Complementary Cone Fields of the Rabbit Retina

Bengt Juliusson,* Anders Bergström,* Pál Röhlich,† Berndt Ehinger,* Theo van Veen,§ and Agoston Szél‡§

Purpose. Complementary cone fields have been considered a unique feature of the mouse retina. In an attempt to map the arrangement of the color-specific cones in other mammals, the authors investigated the rabbit, a commonly used experimental animal for vision research.

Methods. For the identification of the different cone types immunocytochemistry was used with two monoclonal antibodies, each specific to the middle- to long-wave (red-green) and short-wave (blue) sensitive visual pigments, respectively.

Results. The major part of the retinal surface, including the visual streak, exhibited a dominance of M (middle-wave sensitive) cones (6 to 13,000/mm²) versus S (short-wave sensitive) cones (1 to 2,500/mm²). In contrast, the lower 5% to 6% of the total retinal area showed a complete lack of green cones and a high density of blue cones (11,000/mm²). The authors designate this crescent-like area the blue streak of the rabbit retina.

Conclusion. In addition to the visual streak primarily abundant in green cones, there is a specialized area of the rabbit retina that is densely and exclusively populated with blue cones. Although the relative extension of this peculiar cone field is considerably smaller than the S-field of the mouse retina, its position is similar in that it occupies the lowermost part of the retina. The functional implication of this area is unknown. Invest Ophthalmol Vis Sci. 1994;35:811-818.

The topographic proximity of the different cone types enables bipolar cells to receive input from spectrally different elements. In a number of species the ratio of the M/L (middle- to long-wave) and S (short-wave) cones was found to be roughly uniform across the whole retina (rat,1 gerbil2). Although the center of the primate fovea lacks a few blue cones,3,4 the rest of the retina exhibits an even distribution of the various cone types.4 We found unexpectedly that in the mouse the upper half of the retina contains both M and S cones, whereas in the lower half only S cones occur.5 Close relatives of the mouse (wood mouse6) and other rodents (rat,1 gerbil,2 hamster [Szél, unpublished ob-
servations, 1991]) do not possess separate cone fields, so it was reasonable to assume that other mammals also lack this feature. Because the rabbit is frequently used for visual experiments and it is the subject of retinal transplantsations,7,8 we have examined the distribution of the color-specific cone types in this species.

It has been known for a long time that approximately 5% of the photoreceptors in the rabbit retina are cones.9 The presence of cones has been confirmed by electron microscopy10 and lectin cytochemistry.11 The visual streak of the rabbit retina is a horizontal band lying below the optic nerve head, in which the ganglion cell and cone densities are higher than elsewhere in the retina.12 The cone density in this band was found to be as high as 13,000/mm², whereas in the lower periphery it is only 7,500/mm². Various physiologic studies indicate that rabbits have dichromatic vision with a blue (425 nm) and green (520 nm) sensitive spectral peak.13-16 The blue and green cones were found to be organized in receptive fields forming opponent color units.16 No heterogeneity was found in the distribution of the color-coded units.

Two monoclonal antibodies (MAb) generated...
against visual pigments are useful for distinguishing specific cones in vertebrates. MAb OS-2 reacts with short wavelength sensitive cones in mammals, and, in a complementary fashion, MAb COS-1 stains all other cones. In primates, the green and red cones are recognized by COS-1, but in mammals in which only one middle-to-long wavelength (green) sensitive cone type occurs, COS-1 is specific to these M cones. These two antibodies were used to map the distribution of the M and S cones in the rabbit retina.

MATERIALS AND METHODS

Mixed-strain, pigmented, adult rabbits were anesthetized with an overdose of Hypnorm® and subsequently sacrificed with air embolism. The eyes were enucleated and hemisected, the posterior eyecups were immersed in 4% paraformaldehyde dissolved in 0.1 M phosphate buffer. After 18 hours' fixation, the eyecups were infiltrated in 20% sucrose and subsequently embedded in OCT medium (Tissue-Tec, Miles Inc., Elkhart, IN) and frozen.

