Fine Structural Analysis of Extraocular Muscle Spindles of a Two-Year-Old Human Infant

Roland Blumer, Julius-Robert Lukas, Martin Aigner, Reginald Bittner, Isabella Baumgartner, and Robert Mayr

PURPOSE. To clarify whether structural peculiarities formerly described in extraocular muscle (EOM) spindles of aged persons are already present in EOM spindles of a 2-year-old infant.

METHODS. Distal halves of two EOMs obtained from a 2-year-old multiorgan donor were immersion-fixed and prepared for electron microscopy. The fine structure of 10 muscle spindles and of 1 “false spindle” was investigated.

RESULTS. Extraocular muscle spindles of an infant 2 years of age had 2- to 4-layered outer capsules, 376 µm (range, 217-606 µm) long and 97 µm (range, 55-140 µm) wide. In 10 EOM spindles, 4 to 16 intrafusal muscle fibers (mean, 7.9) were present. From a total of 79 intrafusal fibers, 43 (54%) were nuclear chain fibers, and 8 of the 43 exhibited posttraumatic degenerative changes. Thirty-six (46%) intrafusal fibers indistinguishable from extrafusal fibers were called anomalous fibers. No nuclear bag fibers were found. Each muscle spindle contained a variable number of chain fibers and at least one anomalous fiber. Sensory nerve terminals were restricted to the 35 normal chain fibers but were absent from damaged chain fibers and from anomalous fibers. One “false spindle” without a periaxial space was composed of three anomalous fibers and one chain fiber, all of them devoid of sensory terminals.

CONCLUSIONS. Most structural particularities of human EOM spindles described in aged persons are already found in the infant. They cannot be interpreted as age-related changes, but rather they represent specific features of human EOM spindles. (Invest Ophthalmol Vis Sci. 1999;40:55-64)

Proprioception from human extraocular muscles (EOM) is thought to play an important role in the development of a normal binocular vision. Several reports attribute increasing importance to proprioceptive input from EOMs within the oculomotor system. In humans, muscle spindles are accepted to be a regular constituent in EOMs, and their characteristic distribution and high density in different human EOMs are strong indications of the functional importance of these structures.

Ultrastructural observations on human EOM spindles are sparse. Other than a brief description of the fine structure of the sensory endings, only one detailed study of the ultrastructural features of human EOM spindles has been published. This report was exclusively based on material of three aged persons (58 years, 70 years, and 74 years). Transmission electron and light microscopic observations demonstrated that aged human EOM spindles differ in their intrafusal muscle fiber composition from skeletal muscle spindles and from EOM spindles of various mammals. Nuclear bag fibers and nuclear chain fibers are regular constituents of mammalian and human skeletal muscle spindles and of EOM spindles of ungulates. In human EOM spindles, the intrafusal fiber composition is different. Most human EOM spindles lack bag fibers, which represent only 2% of EOM intrafusal fibers of aged persons. Besides chain fibers, unmodified muscle fibers resembling extrafusal muscle fibers were found to be a regular constituent of human EOM spindles, both representing approximately equal numbers of the total intrafusal fiber contingent of aged persons. Unmodified muscle fibers in aged human EOM spindles were designated anomalous muscle fibers.

In addition to a different intrafusal fiber composition, human EOM spindles of old persons vary from skeletal muscle spindles with respect to the periaxial space, which is either ill defined or absent in human EOM spindles. Another difference from skeletal muscle spindles is that in aged human EOM spindles, the inner capsule never invested all intrafusal fibers. Several characteristics of human EOM spindles were considered to be age-related alterations.

Up to now no study has reported on human EOM spindles of younger individuals. The aim of this study was to compare human EOM spindles of a 2-year-old infant with data from those of aged persons to clarify whether the structural characteristics of old human EOM spindles are age-related alterations as suggested by Ruskell, or whether they represent normal but unique features of human EOM spindles.

MATERIALS AND METHODS

Distal halves of a left inferior rectus and a left superior rectus were obtained from a 2-year-old multiorgan donor who died 72 hours after severe head trauma. This study was performed in accordance with the Austrian federal transplantation law.
Methods for securing human tissue were humane, included proper consent and approval, and complied with the tenets of the Declaration of Helsinki.

