Visual Pigment Coexpression in All Cones of Two Rodents, the Siberian Hamster, and the Pouched Mouse

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Purpose. To decide whether the identical topography of short- and middle-wavelength cone photoreceptors in two species of rodents reflects the presence of both opsins in all cone cells.

Methods. Double-label immunocytochemistry using antibodies directed against short-wavelength (S)– and middle- to long-wavelength (M/L)–sensitive opsin were used to determine the presence of visual pigments in cones of two species of rodents, the Siberian hamster (*Phodopus sungorus*) and the pouched mouse (*Saccostomus campestris*) from South Africa. Topographical distribution was determined from retinal whole-mounts, and the colocalization of visual pigments was examined using confocal laser scanning microscopy. Opsin colocalization was also confirmed in consecutive semithin tangential sections.

Results. The immunocytochemical results demonstrate that in both the Siberian hamster and the pouched mouse all retinal cones contain two visual pigments. No dorsoventral gradient in the differential expression of the two opsins is observed.

Conclusions. The retina of the Siberian hamster and the pouched mouse is the first example to show a uniform coexpression of M and S cone opsins in all cones, without any topographical gradient in opsin expression. This finding makes these two species good models for the study of molecular control mechanisms in opsin coexpression in rodents, and renders them suitable as sources of dual cones for future investigations on the role and neural connections of this cone type. (*Invest Ophthalmol Vis Sci.* 2002;43:2468–2473)

In the early 1990s, a general consensus emerged concerning the presence of two different cone types in almost all mammals.[1] The two cone populations are characterized by the expression of either a short-wavelength–sensitive visual pigment of the SWS1 class (S cones) or a middle- to long-wave–sensitive (M/L) visual pigment (M cones). S cones are sensitive to either bluish violet (415–433 nm) or ultraviolet light (360 nm),[2] whereas the peak of M cone sensitivity is mainly in the green-to-yellow part of the spectrum (508–553 nm), dependent on amino acid substitutions at critical sites of the opsin molecule.[3] The appearance of a second M/L visual pigment with an absorption maximum shifted toward longer wavelengths (L visual pigment) was a divergence from the middle-wavelength–sensitive (M) visual pigment in diurnal primates.[4–6]

One of the most potent tools to identify color-specific cone types is immunocytochemistry using visual pigment–specific antibodies. Such antibodies have been developed since the mid 1980s and greatly contributed to distinguishing and mapping color-specific cone types.[7–11] In most mammals, the two cone types were found to be distributed randomly with a ratio in the M and S cone density of usually 10:1. Immunocytochemical and lectin cytochemical studies also revealed that in some mammalian species the S cone population was completely missing.[12–17] However, it was unable to distinguish M and L cones in diurnal primates, because no specific antibodies could be produced due to the 96% identity of the M and L cone opsins.[18]

Among mammals, rodents represent a special group that is characterized by a great variability in the expression and topography of S and M cone opsins. Because many rodent species are important experimental animals in visual research, this variability is of particular significance. The first example of the deviation from the general scheme is the mouse in which a striking topographic separation of S and M cone opsin expression was demonstrated.[19] All cones in the ventral retina are reactive with the S-opsin–specific antibody, whereas the dorsal retina shows an S and M cone distribution pattern similar to that of other mammals. The validity of this finding has been confirmed by regional electroretinography.[20] Subsequently, a similar asymmetrical distribution of S and M cone opsins was found also in the rabbit[21,22] and the guinea pig.[23]

The thorough investigation of the transition zone between the M-cone–rich dorsal and the S-cone–rich ventral retina led to the surprising observation that M and S cone opsins can be expressed in one and the same cone cell.[23] The coexpression of the M and S cone opsins was later corroborated in the mouse[11,24] and completed with the observation that coexpression is not limited to the transition zone but, similar to the guinea pig,[23] is extended to all cones in the ventral retina. The immunocytochemical findings of opsin coexpression in mouse have recently been confirmed by physiologic studies.[25] A further example of cone opsin coexpression in rodents was found in some of those rodent species that do not exhibit an asymmetrical pattern of type-specific cone distribution: in differentiating cones of rat and gerbil, the two cone opsins were coexpressed during development.[26]

Among rodents, the Siberian hamster presents an interesting novel pattern of cone distribution.[27] In this species, the densities of the cones expressing the M and the S cone opsin, respectively, were reported to be present in a 1:1 ratio over the entire retina. This is a surprising finding, because in most mammalian species studied to date, M cones outnumber S cones by a factor of approximately 10. Further, in rodents exhibiting an asymmetrical cone distribution, there is a dorsoventral gradient in the expression of the two cone opsins, so that the densities of cone types and/or the levels of opsin expression differ significantly at any given retinal location. ERG studies in the Siberian hamster confirmed the presence of two

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visual pigments with peaks in the region of 370 and 500 nm, whereas behavioral discrimination using chromatic adaptation failed to show independence of the two systems.[27] The identical density and topography of M and S cones in the Siberian hamster can be compared with the transition zones of the mouse, guinea pig, and rabbit, in which cones reacting with M- and S-specific–antibodies occur in comparable frequencies, because of the coexpression of both opsins in the same cells. The question thus arises whether in the Siberian hamster there are two equivalent but distinct populations of S and M cones or a single-cone population expressing both M and S cone opsins.