Cryostat sections (12 μm) obtained from the vertical meridian were processed for immunocytochemistry. They were briefly incubated in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.25% Triton-X 100. The primary antibodies were diluted in BSA-PBS with Triton. COS-1 (hybridoma supernatant) was diluted 1:100, OS-2 (ascites fluid) 1:10,000. After 18 to 24 hours' incubation, the bound antibodies were detected by avidin-biotin-peroxidase (Vectastain ABC, Vector, Burlingame, CA) and diaminobenzidine in the presence of hydrogen peroxide (H₂O₂). Coverslips were mounted with Permount, and the slides were examined with an Axioskop photomicroscope (Zeiss, Germany). Photographs were taken using Nomarski differential interference optics.

To detect both cone types simultaneously, some sections were reacted with PNA-FITC conjugate (Vector, Burlingame, CA, 200 μg, 1 hour). Lectin specificity was tested with the appropriate competing sugar, D-galactose (0.3 M). Coverslips were mounted with glycerol-PBS and sealed with nail polish. The lectin binding was studied with the same microscope using the appropriate filter set for FITC.

To examine the distribution of cones, retinal wholemounts were prepared. Posterior eyecups derived from six rabbits were immersed in fixative (4% paraformaldehyde). After 10 minutes' fixation, the retina was gently removed from the underlying pigmented epithelium. To ensure the smallest possible damage or loss of the retinal tissue and to minimize the number of cut edges, the retinas were dissected into three pieces cut roughly according to the method of Curcio et al. The superior cap was cut just above the optic nerve head, the middle part ("belt") represented the optic nerve head together with the myelinated optic nerve fibers as they fan out nasally and temporally (myelinated streak). The part of the retina where the optic nerve fibers pass from the visual streak to the optic nerve head was also included in this middle band, together with the upper part of the visual streak. The rest of the retina, including the lower part of the visual streak as well as the lowermost region of the retina, formed the inferior cap. The retina pieces were fixed for 4 to 5 hours in the same fixative and then rinsed in buffer.

The floating retina pieces were processed for immunocytochemistry with antibodies COS-1 and OS-2. After brief preincubation in BSA-PBS with Triton, they were treated with the antibodies for 18 hours. The bound antibodies were detected with an FITC-conjugated anti-mouse secondary antibody (Dako-patts, Hagersten, Denmark). The retina pieces were

![Figure 1](https://via.placeholder.com/150)

**FIGURE 1.** Radial sections of the rabbit retina derived from the superior part (A), visual streak (B), and blue streak (C), respectively. The sections were cut in the vertical meridional plane and reacted with antibody COS-1. Some immunopositive green cone outer segments are marked with vertical arrows. Horizontal arrow points to the junction of the outer and inner segment levels. Note that the number of COS-1 positive cones is the highest in the visual streak. Fewer cones are found in the superior retina, and no green cones are recognized in the blue streak. The short, irregular features originating from the pigmented epithelium are not outer segments; they represent apical processes of the pigment cells. Bar = 100 μm.
Complementary Cone Fields of the Rabbit Retina

The area of the blue streak was also measured. All experimental procedures conformed with the ARVO Resolution on the Use of Animals in Research.

RESULTS

In the sections of the rabbit retina, two cone types could be distinguished by the anti-visual pigment antibodies. The majority of cones in almost the entire retina were recognized by MAb COS-1 (Figs. 1A and 1B). However, in the lowermost part of the vertical sections, COS-1 positive cones were completely missing (Fig. 1C). In this part of the retina, all identifiable cones were stained by MAb OS-2 (Fig. 2C). In the rest of the retina, the OS-2 positive cones were sparse (Figs. 2A and 2B) and were considerably outnumbered by the COS-1 positive cones.