Muscles were cut longitudinally into four strips and cut transversely with a length of 6 mm and a diameter of 3 mm. The tissue was immersion-fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 1 day. After rinsing in the same buffer, specimens were postfixed in buffered 1% osmium tetroxide, dehydrated in graded solutions of ethanol, and embedded in Epon.

Semi-thin cross sections (1 μm) were retained at intervals of 20 μm, stained with toluidine blue, and examined under the light microscope (LM) for the presence of muscle spindles. When muscle spindles were identified in the LM, every semi-thin section was mounted on a slide. Ultrathin sections were cut at appropriate intervals, mounted on dioxane formvar-coated copper grids, immersed in an aqueous solution of 2% uranyl acetate followed by a solution of 0.4% lead citrate in 0.1 M sodium hydroxide, and examined under a transmission electron microscope (TEM, model EM9; Zeiss, Oberkochen, Germany).

Ten human EOM spindles (seven spindles of the left inferior rectus and three spindles of the left superior rectus) and one false spindle of the left inferior rectus were investigated. All muscle spindles and the false spindle were analyzed throughout their capsule length, and in some muscle spindles the investigations were continued up to 100 μm into the extracapsular regions. The lengths of the muscle spindle capsules were calculated by counting both their serial semi-thin sections (1 μm) and their ultrathin sections (100 nm). One tissue block containing muscle spindles was reoriented and cut longitudinally to assess the degree of muscle fiber contraction due to immersion fixation.

Results

Identification of human EOM spindles in cross sections was only possible if the intrafusal fibers were surrounded by a distinct capsule. Polar regions outside the capsules could not be recognized because the extrafusal fibers did not differ from extracapsular intrafusal fibers, neither in diameter (extrafusal fibers, 14.6 ± 3.3 μm; intrafusal fibers, 12.5 ± 4.2 μm) nor in structure (Fig. 1). As was noted in a former study, all muscle spindles investigated were located in the transition zone between the orbital and global layers of the EOMs.

Outer Capsule

The capsule lengths varied from 93 μm to 606 μm with a mean of 376 μm. The largest diameters of all 10 muscle spindles varied between 55 μm and 140 μm with a mean of 97 μm (Table 1).

The intrafusal fibers of the human EOM spindles were encircled by a capsule that consisted of concentric layers of flattened perineurial cells. The perineurial cells were covered with a basal lamina on both sides. and the spaces between adjacent perineurial cell layers contained collagenous fibers. Blood vessels were occasionally observed between neighboring perineurial cell layers. In the polar regions of the muscle spindles, the capsules consisted of one to two cell layers closely encirling the intrafusal fibers. Toward the equatorial regions, the diameter of the spindles and the thickness of their capsules gradually increased, the latter to two to four layers.

In two spindles, all intrafusal fibers in the equatorial region were concentrated in the center of the muscle spindle cross sections, and a well-developed periaxial space separated the intrafusal fibers from the spindle capsule (Fig. 2). In four muscle spindles the intrafusal fibers were uniformly distributed.
on cross sections of the equatorial region, and the periaxial space was less developed. In three muscle spindles a variable number of intrafusal fibers were located in the center within the equatorial region, whereas numerous intrafusal fibers exclusively of the anomalous type had an eccentric position (Fig. 3). A periaxial space was visible only around the intrafusal fibers occupying a central position. In one muscle spindle a periaxial space was absent, and throughout the capsule length between the intrafusal fibers and the capsule only a small cleft was visible that hardly increased in the equatorial region (Table 1).

**Inner Capsule**

All of the 10 human EOM spindles had an inner capsule in the para-equatorial and equatorial regions. LM and TEM observa-

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**Table 1. Morphological Features of 10 Human EOM Spindles of a 2-Year-Old Infant**

<table>
<thead>
<tr>
<th>Intrafusal Fibers per Muscle Spindle</th>
<th>Nuclear Chain Fibers</th>
<th>Anomalous Fibers</th>
<th>Equatorial Diameter (μm)</th>
<th>Capsule Length (μm)</th>
<th>Periaxial Space</th>
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<tbody>
<tr>
<td>4</td>
<td>3</td>
<td>1</td>
<td>65</td>
<td>220</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>2 + 2*</td>
<td>2</td>
<td>77</td>
<td>606</td>
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</tr>
<tr>
<td>6</td>
<td>1 + 2*</td>
<td>3</td>
<td>75</td>
<td>420</td>
<td>+</td>
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<tr>
<td>6</td>
<td>1 + 1*</td>
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<td>55</td>
<td>280</td>
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<td>120</td>
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<td>5</td>
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<td>95</td>
<td>508</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>3 + 2*</td>
<td>3</td>
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</tr>
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</tr>
<tr>
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<td>9</td>
<td>7</td>
<td>140</td>
<td>515</td>
<td>+</td>
</tr>
<tr>
<td>79 (7.9)</td>
<td>35 + 8*</td>
<td>36 (3.6)</td>
<td>967 (97)</td>
<td>3759 (376)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are mean values.