To address this question we performed a detailed immunocytochemical analysis in the Siberian hamster using M- and S-cone–specific antibodies. In addition, when screening further rodent species for a similar cone pattern, we found the pouched mouse (*Saccostomus campestris*) in which densities of immunocytochemically distinguishable cones were fairly equal across the entire retina (Lukáts and Cooper, unpublished data, 2001). In both species, cone distribution and opsin content were studied in retinal wholemounts using double labeling with S- and M-opsin–specific antibodies and peanut agglutinin lectin (PNA). The colocalization of opsins was further examined in adjacent semithin sections which excludes cross reactivities with different antibodies. We found that in both the Siberian hamster and the pouched mouse, all cones express both the M and S cone opsin without any significant dorsoventral gradient. The biological role of this arrangement remains open, we can only speculate about whether the studied species represent a characteristic evolutionary position among other mammals. Part of this study was published in abstract form.[28]

Materials and Methods

Animals

The pouched mouse (*S. campestris*) was collected in Richards Bay, South Africa. Eight adults were anesthetized with halothane and killed with a combination of ketamine (150 mg/kg) and zylazine (30 mg/kg) and perfused intracardially with warm (37°C), heparinized saline followed by Zamboni fixative at 4°C. Five adult Siberian hamsters (*Phodopus sungorus*) were anesthetized and killed with prolonged ether narcosis and then perfused intracardially with a fixative containing 0.1% glutaraldehyde and 2% paraformaldehyde. The eyes of the animals were subsequently enucleated and cut at the ora serrata, and the posterior eyecups were postfixed in 4% paraformaldehyde for 24 hours.

Preparation of the Retina

After a small dorsal cut to mark the orientation of the superior rectus muscle, the retina was carefully detached from the posterior eyecup. For wholemount immunocytochemistry, the retinas were collected in buffer and rinsed for 24 hours before further processing. For tangential semithin sectioning, several samples were cut from different (approximately 10–20) places of the retina, including the peripheral dorsal and ventral parts. The pieces were rinsed overnight in PBS, dehydrated, embedded in Araldite, and flatmounted, and a series of 1-μm-thick tangential sections were cut at the level of the outer segments on an ultramicrotome. Before immunocytochemistry, all retinas and sections were preincubated in 1% bovine serum albumin (BSA) diluted in phosphate-buffered saline (PBS), containing 0.4% Triton X-100 for whole retinas.

Antibodies and PNA Lectin

Two pairs of antibodies were used in this study: two mouse monoclonal antibodies, COS-1 and OS-2, which are specific for M/L and S pigment, respectively,[29] and two rabbit polyclonal antisera, JH455, which recognizes S cones,[9] and CERN956, which is specific for M/L cones.[10] We took advantage of the different sources of antibodies, and used combinations of either OS-2 and CERN956 or COS-1 and JH455 for double labeling. COS-1 is a monoclonal hybridoma supernatant, diluted 1:50, OS-2 is a mouse ascites fluid, diluted 1:5000. The polyclonal JH455 was used in a dilution of 1:5000, and CERN956 was diluted 1:500. To exclude any bias caused by potential minor interspecies differences in affinities of antibodies, the working dilution was carefully selected after each antibody had been tested in a series of five to six graded dilutions. The best dilutions were found to be the same as those used in previous studies on mammals. The incubation time was 5 to 7 days for whole retinas, and 24 hours for sections. Lectin cytochemistry with PNA lectin (*Arachis hypogaea* lectin, PNA), a known general marker of all cones, was used for 2 hours in the form of a biotinylated conjugate diluted 1:20 (Sigma, St. Louis, MO).

Immunocytochemistry on Wholemounts

For single immunolabeling of S and M cones, the retinas were incubated in any of the listed antibodies for 5 to 7 days, then washed in PBS containing 0.4% Triton. Subsequently, the retinas were incubated for 1 day in biotinylated anti-mouse or anti-rabbit antibody diluted 1:150. For detection of the reaction the avidin-biotin complex (ABC) technique (Vectastain; Vector Laboratories, Burlingame, CA) was used with 3,3′-diaminobenzidine (DAB; Sigma) as a chromogen (50 mg DAB and 300 mg nickel-ammonium sulfate dissolved in 50 mL 0.1 M Tris buffer [pH 7.6] in the presence of hydrogen peroxide).

For double-labeling studies, either the OS-2/CERN956 or the JH455/COS-1 combination was used. For the visualization of the bound monoclonal antibodies, OS-2 and COS-1, the retinas were first incubated in biotinylated anti-mouse antibody for 12 to 24 hours and then with either of the two avidin-fluorochrome conjugates: Texas red avidin D (50 μg/mL, Vector Laboratories), or streptavidin conjugate (100 μg/mL, Alexa Fluor 594; Molecular Probes, Eugene, OR). A green fluorescent probe, goat anti-rabbit IgG (H+L) conjugate (100 μg/mL, Alexa 488; Molecular Probes) was used to detect the photoreceptors immunostained by the polyclonal antibodies JH455 and CERN956.

