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The fibrous tapetum of the horse eye

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Abstract

The tapetum lucidum is a light-reflective tissue in the eyes of many animals. Many ungulates have a fibrous tapetum. The horse has one of the largest eyes of any living animal and also has excellent vision in low-light environments. This study aimed to clarify the macroscopic tapetal shape, relationship between the tapetal thickness and the degree of pigmentation of the retinal pigment epithelium (RPE), spatial relationship between the visual streak and the tapetum, and wavelength of the light reflected from the tapetum in the horse. Macroscopically, weak light revealed the tapetum as a horizontal band located dorsal to and away from the optic disc. The tapetum expanded dorsally as the illumination increased. The tapetal tissue consisted of lamellae of collagen fibrils running parallel to the retinal surface; these spread over almost the entire ocular fundus and were thicker in the horizontal band dorsal to the disc. Only the horizontal band of the tapetum was covered by unpigmented RPE, suggesting that this band reflects light and is responsible for mesopic and scotopic vision. The visual streak was located in the ventral part of the horizontal band, ventral to the thickest part of the tapetum. The wavelength of the light reflected from the horizontal band of the tapetum was estimated from the diameter and interfibrous distance of the collagen fibrils to be approximately 468 nm. Therefore, the light reflected from the tapetum should be more effectively absorbed by rods than by cones, and should not interfere with photopic vision.

Key words: collagen fibril; fibrous tapetum; horse; retinal pigment epithelium; visual streak.

Introduction

The eye of the horse is one of the largest among those of the terrestrial mammals (Knill et al. 1977; Waring, 2003). The horse has relatively good visual acuity, approximately half that of humans and twice that of domestic cats (Timney & Keil, 1992; Harman et al. 1999). Horses are active both during the day and at night. This lifestyle requires a sensitive visual system that functions well under low-light conditions. The horse's ability to see under low-light levels is enhanced by its rod-dominant retina and by the presence of a tapetum lucidum (Murphy et al. 2009).

The tapetum lucidum is a light-reflective tissue found in the eyes of both vertebrates and invertebrates. In most mammals, the tapetum is located in the choroid, between the choriocapillary layer and the proper substance of the choroid, and its location is close to the photoreceptors. This is called the choroidal tapetum and can be one of two types: a fibrous tapetum (*tapetum fibrosum*) or a cellular tapetum (*tapetum cellulosum*). A fibrous tapetum consists of layers of regularly arranged collagen fibrils, whereas a cellular tapetum consists of layers of rectangular tapetal cells, including rodlets. Fibrous tapeta are found in the eyes of many ungulates, although not those of pigs and camelids, and cellular tapeta are found in those of many carnivores. The tapetum is thought to reflect light that was not absorbed by the photoreceptors, returning it to the photoreceptors and thus giving them a second chance to perceive the light. The retinal pigment epithelium (RPE) is generally pigmented throughout the retina. However, the RPE in the tapetal area is unpigmented, allowing light to pass through. The tapetum thus increases visual sensitivity under low-light conditions, and improves mesopic and scotopic vision (Walls, 1967; Schwab et al. 2002; Ollivier et al. 2004).

Various structures and materials have been proposed to contribute to tapetal light reflection. However, the major mechanism by which the tapetum reflects light is thought to be thin-film interference produced by alternating high and low refractive layers (Land, 1972; Schwab et al. 2002; Ollivier et al. 2004). The wavelength of maximal reflectance (λ_{max}) of the light reflected by the tapetum can be calculated using the following formula for non-ideal multilayered systems: $\lambda_{max} = 2\ (n_a d_a + n_b d_b)$. In this formula, n_a and n_b represent the refractive indices of the optically light and dense layers, respectively, and d_a and d_b represent the

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actual thicknesses of the optically light and dense layers, respectively (Land, 1972). Therefore, the diameters of the collagen fibrils and the interfibrous distance, which correspond to the optically dense and light layers, respectively, not only generate tapetal reflection but determine the wavelength of the reflected light.

Although there have been several reports on the tapetum, especially the fibrous tapetum, there are only a few very short reports describing the fibrous tapetum of the horse (Bortolami et al. 1979; Ollivier et al. 2004). In our previous study on sheep, we suggested that the distribution of the tapetal tissue is broader than the unpigmented area of the RPE, causing the macroscopic tapetal shape to change with the brightness of the illumination. The functional tapetal area, which is covered by the unpigmented RPE and can be visualized macroscopically under weak light, constitutes only about half of the area of tapetal tissue in the sheep eye (Shinozaki et al. 2010b). Therefore, it is important to investigate the macroscopic tapetal shapes under various levels of illumination, the distribution of the tapetal tissue, and the pigmentation of the RPE through the ocular fundus in order to understand the effects of the fibrous tapetum on vision. However, previous studies of the fibrous tapetum have paid little attention to these points.

The retinal structures of horses have been studied to some extent. In the horse retina, the ganglion cells are densely distributed in the horizontal visual streak (Hebel, 1976; Harman et al. 1999; Guo & Sugita, 2000; Evans & McGreevy, 2007). The visual streak is responsible for better visual acuity and is important for vision in the horse. However, the spatial relationship between the visual streak and the tapetum has not been strictly defined.

In the present study, we investigated the fibrous tapetum in the horse eye by macroscopic and light and electron microscopic methods. We aimed to clarify the macroscopic tapetal shape, relationship between the tapetal thickness and the degree of pigmentation of the RPE, spatial relationship between the visual streak and the tapetum, and wavelength of the light reflected from the tapetum, and to discuss the functional role of the tapetum in vision in the horse.

Materials and methods

Animals

The present study used eyes removed from horses in a slaughterhouse. The horses were healthy Percheron/Norman/Breton crossbreds aged 2–3 years and weighing 900–1000 kg. Their sexes were not considered.

Macroscopic observation

The 21 left eyes were cut along the equatorial plane. After removal of the vitreous body from the posterior half of the eyecup, the ocular fundus was photographed both under available light (i.e. weak light) and with a flash (i.e. strong light). In the three eyecups out of the 21 eyes, the retina, including the RPE, was removed and their ocular fundi were photographed again.

