



Spectral sensitivity of photoreceptors in an Australian marsupial, the tammar wallaby (*Macropus eugenii*)

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Received 17 June 1998; received in revised form 2 December 1998

Abstract

Microspectrophotometric measurements on the rod photoreceptors of the tammar wallaby showed that they have a peak absorbance at 501 nm. This indicates that macropod marsupials have a typical mammalian rhodopsin. An electroretinogram-based study of the photoreceptors confirmed this measurement and provided clear evidence for a single middle wavelength-sensitive cone pigment with a peak sensitivity at 539 nm. The electroretinogram did not reveal the presence of a short-wavelength-sensitive cone pigment as was expected from behavioural and anatomical data. Limitations of the electroretinogram in demonstrating the presence of photopigments are discussed in relation to similarly inconsistent results from other species. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Rods; Cones; Vision; Electroretinogram (ERG); Microspectrophotometry (MSP)

1. Introduction

In recent years our knowledge about colour vision in mammals has greatly increased. A range of species, in addition to primates, have now been shown to possess colour vision and the spectral sensitivity of cones in a substantial number of mammals has been described in detail (reviewed by: Jacobs, 1981, 1993). Apart from the primates, mammals seem to have dichromatic colour vision. As Jacobs points out, information about marsupial colour vision is almost non-existent. The only exception is the Virginia opossum (*Didelphis virginiana*), a very ancient American member of the marsupialia which diverged from the Australian Marsupials around 60 million years ago (Szalay, 1993). Friedman (1967) showed clear behavioural evidence for colour vision in the opossum. For this same species, Jacobs and his co-workers later measured the spectral sensitivity of the cone photoreceptors with an electroretino-

gram (ERG) based method. They found one cone type with a peak spectral sensitivity at about 560 nm, but failed to find any evidence for a short-wavelength-sensitive cone population (S-cones) (Jacobs, 1993). In contrast, immunocytochemical studies in two other American opossum species, the grey short-tailed opossum (*Monodelphis domestica*; Wikler & Rakic, 1990) and the South American opossum (*Didelphis marsupialis aurita*; Ahnelt, Hokoç & Röhlich, 1995), suggest that the retinae of both these species do indeed contain S-cones, although at a low density of less than 300 cells/mm² in the case of the South American opossum, corresponding to about 10% of all cones.

In the following study, we set out to measure the spectral sensitivity of the tammar wallaby's photoreceptors, in order to examine whether Australian Diprotodonts differ from other mammals. The spectral sensitivity estimates also provide an important baseline for behavioural studies that we conducted in this animal (Hemmi, 1999). The tammar wallaby is a kangaroo-like Australian marsupial from the *Diprotodonta*, a group that went through an extensive radiation and produced a range of diverse species including the kangaroos/wallabies, the Australian possum and the koala.

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The adult tammar weighs 4–8 kg and inhabits the southern parts of Australia. On Kangaroo Island, South Australia, where there is a large wild population, the tammar has been seen to forage in the open grass plains at night, but is also active while hidden in the scrub during daylight hours (Inns, 1980). Animals in captivity show some activity throughout the day, but again, are mostly active in the late afternoon and during the night (Blakers, 1972, personal observations). Thus, even though the tammar is often characterised as a nocturnal or crepuscular species, it is active for a significant amount of time during the day.

2. Materials and methods

2.1. Microspectrophotometry of rod photoreceptors

2.1.1. Tissue preparation

The animals came from a breeding colony for experimental animals and were kept at low densities in social groups in outdoor paddocks. Retinae were obtained from six animals sacrificed by cervical displacement for unrelated experiments. The eyes were removed in the dark within 2–3 min after death and put on ice for 2–10 h before the measurements took place. For the preparation, the eyes were transferred into Dulbecco 'A' phosphate-buffered saline (PBS; Oxoid Ltd., UK), pH 7.3. The following preparation was done with the help of an infrared image converter (FJW, USA) under far-red light produced by a long-pass wratten filter (Kodak 87c) in front of a halogen torch. Small pieces of retina (ca. 1 mm²) were placed on a 50 × 25 mm microscope coverslip. The excess PBS was removed and replaced with PBS containing 10% Dextran (250RMM; Sigma, UK) in order to increase the viscosity of the preparation. The tissue was then pulled apart with a needle and a scalpel to separate the photoreceptors, mounted under a coverslip, the edges of which were sealed with paraffin wax.

