Analysis of the Short Wavelength-Sensitive ("Blue") Cone Mosaic in the Primate Retina: Comparison of New World and Old World Monkeys

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ABSTRACT
The distribution of short wavelength-sensitive (SWS or "blue") cone photoreceptors was compared in primates with dichromatic ("red-green colour blind") and trichromatic colour vision. We compared a New World species, the marmoset (Callithrix jacchus), with an Old World species, the macaque monkey (Macaca nemestrina). The SWS cones were identified by their immunoreactivity to an antiserum against the human SWS cone opsin. A single retina from a male capuchin monkey (Cebus apella) also was studied. The SWS cones make up less than 10% of all cone photoreceptors throughout the retina of all animals studied. In marmoset, the peak spatial density of SWS cones is close to 10,000/mm² at the foveola. In macaque, the peak spatial density of SWS cones, close to 6,000/mm², is at the fovea, but SWS cones are absent within 50 µm of the centre of the foveola. In both species, the density of SWS cones is higher on the nasal retinal axis than at corresponding eccentricities on the other retinal axes. The SWS cones in macaque are arranged in a semiregular array, but they are distributed randomly in marmoset. There is no difference in the spatial density or local arrangement of SWS cones between dichromatic and trichromatic marmosets. The results suggest that the SWS cone photoreceptor system is subject to different developmental and evolutionary constraints than those that have led to the formation of the red-green photoreceptor systems in primate vision. J. Comp. Neurol. 406:1–14, 1999.

Indexing terms: colour vision; marmoset; macaque; photoreceptors

The colour vision system of most mammals is dichromatic and is dependent on the presence of two main classes of cone photoreceptors with peak spectral sensitivity in the short wavelength-sensitive (SWS; near 430 nm) and medium-long wavelength-sensitive (ML; 500–570 nm) range (Bowmaker et al., 1991; Jacobs, 1993, 1996). Many of the Old World primate species, including humans, achieve trichromatic colour vision, because they express two cone opsins in the ML range and can utilise the signals arising from all three cone receptor types. Molecular analysis shows that the divergence of the pigments in the ML range is a recent event in the evolutionary history of primates and may have occurred independently for New World and Old World lineages (Shyue et al., 1995), whereas the SWS system is phylogenetically ancient, having diverged from the ML cone pigment before the emergence of primates (Yokoyama and Yokoyama, 1989; Hunt et al., 1995; Jacobs, 1996).

In some nonmammalian vertebrates, such as the teleost fish, the different spectral classes of cones are arranged as interlocking crystalline arrays (Raymond et al., 1995), but the question of whether the SWS and ML cone arrays form independent mosaics in primates is controversial (Ahnelt et al., 1990; Wikler and Rakic, 1990; Curcio et al., 1991; Packer et al., 1996). In the retina of Old World monkeys and humans, the SWS cones are arranged in a semiregular, triangular array (Marc and Sperling, 1977; De Monasterio et al., 1981; Shapiro et al., 1985; Ahnelt et al., 1987;
independent photoreceptor subsystem within the retina. The other nonprimate mammals, the SWS cone array forms an
thermore, the quantitative analysis of SWS cone distribu-
spatial density of SWS cones between marmoset and
is unique to trichromatic primates. Here, we show that
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SWS cones (Jacobs et al., 1993a; Deegan and Jacobs,
SWS cone distribution in all diurnal primates.
Thus, it is not known whether there is a common pattern of
SWS cones altogether (Peichl and Moutairou, 1998). Like-
rodent (African giant rat) and some seals appear to lack
zone where only SWS cones and rods are present (J ulius-
son et al., 1994; Szél et al., 1994, 1996), whereas another
rodent (African giant rat) and some seals appear to lack
SWS cones altogether (Peichl and Moutairou, 1998). Like-
wise, anatomical (Wikler and Rakic, 1990), behavioural,
and electrophysiological evidence shows that two noctur-
nal primates, the owl monkey (Aotus trivirgatus) and the
bush baby (Otolemur crassicaudatus), also lack functional
SWS cones (J acobs et al., 1993a; Deegan and J acobs,
1996). The presence of SWS cones in a New World monkey,
the squirrel monkey (Saimiri sciureus), has been demon-
strated anatomically (Wikler and Rakic, 1990, 1996);
however, to date, there has been no study of the distribu-
tion of SWS cones in any diurnal New World primate.
Thus, it is not known whether there is a common pattern of
SWS cone distribution in all diurnal primates.
In the present study, we compared the distribution of
SWS cones in a trichromatic Old World macaque monkey
(Macaca nemestrina) with a New World monkey, the
common marmoset (Callithrix jacchus). Like many other
New World monkeys studied so far (J acobs, 1984, 1996;
Bowmaker et al., 1985; J acobs et al., 1993b), this species
has both dichromatic and trichromatic individuals (Travis
et al., 1988; Tove´e et al., 1992; Yeh et al., 1995). Thus, the
marmoset provides an opportunity to ask whether the
regular distribution and constant proportion of SWS cones
is unique to trichromatic primates. Here, we show that
there are marked differences in the distribution and
spatial density of SWS cones between marmoset and
macaque retina but that none of these differences is
associated uniquely with the trichromatic phenotype.
Furthermore, the quantitative analysis of SWS cone distribu-
tion in both of these species provides evidence that, like
in other nonprimate mammals, the SWS cone array forms an
independent photoreceptor subsystem within the retina.

