Number, Distribution, and Morphologic Particularities of Encapsulated Proprioceptors in Pig Extraocular Muscles

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PURPOSE. To analyze qualitatively and quantitatively the total complement of encapsulated proprioceptors (Golgi tendon organs [GTOs] and neuromuscular spindles) in pig extraocular muscles (EOMs).

METHODS. EOMs of four pigs of different ages were prepared for light microscopic histochemical and immunohistochemical analysis and for transmission electron microscopy.

RESULTS. GTOs and muscle spindles were numerous in pig EOMs. GTOs were found to be distributed in aponeurotic expansions of the distal and proximal EOM tendons, being more numerous in the distal aponeurosis than in the proximal aponeurosis. The total number of GTOs was higher in the recti EOMs (100–128) than in the oblique EOMs (45–61). Spindles were distributed over the entire muscle length. In each EOM the number of muscle spindles (142–333) exceeded those of GTOs. The morphology of the GTOs was variable. In addition to collagen bundles, approximately one third of the GTOs contained intracapsular muscle fibers that resembled the multiply innervated fiber type. Intracapsular muscle fibers entered the poles of the GTOs and either terminated inside the receptors in collagen bundles or exited the GTOs at the opposite poles. Nerve terminals were numerous in each GTO and established intimate contacts with collagen fibrils.

CONCLUSIONS. Most structural particularities formerly observed in GTOs of rhesus monkey and sheep EOMs are also present in GTOs of pig EOMs. The high number of GTOs with their typical nerve terminals indicates functional importance. During muscle activity, afferent signals from GTOs and muscle spindles may provide sufficient information about eye position. (Invest Ophthalmol Vis Sci. 2001;42:3085–3094)

Proprionicceptive input from extraocular muscles (EOMs) of mammals reaches most regions of the central nervous system involved in vision and oculomotor control.

EOMs of different mammals exhibit considerable species differences in their proprioceptive complement. Muscle spindles have been observed in most mammalian EOMs except in cats, guinea pigs, and rats. In monkey19 and human11–13 EOMs, spindles differ morphologically from their counterparts in other skeletal muscles. In particular, nuclear bag fibers are missing, and a high proportion of unmodified intrafusal muscle fibers with peripheral myonuclei (anomalous muscle fibers) is found in human EOM spindles. In contrast, artiodactyl EOM spindles are plentiful and exhibit a morphology comparable to that of other skeletal muscle spindles in the occurrence of nuclear bag fibers and the size of their periaxial space. Another putative proprioceptive organ, the so-called innervated myotendinous cylinder (IMC) has been described in the myotendinous region in EOMs of rhesus monkeys,16 cats,17 sheep,18 and humans.14 IMCs differ in several aspects from Golgi tendon organs (GTOs). They consist of a single multiply innervated muscle fiber of the global layer and its attached tendon. IMCs are encapsulated by a thin fibrocytic capsule and are supplied with one myelinated axon with preterminals and terminals that establish contacts both with the tendon fibers and the muscle fiber within the IMC capsule. Ultrastructural investigations have shown that myoneural IMC terminals are sensory in cats,17 monkeys,16 and sheep,18 whereas in humans these myoneural synapses are almost certainly motor. Findings also revealed by α-bungarotoxin staining.14

In other skeletal muscles, GTOs are the second classic proprioceptor, but they are scarce (rhesus monkey19) or absent (guinea pig,4 humans14) in EOMs of several mammals. GTOs were found to be numerous in EOMs of cattle,4 sheep,20–22 and camels.8 To date, the fine structure of the GTOs has been investigated in the distal tendon of rhesus monkey EOMs,19 in the proximal tendon of sheep EOMs,23 and in the distal peripheral patch layer24 of sheep EOMs.22 GTOs in the EOMs of these species differ from GTOs in other mammalian skeletal muscles. In a typical GTO of mammalian limb muscles, up to 20 muscle fibers are attached externally to one pole of the GTO.24–26 In contrast, GTOs so far described in EOMs are associated with up to five muscle fibers. In several GTOs of rhesus monkey19 and sheep EOMs,21,22 muscle fibers enter one pole of the proprioceptor and terminate in a collagen bundle within the GTO. In the distal peripheral patch layer of sheep EOMs,25 traversing muscle fibers enter one pole of the GTO and exit the proprioceptor at the opposite pole in 50% of the GTOs.22 Intracapsular muscle fibers in GTOs of rhesus monkey and sheep EOMs always exhibit morphologic features of multiply innervated muscle fibers.19,21,22