2.1.2. The microspectrophotometer

The single beam microspectrophotometer (MSP) has been described in detail by Partridge, Speare, Shand, Muntz and Williams (1992). The light is produced by a 12 V, 100 W quartz-tungsten halogen bulb and is imaged on a holographic grating monochromator (Jobin Yvon, H-1061, France). The light is then projected onto the specimen plane in the shape of a rotatable, variably sized rectangular aperture. The dimensions of this aperture are diffraction limited. The specimen and the measuring beam are then imaged through a 100 × oil immersion objective (Zeiss Neofluar) on to a photomultiplier (Hamamatsu R2928, Japan) or can be viewed through a video-camera/monitor (Insight Vision Systems, UK, 75 series). The speci-

men is illuminated by a 6 V, 15 W light source through a long-pass (50% cut-off at 850 nm) wratten filter (Kodak, 87c). The output of the photomultiplier is converted to a digital signal and logged by a computer. The software to control the MSP and the logging of the data was written by Dr Julian C. Partridge.

2.1.3. Spectral absorbance measurements

To take a measurement, the measuring beam was first set to a wavelength of 750 nm and focused in a cell free area near an outer segment of a photoreceptor to record a baseline scan. The outer segment was then moved into the light beam and a second scan was completed. Both measurements consisted of a sweep from long (750 nm) to short wavelengths (390 nm) and back again, measuring transmitted light intensity in 2 nm steps. Absorption at even wavelengths was measured during the sweep from long to short wavelength, and during the sweep from short to long wavelength, odd wavelengths were measured. The resulting sampling interval was, therefore, 1 nm.

2.1.4. Data analysis

Individual recordings were first digitally filtered with a zero phase-shift filter and then a retinal (A_1) based template curve (Stavenga, Smits & Hoenders, 1993) was fitted to the data. The filtering process removed high frequency noise from the recordings, but did not distort the shape of the signal: when a template was filtered with the same digital filter, the deviations between the filtered and unfiltered version was less than 0.05% of peak sensitivity. A non-linear least squares fitting routine (Levenberg-Marquardt, Marquardt, 1963) was used to fit the template to the data. Measurements between 460 and 750 nm were used for the fit. The best recordings were then selected by the criteria that the sum of the squared residuals in the normalised recordings be less than 0.5 in the range from 40 nm to the short wavelength side of the peak to 120 nm to long-wavelength side of the peak. This selection is purely based on the amount of noise in the data relative to the shape of a template and not on the location of the peak sensitivity.

2.2. Electroretinogram measurements

2.2.1. Animals and apparatus

Four tammar wallabies (three males, one female) weighing between 4.5 and 6 kg were used in this study. The animals were from a different colony to that which supplied animals for the microspectrophotometry. They were also purpose bred for laboratory use and kept in small social groups in outdoor paddocks.

The animals received an intramuscular injection of 0.46–0.65 mg atropine sulphate (Atrosine Mitis, Parnell) 30 min prior to the experiment. They were first

anaesthetised with an intravenous injection of 50 mg Thiopentone Sodium in a 5% solution (Pentothal, Boehringer) through a butterfly needle (23G) inserted into the lateral tail vein. Anaesthesia was continued using thiopentone sodium as required until just prior to beginning recording, when the animal was put on a continuous intravenous infusion of 3.6 mg/kg per h of sodium pentobarbitone (Nembutal, Boehringer) in a compound sodium-lactate solution (Baxter) at 5.6 ml/h. The infusion also contained 7 mg/kg per h of suxamethonium chloride (Scoline, DBL) to maintain paralysis of skeletal muscles. The animals were artificially ventilated with a 1:3 mixture of oxygen and nitrous oxide through a 4 mm Sheridan endotracheal cannula. The animals' temperature was maintained at 37°C by a thermostatically controlled heating blanket. Before the animal was placed into the stereotaxic frame, the hair on its head was cut short, to allow the placement of subcutaneous electrodes. The electrocardiogram was continuously monitored. The CO₂ in the expired air was kept between 4 and 5% throughout the experiment

by adjusting respirator rate or tidal volume. The animals were sacrificed with an overdose of sodium pentobarbitone directly after completion of the experiment and the eyes used for anatomical experiments.