MATERIALS AND METHODS

Tissue preparation

Eyes were obtained from six adult marmosets (Calli-
thrix jacchus), one capuchin monkey (Cebus apella), and
two pigtail macaque monkeys (Macaca nemestrina).
The colour vision phenotype of one female and one male
marmoset and of the (male) capuchin monkey was estab-
lished first in electrophysiological recording experiments
(Yeh et al., 1995; Silveira et al., 1998). The phenotype
identified from those studies is noted where appropriate in
the figure legends. The eyes from the other animals were
taken after experiments that were unrelated to those
described here. The animals were killed with an overdose
of pentobarbitone sodium (80–150 mg/kg, i.v.).

Some animals were perfused intracardially with saline
and then with 2% or 4% paraformaldehyde (PFA) in 0.1 M
phosphate buffer (PB), pH 7.4. The eyes were opened by an
encircling cut and then fixed by immersion in the same
fixative for 4–6 hours. Eyes from the other animals were
treated the same way, except they were removed without
perfusion. The retina was dissected free from the sclera,
pigment epithelium, and vitreous. The isolated retinas
either were stored in PB with 0.01% sodium azide at 4°C
for 2 months to up to 1 year or were immersed in 30%
sucrose in PB overnight, frozen, and stored at −20°C or
−70°C until use. All procedures were approved by the
University of Sydney animal care and ethics committee
and conformed with the provisions of the Australian
National Health and Medical Research Council (NHMRC)
code of practice for the care and use of animals.

Immunocytochemistry

Affinity-purified rabbit antisera directed against the
human SWS cone pigment (J H455) and against the human
MWS/LWS cone pigment (J H492; Wang et al., 1992; gift of
Dr. J. Nathans) were used to label the respective cone
types. Retinal quadrants or whole retinas were immersed
in 30% sucrose in PB overnight. They were rapidly frozen,
thawed, then rinsed (3 times for 10 minutes each) in 0.1 M
phosphate-buffered saline (PBS), pH 7.4. The tissue was
presoaked for 1 hour in 10% bovine serum albumin/0.5%
Triton X-100 in PBS at room temperature before
incubation in the primary antisera. The antisera were
used at a dilution of 1:50,000 or 1:100,000 (J H455) and
1:20,000 (J H492) in 5% bovine serum albumin/0.5%
Triton X-100/0.05% sodium azide in PBS. The tissue was left in
the primary antisera for 5–7 days at 4°C. The following
steps were carried out at room temperature. The tissue
was rinsed in PBS (four times for 15 minutes each) and
then placed in biotinylated anti-rabbit immunoglobulin G
(IgG; 1:300, Vector Laboratories, Burlingame, CA) in 1%
bovine serum albumin/0.5% Triton X-100 in PBS for 3 hours.
The tissue was rinsed in PBS (4 times for 15 minutes each) and
subsequently incubated in the avidin-biotin peroxidase complex (ABC Elite, Vector Laborato-
ries) for 1 hour. After rinsing in PBS (4 times for 15 minutes each), peroxidase was visualised by incubation in
0.05% diaminobenzidine tetrahydrochloride (DAB) in PBS
for 10 minutes, followed by another 10 minutes in fresh
DAB solution to which hydrogen peroxide was added to
obtain a final concentration of 0.01%. The tissue was
washed thoroughly in PBS, postfixed in PFA in PB for 1
hour, rinsed again, and then mounted in 10% Mowiol
(Hoechst, Sydney, Australia) dissolved in a solution contain-
ing glycerol and Tris/HCl buffer (Harlow and Lane, 1988).
This mounting procedure produced minimal shrinkage.