The binding of biotinylated PNA was detected with either Texas red avidin D, or AMCA avidin D (7-amino-4-methyl-3-coumarinylacetic acid 100 μg/mL; Vector Laboratories) as a fluorescent probe. When double labeling with PNA and an S- or M–specific probe, the latter was detected using either a goat anti-rabbit IgG (H+L) conjugate or a goat anti-mouse IgG (H+L; 100 μg/mL; Alexa 488; Molecular Probes).

The retinas were inspected by microscope (DMR; Leica, Heidelberg, Germany) microscope, with the use of Nomarski optics or the appropriate filter set for the fluorescent antibodies. Micrographs were obtained with a cooled color digital camera (Spot II, Diagnostic Instruments) and image-analysis software (Photoshop 5.0; Adobe, San Diego, CA) was used for image processing.

Isodensity Maps

The location of fluorescent or immunoperoxidase-labeled cone photoreceptors in wholemounts was plotted by means of a computerized plotting system. In 70 to 90 sample fields across the retina, cones were counted with a 50× or 100× oil immersion objective. The samples were taken at evenly distributed positions across the retina. Cell densities were calculated by an automated system that was based on a two-dimensional spline surface interpolation algorithm. The algorithm is capable of automatically tracing isodensity contours of the retinal map from the data files. A detailed description of this program is given elsewhere.[30]

Labeling Adjacent Semithin Sections

Consecutive tangential semithin sections were incubated with OS-2, COS-1, and biotinylated PNA, respectively. The bound OS-2 and COS-1 antibodies were labeled with a biotinylated anti-mouse antibody diluted 1:150 for 12 to 24 hours. For visualization the ABC technique was used with DAB, as described earlier. The sections were inspected by microscope (Axiophot; Carl Zeiss, Oberkochen, Germany) with Nomarski optics. Photographs were taken with a digital camera (DP50;

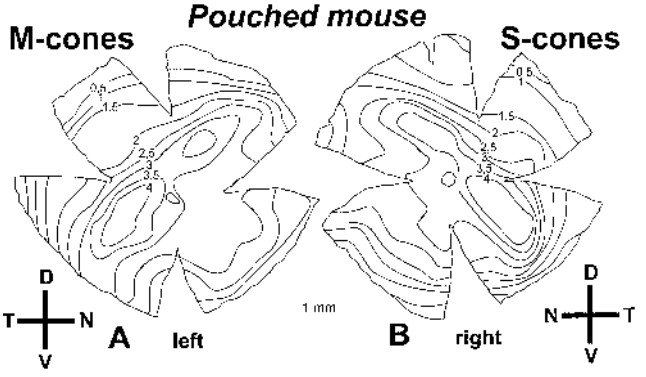


FIGURE 1. Isodensity maps derived from the left (**A**) and the right (**B**) retina of the same specimen of the pouched mouse. On the right eye JH455, a polyclonal antibody specific for S cones, and on the left retina mAb COS-1, selectively labeling M/L cones, were used. Note the symmetrical distribution of the two pigments, with a nearly horizontally aligned visual streak and peak densities in the inferotemporal region. Densities are expressed in 1000 cones/mm^2.

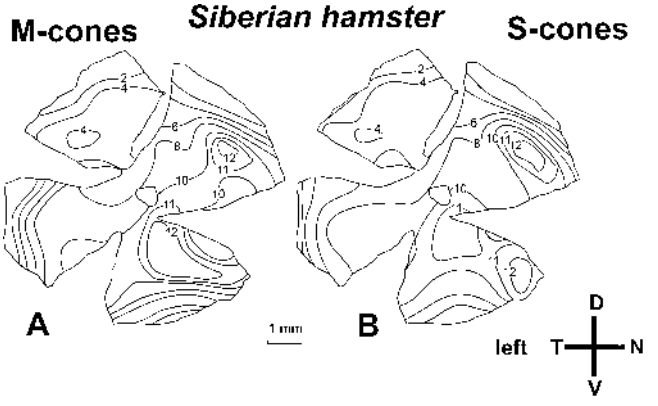


FIGURE 2. Isodensity maps showing the M (**A**) and S (**B**) cone distribution of the same, right retina of a Siberian hamster. The retina was incubated with the combination of OS-2, an anti-S antiserum, and CERN956 specific for the M/L-cones. Note that the derived densities are practically identical. Densities are expressed in 1000 cones/mm^2.

Olympus, Tokyo, Japan) and further processed with image-analysis software (Photoshop 5.0; Adobe). Images of identical areas derived from adjacent sections were compared, so that each cone outer segment was analyzed against the panel of the antibodies and PNA. The pictures were either mounted one under the other, marking the labeled outer segments with arrows, or superimposed on one another. In the latter case, the immunopositive elements were digitally colored.

Confocal Laser Scanning Microscopy

To determine whether there is any spatial difference in the distribution of the bound antibodies along the long axis of the outer segments, images focused on the outer segments at different magnifications were taken with a confocal system (TCS SP; Leica) coupled to a microscope (DMR HC; Leica) using Omega-specific fluorescence filters (Optophotonics, Eauboonne, France).