Although the importance of proprioceptive input from EOMs has been established in many studies,1–3,9,27,28 the significance of species differences in the proprioceptive complement of EOMs is still poorly understood. We therefore analyzed EOMs of several mammals including human, monkey, cat, guinea pig, rabbit, sheep, and pig. Up to now the entire proprioceptive complement of EOMs in any species has never been described in detail. As far as mammalian EOMs have been...
investigated, GTOs have been only found in a few species. To gain more information on the interspecies variation of the proprioceptive complement in mammalian EOMs and its possible variability, we analyzed the encapsulated proprioceptors in pig EOMs. The purpose of this study was to demonstrate GTOs in pig EOMs and to determine whether structural particularities described in former studies also occur in GTOs of pig EOMs. Further, we quantitatively analyzed GTOs and muscle spindles and precisely determined their locations within the muscle. The results of the present study may help to explain the results of previous electrophysiological studies in pigs and may be the basis for further electrophysiological studies. Determining the total proprioceptive complement of EOMs of any species will contribute to solving the major enigma of why species differences occur and to extending the basis for a better understanding of the development of EOM proprioceptors during evolution. This will also provide important new insights into oculomotor control in humans.

**Materials and Methods**

Four pigs were used in the present study. Animals were managed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Two of them (2 months and 1 year old) were killed by a lethal dose of pentobarbital sodium, and the others (3 and 5 years old) were obtained from a local abattoir. The EOMs from the left orbits of three pigs (1, 3, and 5 years of age) were processed for light microscopy. The EOMs from the right orbit of the 1-year-old pig were prepared for transmission electron microscopy. The EOMs from the left orbit of the 2-month-old animal were processed for histochemistry and immunohistochemistry.

**Light Microscopy**

In the 1-year-old pig, the circulatory system of the head was rinsed with a Ringer solution followed by perfusion and fixation with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The orbit was dissected, and the EOMs, including their distal and proximal tendons, were excised. From the 3-year-old and 5-year-old pigs the EOMs were removed in their full lengths. The EOMs, including the levator palpebrae superioris of the three pigs, were immersion fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). The muscles were cut transversely into three pieces, dehydrated in ethanol, and embedded in Epon. Complete series of paraffin cross sections (10 µm) were cut on a microtome and mounted on glass slides. Serial sections were divided into groups of three. Every first section was stained with azan. On every second section, acidic mucopolysaccharides were stained by ferric oxide. Every third section was impregnated with silver.

**Electron Microscopy**

After perfusion fixation, the right medial rectus of the 1-year-old pig was removed and immersion fixed in a solution identical with that used for sections prepared for light microscopy. The distal and the proximal portions of the medial rectus were cut into pieces with a width of 4 mm and a length of 5 mm. Specimens were postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Semithin sections (1 µm) were cut from the tendon ends of the tissue blocks toward the muscle belly. One tissue block was cut longitudinally. When GTOs were identified by light microscope, ultrathin sections were cut at appropriate intervals, mounted on dioxane formvar-coated copper grids, stained in a solution of 2% uranyl acetate followed by 0.4% lead citrate, and examined under an electron microscope (EM 10; Carl Zeiss, Oberkochen, Germany).

**Histochemistry and Immunohistochemistry**

The left lateral, medial, and inferior rectus muscles of the 2-month-old pig were excised and transferred into 20% sucrose solution (pH 7.4), followed by freezing in liquid nitrogen. Transverse sections (10 µm) of the EOMs were cut on a cryostat microtome (KryoCut model 3000; Leitz, Wetzlar, Germany). For histochemistry, sections were processed to demonstrate the enzyme activities of succinic dehydrogenase (SDH) and myofibrillar actomyosin adenosine triphosphatase (mATPase), after acid (pH 4.4) and alkal (pH 10.4) preincubation, according to the method of Guth and Samaha. For immunohistochemistry, unfixed sections were incubated with primary monoclonal antibodies (mouse monoclonal; Novocastra Laboratories Ltd., Newcastle-upon-Tyne, UK) against slow-twitch myosin heavy chain (NCL-MHCs) and fast-twitch myosin heavy chain (NCL-MHCF) at 37°C for 1 to 3 hours. After washing in phosphate-buffered saline (PBS, pH 7.4) sections were incubated with the secondary antibody (goat anti-mouse peroxidase-conjugated immunoglobulin, NCL-GAMP, polyclonal; Novocastra Laboratories Ltd.) at 25°C for 1 hour, followed by washing in PBS (pH 7.4). Diaminobenzidine (DAB) was used as a chromogen.