Light microscopic observation

The posterior halves of the three left eyes from which the neural retina had been removed were fixed in a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The tissue was dehydrated through a graded ethanol series, and the posterior eyecup was divided into 10 parts and embedded in celloidin. Vertical sections 40 μm thick were made every 2 mm, stained with hematoxylin and eosin (HE), and coverslipped with Canada balsam. Transparent films ruled with black lines at 2-mm intervals were pasted under the glass slides. The thickness of the tapetum and pigmentation of the RPE were observed at 2-mm intervals in each section. The tapetal thickness was measured under the 40 × objective using an eyepiece with a scale attachment. The thickness was defined as the distance from the inner point contacting the choriocapillary layer to the outer point at which the melanocytes appeared in the tapetal layers. The pigmentation of the RPE was categorized as one of four grades: unpigmented; and slightly; moderately; and heavily pigmented (Fig. 1). The RPE con-

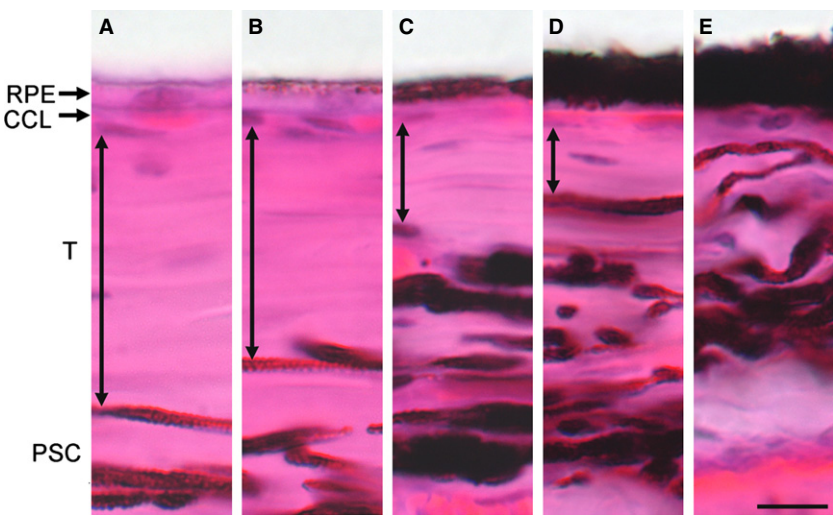

Fig. 1 Photomicrographs of the choroid and retinal pigment epithelium (RPE) in HE-stained vertical sections of a horse eye. The tapetal tissue (T) consists of eosinophilic layers between the choriocapillary layer (CCL) and the proper substance of the choroid (PSC), which includes the melanocytes. (A) Thick tapetal area covered by unpigmented RPE. (B) Relatively thick tapetal area covered by slightly pigmented RPE. (C) Tapetal area covered by moderately pigmented RPE. (D) Thin tapetal area covered by heavily pigmented RPE. (E) Choroid without tapetal tissue covered by heavily pigmented RPE. Scale bar: 10 μm (in E; applies to A–E).

taining very few melanin granules was defined as being unpigmented, containing sparse melanin granules as being slightly pigmented, containing many melanin granules but not filled as being moderately pigmented, and filled with a great amount of melanin granules as being heavily pigmented. The tapetal thickness and the pigmentation of the RPE throughout the ocular fundus were represented as topographical maps.

The relationship between the tapetum and the visual streak was investigated in three right eyes. The anterior half of the eye was removed and the remaining portion fixed as described for the left eyes. Then, the retina and choroid were detached from the sclera, dehydrated, embedded in celloidin, and cut vertically in the longitudinal direction into 50-μm sections, which were stained with thionine.

Digital photomicrographs were taken using an Olympus BH-2 microscope with an Olympus DXC-S500/OL camera.

Electron microscopic observation

The posterior cups of another three right eyes were fixed in a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After fixation, the choroid covered by the RPE was dissected into four parts (P1–P4), as shown in Fig. 2. The pieces were washed in phosphate buffer and post-fixed in 1% OsO_4 for 2 h. The pieces were then washed in distilled water, dehydrated through a graded ethanol series and embedded in epoxy resin. Semithin (1 μm) sections were cut, stained with toluidine blue, and observed by light microscopy to determine the orientation and obtain vertical sections. Ultrathin (80 nm) sections were cut using an ultramicrotome (MT-7000, RMC, USA) and stained, first with 0.2% tannic acid as a mordant for visualization of collagen (Afzelius, 1992; Watanabe et al. 2005), and then with uranium acetate and lead citrate. The ultrathin sections were observed using a JEOL 100CX transmission electron microscope (Japan). The diameter and the interfibrous distance were measured for approximately 200 randomly chosen collagen fibrils. From these data, the wavelength of maximal reflectance in each part was calculated using the following formula: $\lambda_{max} = 2\ (n_a d_a + n_b d_b)$, in which n_a was 1.33, n_b was 1.56, d_a was the mean interfibrous distance and d_b was the mean diameter of the collagen fibrils (Land, 1972; Young et al. 1988).

All values in the present study were expressed as the mean ± SD. The images captured in the photographs were not altered, except for adjustment of contrast and brightness using Adobe Photoshop Elements.

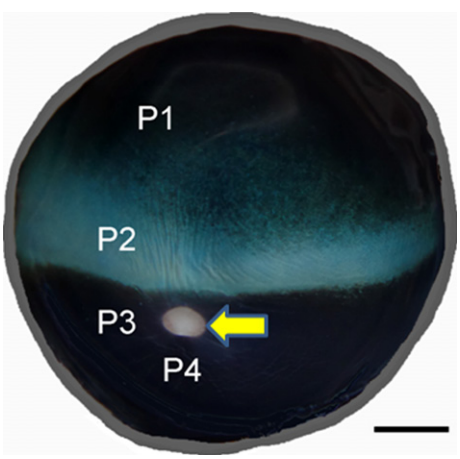

Fig. 2 Photograph of a horse's right ocular fundus under weak light. For electron microscopic examination, the choroid and RPE were dissected into four parts, as indicated by P1–P4. An arrow shows the optic disc. The nasal direction is to the right. Scale bar: 1 cm.