Analysis

The distribution of labelled and unlabelled photorecep-
tors was measured from wholemount preparations with a
computer-assisted camera lucida drawing system (Halasz
and Martin, 1984; Wilder et al., 1996) that enabled accu-
rate mapping of cell distribution over a large range of
retinal eccentricities. The horizontal axis was defined as a line running through the fovea and the centre of the optic disk. The position of each labelled SWS cone was measured in a 100–250 µm wide strip along a chosen (horizontal or vertical) retinal axis. Samples of unlabelled cones were marked also at regular intervals along the chosen axis. Sample sites were chosen where the orientation of receptors was close to vertical (Curcio et al., 1991). In the central retina, only a small number of sample sites satisfied this criterion. To compare the distribution of photoreceptors between species, we express eccentricity as degrees of visual angle. The foveal magnification factor for the schematic marmoset eye is 128 µm/degree (Troilo et al., 1993). The relationship between visual angle and distance on the retina for greater eccentricities can be calculated by trigonometric correction for the difference between the posterior nodal point and the centre of the retinal sphere (Drasdo and Fowler, 1974; Perry and Cowey, 1985). This function is well approximated for marmoset by the cubic polynomial

\[ y = 0.0538x^3 - 0.542x^2 + 9.396x - 0.75, \quad (1) \]

where \( y \) is degrees of visual angle, and \( x \) is distance from the fovea in millimetres (Troilo et al., 1993; Wilder et al., 1996). For macaque monkey, the quadratic polynomial

\[ y = 0.038x^2 + 4.21x + 0.1 \quad (2) \]

was used (Dacey and Petersen, 1992). Density of photoreceptors as a function of eccentricity was estimated by fitting the measured spatial density to a sum of three exponents of the form

\[ Y = C_1e^{l_1x} + C_2e^{l_2x} + C_3e^{l_3x}, \quad (3) \]

where \( X \) is eccentricity in degrees, \( Y \) is cells/mm\(^2\) \times 1,000, and \( C_{1-3} \) and \( l_{1-3} \) are constants (Goodchild et al., 1996b) using a squared error-minimisation search algorithm (MatLab, The Math Works, Natick, MA. Simplex function).

Similar to the results from our previous study of retinal ganglion cell and photoreceptor topography in marmoset (Wilder et al., 1996), in this paper, we use the term “central retina” to refer to the first 10° of visual angle (1.5 mm), “midperipheral retina” refers to eccentricities between 1.5 mm and 4 mm (31°), and “far peripheral retina” refers to eccentricities beyond 4 mm.

**RESULTS**

**Characteristics of labelled SWS cones**

Unlike other methods that have been used previously to label SWS cones (Szél et al., 1988; Lerea et al., 1989; Curcio et al., 1991; Wilder et al., 1996), the polyclonal antiserum J H455 produces a dense reaction product that, except in the central retina, labels the entire SWS cone, including the cone pedicle (Goodchild et al., 1996a; Bumsted et al., 1997; Ghosh et al., 1997; Chan and Grünert, 1998). Figure 1 shows labelled SWS cones in vertical cryostat sections in macaque (Fig. 1A), marmoset (Fig. 1B), and Cebus (Fig. 1C). In macaque and Cebus retina, the SWS cones almost invariably are separated by unlabelled cones, but, in marmoset, examples also were seen of labelled cones that were immediate neighbours. In common with all other species studied so far (Curcio et al., 1991; Ahnelt et al., 1995), the cell bodies of labelled SWS cones in all three species lie close to the outer limiting membrane.
Wholemount preparations of macaque and marmoset retinas are shown in Figure 2. The pattern of labelling with the JH455 (SWS cone) antiserum (Fig. 2A) is obviously different from the pattern seen with the JH492 antiserum to human ML cone opsin (Fig. 2B). The ML cone antiserum labels the majority of cone outer segments very densely. By contrast, the SWS cone antiserum labels a smaller proportion of cones in their entirety and leaves the other cones unlabeled. The pattern of unlabelled cones (Fig. 2B, arrowheads) is complementary to the pattern of cones labelled with the SWS cone antiserum (Fig. 2A, arrowheads) and provides further evidence for the specificity of the SWS antiserum. The SWS cones in macaque, as described previously (Marc and Sperling, 1977; Szél et al., 1988; Wikler and Rakic, 1990), form a semiregular, triangular array (Fig. 2A), but they appear randomly interspersed with unlabelled cones in the marmoset (Fig. 2C,D). A quantitative analysis of these differences in local distribution is provided below.