When both cone types were labeled with PNA, the cone distribution was homogeneous. The entire retina contained a large number of cones (Fig. 3). Although radial sections cannot be used for quantitative evaluation of densities, it appeared that the cone spacing was

FIGURE 2. OS-2 immunocytochemistry carried out on vertical meridional sections of the rabbit retina. The samples originate from the superior retina (A), visual streak (B), and blue streak (C), respectively. Vertical arrows show immunopositive outer segments of blue cones. The outer-inner segment junction is marked with horizontal arrow. Blue cones occur in each part of the rabbit retina. Their frequency is low in the superior part and in the visual streak. In the blue streak, however, they are represented by a considerably higher number. Bar = 100 μm.

placed on microscope slides, photoreceptor layer upward; coverslips were mounted with glycerol-PBS, and the wholemounts were examined with the Axiophot microscope and fluorescent filter set for FITC.

For the reconstruction of the cone distribution, immunopositive cones were counted using a window measuring approximately 0.1 mm². Twenty-five samples equidistant from each other were taken along the vertical meridian. Additional samples derived from other parallel vertical lines were also taken. Five equidistant lines, temporal as well as nasal to the vertical meridian and with at least 15 individual samples each, were included in the reconstruction. The cell counts were performed on enlarged prints of the photographed retinal areas. The calculated density values were processed for drawing of isodensity contours.

Similar calculations were performed on wholemounts from four individual animals. The cell counts derived from defined, corresponding locations of the retinas were compared and averaged. The ratio of blue cones was expressed in percentage of the total cone cell density of the given location. In all four animals,

FIGURE 3. PNA lectin cytochemistry on vertical meridional sections. The arrangement of the pictures is the same as in Figures 1 and 2 (superior retina A, visual streak B, blue streak C). The demonstrated regions of the retina are heavily populated by cones. On each section, vertical arrows point to a few neighboring PNA-positive cones. Note that the lectin labels the outer and inner segments as well. The level of the junction of the two segments is shown by horizontal arrow. Bar = 100 μm.
less close in the superior retina than in either the visual streak or the lowermost region. Accordingly, the cones in the visual streak as well as in the lowermost region were found to be considerably thinner than in the superior retina.

For quantitative analysis, retinal wholemounts were reacted with the two cone antibodies. Immunofluorescence showed that COS-1 positive (M) cones were abundant all over the retina (Figs. 4A and 4B), except for a small, crescent-shaped area in the lowermost region, from which the M cones were completely excluded (Fig. 4C). Immunocytochemistry with antibody OS-2 revealed the presence of only a low number of S cones in the major part of the retina (Figs. 5A and 5B). However, in a small area corresponding to the M-cone free crescent, the density of the S cones was found to be remarkably higher (Fig. 5C).

The distribution of the S cones in the superior and middle part of the retina was apparently nonrandom. The OS-2 positive cones were usually arranged in curved linear arrays surrounding small areas with no positive cones (Figs. 5A and 5B). Other heterogeneities in the cone sizes and distribution could also be observed. The diameter of the cone outer segments in the superior part of the retina was considerably larger than in the middle or lower part (Fig. 6). This difference was more pronounced among the S cones (Fig. 6C and 6D), but the M cones also showed a marked variation of the outer segment size along the vertical meridian (Fig. 6A and 6B).

As can be seen from the isodensity lines (Fig. 7), the density of cones exceeds 6,000/mm² all over the retina. There are two horizontal streaks that exhibit considerably higher densities. One is the visual streak, which occupies an area below the optic nerve head, and the other is the crescent-shaped streak in the lowermost part of the retina. In the former, the M- and S-cone densities are approximately 13,000/mm² and 2,000/mm², respectively, whereas in the latter, the S-cone density exceeds 10,000/mm² and the M cones are totally missing.

If we follow the distribution of S cones downward along the vertical meridian, we find that the superior part and the region with the radiation of the optic nerve head contains roughly 1000 cones/mm². In the
visual streak, the S-cone density starts to increase to the double (2,000/mm²), and in the lowest 5% to 6% of the retinal surface the S-cone density increases up to about 10,000/mm². Comparing these numbers with those of the M cones, it is evident that in the superior part and in the visual streak the M cones outnumber the S cones by a factor of about 6 (Table 1).