Results

Macroscopic observation

The tapetum was located dorsal to and away from the optic disc, and was metallic bluish green to blue in color. The shape of the tapetum changed with the degree of illumination. Under weak light, the tapetum was a horizontal band that narrowed nasally (Figs 2 and 3A). The ventral edge appeared as a clear horizontal line regardless of the brightness, while the dorsal edge was indistinct. As the brightness of illumination increased, the tapetum expanded dorsally and became semicircular at its maximal size, which appeared when it was viewed under strong light such as the flash of a camera (Fig. 3B). The shape of the tapetum differed only slightly among individuals. After the retina, including the RPE, was removed, the tapetum was apparent throughout the ocular fundus, including the part ventral to the optic disc (Fig. 3C).

Histological observations of the tapetum and RPE

In HE-stained sections, the tapetal tissue appeared as eosinophilic layers running parallel to the retinal surface. The tapetal tissue was located just beneath the choriocapillary layer and included a few fibroblasts. The melanocytes were distributed unevenly in the outer layer of the tapetal tissue, so that the outer border of the tapetum was indistinct (Fig. 1).

The RPE contained various amounts of melanin granules. The degree of pigmentation of the RPE was classified as shown in Fig. 1. The RPE containing very few melanin granules was defined as being unpigmented, and containing sparse melanin granules as being slightly pigmented. The RPE containing many melanin granules but detected other intracellular components was defined as being moderately pigmented. The RPE filled with a great amount of melanin granules and observed as a black layer was defined as being heavily pigmented.

Distribution of tapetal thickness

The distribution of the tapetal thickness is shown in Fig. 4A. The tapetal tissue spread over almost the entire ocular fundus. There was no tapetal tissue at the furthest periphery. The mean thickness across the entire tapetal area was 26 ± 2.2 μm. The tapetal thickness was greater in the area of the

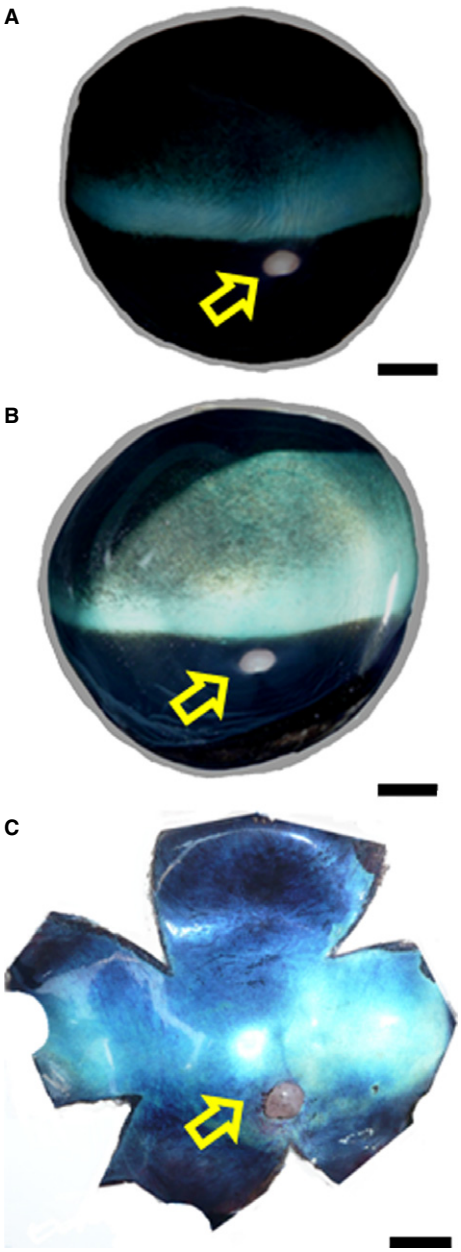

Fig. 3 Photographs of the horse's left ocular fundus under weak light (A), under a flash, i.e. strong light (B), and after the removal of the RPE (C). The tapetum is metallic bluish green to blue in color. Under weak light, the tapetum appears as a horizontal band (A). The tapetum expands dorsally with increasing brightness of illumination (B). In (C), the tapetum is apparent over most of the ocular fundus, including the part ventral to the optic disc (arrow). The nasal direction is to the left. Scale bars: 1 cm.

horizontal band, and decreased gradually in the dorsal direction and abruptly in the ventral direction. The horizontal band was more than 40 μm thick at every point, with a maximum tapetal thickness of 93 ± 5.1 μm. Calculation of the percentages of the area of each thickness showed that the thinner tapetal area predominated, while the thick tapetal area accounted for only a small portion (Fig. 5).

Pigmentation of the RPE

The degree of pigmentation of the RPE is shown in Fig. 4B. The unpigmented area corresponded approximately to the horizontal band of the tapetum and accounted for only a small percentage (11 ± 1.6%) of the entire area of the fundus with tapetal tissue. The slightly pigmented area formed a slim margin around the unpigmented area. The moderately pigmented area corresponded approximately to the area dorsal to the horizontal band, whereas the heavily pigmented area corresponded approximately to the area ventral to the optic disc, where the tapetum could not be detected macroscopically even where tapetal tissue was present.

Relationship of the tapetum to the visual streak

In vertical sections in the longitudinal direction, the visual streak in the retina appeared as a line of successively arranged ganglion cells that was located dorsally to the optic disc, within the ventral part of the horizontal band of the tapetum (Fig. 6). The ventral part of the visual streak was covered by slightly pigmented RPE. The visual streak was ventral to the thickest part of the tapetum in each section, in which the retinal ganglion cells were considerably less densely distributed than in the visual streak. The spatial relationship between the visual streak and the tapetum was approximately constant throughout the ocular fundus.