**Morphometry**

Muscle spindles and GTOs were identified with a light microscope. Their capsule lengths were calculated by counting the number of paraffin sections with known thickness. The maximal width of the GTOS was measured in paraffin sections. The diameters of the nerve fibers (axon plus myelin sheaths) innervating the GTOs were measured in semithin and ultrathin sections. In two muscles, the exact positions of muscle spindles and GTOs were reconstructed from camera lucida drawings.

**Results**

**Topographical Features of the Distal and Proximal Tendon in Pig EOMs**

The analysis of complete serial paraffin cross sections of pig EOMs revealed that both the distal tendons and the proximal tendons had C-shaped aponeurotic expansions that extended on the surfaces of the EOMs. Aponeurotic expansions consisted of densely arranged collagen bundles. These fibrous expansions covered the entire orbital surface and parts of the global surface of each EOM (Fig. 1). Muscle fibers coming from the muscle belly were attached in a staggered way to the collagen bundles of the distal and proximal aponeuroses. Toward the muscle belly more isolated collagen strands were visible that stopped at the point where they were anchored to muscle fibers. The distal aponeuroses extended over approximately half of the EOM's length, whereas the proximal aponeuroses covered approximately one third of the EOM's length.

The levator palpebrae superioris also had a C-shaped aponeurotic expansion of its distal tendon, comparable to those of the other EOMs. The aponeurosis of the proximal tendon surrounded the orbital and global surfaces of the muscle.

**Location and Number of the GTOs**

The GTOs were distributed over the full extent of the proximal and distal aponeuroses. For identification of GTOs in paraffin sections the following criteria published in a previous report were used: continuity of the capsule, presence of collagen bundles inside the organs, occurrence of acidic mucopolysaccharides within the capsule space and nerve fibers innervating the GTOs (Figs. 2A–2C). In three pigs of different age comparable numbers of GTOs were counted in the recti and oblique EOMs (Table 1). In all animals, GTOs were numerous in the distal aponeuroses, but their number was higher in the recti EOMs than in the oblique EOMs. In the proximal aponeuroses...
of the recti muscles GTOs were less numerous, and only three of them were observed in the proximal aponeuroses of the oblique muscles (Table 1).

In the aponeuroses of the levator palpebrae superioris muscles, GTOs were absent with the exception of one GTO detected in the proximal aponeurosis of the 1-year-old pig.

**Morphology of the GTOs**

GTOs had fusiform shapes and were enveloped by a thin capsule consisting of two to four cell layers. The capsule cells passed into the perineurium of the nerves innervating the GTOs and had a basal lamina investment. Thus, similar to limb
muscles, GTO capsules consisted of perineurial cells. Adjacent capsule cell layers were separated by collagen fibrils. Inside, the GTOs’ collagen bundles were arranged in parallel with the longitudinal axis of the receptors (Fig. 2D). In addition to collagen bundles, intracapsular muscle fibers were observed in approximately one third of the GTOs (Figs. 2E, 2F). In their pole regions, the capsule tightly enclosed the collagen bundles and the intracapsular muscle fibers (if present). In the GTO equatorial region a tissue-free space containing acidic mucopolysaccharides separated the collagen bundles and the intracapsular muscle fibers from the capsule in most GTOs. This tissue-free space was absent in only a few proprioceptors (Fig. 2D). The collagen bundles and intracapsular muscle fibers were incompletely encircled by thin processes of fibroblasts. In electron micrographs these fibroblasts had no basal lamina. Two poles can be distinguished in each GTO. In the following, the pole directed toward the muscle belly will be referred to as the muscular pole and the opposite pole as the tendinous pole. Reconstructions of serial cross sections allowed to identify four types of GTOs in the distal and proximal aponeuroses of each EOM as demonstrated schematically in Figure 3. A total of 534 GTOs in the EOMs of the 1-year-old pig formed the basis of this classification which was confirmed by the analysis of the three other pigs. The first type was represented by GTOs that contained exclusively collagen bundles. Between 46% and 82% of the GTOs in each EOM exhibited type 1 characteristics. One to three muscle fibers were observed to terminate in collagen bundles 50 to 200 μm outside the capsules (Fig. 3A). In some cases, the GTO collagen bundles could be followed outside the GTO for approximately 500 μm where they were not directly attached to muscle fibers but intermingled with other collagen bundles. In type 2 characteristics, one to three muscle fibers entered the muscular pole of the receptors and exited the GTOs at the tendinous pole. These traversing muscle fibers entered the muscular pole of the receptors and exited the GTOs at the tendinous pole. These traversing muscles always occupied an eccentric position within the GTOs. Traversing muscle fibers were either embedded in collagen bundles or separated from the latter (Fig. 3C).