**SWS cones in the fovea**

Previous analyses of SWS cone distribution in Old World primates (Marc and Sperling, 1977; Szél et al., 1988; Curcio et al., 1991; Wikler and Rakic, 1996) have shown that the maximum density of SWS cones is immediately adjacent to the foveola, but there is a small “gap” at the very centre of the fovea. We find the same result in
macaque retina (see below). By contrast, in the marmoset retina, there is no SWS cone-free zone at the foveola. Figure 3 shows wholemount preparations of the fovea in macaque (Fig. 3A,B), marmoset (Fig. 3C), and Cebus (Fig. 3D). In the macaque and marmoset preparations, the fovea is intact, but there is some disruption at the foveola in the Cebus preparation. Nevertheless, there is a clear difference in the distribution of foveal SWS cones between marmoset and the other two species. In macaque (Fig. 3B), the SWS cones are absent from a region of about 50 µm in diameter at the foveola (Szél et al., 1988). By contrast, in marmoset, the foveola is marked by a dense aggregation of labelled SWS cone outer segments (Fig. 3C). In Cebus, there is a clear decrease in the density of labelled cones within 100 µm of the foveola (Fig. 3D), but the exact diameter of the SWS cone-free zone could not be measured. These observations show that the foveola is not a zone of exclusion for SWS cones in marmoset, despite the fact that the total density of foveal cones in that species is comparable to that in macaque or human (Trollo et al., 1993; Wilder et al., 1996). That this arrangement is not a general difference between New World and Old World primates is shown by the lack of SWS cones in the centre of the foveola in Cebus.

**Distribution of SWS cones in marmoset**

Next, we quantified the distribution of SWS cones in marmoset. The method of quantification is illustrated in Figure 4. An outline of the retina was drawn at low magnification, then the location of each labelled cell body was marked in a sample strip along one retinal axis. The data were transformed to fovea-centred coordinates (Fig. 4A), and the number of cells was measured at regular intervals along a subsample of constant width (Fig. 4B) to give a high-sampling-density graph of cell distribution (Fig. 4C).

The distribution of SWS cones on four axes of the retina of one trichromatic female marmoset is shown in Figure 5. The peak density close to 10,000 cells/mm² is at the foveola, with density dropping rapidly over the first 2 mm...
and less rapidly thereafter. The nasal axis (Fig. 5A) clearly is different from the other three axes measured, with an almost constant density of SWS cones between the optic disk and the edge of the retina.

Comparison of dichromatic and trichromatic marmosets

There is no difference between the local order of the SWS cone mosaic or the overall SWS cone distribution between retinas from marmosets that were identified as possessing either one or two pigments in the ML range. The graph in Figure 6A compares the spatial density of SWS cones along the horizontal axis of a dichromatic male and a trichromatic female marmoset. A: Horizontal axis. B: Vertical axis. The peak density close to 10,000 cells/mm² is at the fovea. The density in nasal retina is almost constant from the optic disk and the edge of the retina, where a slight increase is apparent. Density falls more rapidly in the other axes.

Fig. 4. Analysis of the spatial density of SWS cones. A: Outline drawing of a wholemounted marmoset retina transformed to fovea-centred coordinates. The solid circle shows the position of the optic disk. The open circle shows the position of the fovea. The position of each labelled SWS cone along the temporal axis was marked (gray shading). B: A sample window (rectangle) is superimposed on the band of marked cells. C: The number of marks in 100-µm intervals along the sample window is counted to give the spatial density distribution of labelled cells.