Ultrastructure of tapetal tissue

The tapetal tissue had a lamellar structure running parallel to the retinal surface. Each lamella consisted of collagen fibrils that ran parallel in the same direction at regular intervals. There were a few fibroblasts between contiguous lamellae (Fig. 7A). The lamellae were variable in thickness and frequently bifurcated or fused with other lamellae (Fig. 7B). Tapetal tissue was observed beneath the choriocapillary layer (Fig. 8). The scleral side of the tapetal tissue contained many melanocytes and fibroblasts between the lamellae, and the border between the tapetum and the proper substance of the choroid was indistinct (Fig. 7C). The area ventral to the optic disc contained only thin, fragmentary tapetal tissues that frequently mixed with melanocytes and fibroblasts (Fig. 8D). There were few melanin granules in the RPE over the horizontal band of the tapetum (Fig. 8B).

Diameter and interfibrous distance of the collagen fibrils

The mean diameter of the collagen fibrils was 114.0 ± 2.0 nm in P1, 127.8 ± 2.3 nm in P2, 116.4 ± 1.8 nm in P3 and 88.2 ± 4.9 nm in P4. The diameter tended to be greater in P2, which corresponded to the horizontal band, and

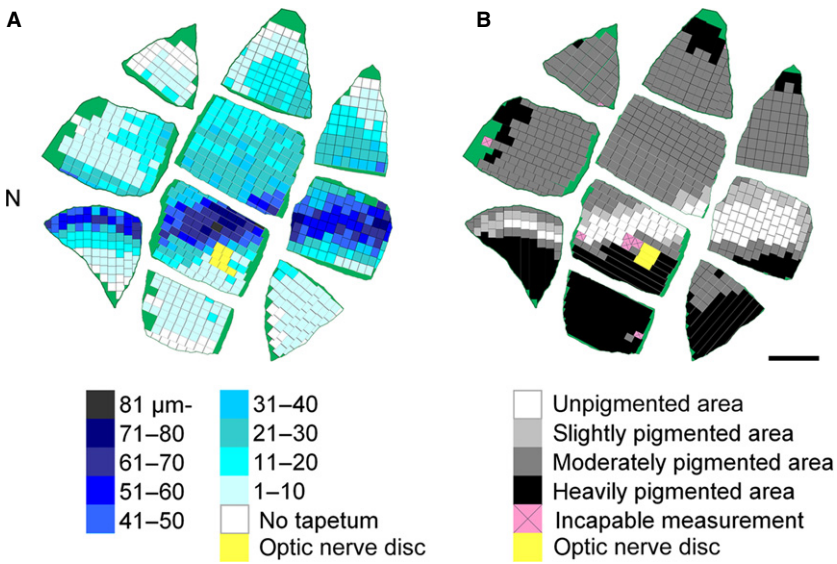

Fig. 4 Topographical maps of the tapetal thickness (A) and pigmentation of the RPE (B) of a horse's left eye. The thickness and degree of pigmentation are color-coded as shown in the insets. N, nasal. Scale bar: 1 cm (in b; applies to A, B).

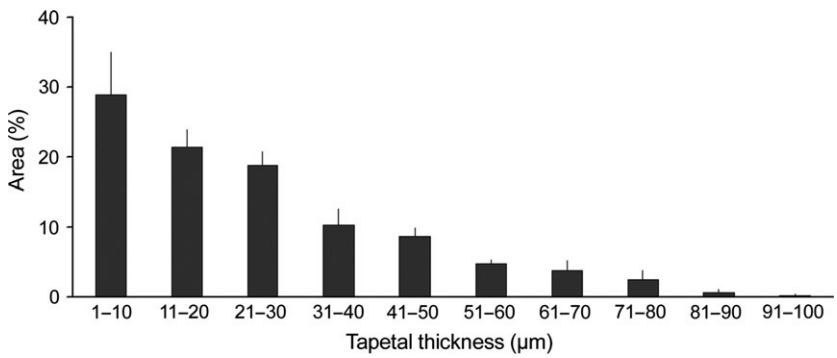

Fig. 5 Percentages of the area of the tapetum of each thickness. The thinner tapetal area predominates, with the thicker tapetal area occupying only a small proportion. The values are the means ± SDs of three horses.

smaller in P4 (Fig. 9A). The mean interfibrous distance of the collagen fibrils was 28.2 ± 6.2 nm in P1, 25.9 ± 2.9 nm in P2, 31.3 ± 4.9 nm in P3 and 25.6 ± 4.3 nm in P4. The interfibrous distance did not differ greatly among the parts (Fig. 9B).

Wavelength of the light reflected from the tapetum

The mean wavelength of maximal reflectance of the light reflected from the tapetum, as estimated from the diameter and interfibrous distance of the collagen fibrils, was 430.5 ± 10.4 nm in P1, 467.7 ± 13.0 nm in P2, 446.4 ± 15.0 nm in P3 and 343.3 ± 25.4 nm in P4. The wavelength tended to be longer in P2 and shorter in P4.

Discussion

In the present paper, we have described the macroscopic appearance of the tapetum, distributions of tapetal tissue and unpigmented RPE throughout the ocular fundus, and fine structure of collagen fibrils of the tapetum of the horse eye. These components are fundamental to understanding tapetal function.

Macroscopic appearance of the horse tapetum

The tapetum of the horse was located dorsal to and away from the optic disc, and when viewed under weak light appeared as a horizontally straight band that narrowed nasally. The tapetum expanded dorsally as the brightness of the illumination increased and became semicircular in shape at its maximal size. The horse tapetum has previously been reported to resemble a rounded triangle or semicircle (Seiferle, 1975; Ollivier et al. 2004), or to occupy the dorsal two-thirds of the ocular fundus in the ophthalmoscopic appearance (Cutler et al. 2000). These reports are similar to the shape of the maximum-sized tapetum observed under strong light in this study. The tapetum in sheep changes in shape with the brightness of illumination (Shinozaki et al. 2010b). Thus, the tapetal shape can change with the brightness of illumination. However, the ocular fundus would rarely be exposed to strong light, such as that produced by a flash or ophthalmoscopy, under natural conditions. The functional tapetal area used for mesopic and scotopic vision should correspond to the horizontal straight band of the tapetum apparent under weak light. The macroscopic tapetal shape should be observed under various levels of