This ratio, however, changes below the visual streak. In an area delineated from above by a nearly horizontal imaginary line, no M cones can be found at all, but the number of S cones are found to be the highest here. The complementarity of the M- and S-cone distribution patterns is obvious from the comparison of the two isodensity maps (Fig. 7). The size of the M-cone-free and S-cone-rich area was found to be roughly 5% to 6% of the total retinal area (Table 1).

DISCUSSION

Cone photoreceptors are found throughout the rabbit retina, as shown by cytochemistry with PNA (Fig. 3), a lectin selective to retinal cones in vertebrates. Immuno-cytochemistry revealed the presence of two immunologically identifiable cone types in the rabbit retina, supporting earlier results indicating dichromatic vision in this animal. In accordance with our earlier findings in the rabbit, the green-sensitive cones (M cones) are stained by antibody COS-1, whereas the blue-sensitive (S cones) are recognized by antibody OS-2. In the major part of the retinal surface, the M cones outnumber the S cones by almost one order of magnitude (Figs. 8A and 8B) That ratio (1:10) between the two cone types corresponds well to the quantitative relationship of the M and S cones observed in a number of mammalian species. Because the spatial arrangement of the two cone types is roughly uniform in the major part of the retinal surface, the morphologic basis of true color vision, namely the mosaic-like pattern of the spectrally different elements, is given.

The visual streak, characterized by the highest ganglion and cone cell densities, was not found to differ in the M/S cone ratio from the superior part of the retina. The only difference in the photoreceptors was the...
FIGURE 6. High-magnification photographs derived from similar preparations as shown in Figures 4 and 5. The antibodies used (COS-1 on A and B; OS-2 on C and D) are marked. A and C show the superior area, B shows the visual streak, D the blue streak. Note that the green cones are more frequent in the visual streak than in the superior retina. Similarly, the frequency of blue cones in the blue streak exceeds that of the superior retina. Bar = 50 μm.

approximately double density of cones with the corresponding smaller outer segment diameter in this area. Therefore, the visual streak (area centralis) can be considered an area of higher visual acuity\textsuperscript{12} with a normal proportion of M and S cones.

The M/S cone ratio is, however, drastically changed in the lowermost part of the retina. This crescent-like field immediately above the ora serrata contains exclusively blue-sensitive cones and can be called the “blue streak” of the rabbit. The blue-sensitive cones are relatively thin and closely packed in the blue streak with a density that is only slightly below that of the M cones in the visual streak (Figs. 8A and 8B).

The presence of the blue-streak is practically equivalent to the S-field of the mouse retina.\textsuperscript{5} However, the latter occupies the total lower half of the retina, whereas the former contributes only 5\% to 6\% of it. As in the mouse, the functional implication of the presence of the blue field is unknown in the rabbit. One might easily find a teleological explanation for the advantage of such a field, which is the greater perceptibility of predators coming from above. Their image is formed against the background of the sky. The high density of blue-sensitive cones might render the lower part of the retina a very sensitive monitor to screen the sky. The visual streak and the superior retina, with a considerably larger number of M cones, serve to scan the ground and the level of the horizon that are rich in green color.

Electrophysiologic experiments did not show any remarkable topographic differences in the distribution of the color-coded units.\textsuperscript{15} However, the total retinal surface was not thoroughly mapped. Based on our findings, we interpret the chromatic organization of
the rabbit retina as a set of horizontal and parallel bands or streaks playing different roles in visual performance (Fig. 8C). The visual streak is the area of the highest ganglion cell and cone density, in which the numbers of the spectrally different cone types correspond to the usual ratio of 1:10. The other outstanding zone is the blue streak, in which the cone cell density is in the same order of magnitude as that of the visual streak. This area is, however, totally devoid of green cones. Above the visual streak, there is the myelinated streak in which the radiation of the optic nerve fibers lies. That area is the blind spot of the rabbit. In addition, there are other regions of the rabbit retina—a superior crescent and a narrow band between the visual streak and blue streak—that are apparently less specialized in their visual functions.