Control experiments were performed by omitting the primary antibodies or by replacement with normal serum at the same concentration as the antibody. No labeling was observed in any control sections or whole retinas.

Experimental animals in this study were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. *S. campestris* were captured under permit form the Department of Nature Conservation for the Cape Province, South Africa, and were perfused under ethics clearance from the University of Pretoria Ethics Committee (order 000406-004). The design and conditions of the animal experiments performed on Siberian hamsters were approved by the Animal Ethical Committee of Semmelweis University, Budapest, Hungary (BFAEA 2737/99).

RESULTS

Retinal Topography and Densities of M and S Cones

In both rodent species examined, a striking agreement between S- and M-cone densities was found at all retinal locations. Immunocytochemistry on the pouched mouse retinal wholemounts revealed a roughly centroperipheral gradient for both cone types with a near-horizontally aligned visual streak of high cone density. Due to the sloping of the visual streak, the highest densities were found in the inferotemporal quadrants with both M- and S-specific antibodies (Figs. 1A, 1B). Similar immunocytochemical reactions and mappings were also performed on retinas derived from the Siberian hamster. The visual area was not nearly as regularly oriented as in the pouched mouse, the actual figures were somewhat different, and slightly different affinities to the antibodies were also noticed (M cones were stained slightly weaker in the pouched mouse, whereas S cones were less intensely labeled in the hamster). The isodensity maps of the M cones and S cones, however, were found to be practically identical with one another (Figs. 2A, 2B). Note that in neither of the two species has any significant dorsoventral polarization in the expression level of the M and S pigment been observed. The described pattern was not subject to any change caused by the modification of the antibody dilutions: higher and lower concentrations caused a uniform intensification and weakening of the reaction, respectively.

The sloping of the visual streak on our isodensity maps showed a slight within-species variation. The occasional 10° to 15° difference can be attributed partly to the interindividual variations and partly to the insignificant error of the orientation method.

PNA is a well-known marker of all cones. If antibodies COS-1 and OS-2 recognized distinct cone types in these species, isodensity maps of PNA-positive cones should show roughly twice as high densities as those found with either of the two color-cone–specific antibodies alone. When the same retinal wholemount of the pouched mouse was double-labeled with an S-cone–specific antibody (Fig. 3B) and with the all-cone–specific PNA (Fig. 3A), the isodensity maps were close to

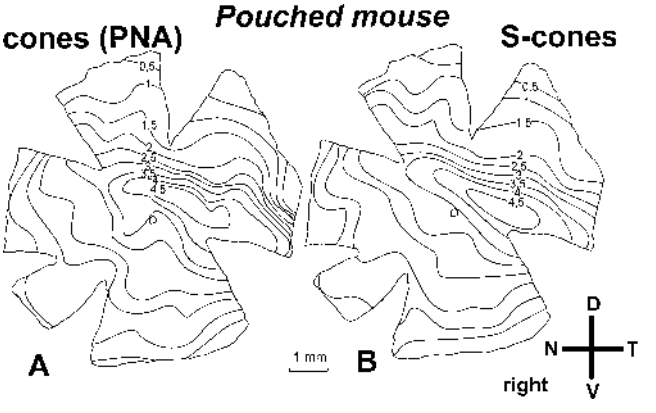


FIGURE 3. Isodensity maps derived from the same retina of the pouched mouse showing density values obtained using an all cone marker PNA (**A**) and a blue-cone–specific antibody JH455 (**B**). The two maps are practically identical. Densities are expressed in 1000 cones/mm^2.

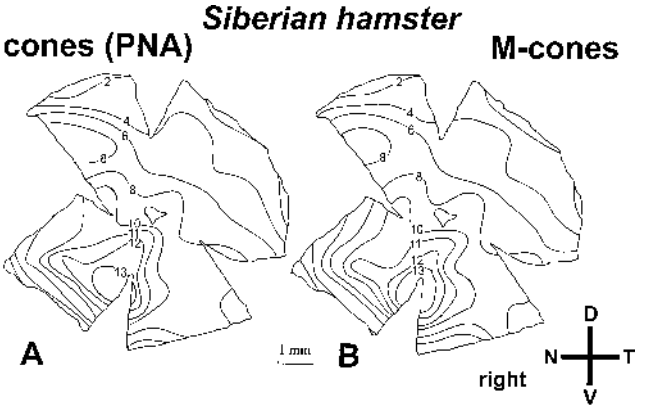


FIGURE 4. Isodensity maps, showing the similar distribution of PNA-positive cones (**A**) and M cones (**B**) in the Siberian hamster retina. Immunocytochemistry with mAb COS-1 and lectin cytochemistry with PNA were subsequently used on the same retina. Densities are expressed in 1000 cones/mm^2.

identical and the actual cone densities also were the same. Exchanging the S-specific antibody for the M-specific mAb COS-1 in similar double-labeling experiments led to the same results (not shown).

The isodensity maps of the M cones, S cones, and all cones of the Siberian hamster were also found to be very similar with almost identical density numbers (Fig. 4). This finding clearly indicates the presence of a uniform cone population with similar affinity to both M- and S-specific antibodies.