Fig. 5. Spatial density of SWS cones in the retina of a trichromatic female marmoset. A: Horizontal axis. B: Vertical axis. The peak density close to 10,000 cells/mm² is at the fovea. The density in nasal retina is almost constant from the optic disk and the edge of the retina, where a slight increase is apparent. Density falls more rapidly in the other axes.
trichromatic female marmoset, with exponential functions (smooth curves) fitted. The data sets are practically identical. The local SWS cone distribution in central and peripheral retina, likewise, is similar in these two animals (Fig. 6B). The SWS cones are distributed randomly in both animals. We made qualitative observations on several retinas apart from those analysed quantitatively here: No obvious differences in spatial distribution or local order of SWS cones were seen. Because, in many preparations, the foveola was disrupted during the dissection process, we cannot rule out the possibility that differences exist within 50 µm of the fovea.

Proportion of SWS cones in marmoset

To compare the density of SWS and ML cones, the density of unlabelled cones also was measured at some of the SWS cone sample points. This is shown for the fovea of one female marmoset in Figure 7. The position of SWS cones in a sample along the horizontal axis is shown in Figure 7A. The percentage of SWS cones is shown in Figure 7B. Within the fovea, the proportion of SWS cones shows a localised decrease, from nearly 10% at 200 µm to under 3% at 15 µm eccentricity (Fig. 7B). The peak density of unlabelled cones in this marmoset retina (close to 350,000 cells/mm²; Fig. 7B) is higher than that measured for any macaque retina in the current study or reported previously for macaque or human retina (Cucui et al., 1987; Packer et al., 1989; Wässle et al., 1989; Wikler et al., 1990). This makes it unlikely that the SWS cones are excluded from the foveola of macaque and human because of the high packing density of ML cones.

The total cone density and the ratio of cones to rods in peripheral retina of marmoset are higher than in macaque or human (Troilo et al., 1993; Wilder et al., 1996). We asked whether this difference is due to a proportionate increase in the density of all cone types or to a specific increase of SWS or ML cone populations. Density measurements for ML and SWS cones at the same sample points on the horizontal axis of one marmoset are shown in Figure 8. The data points for each axis are fit with a sum of three exponentials (see Materials and Methods). These functions are shown as the solid curves in Figure 8A, and the values of the parameters for these curves are shown in Table 1. The ratio of labelled (SWS) cones to unlabelled (ML) cones shows an initial rise over the first 10° (Fig. 8B), because the density of ML cones falls more rapidly than the density of SWS cones. Thereafter, the proportion of SWS cones falls slightly to form about 3% of all cones in the far peripheral retina. The curves have the same shape for temporal and nasal retina, showing that the high total cone density in nasal retina is not due to a disproportionate increase in the number of SWS cones.

Distribution and proportion of SWS cones in macaque.

Figure 9A shows SWS cone density on the horizontal and vertical axes of one macaque retina derived from exponential fits, as described above (see Eq. 3 in Materials and Methods). The main difference between marmoset and macaque retina is that the SWS cone density decreases more rapidly outside the fovea in macaque. As in marmoset, the SWS cone density drops most steeply within the central 2 mm.

Although the highest SWS cone density for macaque is seen at the fovea, a fine-grained analysis of the foveal SWS cone mosaic shows that there is always a region about 50 µm in diameter that contains at most one or two labelled cones. Outside this region, the SWS cone density rises very sharply to a peak close to 6,000 cells/mm² at 100 µm from the centre of the fovea. This is shown in Figure 9B for an animal in which both the labelled cones and the unlabelled...
cones could be marked within the foveola. The SWS cone-free zone coincides with the point of maximum ML cone density. Figure 9C,D shows the proportion of SWS cones throughout the retina of macaque. Similar to the marmoset (cf. Fig. 8A), the ML and SWS cone distributions follow the same overall pattern, with a peak in central retina (Fig. 9C). The SWS cones in macaque form close to 9% of cones in the peripheral retina, but the proportion drops within the central 20° (Fig. 9D). Our SWS and total cone density values for peripheral retina in M. nemestrina (current results) are comparable to those given for rhesus macaque monkey (M. mulatta) by Wikler and Rakic (1990) and for the total cone population in M. nemestrina (Packer et al., 1989), but we measured somewhat higher densities of SWS cones than the former authors did.