Electroretinograms were differentially recorded using three silver/silver-chloride wires. The recording electrode was placed 1 mm inside the vitreous through a small incision in the cornea. The other two electrodes were placed subcutaneously, the reference electrode just temporal of the recording eye and the common earth on the animal's cheek about equidistant to the other two electrodes. All electrodes were connected to a two stage preamplifier with a gain of 50 000, the output of which was fed into an A/D-converter. Visual stimuli were generated on a computer controlled imaging projector (Barco, DATA800) driven by a Truevision ATVista graphics board at 80 Hz frame rate. Retinal voltage responses were sampled at 2.56 kHz, exactly 32 times the frame rate. Stimulation and recording were both run from the same clock for exact synchronisation.

In the stereotaxic frame, the animal's head was held in such a way that the area of highest ganglion cell density, the visual streak, projected approximately to the horizon (Tancred, 1981; Wong, Wye-Dvorak & Henry, 1986; Mark, James & Sheng, 1993). A tangential screen was placed 0.5 m from the animal such that the centre of the stimulus square was aligned with the horizon. The horizontal placement of the viewing screen did not change the response amplitude by a large amount and we therefore chose a final position of about 30° offset from the vertical meridian (directly in front of the animal) that gave good responses for all animals. The opposite eye was kept closed by taping its lid with adhesive tape.

2.2.2. Design and procedure

The experimental design employed to measure the spectral sensitivity of the wallabies' photoreceptors is a variation of the flicker photometric procedure introduced by Neitz and Jacobs (1984). Rather than measuring the response amplitude to monochromatic light stimuli, we measured the effectiveness of monochromatic lights in reducing the ERG response to a flickering broadband light stimulus. Fig. 1 shows a plan view of the experimental arrangement. The animal (top of figure) is looking at a tangential screen (middle). The stimuli were projected onto the screen from the opposite side (bottom). We measured the response amplitude of the wallaby's eye to a sinusoidal contrast modulation of a single, white or broadband coloured, square check, presented on a grey background with equal time-averaged luminance. The check subtended an area of 30 × 30° at a viewing distance of 0.5 m and its contrast, with respect to the background, was sinusoidally reversed at either 20 or 40 Hz.

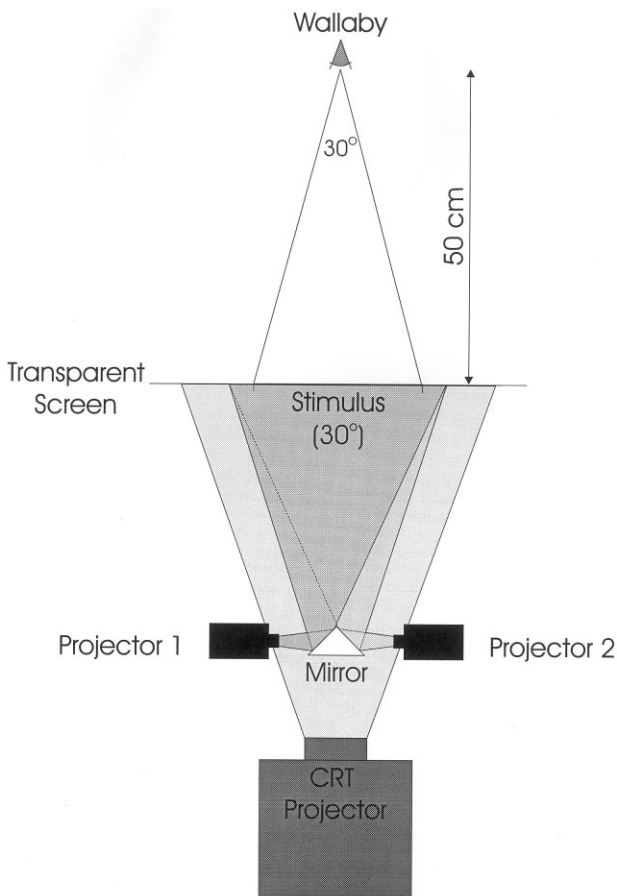


Fig. 1. A plan view of the experimental apparatus. The animal (at the top) is viewing a tangent screen (centre). A CRT-projector and two slide projectors (bottom) were used to back-project the stimulus onto the same screen. The three different light sources allowed a variety of light conditions to be produced.