<table>
<thead>
<tr>
<th>EOM</th>
<th>One-Year-Old Pig</th>
<th>Three-Year-Old Pig</th>
<th>Five-Year-Old Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distal Tendon</td>
<td>Proximal Tendon</td>
<td>GTOs per EOM</td>
</tr>
<tr>
<td>Superior rectus</td>
<td>81</td>
<td>28</td>
<td>109</td>
</tr>
<tr>
<td>Inferior rectus</td>
<td>90</td>
<td>16</td>
<td>106</td>
</tr>
<tr>
<td>Medial rectus</td>
<td>89</td>
<td>25</td>
<td>114</td>
</tr>
<tr>
<td>Lateral rectus</td>
<td>83</td>
<td>21</td>
<td>104</td>
</tr>
<tr>
<td>Superior oblique</td>
<td>47</td>
<td>1</td>
<td>48</td>
</tr>
<tr>
<td>Inferior oblique</td>
<td>52</td>
<td>1</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>442</td>
<td>92</td>
<td>534</td>
</tr>
</tbody>
</table>

Figure 3. (A–D) Schematic diagrams of four types of GTOs. (A) Type 1. The GTO contains exclusively collagen bundles. Two muscle fibers (MF) are attached outside the capsule to the GTO traversing collagen bundles. Nerve (N), collagen bundles (COL), capsule (C). (B) Type 2. Three muscle fibers terminate inside the GTO in collagen bundles. (C) Type 3. One muscle fiber traverses the GTO. (D) Type 4. One muscle fiber terminates inside the GTO, and another muscle fiber traverses the GTO.
One to 4% of the GTOs in each EOM combined type 2 and type 3 characteristics (type 4; Fig. 3D).

All intracapsular muscle fibers of the GTOs were morphologically indistinguishable from adjacent extracapsular muscle fibers and exhibited subsarcolemmal myonuclei. Electron micrographs demonstrated that all intracapsular muscle fibers of the GTOs had densely arranged myofibrils, few small mitochondria, and a poorly developed sarcoplasmic reticulum. In longitudinal sections of the sarcomeres the M-line was absent within the H-band. Intracapsular muscle fibers exhibited a high activity for mATPase after acid preincubation and displayed no mATPase activity after alkali preincubation (Figs. 4A, 4B). In sections stained for SDH, intracapsular muscle fibers showed a fine granular pattern of sparse formazan particles (Fig. 4C). For immunohistochemistry GTOs were identified with azan staining (Fig. 4D). Intracapsular muscle fibers were immunoreactive for anti slow-twitch MHC antibody and negative for anti-fast-twitch MHC antibody (Figs. 4E, 4F).

Blood vessels were found in some GTOs. They entered the receptors together with the innervating nerves or separately (Fig. 2E).

Morphometry

The lengths of the GTO capsules were measured in three EOMs of the 1-year-old pig (medial rectus, lateral rectus, and inferior oblique). In the distal aponeuroses the capsule lengths varied between 180 and 810 μm (mean, 387 ± 123 μm [± SD], n = 224) and their maximal widths between 40 and 130 μm (mean, 67 ± 25 μm, n = 224). GTOs in the proximal aponeuroses

![Figure 4](https://example.com/figure4.png)

**Figure 4.** (A–C) Consecutive frozen sections through a GTO with one intracapsular muscle fiber in the distal aponeurosis of the left lateral rectus. (A) Capsule (C). The intracapsular muscle fiber (arrow) is positive for ATPase after acid preincubation and (B) negative for ATPase after alkali preincubation. (C) Staining for SDH shows sparse formazan particles in the intracapsular muscle fiber. (D–E) Consecutive frozen sections through a GTO with two intracapsular muscle fibers in the distal aponeurosis of the left medial rectus. (D) The GTO was identified by azan staining. Intracapsular muscle fibers (arrow). (E) The intracapsular muscle fibers are positive for anti slow-twitch MHC antibody and (F) negative for anti fast-twitch myosin antibody. Scale bars, 10 μm.
were smaller, 240 μm up to 510 μm long (mean, 351 ± 67 μm, 
n = 47), and their maximal widths varied between 30 and 70 μm (mean, 38 ± 9 μm, n = 47).