Presence of Both Visual Pigments in All Cones

To show that the very same elements are indeed stained by each cone-marker used, double-labeling studies on retinal wholemounts and on consecutive sections were performed. To avoid possible cross reactions, antibodies produced in different species were used in a pair-wise manner. As shown by micrographs taken from identical retinal areas with filter sets for the specific fluorochromes, the very same elements on the picture pairs were found to be labeled in the pouched mouse (Figs. 5A, 5B).

Similar picture pairs obtained from the Siberian hamster retina revealed the same colocalization of the two cone markers. Each cone outer segment was labeled by both antibodies (Figs. 5C, 5D).

Picture pairs obtained with a confocal laser scanning microscope in the pouched mouse (Figs. 6A, 6B) and the Siberian hamster (Figs. 6C, 6D) also show that the cone populations recognized by M-pigment– and S-pigment–specific antibodies, respectively, are identical. Note that the distribution of both visual pigments along the long axis of the outer segments was found to be similar, and no significant proximodistal heterogeneity was observed.

To exclude the possibility of any kind of cross reaction or interference with either primary or secondary antibodies and detection systems, we also used the consecutive tangential section method. With this approach 0.5- to 1-μm-thin adjacent sections of the same outer segments were individually exposed to M- or S cone–specific antibodies, and the micrographs were compared. Comparison of identical retinal areas from semithin sections labeled by PNA, S- and M-cone–specific antibodies clearly demonstrates the colocalization of all three markers in the same cells, irrespective of whether the samples were taken from the upper or lower retinal quadrants (Fig. 7).

DISCUSSION

In all mammalian species studied to date, marked differences have been reported in the distribution of color-specific cone types. From the early histochemical experiments of De Monasterio et al.[31] and Ahnelt[32] to the first immunocytochemical analyses,[8,33] putative, then identified, S cones were reported to make only approximately 5% to 10% of all cones. Later, the total absence of short-wave cones was reported in a number of species,[12–17] and marked topographical asymmetries of cone densities were found in the mouse, guinea pig and the rab-

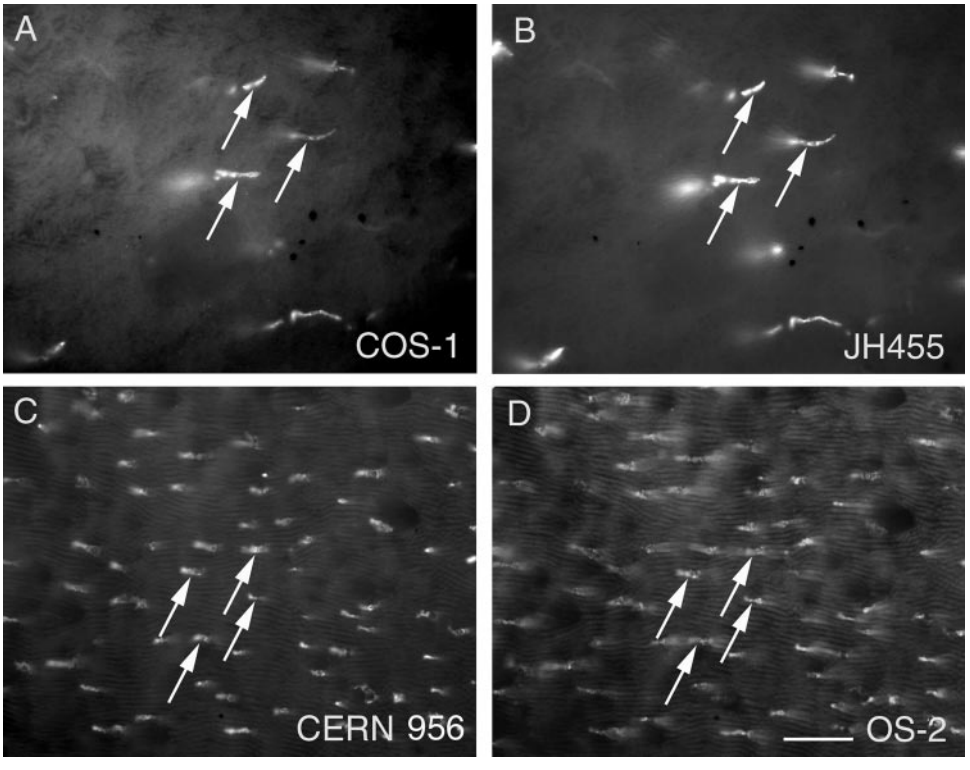


FIGURE 5. Fluorescence photomicrographs showing M and S cones of the pouched mouse (**A**, **B**, respectively) and the Siberian hamster (**C**, **D**, respectively). Note that all cones are labeled by both antibodies. The photographs were taken from the superior-temporal quadrant, approximately halfway between the optic nerve head and the ora serrata. Some dual elements are marked with *arrows*. Bar, 20 μm.