By projecting secondary, unmodulated lights onto the same screen (Fig. 1, projector 1), we were able to reduce the contrast of the modulated check and thereby the amplitude of the animal's response. The secondary light was either an achromatic, white light or a monochromatic light with a half band width of 10–20 nm. In order to measure the spectral sensitivity of the eye for a given wavelength, we recorded the responses of the eye to the modulated check in the case where either a monochromatic light or an achromatic secondary light, was projected onto the same screen. We then changed the intensity of either the monochromatic or the white secondary light in order to match the recorded response amplitude under the two conditions. The intensities of the secondary lights were recorded simultaneously with the animals response by using the analogue output of a radiometer (International light: IL 700 Research Radiometer). Radiometric measurements were converted to number of photons for all calculations. In order to make it easier for the reader to appreciate the conditions used, intensities are reported in photometric units. The ratio of the number of photons contained in the two secondary lights (monochromatic and white) at the point of equal response, was used as a relative measure of the animal's sensitivity towards this particular wavelength. The two lights were projected in sequence by the same projector and thus had the same relative spatial intensity distribution. In order to measure this point of equal response, 10–12 consecutive recordings, alternating between the monochromatic and the white light, were completed. Each recording was 2 s long. The ratio of the mean responses for the two conditions was calculated and the light intensities were adjusted accordingly. A new set of 10–12 recordings was then taken. Recording stopped after several recordings on either side of the point of equal response were completed. A linear fit through the ratios of the photon flux of the secondary lights plotted against the response ratios was then used to determine the photon flux ratio at the point of equal response amplitude. This design does not make any assumptions about the relationship between response amplitude and stimulus contrast apart from assuming the relationship to be monotonic.

The high contrast modulated check-stimulus and its time modulation was produced by a CRT-projector (Barco, DATA800) at 80 cd/m² mean luminance. The secondary lights were produced by two slide projectors equipped with 250 W halogen lamps (Philips; 6550). The spectral secondary lights varied between 10 and 100 cd/m² depending upon wavelength. The spectral response properties of all filters, lights and the phosphors of the CRT-projector were measured with two calibrated spectrophotometers (casi, ITRES instruments Inc.; s1000, Ocean Optics Inc.)

Different cone types were isolated by following the general protocol outlined in Jacobs, Neitz and Crognale (1985). In order to measure the wallabies' middle to long-wavelength-sensitive cones (M-cones) a white stimulating check was used, the contrast of which was reversed at 40 Hz. The maximum contrast without a superimposed secondary light, was about 80%. The mean luminance of the final stimulus (including secondary lights) varied between 80 and 180 cd/m².

We attempted to measure the short-wavelength-sensitive cones (S-cones) by changing the recording procedures to a slower stimulation rate, concurrent intense long-wavelength adaptation and a blue-dominated stimulation light (Crognale, Jacobs and Neitz, 1991). This was done by modulating only the blue phosphor of the projection monitor and keeping the green phosphor at mean luminance and the red phosphor at its highest luminance at all times. Thus, we had a blue stimulating light on a yellow background. In addition, we used either a 590 nm band pass adaptation light (12 nm half energy band width) with an intensity of 104 cd/m² (animal f430I), or a 600 nm long-pass adaptation light (Kodak cut-off filter no. 25 with 50% transmission at 600 nm) with an intensity of 690 cd/m² (animal m151096). The contrast of the blue check was reversed at 20 Hz. The photon contrast as seen by a pigment with peak sensitivity at 540 nm would have been reduced by a factor of 9 to about 9% (animal f430I) or by a factor of 22 to about 3.6% for animal m151096 as compared to the previous condition without the concurrent adaptation light and a white flickering check. For a short-wavelength-sensitive pigment at 440 nm on the other hand, the photon contrast would only have dropped by about 12% to a final contrast of 70% in both cases. None of these contrast measurements incorporate the secondary lights used to determine the point of equal response (white or monochromatic), that would further reduce the contrast seen by the photoreceptors.

2.2.3. Data analysis

Sensitivity measurements for individual animals were fitted with a retinal (A_1) based template (Stavenga et al., 1993). A non-linear least squares fitting routine (Levenberg-Marquardt, Marquardt 1963) was used to fit the template to the data. Error estimates were obtained with a bootstrap method (Moulton & Zeger 1991). Fitting was straightforward and all results have been visually inspected. Due to the use of halogen light bulbs for the secondary lights, there was more light available for the longer wavelength, which made these measurements more reliable. Fits to the data were, therefore, confined to wavelength between 470 and 610 nm.