### Innervation of the GTOs

A single myelinated nerve fiber 6 to 8 μm in diameter (6.7 ± 0.7 μm, n = 15) entered each GTO approximately centrally. Inside the GTOs, these nerve fibers divided into two to three myelinated nerve branches which extended toward the receptor poles. In some instances, splitting of the myelinated nerve fiber occurred shortly before its entrance into the receptor. Within the GTO, myelinated nerve fibers running toward both GTO poles gave rise to smaller nerve branches. These smaller, still myelinated nerve fibers entered the longitudinally oriented collagen bundles. Within the collagen bundles the nerve fibers lost their myelin sheaths and divided into preterminal axons which showed a complete Schwann cell envelope. A basal lamina separated the outer surface of the Schwann cell from surrounding collagen bundles. The axoplasm of preterminal axons contained mitochondria, neurotubules, and neurofilaments. Preterminal axons branched extensively to form numerous nerve terminals. The relationship between nerve terminals and collagen bundles was investigated using reconstructions of ultrathin serial sections. Some nerve terminals were located between collagen bundles that divided and reunited again. Other nerve terminals were found between collagen bundles that twisted around each other like braided strands in a rope. Nerve terminals were oval or sickle shaped. Some sickle-shaped nerve terminals were completed by Schwann cells to form annular enclosures around collagen fibrils (Figs. 5A, 5B). Only parts of the nerve terminals were covered by Schwann cell processes. Areas devoid of a Schwann cell investment were usually coated by a basal lamina. In some nerve terminals the basal lamina was interrupted so that the axolemma established intimate contacts with the neighboring collagen fibrils. The flocculent axoplasm of the nerve terminals contained a large number of mitochondria that were either evenly distributed or arranged in clusters. Few clear vesicles were observed in some nerve terminals (Figs. 5B–5D).

In case the GTOs contained intracapsular muscle fibers, no myoneural synapses were found because collagen fibrils separated the nerve terminals from the muscle fiber (Fig. 5D).

### Number and Morphology of Muscle Spindles

Muscle spindles were counted in all EOMs of three pigs (Table 2). The superior oblique muscles had most muscle spindles, whereas the inferior obliques had the fewest (Table 2). The number of muscle spindles exceeded those of the GTOs in each EOM (Tables 1, 2). However, the ratio of muscle spindles.

Figure 5. (A) Ultrathin cross section through a GTO in the distal aponeurosis of the right medial rectus. Numerous nerve terminals (arrowhead) are among the collagen bundles. Capsule (C). (B) Detail of (A). A nerve terminal (T) completely encloses collagen fibrils. Annular enclosures are completed by Schwann cell processes (S). A basal lamina (arrowhead) which is interrupted (arrow) lies between the axolemma and the collagen. The nerve terminal contains mitochondria (M). (C) Ultrathin longitudinal section through a nerve terminal. Basal lamina (arrowhead). (D) Ultrathin cross section. A nerve terminal lies close to the surface of an intracapsular muscle fiber. Basal lamina (arrowhead). Scale bar, 1 μm.
to GTOs was higher in the oblique muscles than in the recti muscles (Table 3).

Most muscle spindles contained four to five intrafusal muscle fibers. Others had up to nine intrafusal muscle fibers. Each muscle spindle possessed a single nuclear bag fiber that, similar to other skeletal muscle spindles, exhibited an accumulation of myonuclei in its equatorial region. The remaining intrafusal muscle fibers were of the nuclear chain fiber type with a single row of central myonuclei in their equatorial region. Nuclear bag and nuclear chain fibers received sensory terminals. In the equatorial region of the muscle spindles a large periaxial space separated the intrafusal muscle fibers from the capsule.

**Distribution Pattern of Muscle Spindles and GTOs**

The distribution of muscle spindles and GTOs within the left superior rectus and the left superior oblique of the 1-year-old pig is shown in Figure 6. In both EOMs muscle spindles were distributed over the entire muscle length. Only the most distal and the most proximal portions were devoid of spindles. Those parts of the EOMs that were covered by the aponeuroses of the distal and proximal tendons contained both muscle spindles and GTOs. GTOs were located at the muscle surface, whereas most muscle spindles were found in the orbital layer or in the global layer. Some muscle spindles were observed in the transition zone between the orbital and global layers.

**Innervated Myotendinous Cylinders**

Despite detailed investigations, no IMCs could be found in this tissue.