* Damaged chain fibers without sensory terminals.
tions revealed that in one muscle spindle this inner capsule consisted of two cell layers: the outer layer surrounded all intrafusal fibers and the inner one invested a different number of intrafusal fibers (Fig. 2). Nine muscle spindles exhibited a one-layered inner capsule ensheathing a variable number of intrafusal fibers. Anomalous muscle fibers located adjacent to the spindle capsule were not enveloped by inner capsule cells (Fig. 3).

The cells of the inner capsule consisted of a prominent cell body that appeared to be rounded or irregularly shaped. A variable number of thin cytoplasmic cell processes emanated from the cell body to encircle individual intrafusal fibers. Adjacent cell processes had cell-to-cell contacts at numerous points. TEM demonstrated that the cells of the inner capsule lacked a basal lamina investment.

Intrafusal Fibers
Ten EOM spindles of the infant contained between 4 and 16 intrafusal fibers, with a mean of 7.9 intrafusal fibers. A total of 79 intrafusal fibers were observed, 43 nuclear chain fibers (54%) and 36 anomalous fibers (46%). In addition to nuclear chain fibers, anomalous fibers were a regular constituent of the infant spindles. Each muscle spindle contained at least one anomalous fiber (Table 1).

Nuclear Chain Fibers
Thirty-five of 43 nuclear chain fibers exhibited a normal morphology. In the polar regions of the muscle spindles most of these typical nuclear chain fibers had subsarcolemmal myonuclei, and their diameters were similar to those of the anomalous fibers. A small number of nuclear chain fibers had single central myonuclei in their polar regions and also in parts of their extracapsular regions. In the para-equatorial regions the myonuclei of all nuclear chain fibers were found in a central position, separated from each other by interspaces filled with undifferentiated electron translucent sarcoplasm. In the equatorial regions these interspaces gradually diminished in such a way that adjacent myonuclei of the chain were touching each other. In the equatorial regions the mean diameter of the nuclear chain fibers was significantly smaller than that of the spindle poles (equator: 8.5 ± 2.2 μm [mean ± SD], pole: 10.8 ± 2.8 μm, Student’s t-test, P < 0.001, n = 35), and the central myonuclei were only surrounded by a thin layer of sarcoplasm (Figs. 4, 5). Four nuclear chain fibers exhibited short areas in their equatorial regions in which two myonuclei were lying side by side (Fig. 6). Length measurements of sarcomeres in longitudinally cut extrafusal fibers revealed that numerous muscle fibers exhibited contracted sarcomeres (length, 1.6 μm). In one nuclear chain fiber the sarcomeres were contracted to an extent that no I-bands were visible (Fig. 7). The contraction of the muscle fibers might have been caused by the fixation procedure. Thereby in nuclear chain fibers, myonuclei that were originally arranged in a chain came to lie side by side.

Ultrastructural images revealed that throughout their capsule length the sarcomeres of the nuclear chain fibers consisted of thick Z-lines and small I-bands. Within the H-bands, a distinct M-line was present (Fig. 7). All nuclear chain fibers were endowed with sensory terminals. Three muscle fibers received a single motor terminal within the capsules of their muscle spindles.

Eight of 43 nuclear chain fibers (Table 1) exhibited degenerative changes that may have been induced by the severe head trauma of the donor. In these damaged nuclear chain fibers clustered myofilaments, areas of myofibrillolysis, swelling of the mitochondria, and dilatation of the sarcoplasmic reticulum were present; in addition, karyorrhexis of central myonuclei and pyknotic central myonuclei were evident (Fig. 8). In these nuclear chain fibers, three to nine single central myonuclei were present on cross sections throughout their capsule length. They never formed a continuous chain but were always separated by variable intervals of sarcoplasm. A small number of extrafusal muscle fibers exhibited comparable ultrastructural changes. All damaged nuclear chain fibers were devoid of sensory terminals (Table 1, Fig. 8). On two of these muscle
fibers single motor terminals were present within their intracapsular course.