**TABLE 1. Cone Densities in Various Locations Along the Vertical Meridian of Retinas from Four Individual Animals**

<table>
<thead>
<tr>
<th>Location</th>
<th>Animal 1</th>
<th>Animal 2</th>
<th>Animal 3</th>
<th>Animal 4</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior (halfway between optic nerve head and ora serrata)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COS-1</td>
<td>5.9</td>
<td>7.2</td>
<td>7.5</td>
<td>6.8</td>
<td>6.85 ± 0.69</td>
</tr>
<tr>
<td>OS-2</td>
<td>0.85</td>
<td>0.65</td>
<td>0.90</td>
<td>0.82</td>
<td>0.80 ± 0.11</td>
</tr>
<tr>
<td>COS-1 + OS-2</td>
<td>6.75</td>
<td>7.85</td>
<td>8.40</td>
<td>7.62</td>
<td>7.70 ± 0.69</td>
</tr>
<tr>
<td>OS-2 (%)</td>
<td>12.5</td>
<td>8.30</td>
<td>10.7</td>
<td>10.8</td>
<td>10.6 ± 1.7</td>
</tr>
<tr>
<td>Visual streak (5 mm below optic nerve head)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COS-1</td>
<td>12.6</td>
<td>12.5</td>
<td>11.6</td>
<td>13.5</td>
<td>12.5 ± 0.78</td>
</tr>
<tr>
<td>OS-2</td>
<td>1.80</td>
<td>1.20</td>
<td>1.50</td>
<td>1.70</td>
<td>1.55 ± 0.26</td>
</tr>
<tr>
<td>COS-1 + OS-2</td>
<td>14.4</td>
<td>13.7</td>
<td>13.1</td>
<td>15.2</td>
<td>14.1 ± 0.90</td>
</tr>
<tr>
<td>OS-2 (%)</td>
<td>12.5</td>
<td>8.80</td>
<td>11.5</td>
<td>11.2</td>
<td>11.0 ± 1.6</td>
</tr>
<tr>
<td>Inferior (blue streak, 3 mm above the ora serrata)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COS-1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>9.10 ± 1.0</td>
</tr>
<tr>
<td>OS-2</td>
<td>10.5</td>
<td>8.25</td>
<td>8.40</td>
<td>9.10</td>
<td>9.10 ± 1.0</td>
</tr>
<tr>
<td>COS-1 + OS-2</td>
<td>10.5</td>
<td>8.25</td>
<td>8.40</td>
<td>9.10</td>
<td>9.10 ± 1.0</td>
</tr>
<tr>
<td>OS-2 (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>9.10 ± 1.0</td>
</tr>
<tr>
<td>Sizes of green-cone free areas (%)</td>
<td>5.3</td>
<td>4.8</td>
<td>6.3</td>
<td>5.8</td>
<td>5.6 ± 0.65</td>
</tr>
</tbody>
</table>
FIGURE 8. Schematic diagrams of the rabbit retina showing the global densities of M and S cones (A and B, respectively). The density ranges are given in thousands of cells/mm². Figure C shows the distinguished areas of the retinal cone mosaic in the rabbit. The visual streak with many green cones and fewer blue cones occupies the middle horizontal band of the eye. The blue streak, an area with many blue but no green cones, is found in the lowermost crescent of the rabbit retina.

Key Words
immunocytochemistry, color vision, photoreceptor topography, rabbit, visual streak

Acknowledgments
The authors thank Karin Arner and Katarina Ryden for their expert technical assistance.

References
9. Davis FA. The anatomy and histology of the eye and orbit of the rabbit. Trans Am Ophthalm Soc. 1929;27:401-441.