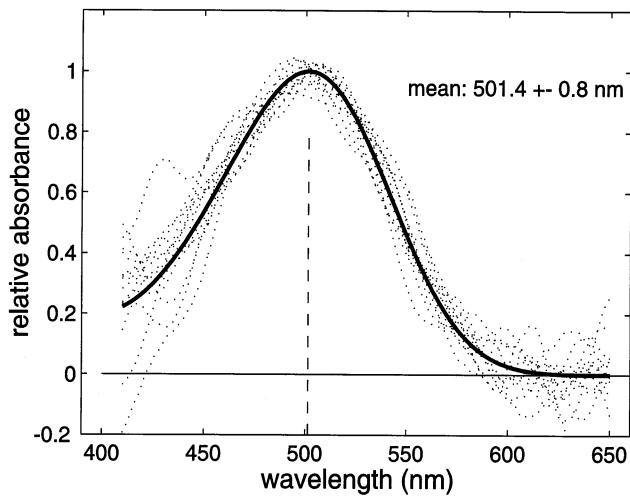


Fig. 2. Microspectrophotometric measurements from 14 rod photoreceptors (dotted lines) and a retinal based (A1) template (solid line) with a peak in absorbance at the mean (dashed line) for the 14 measurements.

3. Results

3.1. Microspectrophotometry

The MSP measurements were restricted to rod mea-

surements because it was found that the cones were so tightly associated with the pigment epithelium in the unfixed retina that removal of the pigment epithelium also removed the cone outer segments from the preparation. We therefore could not record from cone cells. Fourteen recordings satisfied the selection criteria based on the noise in the recording as outlined in the methods section. Fig. 2 shows the 14 selected recordings (dotted lines). The mean peak location is 501.4 ± 0.8 nm (mean \pm SE). The solid curve shows a template with peak location at 501.4 nm. The maximum deviation between the recordings and the fitted templates in the region used to select the recordings is less than 16% of the peak sensitivity.

3.2. Electroretinogram measurements

3.2.1. Middle-wavelength-sensitive cones

Fig. 3 shows the spectral sensitivity curves obtained for four tammar wallabies when we recorded under conditions that were shown in other mammals to favour middle- to long-wavelength-sensitive cones (e.g. Jacobs et al., 1985; Jacobs & Deegan II, 1992). The circles and stars represent the computed sensitivity measurements. Only the data points marked with stars were used in fitting a standard retinal based mammalian

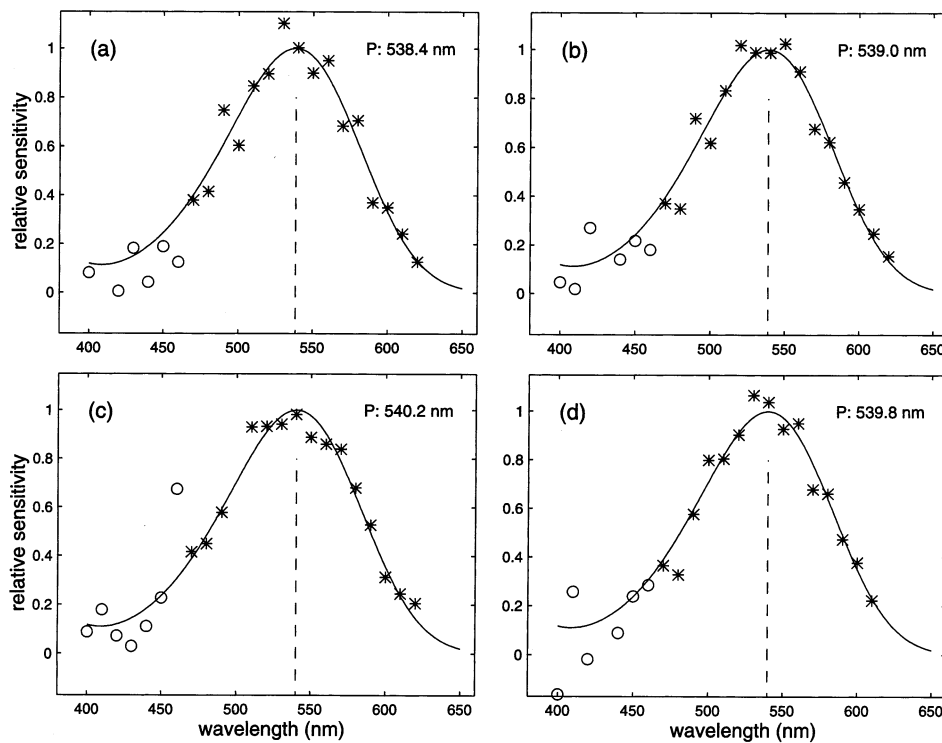


Fig. 3. Spectral sensitivity measurements for four animals (female: a, males: b–d), under conditions that should favour the responses of M-cones (see text for details), using an electroretinogram based procedure. Solid lines represent the best fitting retinal based template curve. Only measurements above 460 nm (*) were used for the fits. The peak sensitivity of the fitted templates all fall within 2 nm at about 539 nm and the largest standard deviation for any peak is 1.6 nm. The peak sensitivity for each template is given in the top right hand corner of each graph (P:).