**DISCUSSION**

This study presents the results of a qualitative and quantitative investigation of the entire complement of encapsulated proprioceptors in pig EOMs. For the first time, the distribution of all encapsulated proprioceptors in a certain species is precisely shown over the whole muscle length. In addition to a high number of muscle spindles, GTOs are consistently present in the recti and the oblique EOMs in pigs. In all EOMs of three pigs the quantity of these two proprioceptors is rather constant. All GTOs are distributed in aponeurotic expansions of the distal and proximal tendons. GTOs are more numerous in the recti EOMs than in the oblique EOMs. In each EOM, their number was higher in the distal aponeurosis than in the proximal aponeurosis. In the EOMs muscle spindles outnumber GTOs. The ratio of muscle spindles to GTOs is different in the recti and oblique muscles. In particular, values are very similar in the recti muscles, whereas they are remarkably high in the oblique ones. In pig levator palpebrae superioris muscles GTOs are extremely rare or absent, whereas more GTOs were found in the levator palpebrae superioris muscles of sheep and camels. In the current study GTOs are described for the first time in pig EOMs and the morphology of these receptors is presented in detail.

Two previous studies supplied data on muscle spindles in pig EOMs. In a quantitative analysis of EOM spindles in several mammals, Maier et al. counted the muscle spindles in four EOMs (medial rectus, lateral rectus, inferior rectus, and inferior oblique) of one neonatal pig. In the present study, we investigated all EOMs of three pigs. We found a comparable number of muscle spindles in the recti EOMs, whereas the number of muscle spindles in the inferior oblique is higher than previously reported. The superior oblique muscles are unique in containing a surprisingly high number of muscle spindles. Kubota described the ultrastructure of pig EOM spindles and found most of them to contain a single nuclear bag fiber and three to four nuclear chain fibers. These observations are congruent with the results of the present study.

As sheep and camel EOMs, we found the muscle spindles to be distributed over the whole muscle lengths in pig EOMs. In contrast, the midbelly region of human EOMs contained no spindles. These spindle-free zones coincided with the zones of en plaque motor endplates. This study is in line with previous investigations that described the presence of GTOs and muscle spindles in EOMs of artiodactyls (i.e., cattle, camels, and sheep) although the presence of spindles in pig EOMs has been reported. The occurrence of GTOs was established in the current study. Evidence at hand suggests that a high number of both proprioceptors in the EOMs of artiodactyls is a unique feature among mammals.

**Table 2. Number of Muscle Spindles in the Pig EOMs of the Left Orbit**

<table>
<thead>
<tr>
<th>EOM</th>
<th>One-Year-Old Pig</th>
<th>Three-Year-Old Pig</th>
<th>Five-Year-Old Pig</th>
<th>Number of Muscle Spindles (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior rectus</td>
<td>212</td>
<td>208</td>
<td>213</td>
<td>211 ± 2.6</td>
</tr>
<tr>
<td>Inferior rectus</td>
<td>184</td>
<td>210</td>
<td>181</td>
<td>191.7 ± 15.9</td>
</tr>
<tr>
<td>Medial rectus</td>
<td>229</td>
<td>195</td>
<td>201</td>
<td>208.4 ± 18.1</td>
</tr>
<tr>
<td>Lateral rectus</td>
<td>205</td>
<td>186</td>
<td>179</td>
<td>190 ± 13.5</td>
</tr>
<tr>
<td>Superior oblique</td>
<td>333</td>
<td>314</td>
<td>284</td>
<td>310.3 ± 24.7</td>
</tr>
<tr>
<td>Inferior oblique</td>
<td>156</td>
<td>142</td>
<td>148</td>
<td>148 ± 7.0</td>
</tr>
<tr>
<td>Total</td>
<td>1319</td>
<td>1255</td>
<td>1206</td>
<td>1260 ± 56.7</td>
</tr>
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</table>

