some fibres contained central nuclei along their whole length with little myofilamentous material throughout. The latter possessed few, if any, contractile organelles in their polar regions. None of the spindles contained intrafusal fibres with modified regions in register. For example, all of the intrafusal muscle fibres shown in figure 3 were nuclear chain fibres but only 3 of 11 contained central nuclei, each surrounded by fragments of inner capsular membrane at the level shown.

All fibres with conventional intrafusal features were identified as being of nuclear chain type (fig. 4). In a few

fibres two nuclei could be seen lying abreast for a distance equal to their own length, but otherwise, no accumulation of nuclei or regional thickening were found to be sure of the presence of 'bag' fibres.

The spindle capsule was made up of two to four laminae of cells. Enclosure of adjacent extrafusal fibres between capsular layers occurred in a majority of spindles (fig. 4-6) and some penetrated the capsule and entered the capsular space. The latter usually continued as intrafusal fibres, leaving the spindle at its pole, or they terminated abruptly.

Independent of topographical spindle distribution, most intrafusal fibre diameters fell within the range of 5-25 μm . However, among a total of 175 intrafusal fibres, 45 (25.7%) were similar in diameter to extrafusal fibres. They had peripherally placed nuclei, had no equatorial modification and received no nerve terminals. The majority of them entered the capsule at the poles along with the other intrafusal fibres. They either ran the full length of the spindle or they ran only short distances. In the latter case they, most commonly, could be traced from one pole, terminating rather abruptly near the middle region of the spindle without changing appearance.

By analysing successive sections throughout the length of the intrafusal fibres with nuclear chain characteristics (130 in number, constituting 74.3% of the total) it became clear that a large number of them were interrupted, fragmented or terminated abruptly. Consequently, the number of intrafusal fibres seen in transverse section varied at different positions along spindles. Interruptions or termination of intrafusal fibres could occur anywhere along the length of the spindle but most commonly at the level of the equatorial region.

The fractured ends were separated by gaps varying from <10 to 300 μm in length. Where fibres were interrupted by a short gap there was little difficulty in concluding that they represented an interruption of the same fibre because its position, relative to its neighbours, was unchanged and the gap was often bridged by slender connective tissue strands. A few fibres were fragmented with breaks in 2 or 3 places. With larger interruptions the pieces belonging to the same fibre were less confidently identified but, using the criteria of position and continuity of connective tissue, in most cases an interrupted fibre, rather than the attenuation of two separate fibres was indicated. Sixty-eight (38.8%) of the fibres were fragmented or terminated abruptly. They were fairly evenly distributed so only 5 of 33 spindles were free of such fibres while in 8 spindles, fibres were exclusively of this type. In a few instances fibre breaks were coincident at the equato-

occurrence of these features in adult muscles was argued to be a legitimate characteristic of extraocular muscle spindle structure [Ruskell, 1989] and we take a similar view regarding the present infant material.

The richness of EOM spindles has been commented upon by numerous authors and is regarded as important in the achievement of fine oculomotor control. Spindles were numerous in the pieces of muscle used in this study, but as noted by others, the frequency of false spindles suggests that earlier estimates of spindle numbers were subject to significant error.

It is pertinent that the lowest incidence of spindles was reported by Lukas et al. [1994] who identified and excluded false spindles in the muscles of adults. They are evidently characteristic of infant and adult muscles alike, as seven of 40 encapsulated structures were identified as false spindles in the present study and one of 11 in the muscles of a two-year-old reported by Blumer et al. [1999]. False spindles may be of little significance, indicating only a propensity for the perineurium to transiently enclose adjacent muscle fibres. The suggestion that they might be erstwhile true spindles deprived of specialised fibres by degeneration [Ruskell, 1989] is inconsistent with their presence in infant muscles.

The main purpose of the study was to determine whether or not the unusual features of the adult EOM spindle are also expressed in infants and, apart from a possible minor item, discussed below, it has been shown that they are. Blumer et al. [1999] came to the same conclusion and it is relevant that in an earlier study Cooper and Daniel [1949], who included infant muscle among their preparations, did not report any differences.