In Figure 10, the differences between marmoset and macaque are summarised. In marmoset, the peak density of SWS cones is higher, and density drops less steeply with visual angle (Fig. 10A). The peak density of ML cones is similar in both species, but ML cone density is higher in the peripheral retina of marmoset (Fig. 10B). This means that the proportion of SWS cones in the far peripheral retina of macaque (8-9%) is higher than for marmoset (1.5-2.3%; Fig. 10C).

**Regularity of the SWS cone mosaic**

A number of previous studies have derived useful quantitative measures of the spatial distribution of cell populations in the retina (Wassle and Riemann, 1978; Shapiro et al., 1985; Ahnelt et al., 1987; Szél et al., 1988; Curcio et al., 1991; Cook, 1996). For the present study, we used the recently developed density recovery profile (DRP; Rodieck, 1991). The particular advantage of the DRP analysis is that it can be used to derive a “packing factor,” which is a scalar measure of deviation from a triangular array (Rodieck, 1991; Koyama and Marshak, 1997). The packing factor varies between 0 (random distribution) and 1 (triangular array). This allows the degree of regularity of the SWS cone mosaics to be compared quantitatively. DRP histograms and the local distribution of SWS cones in marmoset and macaque are shown in Figure 11. The sample areas were from midperipheral retina and were approximately matched for SWS cone density (marmoset, 1,382 cells/mm²; macaque, 793 cells/mm²). Both of the samples show significant deviation from a random array (marmoset: re/Dr = 1.94; P < 0.01; macaque: re/Dr = 4.46; P < 0.01; probabilities calculated according to Rodieck, 1991). We measured the soma diameter of 100 labelled SWS cones at 4.96 mm in peripheral marmoset retina. The average diameter was 6.3 µm, S.D. 0.79 µm. This value is close to the effective radius of 9.7 µm (Fig. 11B). The effective radius is a measure of the average “dead space” around the centre of each SWS cone. In other words, the deviation from a random array for marmoset SWS cones can be accounted for by the simple fact that the SWS cone somata do not overlap their position in the outer nuclear layer, so their centre-centre spacing cannot be less than their soma diameter. The residual difference (3.4 µm) between the effective radius and the soma diameter probably can be
accounted for by the processes of Müller cells, which are interposed between the cone somata. By contrast, the regularity of the mosaic in macaque is close to three times that for marmoset, and the effective radius (22.3 µm) cannot be accounted for by the size of the SWS cone somata (Figs. 1, 11, 12).

**Regularity of SWS cone pedicles**

The distribution of the cone pedicles in marmoset, is less ordered than in macaque. Figure 12 shows three different planes of focus through the photoreceptors in wholemount preparations of macaque (Fig. 12A–C) and marmoset (Fig. 12D–F) peripheral retina. The through-focus series reveals that the labelled somata and cone pedicles maintain the same spatial relations as the cone outer and inner segments; that is, the difference between the species is maintained at the level of synaptic contact with the postsynaptic neurones. We conclude that there is a significant difference in the degree of regularity of SWS cones and cone synaptic terminals between marmoset and macaque retinas.

**DISCUSSION**

Our results show that the overall distribution of SWS cones in marmoset fits clearly into the pattern described for diurnal Old World primates, but there are quantitative differences in the proportion and local spatial distribution of SWS cones in the marmoset compared with the macaque. The following discussion addresses three key questions. First, we ask whether a general scheme for distribution of cone photoreceptors in primates can be drawn. Second, we ask whether the features we see are associated with the differences in colour vision of New World and Old World primates. Third, in the light of our results, we review the developmental mechanisms currently proposed for generating the adult pattern of photoreceptors.

**SWS cone distribution in primates**

In both the marmoset and the macaque, SWS cones are concentrated near the fovea, and their density near the fovea is over 50-fold that in the peripheral retina. Although some retinas showed a slight increase in both SWS and unlabelled cone density at the peripheral margin (see, e.g. Fig. 5; see also Curcio et al., 1987; Wikler et al., 1990; Williams, 1991), we see no evidence for a zone of increased SWS cone density in the ventralmost retina, as seen in some nocturnal rodents (Szél et al., 1996) and in rabbit (Famiglietti and Sharpe, 1995). The peak density of SWS cones in marmoset, close to 10,000 cells/mm^2, is the highest for any species reported so far (cf. human peak: 2,000 cells/mm^2; Curcio et al., 1991; baboon peak: 6,000 cells/mm^2; Marc and Sperling, 1977; macaque peak: 4,000-6,000 cells/mm^2; De Monasterio et al., 1981; Wikler and Rakic, 1990). The differences in density are approximately inversely proportional to eye size, so that the theoretical maximum array sampling frequency (Nyquist frequency) should be similar for the three species.