**Table 3. Ratio of Muscle Spindles to GTOs in the Pig EOMs of the Left Orbit**

<table>
<thead>
<tr>
<th>EOM</th>
<th>One-Year-Old Pig</th>
<th>Three-Year-Old Pig</th>
<th>Five-Year-Old Pig</th>
<th>Ratio (Mean ± SD)</th>
</tr>
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<tbody>
<tr>
<td>Superior rectus</td>
<td>1.9</td>
<td>1.7</td>
<td>2.1</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Inferior rectus</td>
<td>1.7</td>
<td>2.1</td>
<td>1.7</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Medial rectus</td>
<td>2.0</td>
<td>1.5</td>
<td>1.8</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Lateral rectus</td>
<td>1.9</td>
<td>1.6</td>
<td>1.6</td>
<td>1.7 ± 0.2</td>
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<tr>
<td>Superior oblique</td>
<td>6.9</td>
<td>6.9</td>
<td>4.6</td>
<td>6.1 ± 1.3</td>
</tr>
<tr>
<td>Inferior oblique</td>
<td>2.9</td>
<td>3.1</td>
<td>3.0</td>
<td>3.0 ± 0.1</td>
</tr>
</tbody>
</table>
Each GTO in pig EOMs has a perineurial capsule and shows an intracapsular bidirectional arborification of nerve fibers. Nerve terminals establish contacts with collagen fibrils. These features are consistent with GTOs of mammalian limb and trunk muscles and provide sufficient evidence to classify these encapsulated organs of this study as GTOs. However, fluid-filled spaces separating collagen bundles from the GTO capsule and intracapsular muscle fibers observed in most GTOs of pig EOMs are absent in GTOs of mammalian limb and trunk muscles. The structural particularities of GTOs in mammalian EOMs described in previous reports are also present in numerous GTOs of pig EOMs. In accordance with findings in GTOs of rhesus monkey and sheep EOMs, this investigation demonstrates the occurrence of intracapsular muscle fibers in one third of pig GTOs. As in the distal peripheral patch layer of sheep EOMs, we found GTOs with traversing muscle fibers also in pig EOMs. Another similarity between GTOs of both species is the presence of a fluid-filled space which, however, appears less pronounced in pigs. Consequently, equatorial diameters of pig GTOs (67 ± 25 μm) are smaller than their counterparts in sheep EOMs (101 ± 18 μm).

Recently, we were able to distinguish four morphologic types of GTOs in the distal peripheral patch layer of sheep EOMs. These four types correspond to the GTO types presented herein. However, differences in the numerical proportion have to be emphasized. In the distal peripheral patch layer of sheep EOMs, GTOs containing exclusively collagen bundles (GTO type 1) are few, and the majority of the GTOs contains traversing muscle fibers (GTO type 3). In contrast, most GTOs in pig EOMs exhibit type one characteristics and few GTOs are consistent with type 3. The remaining GTO types 2 and 4 are present in comparable percentages in both species.

Intracapsular muscle fibers in the GTOs of rhesus monkey and in sheep EOMs resembled the multiply innervated type in their fine structure. This was further confirmed by an immunohistochemical analysis of the MHC pattern of intracapsular muscle fibers in sheep EOMs. In the present study, intracapsular muscle fibers in GTOs of pig EOMs were analyzed by transmission electron microscope and light microscope, by means of histochemistry and immunohistochemistry. Intracapsular muscle fibers in pig GTOs have few and small mitochondria, sparse sarcoplasmic reticulum, and densely arranged myofibrils. These fine structural features correlate with multiply innervated muscle fibers in EOMs of various mammals. Further, it was shown that intracapsular muscle fibers in GTOs of pig EOMs were positive for mATPase after acid preincuba-
tion and positive for slow-twitch MHC antibody. From studies on other mammalian species and humans it is well known that muscle fibers exhibiting this staining pattern for mATPase and slow-twitch MHC belong to the multiply innervated type. Recently, it was demonstrated in rat EOMs that multiply innervated muscle fibers of the orbital layer contain slow-twitch MHC and additionally embryonic MHC. We cannot exclude that multiply innervated intracapsular muscle fibers in GTOs of pig contain other MHC isoforms. Based on fine structure and on histochemical staining for mATPase and SDH, intracapsular GTO muscle fibers of pigs correspond to the multiply innervated muscle fibers in the distal peripheral patch layer of sheep EOMs. Further, based on mATPase and SDH activity and MHC expression, intracapsular muscle fibers in GTOs of pig EOMs resemble the low-oxidative, multiply innervated muscle fibers in the marginal zone of human EOMs. In conclusion, these results indicate that intracapsular GTO muscle fibers of pig EOMs may derive from a particular muscle layer comparable with the peripheral patch layer in sheep EOMs and the marginal zone in human EOMs.

GTOs containing intracapsular muscle fibers with sensory myoneural contacts have been observed in developing limb muscle of rats. A few days after birth, these muscle fibers withdraw from the receptors and in mature GTOs sensory nerve terminals is exclusively found among the collagen bundles. Ruskell argued that the occurrence of intracapsular muscle fibers observed in numerous GTOs of young sheep (11–14 months old) and young rhesus monkeys EOMs may be a sign of a prolonged GTO maturation. These intracapsular muscle fibers may get lost with advancing age. If, however, this particular morphology were also observed in GTOs of old animals of the same species, Ruskell stated that this would indicate the retained development of these receptors. Recently, we compared GTOs in the EOMs of a 1-year-old and a 5-year-old sheep but found no differences in the morphology of their GTOs. The results of the present study are in line with this investigation. The incidence of intracapsular muscle fibers is almost the same in GTOs of four pigs ranging from 2 months to 5 years of age. Moreover, sensory neuromuscular contacts observed in developing GTOs of rat are not found in the GTOs of pig EOMs. We conclude that the particular morphology of numerous GTOs in pig EOMs indicates that these receptors are retained in development, as we previously observed in sheep EOMs.