**Anomalous Fibers**

All intrafusal fibers that lacked equatorial modification of their myonuclei were ascribed to the anomalous type. The anomalous fibers had typical subsarcolemmal myonuclei throughout their lengths, and, thus, their appearance was indistinguishable from extrafusal fibers. Throughout the capsule length of the human EOM spindles, the diameter of the anomalous fibers remained rather constant (equator: 14.7 ± 4.0 μm [mean ± SD], pole: 14.3 ± 4.3 μm, n = 36). Most of the anomalous fibers exhibited larger diameters in their equatorial regions.
than normal chain fibers. In seven muscle spindles the anomalous fibers remained within their capsule space throughout the whole capsule lengths. In three muscle spindles anomalous fibers were penetrating at least the inner layer of the outer capsule at variable points: In one muscle spindle, three anomalous fibers coming from outside entered the muscle spindle and, after running a short distance within the capsule space, these anomalous fibers exited the muscle spindle. In another muscle spindle serial sections demonstrated that two anomalous fibers were running in the equatorial zone for a distance of 120 μm between adjacent capsule layers. In a third muscle spindle, three anomalous fibers successively penetrated the muscle spindle capsule in the polar region.

TEM images revealed that the anomalous fibers contained numerous mitochondria with occasional subsarcolemmal accumulations. Sensory terminals were never found on anomalous fibers. Single motor terminals were observed on six anomalous fibers within the capsular regions.

Breakage, Interruption, and Intrafusal Fiber Splitting
Four nuclear chain fibers with areas of sensory innervation in three muscle spindles abruptly ended at variable points within the encapsulated regions. TEM pictures demonstrated that shortly before breakage, these muscle fibers contained swollen mitochondria, and their myofibrillar material appeared dispersed (Fig. 9). One nuclear chain fiber with sensory terminals was interrupted for a distance of 35 μm. Another nuclear chain fiber with sensory terminals split after having passed the equatorial region.

Associated Muscle Fibers
Muscle fibers running for variable distances between neighboring outer capsule layers without entering the capsule space were called associated muscle fibers. Associated muscle fibers were observed in two human EOM spindles (Fig. 2). As associated muscle fibers had subsarcolemmal myonuclei throughout their length they were indistinguishable from anomalous fibers and extrafusal fibers. Neither sensory nor motor terminals were found on associated muscle fibers within their course between outer capsule layers.

Myosatellite Cells
All chain fibers outside their areas of sensory innervation, all damaged chain fibers, anomalous fibers, and associated muscle fibers throughout their lengths had typical satellite cells like extrafusal fibers. Satellite cells were located on the intrafusal fiber surface and covered by the basal lamina of the muscle fiber as in skeletal muscle. They had typical oval shapes and moderately indented the surface of their intrafusal fibers and associated muscle fibers. They possessed a large nucleus that was surrounded by a thin layer of cytoplasm.

Sensory Terminals
Sensory terminals were present on all normal chain fibers, and they enwrapped the individual fibers, forming irregular coils. Their areas of sensory innervation varied between 30 μm and 360 μm. Sensory terminals were located not only in the equatorial regions, but in most chain fibers they also covered at least one side of the para-equatorial regions. In one muscle spindle the sensory terminals on two chain fibers extended on one side into the extracapsular region of the muscle spindle. TEM observations indicated that the sensory terminals often deeply indented the surfaces of the chain fibers. Rarely, the sensory terminals extended from the surface into the intracapsular regions (Fig. 4). Sensory terminals contained numerous mitochondria that were either distributed uniformly or were concentrated near the synaptic cleft. Their axoplasm was electron translucent. The intracapsular fiber basal lamina covered the outer surfaces of the sensory terminals but was absent within the synaptic clefts. The synaptic clefts measured 25 nm. In a small number of terminals, there were junctional complexes that were shaped like discs between opposed plasma membranes of sensory terminals and intracapsular fibers (Figs. 5, 6, 7).