Several of the morphological features led Ruskell [1989] to suggest that human EOM spindles are incapable of generating adequate proprioceptive signals. In contrast, Blumer et al. [1999], while confirming most of the atypical features, speculated that they might represent special functional properties. These different points of view are clearly unreconcilable and demand consideration. One factor used to question normal functional capacity of spindles is the tight packing of the equatorial region. In agreement with Blumer et al. [1999], we have shown that infant muscles share this feature with adult muscles. Accepting that the function of the broad fluid-filled equatorial expansion found in non-human EOM is to protect the intrafusal fibres by damping the effect of extrafusal fibres during muscle contraction [Brzezinski, 1961], then this facility is commonly denied to human EOM spindles.

In seeking further information regarding the unusually large muscle fibres commonly found in EOM spindles [Cooper and Daniel, 1949; Merrillees et al., 1950; Lukas et al., 1997], most of them were shown to lack nerve terminals in their intrafusal passage [Ruskell, 1989; Blumer et al., 1999] and were indistinguishable from extrafusal fibres. They are referred to as anomalous spindle fibres and about one third of intrafusal fibres were found to be of this type – fewer than in the earlier studies. Assuming that they conform functionally as well as structurally to the general population of extrafusal fibres, their activity will dictate the length of the spindle and, by contraction have the capacity to off-load spindle fibres, disturbing the normal cycle of receptor excitation. Moreover, with contraction-induced increment in diameter, the anomalous fibres could directly interfere with receptor responses. Blumer et al. [1999], rather than considering the constraints that anomalous fibres might impose on spindles, addressed the problem by noting the differentiation of spindle fibres in early development and suggested that their presence might indicate a continuing capacity for differentiation. They supported the notion by referring to the presence of mature extrafusal muscle fibres in EOM expressing heavy chain myosin normally found in neonatal or embryonic muscle or in mature intrafusal fibres. They argue that their presence in adult EOM represents a potential store for fibre incorporation in spindles. Were this the case, the contention that their presence is an encumbrance to normal spindle activity still obtains.

Perhaps the most obvious factor inconsistent with normal spindle morphology is the discontinuity of numerous chain fibres reported by Ruskell [1989]. A large majority of spindles examined in the present study contained modified fibres of this kind and in eight cases all the fibres were fractured or terminated abruptly. Such fibres were noted in 5 of 10 spindles described by Blumer et al. [1999] who attributed their appearance primarily to damage caused by the severe trauma sustained by the donor. We consider that extraneously induced tissue damage cannot account for the present findings or in the earlier study [Ruskell, 1989], and we maintain that they represent a naturally occurring condition. It would be of interest to examine fetal material to check if these features are present in early development or represent a developmental or degenerative change.

A novel group of anomalous fibres amounting to 7% of the total, received nerve terminals in adult spindles [Ruskell, 1989]. They had morphological characteristics similar to those of the chain fibres at the equator and were therefore presumably sensory. This type of fibre, unob-

served in the present study, was also noted by Lukas et al. [1997], although none were found in the infant muscles examined by Blumer et al. [1999]. But one cannot discount the possibility of their later development.

When these various peculiar features of EOM spindles were first noted in older tissue the question was posed whether or not they were attributable to ageing, but their presence in infant tissue rules out that possibility. It follows that the arguments presented here, questioning spindle capacity to fulfil the role of proprioceptors are equally applicable to young muscle, and the evidence of degeneration and disorganisation sustain the consideration [Ruskell, 1989] that EOM spindles have a questionable fidelity in their response to muscle stretch.

Finally, in view of the present finding it is appropriate to comment on the proposed role for muscle spindle pro-

prioception in the development of binocular vision [Buisseret and Maffei, 1977; Hein and Diamond, 1983]. EOM spindles are the receptors thought to provide the proprioception important in the development of binocular vision in various animals [Hein and Diamond, 1983]. This view could be sustained in man despite the features suggesting spindle redundancy in mature muscles, provided the anomalies were acquired with age. But as we have shown they are already present in infancy.

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