It was proposed by Ruskell that in case of a retained development in GTOs of mammalian EOMs, the utility of GTOs may be questionable. However, the following arguments favor the endowment of pig EOMs with special but functioning GTOs. The number of GTOs is high in those pig EOMs that enable movements of the globe. Moreover, all GTOs in pig EOMs are richly supplied with nerve terminals that are distributed in each collagen bundle. These nerve terminals contain numerous mitochondria and are partly invested with Schwann cells. Because large portions of the nerve terminals are covered only with a basal lamina they establish intimate contacts with the neighboring collagen fibrils. In some areas, the basal lamina of nerve terminals is interrupted. These findings are consistent with those of nerve terminals in GTOs of other mammalian skeletal muscles.

**Functional Considerations**

Electrophysiological studies focusing on afferents from EOMs are rare. Interesting studies have been performed in various artiodactyls including pigs. After muscle stretch, afferent signals from pig EOM spindles were recorded in the ipsilateral trigeminal ganglion. The discharge characteristics of EOM muscle spindles were analogous to those recorded from limb muscle spindles. At least in mammalian non-EOM spindles, the ability to monitor contraction velocity is ascribed to sensory endings contacting bag fibers. Because of the absence of bag fibers in human and monkey EOM spindles, they are thought to monitor muscle length without any velocity component. In contrast, other skeletal muscle spindles and artiodactyl EOM spindles share many morphologic similarities. Therefore, they are likely to possess the full range of functional capacities of spindles in other skeletal muscles.

The functional importance of GTOs has never been investigated in mammalian EOMs. In mammalian limb and trunk muscles, active muscle contraction is the effective stimulus for GTO excitation. The contraction of the muscle fibers attached to the GTOs tightens the collagen bundles, which entails deformation of the nerve terminals. The distorted nerve terminals are thought to generate an action potential. Along these lines, we assume that contraction of muscle fibers that terminate inside the GTOs or that are attached to traversing GTO collagen bundles outside the receptors would deform the nerve terminals between braided and splitting collagen bundles in GTOs of pig EOMs. Contraction of traversing muscle fibers in GTOs is thought to decrease the sensitivity of the receptors by reducing the strain on the GTO collagen bundles. Thus, a more powerful contraction of muscle fibers associated with GTOs would be necessary to stretch the collagen bundles and to deform the nerve terminals.

GTOs in pig EOMs are associated with one to three muscle fibers, most likely of the multiply innervated type. After stimulation, multiply innervated muscle fibers were reported to exhibit slow, longlasting local contractions. We assume that GTOs in pig EOMs would register minute contractions of single or very few multiply innervated muscle fibers. Moreover, the widespread distribution of GTOs over the length of the recti EOMs indicates that these receptor monitor contractions in most portions of the muscles. We therefore conclude that GTOs in pig EOMs are capable of registering fine eye movements. Along these lines, the question arises whether IMCs in those mammals without GTOs in their EOMs play a similar functional role. If this is true, IMCs would be even more sensitive in registering fine eye movements, because they are associated with only a single multiply innervated muscle fiber.

The results of the present study indicate that GTOs in pig EOMs would respond to muscle contraction, and electrophysiological studies demonstrate that muscle spindles are sensitive to muscle stretch. Thus, EOMs of pigs may be supplied with two feedback systems. During contraction of an EOM, GTOs are thought to register muscle tension, whereas muscle spindles monitor muscle length. We conclude that afferent signals from both proprioceptors may provide sufficient information about the direction of ocular movement. This may also be of importance for movement vision in these animals.

To date, the nature of afferent signals that can be assigned to GTOs in mammalian EOMs has been unknown. This study presents two EOM nervous end organs that are unequivocally sensory. Thus, it may be the basis for further electrophysiological studies to investigate afferents from GTOs and to distinguish between afferent signals from two kinds of proprioceptors. After all, these highly warranted studies will contribute to important new insight in the general organization of oculomotor control.

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References