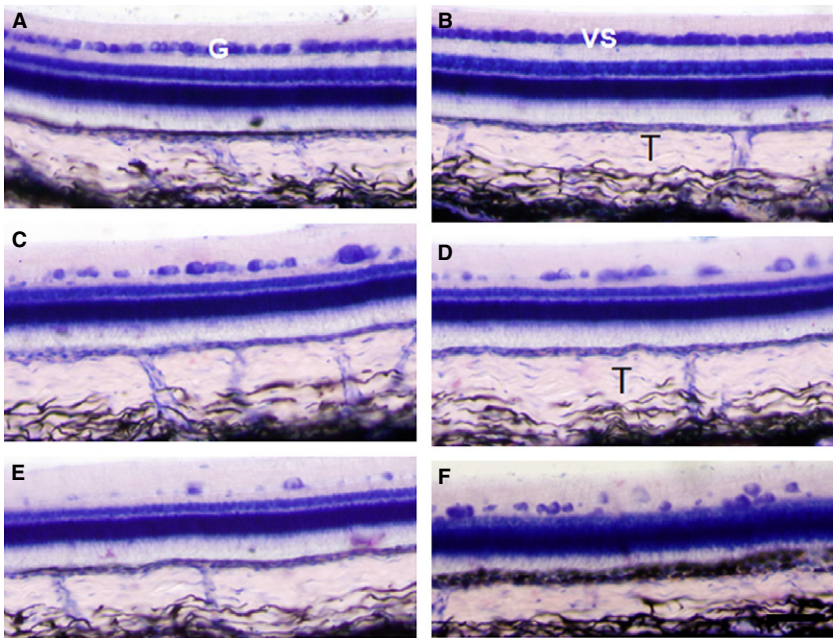

Fig. 6 Photomicrographs of the horse choroid and retina in a thionine-stained longitudinal section taken slightly temporal to the optic disc. Images (A–F) are from the same section and are listed in ventral to dorsal order. The ventral side corresponds to the left side of all images. (A) The ventral boundary part of the horizontal band of the tapetum. Although the tapetum is relatively thick, the RPE is pigmented. The retinal ganglion cells are relatively densely distributed, and the visual streak, covered by slightly pigmented RPE, is found on the dorsal (right) side. (B) Showing the visual streak. The tapetum is thick. The RPE is slightly pigmented on the ventral (left) side and unpigmented on the dorsal (right) side. (C, D) The thickest part of the tapetum, which is covered by the unpigmented RPE. The density of the retinal ganglion cells decreases markedly. The slightly pigmented RPE appears on the dorsal (right) side in (D). (E) Although the tapetum is thick, the RPE is slightly pigmented. (F) Although the tapetum is still relatively thick, the RPE has become moderately pigmented. In (E) and (F), the retinal ganglion cells are notably sparse. G, retinal ganglion cells; T, tapetal tissue; VS, visual streak. Scale bar: 50 μm (in F; applies to A–F).

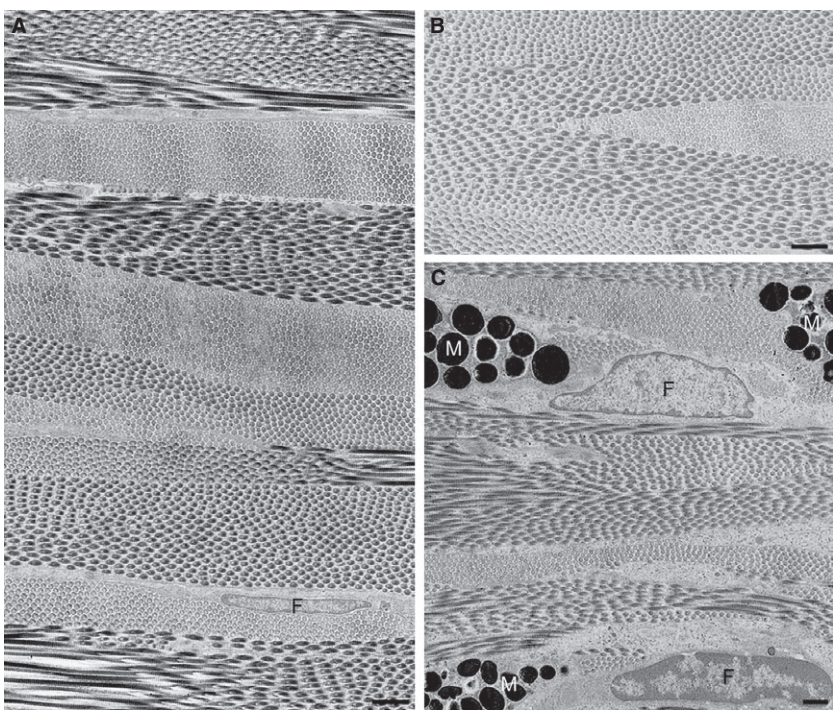

Fig. 7 Electron micrographs of the horse tapetum. The tapetal tissue consists of collagen fibrils running parallel to the retinal surface and grouped in lamellae. There are a few fibroblasts between contiguous lamellae (A). Lamellae often bifurcate or fuse (B). In the scleral side of the tapetal tissue, the profusion of melanocytes and fibroblasts between the lamellae obscures the border between the tapetum and the proper substance of the choroid (C). F, fibroblast; M, melanocyte. Scale bars: 1 μm.