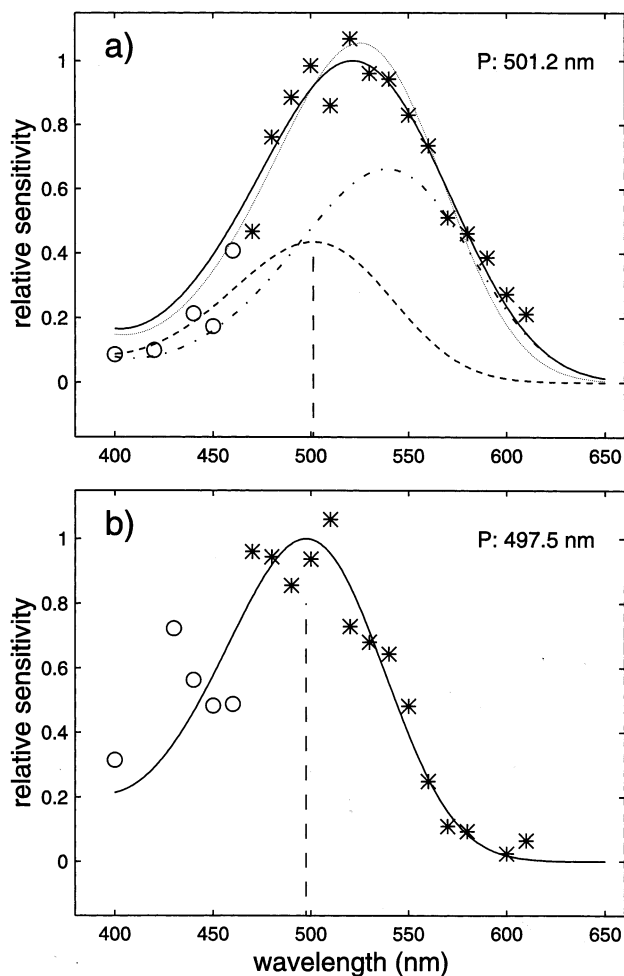


Fig. 4. Spectral sensitivity measurements for two animals under conditions that should favour the S-cone response (see text for details). (a) Responses for the animal f430. The fine dotted line shows that a single template does not fit the data very well, especially in the long-wavelength region where the template curve is too narrow. As the same animal was shown previously to have a photoreceptor of the 539 nm cone type (Fig. 3a), we fitted the same data with a combination of two templates with the peak of one template (dashed-dotted line) fixed at 539 nm. The combined sensitivity of the two templates (solid line) fits the long-wavelength region of the data more accurately. The template curve selected by the fitting algorithm has a peak at 501 ± 9 nm (mean \pm SE), the same spectral sensitivity as the rod photoreceptors (Fig. 2). (b) By increasing the concurrent long-wavelength adaptation, we were able to completely suppress the response of the 539 nm cone type in another animal (m151096) and the data is now well fitted by a single template with a peak sensitivity at about 498 ± 2 nm (mean \pm SE). The peak sensitivity for each template is given in the top right hand corner of each graph (P:).

template (solid curve; Stavenga et al., 1993) to the data. For all four animals the peak sensitivity obtained fell within 1 nm of the mean at 539.4 nm. The largest standard deviation for the individual peak locations is 1.6 nm. The fits obtained for single templates are good and there is no indication that the data are the result of superimposed responses from two different pigments. It appears that one pigment is

strongly dominating the response. When trying to fit two templates to the data, the fitting algorithm invariably fitted a positive and a negative template, both located around the peak for the single template, again suggesting that the recordings contained significant input from only one photoreceptor type.