In the marmoset, the highest density of SWS cones is in the centre of the fovea: there is no SWS cone-free zone. In macaque, the SWS cone-free zone is very small (Figs. 3, 9; see also Szél et al., 1988; Wikler and Rakic, 1990), and the highest density of the SWS cones is within the fovea, immediately surrounding the SWS cone-free zone. In human and baboon, the SWS cone-free zone is larger, and the absolute density of SWS cones rises more slowly to reach a peak at 1°–2° from the fovea (Marc and Sperling, 1977; Curcio et al., 1991). The size of the SWS cone-free zone cannot be explained by a general mechanism whereby the SWS cones are excluded from areas where the spatial density of ML cones is very high, because the absolute ML cone density in the marmoset fovea (200,000–300,000 cells/mm^2) is at least as high as that in the Old World primates.

We hypothesise that the size of the SWS cone-free zone is determined by eye size in foveate primates. The small size of the marmoset eye means that the angular sampling aperture of foveal receptors should be about twice that for the human eye, and the defocusing of shorter wavelengths as a result of chromatic aberration of the lens, likewise, would be reduced in the smaller eye of the marmoset. These factors both would bring the spatial signal-transmission characteristics of the SWS cone array closer to that of the ML cone array in the smaller eye of the marmoset. In accord with this pattern, the macaque eye is smaller than the human or baboon eye, and the SWS cone-free zone in macaque is smaller than in these latter species (Marc and Sperling, 1977; Curcio et al., 1991). Furthermore, the fact that the Cebus retina (which is similar in size to the macaque retina) also exhibits an SWS cone-free zone (Fig. 4D) shows that the presence of SWS cones in the foveola is not simply a characteristic of New World monkeys, per se.

**SWS cones and primate colour vision**

The SWS cone pigment encoding gene is located on chromosome 7, whereas the ML cone pigment encoding gene(s) is located on the X chromosome (Nathans et al., 1992; Jacobs, 1996). For all diurnal primates studied so far, immunocytochemical and behavioural evidence suggests that the SWS cone pigment is expressed and can be used to make dichromatic colour discriminations (Wikler and Rakic, 1990; Kalloniatis and Harwerth, 1991; Tovee et al., 1992; Jacobs, 1996). For all diurnal primates studied so far, immunocytochemical and behavioural evidence suggests that the SWS cone pigment is expressed and can be used to make dichromatic colour discriminations (Wikler and Rakic, 1990; Kalloniatis and Harwerth, 1991; Tovee et al., 1992; Jacobs, 1996).
al., 1992; Jacobs et al., 1996; Szel et al., 1996). That the postreceptoral neurones transmitting SWS cone signals are different from those carrying ML cone signals is clear for all primates studied so far: specific SWS cone-contacting bipolar cells have been identified in both New World and Old World primates (Mariani, 1984; Boycott and Wässele, 1991; Koyama and Marshak, 1992; Ghosh et al., 1997), and the cone contacts of H2 horizontal cells also are biased toward SWS cones in both species (Ahnelt and Kolb, 1994; Dacey et al., 1996; Goodchild et al., 1996a; Chan and Grünerht, 1998). A specific (small bistratified) ganglion cell type transmits excitatory signals from SWS cones (Dacey and Lee, 1994; Ghosh et al., 1997), and the thalamic pathway for SWS opponent signals is distinct from that for ML opponent cells in the marmoset (Martin et al., 1997). Our results support all of this evidence that the SWS and ML cone systems are independent entities. First, the similar connec-

Fig. 9. Spatial density of SWS cones in macaque. A: Comparison of the different retinal axes. The solid lines are three stage exponential fits, as in Figure 8, except that this graph is replotted in millimetres of eccentricity. B: A patch of the foveola in macaque retina where both labelled and unlabelled cones could be identified. Circles mark the location of SWS cones. Single dots mark the location of unlabelled ML cones. SWS cones are absent from the central 40 µm. C,D: The proportion of SWS cones along the horizontal axis. Both SWS and ML cones were measured at each sample point. The solid lines show exponential fits, as in Figure 8.