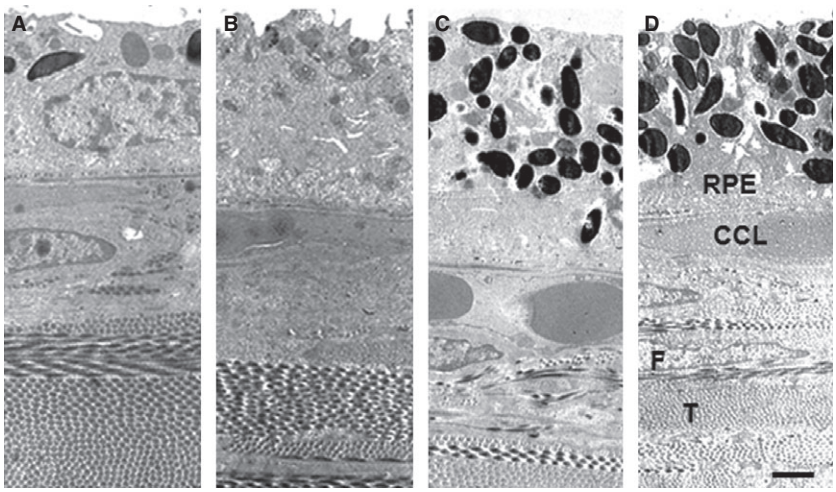

Fig. 8 Electron micrographs from the RPE to the tapetal tissue in the horse eye. (A) An electron micrograph from P1; (B) from P2; (C) from P3; and (D) from P4 in Fig. 2. The tapetal tissue is beneath the choriocapillary layer. In P2 (B), the RPE is unpigmented. In P4 (D), fibroblasts appear frequently in the tapetal tissue. CCL, choriocapillary layer; F, fibroblast; RPE, retinal pigment epithelium; T, tapetal tissue. Scale bar: 1 μm (in D; applies to A–D).

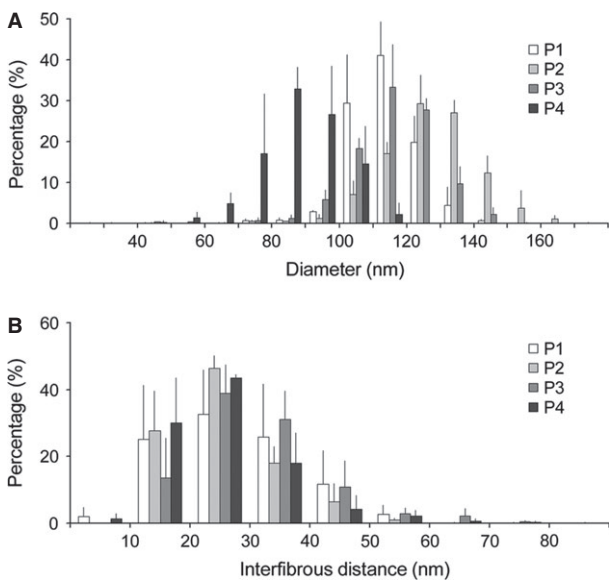

Fig. 9 Percentages of the diameter (A) and interfibrous distance (B) of the collagen fibrils in each part (P1–P4). The diameter tends to be larger in P2 and smaller in P4. The interfibrous distance shows no trends among the parts. The values are the means ± SDs of three horses.

illumination in an investigation of tapetal function. After the retina, including the RPE, was removed, the tapetum was found throughout the ocular fundus of the horse eye, including the part ventral to the optic disc. This indicates that the pigmentation of the RPE determines the macroscopic area of the tapetum.

The fibrous tapetum in the horse eye

The tapetal tissue of the horse showed a layered structure, with lamellae consisting of collagen fibrils running parallel to the retinal surface. The fibrils within each lamella ran parallel to each other and were arranged regularly. These findings were typical of the fibrous tapetum, and resembled those of previous reports on whales and on ungulates such as sheep, bovines and horses (Bellairs et al. 1975; Bortolami et al. 1979; Braekevelt, 1986; Young et al. 1988).

Histologically, the tapetal tissue in the horse eye spread over almost the entire ocular fundus except for the most peripheral areas. The tapetal tissue was moderately thick (> 10 μm) over the broad area dorsal to the optic disc. In particular, the tapetal tissue in the horizontal band located dorsal to the disc was thicker (> 40 μm throughout) and reached a maximum thickness of approximately 90 μm. The tapetal thickness has been reported to be up to 30 μm in horses (Ollivier et al. 2004), 50–60 μm in bovines (Braekevelt, 1986) and 70 μm in our previous study on sheep (Shinozaki et al. 2010b). The maximum tapetal thickness of the horse eye in the present study was thicker than those measured in previous reports on horses, bovines and sheep. At the very least, it is highly likely that the fibrous tapetum of the horse is indeed thicker than that of sheep, as we used the same method to measure the thickness of the tapetum in both this study and the sheep study.

The tapetum and the RPE

According to Land (1972), the reflectance of a thin-film system increases with the number of layers. The RPE that covers the tapetum is unpigmented in order to allow light to pass through the RPE and be reflected by the tapetum (Walls, 1967; Schwab et al. 2002; Ollivier et al. 2004). The combination of thick tapetal tissue and the unpigmented RPE produces maximal reflectance, as the former has higher reflective power and the latter allows more light to pass through. In the horse eye, the tapetal tissue was especially thick in the horizontal band, where the majority of it was also covered by the unpigmented RPE. Therefore, the horizontal band of the tapetum must be highly functional as a light reflector and in fact is macroscopically observable even under weak light. The dorsal tapetal area was moderately thick and was covered by moderately pigmented RPE. Only

especially strong light can pass through the moderately pigmented RPE and be reflected by the moderately thick dorsal tapetal tissue. This explains why the macroscopic tapetum expanded dorsally as the brightness of illumination increased. Ventral to the horizontal band, the tapetal thickness abruptly decreased, and the melanin content of the RPE simultaneously increased rapidly. As result, the ventral tapetal area, being thin and covered by heavily pigmented RPE, could not be observed macroscopically even under strong light, and the ventral border of the horizontal band was macroscopically clear and constant regardless of the brightness of illumination. The tapetal thickness and the pigmentation of the RPE seem to determine the functional tapetal area. In the horse eye, the functional tapetal area for mesopic and scotopic vision would be limited to the horizontal band in which the thick tapetal tissue is covered by the unpigmented RPE.