On four chain fibers in three muscle spindles sensory nerve endings exhibited a dense axoplasm full of mitochondria with swelling of the matrix and reduction of their cristae, which may be interpreted as Wallerian degeneration. Three chain fibers were exclusively endowed with such degenerated sensory endings. On one chain fiber normal and degenerated sensory terminals were observed. Degenerated sensory terminals covered fiber lengths between 30 μm and 130 μm (Fig. 10).

Motor Terminals
On two chain fibers single motor terminals were found in the immediate vicinity of the sensory regions. TEM images revealed that they were positioned superficially without causing a depression in the surface of the intrafusal fibers. Their subsynaptic membranes were smooth.

Single neuromuscular junctions were observed on two chain fibers and on six anomalous fibers at variable points within the spindle capsules. On three chain fibers single motor terminals were located in their extracapsular regions. All these motor terminals had numerous synaptic knobs. These knobs
indented the fibers' surfaces and the subsynaptic membranes were folded (Fig. 11).

**False Spindle**

One encapsulated structure contained four muscle fibers but lacked sensory terminals throughout the capsule length (330 μm). It is extremely unlikely that sensory endings have been missed, because they always covered a substantial length of the muscle fiber, and because ultrathin sections were taken at very short intervals (5-7 μm). This structure without sensory investment was therefore classified as a false spindle. The capsule consisted of three cell layers and was always in close contact to the muscle fibers throughout its length. A periaxial space was absent, and its maximum width was 35 μm. The capsule cells were covered on both sides with basal laminae. Individual muscle fibers were encircled by processes that extended from the innermost layer of the capsule. Reconstruction of serial sections of the encapsulated region revealed that there were three muscle fibers of the anomalous type with only subsarcolemmal myonuclei. One muscle fiber exhibited a row of five central myonuclei with variable amounts of sarcoplasm between them. Thus, it is questionable whether this fiber should be classified as a chain fiber.

Motor terminals were found within the encapsulated region of this false spindle on two anomalous fibers. One anomalous fiber was multiply innervated with three neuromuscular contacts, whereas the other anomalous fiber had a single motor terminal. Apart from their larger content of densely packed vesicles, motor terminals in the false spindle did not differ in their morphology from extracapsular motor endings on chain fibers and those on anomalous fibers in infant EOM spindles (Figs. 12, 13).

**DISCUSSION**

This study describes for the first time the ultrastructure of human EOM spindles at an age at which the development of binocular vision and interocular alignment were not yet complete. Most morphologic characteristics of human EOM spindles as described by Ruskell and Lukas et al. in aged persons were also present at the age of 2 years. In particular, EOM spindles of the 2-year-old infant and those of old individuals...
fibers and chain fibers, with the regular presence of at least one contrast to that, mammalian skeletal muscle spindles and EOM the aged persons 7-9 were devoid of nuclear bag fibers. In equatorial nucleation were a regular constituent of human spindles of ungulates were reported to contain exclusively bag fibers was only 2% of the total; anomalous fibers were 50% to 73% in one study 7 and 43% in another 8 (Table 2). Another characteristic of EOMs described by Ruskell7 and confirmed by Lukas et al. 8 was the occurrence of false spindles, encapsulated structures containing muscle fibers without any sensory innervation. One false spindle was identified and analyzed in the infant.

Other features distinguishing human EOM spindles from mammalian muscle spindles are the limited or absent periaxial space 7 and the fact that intrafusal fibers and adjacent extrafusal fibers exhibit similar diameters. Although measurements (Table 1) revealed that the mean equatorial diameter and the mean number of intrafusal fibers per spindle were similar in the infant and in aged persons, the periaxial space seemed to be more pronounced in infant spindles. The absolute diameter of the periaxial space remained constant with increasing age, whereas intrafusal fibers in the infant are smaller (12.5 µm) compared with the aged (15.3 µm). 7 The periaxial space protects the sensory nerve terminals of the intrafusal fibers from nonspecific stimuli like contraction of neighboring extrafusal fibers. 16 Thus, because of a relatively larger periaxial space, infant muscle spindles appear to be better protected from mechanical interference.