Fig. 10. Quantitative comparison of SWS cones in macaque and marmoset. Solid curves are exponential fits to average data, as in Figures 8 and 10. A: SWS cone density is slightly higher over most of the marmoset horizontal axis, but the distribution is similar in both species. B: Comparison of SWS and total cone density (SWS + ML) on the temporal axis. The greater value for total cone density in marmoset cannot be accounted for by a greater density of SWS cones. C: Comparison of the proportion of SWS cones in macaque and marmoset. SWS cones form a lower proportion of all cones in the peripheral retina of marmoset but a higher proportion in central retina.
Activity of SWS cone pathways in New World and Old World monkeys is present, despite the fact that the local photoreceptor distribution is random in marmoset but regular in the macaque (Figs. 2, 11, 12). Second, the presence of two or one ML pigments has no effect on the spatial density or distribution of SWS cones in marmosets (Fig. 6).

Specification of the SWS and ML cone mosaics

Our results argue for a relative independence of the factors that specify the SWS and ML cone mosaics in primates. Although the overall distribution of SWS and ML cones is similar, the proportion of SWS cones at any given eccentricity is lower in marmoset, and the proportion of SWS cones varies with eccentricity for both species (Fig. 10). Furthermore, there are dramatic differences in local order of SWS cones in the two species (Figs. 11, 12). The possibility that the SWS cone mosaic interacts with the ML cone mosaic during development to generate the adult pattern of cone spacing has been discussed by a number of authors (Curcio et al., 1991; Wikler and Rakic, 1991, 1997; Bumsted et al., 1997; Wikler et al., 1997). Wikler and Rakic and Wikler et al. identified an array of early differentiating cones in macaque that they suggested could act as organisers of the adult cone mosaic. Bumsted et al. showed that the early differentiating cones are almost certainly the SWS cones and argued for independent developmental mechanisms to specify SWS or ML cone.

Fig. 11. A–D: Analysis of the spatial distribution of SWS cones in macaque and marmoset. A,C: Sample fields showing the location of labelled SWS cones in marmoset (A) and macaque (C). B,D: Density recovery profile (DRP) for these two samples. The DRP gives a measure of the average "dead space" around the cones; this is shown by the light-shaded histogram. The vertical bar shows the effective radius (ER); this value increases with increasing average intercell distance.

The ER values are 9.7 μm for marmoset and 22.3 μm for macaque. The dark-shaded histograms show the nearest-neighbour distribution (Wässle and Riemann, 1978), which, likewise, has a greater average value for macaque. The packing factor is a measure of the regularity of the mosaic (0, random distribution; 1, triangular array). Its value for marmoset is less than one-third that of macaque, further evidence that there is less order in the marmoset SWS cone array.
Fig. 12. Wholemount view. Focus series of labelled SWS cones in macaque (A–C) and marmoset (D–F) retina showing labelled inner and outer segments (A,D), cell bodies (B,E), and Henle fibres with cone pedicles (C,F). The arrowheads point to the same two cells at each level of focus. The relative location of labelled outer segments is preserved at the level of the cell bodies. The Henle fibres produce a uniform shift of the SWS pedicle array relative to the outer segments but do not lead to changes in the spacing or local distribution. Scale bar = 50 µm.
identity. Our results provide circumstantial evidence in favour of this proposal if one considers the simple fact that the receptive field properties of cells in the SWS cone pathway in both dichromatic and trichromatic New World primates are essentially the same as those of Old World monkeys (Martin et al., 1997; Silveira et al., 1998). This means that the differences in local SWS cone distribution have no great functional consequences for the chromatic response properties of blue-ON cells. The local disorder of SWS cones near the fovea in macaque and human (Williams et al., 1981b; Curcio et al., 1991), likewise, has been invoked to suggest that the orderly arrangement of SWS cones is of no consequence for SWS cone-mediated spatial vision and simply may reflect the spatial and developmental independence of the SWS cone subsystem.

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