According to Braekevelt (1986), the RPE over the peripheral tapetum is moderately to heavily pigmented in the bovine eye. In sheep, the functional tapetal area, which is covered by the unpigmented RPE and is macroscopically visible under available light (i.e. weak light), is located in the part of the ocular fundus dorsal to the optic disc and is L-shaped, with the nasal part elongated horizontally and the temporal part expanded dorsally. However, the distribution of tapetal tissue is wider than the functional tapetal area. The functional tapetal area covered by the unpigmented RPE is only about 55% of the area with tapetal tissue (Shinozaki et al. 2010b). In horses, tapetal tissue was found over most of the ocular fundus. However, the functional tapetal area that was both thick and covered by the unpigmented RPE was limited to the area of the horizontal band, which constituted just 11% of the entire area with tapetal tissue. Therefore, in spite of the wide distribution of tapetal tissue in the horse eye, the functional tapetal area is considerably narrower than those of sheep and bovines. The non-functional tapetal area covered by the pigmented RPE seems to be present to a greater or lesser extent in the fibrous tapetum of many ungulates. However, it is unclear why the non-functional tapetum is retained. It may be related to the development of the eye or be an evolutionary remnant.

Relation of the tapetal collagen fibrils to color of the tapetum

The mean diameter of the fibrils in the horizontal band of the horse tapetum was approximately 128 nm. The mean interfibrous distance in the band was approximately 26 nm. In other species with a fibrous tapetum, the diameter of collagen fibrils ranges from 200 nm in bovines (Braekevelt, 1986) to 150 nm in sheep (Bortolami et al. 1979), and 124 nm in the green to blue region or 113 nm in the blue region in whales (Young et al. 1988). The tapetal fibrils of the horse were thinner than those of bovines and sheep, and were similar in thickness to those of whales. The interfibrous distance is 67 nm in sheep (Bortolami et al. 1979) and 46 nm in the blue region or 40 nm in the green to blue region in whales (Young et al. 1988). The interfibrous distance in horses was greatly shorter than that in sheep and somewhat shorter than that in whales.

In the present study, we estimated the wavelength of maximal reflectance of the light reflected from the tapetum from the diameter and interfibrous distance of the collagen fibrils. In the horizontal band of the tapetum, this wavelength was approximately 468 nm, corresponding to blue (435–480 nm). By macroscopic observation, the horse tapetum appeared bluish green to blue in color. Therefore, the estimated color of the horse tapetum was similar to the observed color. The color estimated by the same method of calculation was also consistent with the observed tapetal color in whales, in which the wavelength was 473 nm in the blue region or 493 nm in the green to blue region (Young et al. 1988). The wavelengths of maximal reflectance were similar between horses and whales.

The horse retina has three types of photoreceptors: rods; middle- to long-wavelength-sensitive (M/L) cones; and short-wavelength-sensitive (S) cones. Rods contribute to visual sensitivity in mesopic and scotopic vision, M/L cones to visual resolution and color vision in photopic vision, and S cones to color vision in photopic vision. In horses, the wavelength of maximum absorption of the rod photopigment is predicted to be 499 nm (Zhao et al. 2009), and the spectral peak of cone photopigment sensitivity is 539 nm for M/L cones and 428 nm for S cones (Carroll et al. 2001). Therefore, the horizontal band of the tapetum would effectively reflect light that is usable by rod photopigment, and thus increase the sensitivity of mesopic and scotopic vision. The estimated wavelength of the light reflected by the other parts of the tapetum is too short to be received by rods. This suggests that the ultrastructure, as well as the macroscopic and histological evidence, is consistent with only the horizontal band of the tapetum being functional.

In clinical ophthalmoscopy, the tapetal color is typically greenish yellow in horses (Matthews et al. 1990; Cutler et al. 2000). The tapetal reflection in ophthalmoscopy is somewhat different in color from that in the macroscopic observation of the present study. As described above, the tapetal shape can change with the brightness of illumination. The tapetum not only in shape but also in color may change by observational methods. In ophthalmoscopy with very strong brightness, observers would see the light passing through, reflected by and scattered by some ocular structures such as the cornea, the lens, the vitreous body and the retina in addition to the tapetum. However, the ocular fundus would rarely be exposed to strong light under natural conditions. From a functional standpoint, the light reflected from the tapetum is directly absorbed by photoreceptors, and its wavelength can be estimated from the anatomical data, independently of observational

methods of the ocular fundus. Therefore, the anatomical estimation of the wavelength of the light reflected from the tapetum is very important for understanding of the tapetal effect on the horse vision.

Effects of the tapetum on vision in the horse

Horses are active both during the day and at night (Murphy et al. 2009). As they spend a great deal of time grazing and have many predators, they must always scan the surrounding environment. At night, the horse exhibits excellent scotopic vision not only for stimulus shapes but also for navigation within a very dark confined environment (Hanggi & Ingersoll, 2009). The horse retina is rod-dominant (Wouters & De Moor, 1979; François et al. 1980), and retinal ganglion cells accumulate in the horizontal visual streak (Hebel, 1976; Harman et al. 1999; Guo & Sugita, 2000; Evans & McGreevy, 2007). The present study found the visual streak to be located in the ventral part of the horizontal band of the tapetum. Cooperation between these retinal features and the horizontal band of the tapetum should provide horses with good mesopic and scotopic vision in the horizontal visual field.