In the investigation by Ruskell, 7 five nuclear bag fibers were described. Only one of the bag fibers had an accumulation of myonuclei in its “bag” region, but four of them had two myonuclei abreast over a short length of each fiber. The classification of intrafusal fibers with two nuclei abreast as bag fibers needs attention. In skeletal muscle spindles a bag fiber is defined as an intrafusal fiber with an accumulation of myonuclei in its “bag” region. 17 In the tenuissimus of the cat, Adal 18 qualified intrafusal fibers with two nuclei side by side as nuclear chain fibers. In most skeletal muscle spindles the bag fibers are of a larger diameter throughout their length than chain fibers. 17 Furthermore, in skeletal muscle spindles, bag fibers and chain fibers differ in the M-band structure of the equatorial sarcomeres. Bag fibers contain two faint M-lines within the H-band, whereas chain fibers show one single well-defined M-line. 19,20 In the present study four intrafusal fibers were found with two myonuclei abreast. However, apart from that, these four fibers were indistinguishable in their morphology from chain fibers. We concluded that intrafusal fibers with only two nuclei abreast are most likely chain fibers that were artificially changed during the fixation procedure.

The localized pathologic changes observed in eight chain fibers, and scattered extrafusal fibers can be explained by the severe head trauma that caused the death of the infant 72 hours after injury. 21 Damages of the myelin sheaths of single axons and electron dense axoplasm and altered mitochondria observed in four sensory endings are considered to be signs of Wallerian degeneration, probably caused by a rupture of the axolemma (Lassmann H, personal communication, March 1997). The majority of muscle fibers, axons, and nerve terminals have not been visibly damaged by this trauma. We believe that, in general, the severe head trauma did not affect the reliability of the results presented in this study.

In humans 22 in contrast to other species (e.g., rats), 23-24 the development of skeletal muscle spindles is completed before birth. So far, data on the development of human EOM spindles are not available in the literature. In the present study we did not observe any features of continuing spindle development such as an incomplete capsule, an ongoing fusion of myoblasts, or close apposition of intrafusal fibers. However,

Table 2. Intrafusal Fiber Composition in Human EOM Spindles of Persons of Different Ages

<table>
<thead>
<tr>
<th>Author</th>
<th>Age of the Individuals (y)</th>
<th>% of Nuclear Chain Fibers</th>
<th>% of Anomalous Fibers</th>
<th>% of Nuclear Bag Fibers</th>
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<tr>
<td>Ruskell 7</td>
<td>70, 74</td>
<td>25</td>
<td>73</td>
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<td>48</td>
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<td>67, 72, 83</td>
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<td>This study</td>
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postnatal maturation of extrafusal fibers with respect to their fiber thickness and their mitochondrial pattern occurs in cat and rat EOMs. Maturation of extrafusal fibers continues up to the adult age of these animals. In monkeys, the singly innervated muscle fibers of the orbital layer attain their definitive size and mitochondrial content within the first 6 months. By comparing the mean intrafusal fiber diameter in the EOMs of the infant (12.5 μm) with that in the EOMs of aged persons (15.3 μm), an increase in the intrafusal fiber thickness is noted.

The circumstances of a high incidence of anomalous fibers in aged human EOMs, such as frequent lipofuscin deposits, loss of fiber length, and fiber fragmentation of regular intrafusal fibers, stimulated suggestions by Ruskell that anomalous fibers are arguably incursions of extrafusal fibers replacing degenerated intrafusal fibers and that false spindles may represent the culmination of spindle degeneration, with all nuclear bagged intrafusal fibers and that false spindles may represent the size and mitochondrial content within the first 6 months. By activated muscle fibers of the orbital layer attain their definitive size and mitochondrial content within the first 6 months.

In this study in the infant, anomalous fibers were almost equal in number to chain fibers (Table 2). Loss of fiber length was observed in four chain fibers, fragmentation in one chain fiber, and splitting in another chain fiber. However, lipofuscin was not present at the age of 2 years. The false spindle examined was composed of three anomalous fibers and one chain fiber, with all four lacking any sensory innervation. Apart from the presence of lipofuscin most aberrant features of human EOM spindles cannot be interpreted as age-related changes. It has been suggested that the specific muscle spindle morphological features of human EOM spindles cannot be interpreted as age-related changes. However, it seems very unlikely that evolutionary redundant receptors would be seen so frequently. As previously reported, muscle spindles were at least as frequent in human EOMs as in skeletal muscles, with known high muscle spindle density when compared gram for gram.