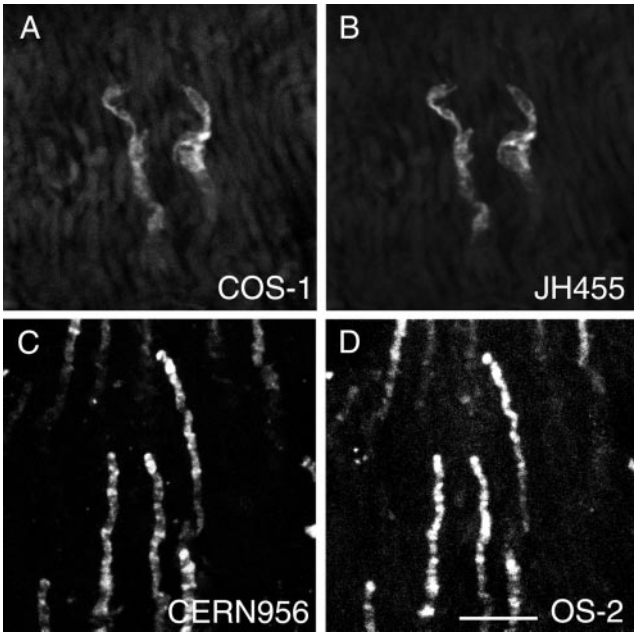


FIGURE 6. Confocal images demonstrating the identical distribution of M and S pigments in the cone outer segments as labeled by antibodies COS-1 (**A**) and JH455 (**B**) in the pouched mouse and by CERN956 (**C**) and OS-2 (**D**) in the Siberian hamster. Note that the distribution of the two pigments along the long axis of the outer segments is homologous. The pictures were taken from the inferior-temporal periphery, at the junction of the distal and middle thirds of the line connecting the optic nerve head and the ora serrata. Bar, 10 μm.

bit.[19,21–23,34] In these latter reports, a common feature of the investigated species was the occurrence of smaller or larger retinal areas with the underexpression or absence of M-cone visual pigment.

More recently, Calderone and Jacobs[27] have contrasted the retina of the Siberian hamster with that of the Syrian hamster—the latter species being free of any S cones. Especially interesting is the retina of the Siberian hamster in which M- and S-cone densities were found to be practically identical. This phenomenon can be explained by assuming either a similar distribution of distinct M and S cone populations or the simultaneous presence of S and M cone visual pigments in a single cone population.

The present study provides evidence for the second possibility. We showed the coexpression of two cone opsins in all cones, by using different approaches, such as double staining with either PNA and one-cone-type–specific antibody or with two cone-type–specific antibodies produced in different species. The colocalization of both markers in these labeling studies as well as in those in which the two markers were applied on consecutive semithin sections provides irrefutable evidence for the existence of dual cones all over the retina of the Siberian hamster. When screening other rodent species for a similar coexpression pattern, we found the pouched mouse (*S. campestris*) to possess cones expressing both M- and S-cone opsins, very similar to those in the Siberian hamster. We must emphasize that the present findings were obtained with the same antibodies and dilutions as those used in our previous studies.[15,19,23,34,35] In these two species other antibodies[9,10] have produced equivalent results. Therefore, the cone pattern presently described in the Siberian hamster and in the pouched mouse is not an artifact due to a possible cross reaction or poorly characterized antibodies.

When comparing this cone opsin expression pattern with that of other rodents, it becomes clear that the uniform expression of both M and S opsins in all cones of the Siberian hamster and the pouched mouse is novel and unique. The first report describing the coexistence of two cone opsins in one cone cell (dual cone) was published by Röhlich et al.[23] who identified these cones in the transition zone between the dorsal and ventral part of the retina in mouse, rabbit, and guinea pig. In the latter species, the presence of dual cones was not confined only to the transition zone, but these cones were present also in the lower half of the retina. Later, others reported a similar pattern in the mouse as well.[24] The elegant physiological experiments of Lyubarsky et al.[25] were also more compatible with a higher occurrence of dual cones in the lower half of the retina in this species. Applebury et al.[11] have presented yet another model for the expression of mouse cone visual pigments. In their study a dorsoventral M-pigment gradient was proposed with all cones expressing this pigment, albeit at various levels. The S pigment, in contrast, was reported to be expressed in a constant manner with the exception of a few dorsal cones that apparently failed to synthesize this pigment. These studies show that in species in which dual cones have been described, these cones are as a rule confined to certain retinal areas[23] or restricted to a narrow developmental time window,[26] and there is usually a marked dorsoventral gradient in the expression of type-specific cone opsins.[11,23] In contrast, dual cones in the Siberian hamster and pouched mouse have a single cone population with each cone expressing both M and S opsins. A dorsoventral gradient in the expression of color-specific opsins is missing.