The functional tapetal area and the dense area of retinal ganglion cells seem to approximately coincide in horses as well as in sheep (Shinozaki et al. 2010A,B). There is some concern that the tapetum may not only increase mesopic and scotopic visual sensitivity, but also decrease visual resolution due to scattered light and glare in a bright environment (Hebel, 1976; Braekevelt, 1986; Timney & Macuda, 2001). However, we think that the adverse effect of the tapetum on photopic visual resolution in the horse should be quite small. Our reasons are as follows. First, the wavelength of the light reflected from the tapetum is far from the spectral peak of photopigment sensitivity of M/L cones, which are responsible for photopic visual resolution and color vision. The wavelength of the light reflected from the tapetum is closer to the spectral peak of photopigment sensitivity of S cones, but these are responsible for color vision rather than for photopic visual resolution. Cones specifically are more densely distributed in the visual streak of the horse than in the more peripheral retina (Sandmann et al. 1996). Therefore, in photopic vision, the light reflected from the tapetum may not seriously degrade the good visual resolution achieved by the cones and retinal ganglion cells in the visual streak. Second, the location of the visual streak ventral to the thickest part of the horizontal band of the tapetum and its partial coverage by slightly pigmented RPE may attenuate any adverse effect of the tapetum. Third, visual acuity in animals can be predicted from anatomical measurements of the retinal ganglion cell density and the axial length, or from quantitative behavioral tests. The visual acuity measured using standard psychophysical procedures accords well with anatomically based estimates of visual acuity (Timney & Keil, 1992). If this is true, the tapetum may not greatly reduce visual resolution, as the visual acuity predicted by the anatomical data is determined independently of the tapetum.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Author contributions

A.S. and S.T. carried out the experiments, the data analysis and drafted the manuscript. Y.Z.H. helped to draft the manuscript. M.U. participated in the design and coordination of the experiment, and aided in drafting of the manuscript. All authors read and approved the final manuscript.

References

Afzelius BA (1992) Section staining for electron microscopy using tannic acid as a mordant: a simple method for visualization of glycogen and collagen. *Microsc Res Tech* **21**, 65–72.

Bellairs R, Harkness ML, Harkness RD (1975) The structure of the tapetum of the eye of the sheep. *Cell Tissue Res* **157**, 73–91.

Bortolami R, Callegari E, Lucchi ML (1979) Ultrastructural observations on the tapetum lucidum fibrosum. *Anat Histol Embryol* **8**, 72–78.

Braekevelt CR (1986) Fine structure of the bovine tapetum fibrosum. *Anat Histol Embryol* **15**, 215–222.

Carroll J, Murphy CJ, Neitz M, et al. (2001) Photopigment basis for dichromatic color vision in the horse. *J Vis* **1**, 80–87.

Cutler TJ, Brooks DE, Andrew SE, et al. (2000) Disease of the equine posterior segment. *Vet Ophthalmol* **3**, 73–82.

Evans KE, McGreevy PD (2007) The distribution of ganglion cells in the equine retina and its relationship to skull morphology. *Anat Histol Embryol* **36**, 151–156.

François J, Wouters L, Victoria-Troncoso V, et al. (1980) Morphometric and electrophysiologic study of the photoreceptors in the horse. *Ophthalmologica* **181**, 340–349.

Guo X, Sugita S (2000) Topography of ganglion cells in the retina of the horse. *J Vet Med Sci* **62**, 1145–1150.

Hanggi EB, Ingersoll JF (2009) Stimulus discrimination by horses under scotopic conditions. *Behav Processes* **82**, 45–50.

Harman AM, Moore S, Hoskins R, et al. (1999) Horse vision and an explanation for the visual behaviour originally explained by the 'ramp retina'. *Equine Vet J* **31**, 384–390.

Hebel R (1976) Distribution of retinal ganglion cells in five mammalian species (pig, sheep, ox, horse, dog). *Anat Embryol* **150**, 45–51.

Knill LM, Eagleton RD, Harver E (1977) Physical optics of the equine eye. *Am J Vet Res* **38**, 735–737.

Land MF (1972) The physics and biology of animal reflectors. *Prog Biophys Mol Biol* **24**, 75–106.

Matthews AG, Crispin SM, Parker J (1990) The equine fundus II: normal anatomical variants and colobomata. *Equine Vet J Suppl* **22**, 50–54.

Murphy J, Hall C, Arkins S (2009) What horses and humans see: a comparative review. *Int J Zool* **2009**, 1–14.

Ollivier FJ, Samuelson DA, Brooks DE, et al. (2004) Comparative morphology of the tapetum lucidum (among selected species). *Vet Ophthalmol* **7**, 11–22.

Sandmann D, Boycott BB, Peichl L (1996) Blue-cone horizontal cells in the retinae of horses and other equidae. *J Neurosci* **16**, 3381–3396.

Schwab IR, Yuen CK, Buyukmihci NC, et al. (2002) Evolution of the tapetum. *Trans Am Ophthalmol Soc* **100**, 187–200.

Seiferle E (1975) Sinnesorgane. In: *Lehrbuch der Anatomie der Haustiere, Band 4, Nervensystem, Sinnesorgane, Endokrine Drüsen*. (eds Nickel R, Schummer A, Seiferle E), pp. 337–398. Berlin: Paul Parey.

Shinozaki A, Hosaka Y, Imagawa T, et al. (2010a) Topography of ganglion cells and photoreceptors in the sheep retina. *J Comp Neurol* **518**, 2305–2315.

Shinozaki A, Hosaka Y, Imagawa T, et al. (2010b) Relationship between distribution of tapetum fibrosum and retinal pigment epithelium in the sheep eye. *J Vet Med Sci* **72**, 211–215.

Timney B, Keil K (1992) Visual acuity in the horse. *Vision Res* **32**, 2289–2293.

Timney B, Macuda T (2001) Vision and hearing in horses. *J Am Vet Med Assoc* **218**, 1567–1574.

Walls GL (1967) *The Vertebrate Eye and Its Adaptive Radiation*. New York: Hafner.

Waring GH (2003) Perception and orientation. In: *Horse Behavior*, 2nd edn., pp. 18–35. New York: Noyes Publications/William Andrew Publishing.

Watanabe T, Hosaka Y, Yamamoto E, et al. (2005) Control of the collagen fibril diameter in the equine superficial digital flexor tendon in horses by decorin. *J Vet Med Sci* **67**, 855–860.

Wouters L, De Moor A (1979) Ultrastructure of the pigment epithelium and the photoreceptors in the retina of the horse. *Am J Vet Res* **40**, 1066–1071.

Young NM, Hope GM, Dawson WW, et al. (1988) The tapetum fibrosum in the eyes of two small whales. *Mar Mamm Sci* **4**, 281–290.

Zhao H, Ru B, Teeling EC, et al. (2009) Rhodopsin molecular evolution in mammals inhabiting low light environments. *PLoS One* **4**, e8326.