Experimental studies in skeletal muscles gave evidence that devascularization and application of myotoxic substances could induce muscle spindle degeneration with subsequent regeneration. It is important to note that in all these studies regeneration of intrafusal fibers was found to originate from myosatellite cells. Necrotic sarcoplasm of the degenerated intrafusal fibers is removed by phagocytes. The myosatellite cells survive, and during regeneration they segregate myoblasts, which fuse to myotubes. Analogous to the transformation of undifferentiated intrafusal fibers into bag fibers and chain fibers by primary sensory nerve terminals in normal development, innervation with sensory terminals was reported to induce maturation to bag fibers and chain fibers with typical equatorial nucleation in spindle regeneration even in adult rats. In human EOM spindles of the infant, myosatellite cells were regularly present in intrafusal fibers except in their equatorial regions, which are endowed with sensory terminals. These facts do not support the hypothesis of replacement of degenerated intrafusal fibers in human EOMs by incursion of extrafusal fibers.

On the other hand, reconstructions of 10 muscle spindles of the 2-year-old infant revealed that muscle fibers indistinguishable from adjacent extrafusal fibers were found to be interposed for variable distances between the layers of the outer capsule (= associated muscle fibers) and so-called "anomalous" fibers in an intrafusal position. In contrast to typical intrafusal fibers, most anomalous fibers were not ensheathed by an inner capsule. In the infant, two muscle spindles contained one associated muscle fiber each, but all 10 muscle spindles had anomalous fibers (1-7 per spindle) together with nuclear chain fibers as regular constituents. Eight of 36 anomalous fibers did not run the whole length within the capsule space, but rather penetrated one muscle spindle capsule in the para-equatorial region and left the capsule space in another muscle spindle to run between outer capsule layers before reentering at an equatorial level. Furthermore, anomalous fibers entered one muscle spindle from outside and, after running within the capsule space, left the muscle spindle again. These findings together with abrupt ending and fragmentation of intrafusal fibers with typical equatorial nucleation are in conformity with the situation in the aged adult. In contrast to aged human EOM spindles, where 8 of 120 anomalous fibers were generously supplied with sensory endings, all 36 anomalous fibers of the infant were lacking sensory terminals. However, three anomalous fibers endowed with sensory endings were observed in two human EOM spindles of a 17-year-old boy (authors' unpublished observation, June 1997).

Anomalous fibers supplied with sensory terminals were a major argument for Ruskell's hypothesis of spindle reorganization imposed by degeneration. Although they were not observed at 2 years of age, the question remains as to whether anomalous fibers are an expression of a regular turnover of intrafusal fibers or whether they exert a special function of their own. In a similar vein, evidence for the continuing plasticity of EOM fibers during postnatal life was given by myosin immunohistochimistry. In EOMs of adult rats and in EOMs of humans numerous morphologically mature extrafusal fibers of the orbital layer coexpress both fast and neonatal, or neonatal and embryonic, myosin heavy chain (MHC). In adult mammalian skeletal muscles, expression of embryonic and neonatal MHC is confined to the intrafusal fibers. Because many extrafusal fibers in human EOMs imitate a MHC expression only found during development and in intrafusal fibers in other mammalian skeletal muscle, one might speculate that this indicates a continuing capacity for differentiation. By incorporation of such extrafusal fibers into human EOM spindles, sensory contacts on anomalous fibers (crystalline extrafusal fibers) might induce their modification into chain fibers in a manner similar to that in skeletal muscle spindle development. The caveat here remains, that EOM fibers expressing embryonic and neonatal MHC are otherwise completely mature muscle fibers.

Because of the unique morphology of human EOM spindles, previous reports doubted their capacity to provide useful proprioceptive information. In contrast, the following observations clearly indicate their functional importance. First, a high and constant number and a characteristic distribution of spindles within each of the six different EOMs were demonstrated. Second, the morphology of terminals on chain fibers was similar to that of terminals in skeletal muscle spindles. Third, the characteristic morphology of EOM spindles is also present at the age of 2 years and must not be interpreted as age-related degeneration. More likely, their unique morphology represents special functional properties. The lack of nuclear bag fibers in human EOM spindles might indicate that human EOM spindles have predominantly a static function and that they monitor the degree of muscle length changes rather than the contraction velocity. Finally, widespread afferent input from EOMs to the central nervous system suggests proprioceptor activity whereby muscle spindles and myotendi-
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References