3.2.2. Other photoreceptors

Animal f430 was also tested under conditions that should have favoured the S-cone response by reducing the stimulus contrast for the M-cones (539 nm) by a factor of nine to a final contrast of 9%. Any hypothetical short-wavelength pigment would have been exposed to virtually unchanged contrast of about 70% (see Section 2). The results are shown in Fig. 4a. Under the assumption that only one pigment is present, the best fitting template shows a peak sensitivity of about 525 nm (dotted line). The fit is not ideal, however. The template is slightly too narrow and there are strong systematic deviations in the long-wavelength region that we did not observe in the previous recordings. We knew at this point that this particular animal (f430) had a photoreceptor of the 539 nm type (Fig. 3a). We therefore fitted two templates and fixed one at 539 nm (dashed-dotted line). The algorithm then selected the second template to have a peak sensitivity at 501 ± 9 nm (dash line). The resulting fit (solid line) is better than the fit of the single template, especially in the long-wavelength region. This suggests that we indeed recorded from two different photoreceptor types at the same time. The fitted pigment has the same spectral absorbance as the rod photoreceptors (Fig. 2). In order to confirm this conclusion, we repeated the recordings in a second animal, which had also been shown to possess a cone photoreceptor of the 539 nm type (Fig. 3b). This time though, we increased the intensity of the long-wavelength adaptation such that the contrast seen by a 539 nm cone photoreceptor was reduced to 3.6%. As can be seen from Fig. 4b, the response of the 539 nm cone type has now completely disappeared and the result is well fitted by a single template with a peak at 498 ± 2 nm (mean \pm SE). This is in good agreement with the peak of the second template measured for animal f430 (Fig. 4a) and with the MSP rod measurements (Fig. 2).

When we used an even stronger long-pass adaptation light, with 50% transmission at about 558 nm with an intensity of 1700 cd/m^2 , we were not able to produce any consistent response changes associated with the secondary light. Under these conditions the contrast for a photoreceptor at 500 nm, as measured above, would have had its stimulus contrast reduced by a factor of 6 to about 7% (without secondary lights), whereas a blue pigment of about 440-nm peak sensitivity would still have seen about 70% contrast.

4. Discussion

4.1. Rod photoreceptors

The MSP measurements (Fig. 2) show that the wallabies have a rhodopsin with a peak absorption at about 501 nm, as is typical for placental mammals (Lythgoe, 1972). This measurement was confirmed by the ERG recordings where we have two estimates of peak absorption at 498 and 501 nm, respectively (Fig. 4a, b). The close correspondence between the MSP and the ERG recordings supports the impression of a human observer that the cornea and the lens in the tammar wallaby are colourless. The MSP measurements were not influenced by these structures, whereas the ERG recordings could potentially have been, yet the two methods produced similar results.

4.2. Cone photoreceptors

The ERG measurements allowed us to measure the spectral sensitivity of the wallabies' middle- to long-wavelength-sensitive photoreceptors, having a peak sensitivity located at about 539 nm. The results from the four animals are in close agreement, with standard deviations of less than 1.6 nm. The spectral sensitivity of this M-cone pigment falls within the range typical for placental dichromats of between 500 and 540 nm (Jacobs, 1993), but is shorter than that of the Virginia opossum (560 nm).

Our attempts to measure a short-wavelength-sensitive cone pigment were unsuccessful. We were able to suppress the responses of the M-cones to such a degree, that we either picked up a faint combined signal from the rods and the M-cones (Fig. 4a), or even completely abolished the M-cone response and only recorded a weak rod signal (Fig. 4b). In neither recording is there any sign of S-cones. When we suppressed the rod signal even further by using a strong long-pass adaptation light with 50% transmission at 560 nm, we failed to produce any consistent changes in the response level associated with the secondary lights at all.

There are several possible explanations for this result:

(1) Since we used a halogen light source, the monochromatic test lights in the blue region of the spectrum were weaker than in the green–red area and would have reduced the reliability of the results in the blue range of the spectrum. Above 450 nm, however, we arrived at good, repeatable estimates for the M-cones and thus should have had enough light to detect the presence of an S-cone type, unless its peak fell at a wavelength shorter than 400 nm. Our experiments could not preclude the presence of a UV sensitive cone population.

(b) A second possible explanation is concerned with the density of the S-cones. A recently concluded im-

munocytochemical study of the tammar retina and found clear evidence for two distinct cone types (Hemmi & Grünert, 1999). The two populations have very different spatial distributions, but both are present throughout the retina. One of the two types was labelled by the antibody JH455 which labels the short-wavelength-sensitive cones in other mammalian retinae (Wang, Macke, Merbs, Zack, Klaunberg, Bennet et al., 1992; Goodchild, Chan & Grünert, 1996; Sandmann, Boycott & Peichl, 1996; Chan & Grünert, 1998). In the area of the retina stimulated in the ERG recordings, these S-cone candidates have a low density of 500–1000 cell/mm², or roughly 5–10% of the M-cone density. It is possible that these two reasons, lower sensitivity in the blue range and low density of the S-cones, together could have reduced the amplitude of the S-cone response so that it could not be detected in the ERG.