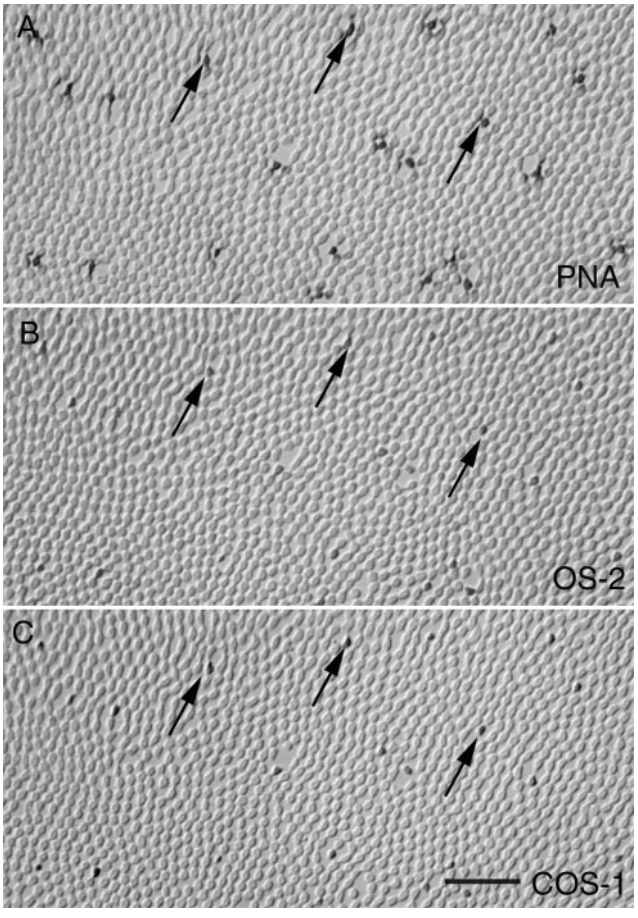


FIGURE 7. Identical areas of three consecutive tangential semithin sections reacted for PNA (**A**, all cones), monoclonal antibody OS-2 (**B**, S cones) and mAb COS-1 (**C**, M cones) in the Siberian hamster. The sections were taken from the superior-nasal periphery, approximately at the junction of the distal and middle thirds of the line connecting the optic nerve head and the ora serrata. The population labeled by each probe is identical. Some positive elements are marked with *arrows*. Bar, 10 μm.

When the Syrian hamster is compared with the Siberian hamster,[16,27] it becomes clear that in both species the pure S cone population is missing. Both species have a single cone population that expresses only the M opsin in the Syrian hamster and both M and S opsins in the Siberian hamster. Because there is only one cone population, real color vision cannot be present. Although in the Syrian hamster the photopic sensitivity is confined to the green range of the spectrum, in the Siberian hamster, this range is extended toward the blue and UV part.[27] Whether the broadening of the spectral sensitivity of this single cone population offers any advantage for the Siberian hamster or not, remains to be elucidated. A further question is whether the hamster can use this photopic sensitivity in addition to the dominating scotopic sensitivity to collect additional visual information.

During ontogenesis, the determination of the cone cell line from progenitor cells, the separation of the M- and S-cone population and subsequently the expression of the cone opsins are probably controlled by sequential, complex gene regulations.[36–38] In the M-cone type, one of the last steps seems to be the turning on of the M opsin gene with the turning off of the S opsin gene. If this control mechanism is not working, both M and S opsins are expressed, or the expression of the opsins is shifted toward the blue (or UV) range. From an evolutionary point of view, it would be interesting to reveal the control factors that govern the opsin switch in cones. In this respect the Siberian hamster (together with the pouched mouse) deserves special attention as a suitable model, because it possesses a single cone population which coexpresses both M and S opsins. The molecular genetic comparison with the Syrian hamster, in which this single cone population expresses the M opsin only, offers a favorable opportunity to study molecular control mechanisms in a pure system and to understand developmental, genetic and evolutionary aspects of opsin coexpression.