(c) The immunocytochemical study suggests yet another possible explanation for the failure to record S-cone activity even after abolishing M-cone responses. We noticed that all suspected S-cones could also be more faintly labelled with the antibody against M-cones, with clear differences in the strength of labelling between different retinal regions. This raises the possibility that the S-cones also contain a certain amount of M-cone pigment in this species. There have indeed been suggestions for other mammals that there are cones that express two different visual pigments in the adult animal, although only in limited areas of the retina (e.g. Röhlich, van Veen & Szél, 1994; Glösmann & Ahnelt, 1998). If this were true in the tammar wallaby it would mean that, by adapting the M-cone pigment we would also have adapted the S-pigment containing cones and, as a consequence, reduced their responses. Taken in conjunction with the above two points, this would certainly have been enough to prevent us from recording any S-cone responses. Two visual pigments in one cone population would not preclude the animal from having dichromatic colour vision. The two photoreceptor types would still provide unique spectral responses. The signal to noise ratio in the chromatic channel would be reduced, however.

(d) Our ERG results could, of course, also be taken as evidence that wallabies do not actually have S-cones. This conclusion, however, does not agree with the immunocytochemical results and more importantly is at odds with behavioural experiments, clearly showing that the wallabies have dichromatic colour vision with a null-point at about 485 nm (Hemmi, 1999). With the knowledge that the wallabies have M-cones with a peak spectral sensitivity at 539 nm, the results from these behavioural studies can only be explained if we postulate the presence of a short-wavelength-sensitive cone type with a peak sensitivity at about 420 nm. For the same reason, the behavioural results also rule out the possibility that the 'rod' responses measured in our

ERG experiments were produced by an unusual S-cone population with a peak sensitivity around 500 nm.

The situation in the tammar wallaby is therefore comparable to that reported across various opossum species (Jacobs 1993), showing the same conflicting information between the ERG, the immunocytochemical and the behavioural results. In the case of the tammar wallaby, however, all three studies were completed in the same species and the evidence clearly shows that the wallabies do have a functional, low density S-cone population, indicating that the ERG method used, for reasons discussed above, was not sensitive enough to measure their weak responses in this animal.

Another issue is the presence of retinal oil droplets in the retina of the tammar wallaby, as has been found in other marsupials (Hoffmann, 1876-77; O'Day, 1935). Oil droplets are often discussed in relation to colour vision (e.g. Muntz, 1972; Jacobs, 1981). In the tammar, however, these oil droplets are all transparent to the human observer and in our immunocytochemical study we found that all cones contain oil droplets (Hemmi & Grünert, 1999). Also, the good fit between the templates and the M-cone responses show that these responses are not strongly distorted by the oil droplets. For these reasons, we do not think that oil droplets are involved in colour vision in this species. Their role is more likely to increase the light sensitivity of the cone photoreceptors (Young & Martin, 1984).

5. Conclusion

In this study we showed that the tammar wallaby has a typical mammalian rod visual pigment with a maximum absorbance at about 500 nm. We also measured the spectral response of a single middle wavelength-sensitive cone type with a peak spectral sensitivity at about 539 nm. This peak is at a shorter wavelength than that reported for the Virginia opossum, the only other marsupial species for which data are available. The spectral sensitivity of the wallabies' presumed short-wavelength-sensitive cone class (Hemmi, 1999; Hemmi & Grünert, 1999) could not be measured. There is a clear need to undertake further studies to establish whether the wallabies' S-cones do in fact contain two visual pigments and to measure their spectral sensitivity. It would also be interesting to test whether the S-cones found in the opossums also double label with the antibody against the M-cones or not.

Acknowledgements

Our special thanks go to Dr L.R. Marotte for her help with the electrophysiological experiments, to Prof.

W.R.A. Muntz for his help with the MSP study and to Dr G. Shaw for providing the retinae for the MSP measurements. We also thank Dr A. James for allowing us to use his software routines to interface the hardware, Dr J. Zeil for his thorough criticism of the manuscript and A. Devlin and K. Williams for taking care of the animals. The experiments comply with the 'principles of animal care', publication no. 86-23, revised 1985 of the National Institute of Health. They are also in compliance with the Australian Capital Territory Animal Welfare Act (1992) and were covered by two ethical protocols (RDN.44.95 & RDN.31.93) approved by the animal experimentation ethics committee of the Australian National University.

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