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References

1. Jacobs GH. The distribution and nature of colour vision among the mammals. *Biol Rev.* 1993;68:413–471.
2. Jacobs GH, Neitz J, Deegan JF. Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature.* 1991;353:655–656.
3. Yokoyama S. Molecular evolution of vertebrate visual pigments. *Prog Retinal Eye Res.* 2000;19:385–419.
4. Yokoyama S, Yokoyama R. Molecular evolution of human visual pigment genes. *Mol Biol Evol.* 1989;6:186–197.
5. Hunt DM, Cowing JA, Patel R, Appukuttan B, Bowmaker JK, Mollon JD. Sequence and evolution of the blue cone pigment gene in Old and New World primates. *Genomics.* 1995;27:535–538.
6. Jacobs GH, Neitz M, Deegan JF, Neitz J. Trichromatic colour vision in New World monkeys. *Nature.* 1996;382:156–158.
7. Lerea CL, Bunt-Milam AH, Hurley JB. Alpha-transducin is present in blue-sensitive, green-sensitive and red-sensitive cone photoreceptors in the human retina. *Neuron.* 1989;3:367–376.
8. Szél Á, Diamantstein T, Röhlich P. Identification of the blue-sensitive cones in the mammalian retina by anti-visual pigment antibody. *J Comp Neurol.* 1988;273:593–602.
9. Wang YS, Macke JP, Merbs SL, et al. A locus control region adjacent to the human red and green visual pigment genes. *Neuron.* 1992; 9:429–440.
10. Vissers PM, DeGrip WJ. Functional expression of human cone pigments using recombinant baculovirus: compatibility with histidine tagging and evidence for N- glycosylation. *FEBS Lett.* 1996; 396:26–30.
11. Applebury ML, Antoch MP, Baxter LC, et al. The murine cone photoreceptor: a single cone type expresses both S and M opsins with retinal spatial patterning. *Neuron.* 2000;27:513–523.
12. Wikler KC, Rakic P. Distribution of photoreceptor subtypes in the retina of diurnal and nocturnal primates. *J Neurosci.* 1990;10:3390–3401.
13. Jacobs GH, Deegan JF. Cone photopigments in nocturnal and diurnal procyonids. *J Comp Physiol.* 1992;171:351–358.
14. Jacobs GH, Neitz M, Neitz J. Mutations in S-cone pigment genes and the absence of colour vision in two species of nocturnal primate. *Proc R Soc Lond B Biol Sci.* 1996;263:705–710.
15. Szél Á, Röhlich P, Caffé AR, van Veen T. Distribution of cone photoreceptors in the mammalian retina. *Microsc Res Tech.* 1996; 35:445–462.
16. von Schantz M, Argamaso-Hernan SM, Szél Á, Foster RG. Photopigments and photoentrainment in the Syrian golden hamster. *Brain Res.* 1997;770:131–138.
17. Fasick JI, Cronin TW, Hunt DM, Robinson PR. The visual pigments of the bottlenose dolphin (*Tursiops truncatus*). *Vis Neurosci.* 1998;15:643–651.
18. Nathans J, Piantanida TP, Eddy RL, Shows TB, Hogness DS. Molecular genetics of inherited variation in human color vision. *Science.* 1986;232:203–211.
19. Szél Á, Röhlich P, Caffé AR, Juliusson B, Aguirre GD, van Veen T. Unique topographic separation of two spectral classes of cones in the mouse retina. *J Comp Neurol.* 1992;325:327–342.
20. Calderone JB, Jacobs GH. Regional variations in the relative sensitivity to UV light in the mouse retina. *Vis Neurosci.* 1995;12:463–468.
21. Juliusson B, Bergström A, Röhlich P, Ehinger B, van Veen T, Szél Á. Complementary cone fields of the rabbit retina. *Invest Ophthalmol Vis Sci.* 1994;35:811–818.
22. Famiglietti EV, Sharpe SJ. Regional topography of rod and immunocytochemically characterized "blue" and "green" cone photoreceptors in rabbit retina. *Vis Neurosci.* 1995;12:1151–1175.
23. Röhlich P, van Veen T, Szél Á. Two different visual pigments in one retinal cone cell. *Neuron.* 1994;13:1159–1166.
24. Glösmann M, Ahnelt PK. Coexpression of M- and S-opsin extends over the entire inferior mouse retina (Abstract). *Eur J Neurosci.* 1998;10:357.
25. Lyubarsky AL, Falsini B, Pennesi ME, Valentini P, Pugh EN Jr. UV- and midwave-sensitive cone-driven retinal responses of the mouse: a possible phenotype for coexpression of cone photopigments. *J Neurosci.* 1999;19:442–455.
26. Szél Á, van Veen T, Röhlich P. Retinal cone differentiation. *Nature.* 1994;370:336.
27. Calderone JB, Jacobs GH. Cone receptor variations and their functional consequences in two species of hamster. *Vis Neurosci.* 1999;16:53–63.
28. Szél Á, Lukáts Á, Szepessy Zs, et al. Visual pigment coexpression in all retinal cones of the Siberian hamster [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 2001;42(4):**S**360. Abstract nr 1948.
29. Röhlich P, Szél Á. Binding sites of photoreceptor-specific antibodies COS-1, OS-2 and AO. *Curr Eye Res.* 1993;12:935–944.
30. Dkhissi-Benyahya O, Szél Á, DeGrip WJ, Cooper HM. Short and mid-wavelength cone distribution in a nocturnal Strepsirrhine primate (*Microcebus murinus*). *J Comp Neurol.* 2001;438:490–504.
31. De Monasterio FM, Schein SJ, McCrane EP. Staining of blue-sensitive cones of the macaque retina by a fluorescent dye. *Science.* 1981;213:1278–1281.
32. Ahnelt PK. Characterization of the color related receptor mosaic in the ground squirrel retina. *Vision Res.* 1985;25:1557–1568.
33. Curcio CA, Hendrickson A. Organization and development of the primate photoreceptor mosaic. *Prog Retinal Eye Res.* 1991;10:89–120.
34. Szél Á, Csorba G, Caffé AR, Szél G, Röhlich P, van Veen T. Different patterns of retinal cone topography in two genera of rodents, Mus and Apodemus. *Cell Tissue Res.* 1994;276:143–150.
35. Szepessy Zs, Lukáts Á, Fekete T, Barsi Á, Röhlich P, Szél Á. Cone differentiation with no photopigment coexpression. *Invest Ophthalmol Vis Sci.* 2000;41:3171–3175.
36. Neitz M, Neitz J. The uncommon retina of the common house mouse. *Trends Neurosci.* 2001;24:248–249.
37. Applebury ML. Response: the uncommon retina of the common house mouse. *Trends Neurosci.* 2001;24:250.
38. Szél Á, Lukáts Á, Fekete T, Szepessy Zs, Röhlich P. Photoreceptor distribution in the retinas of subprimate mammals. *J Opt Soc Am A.* 2000;17:568–579.