J Comp Physiol A (1993) 172:295–301

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Colour vision in the passeriform bird, *Leiothrix lutea*: correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity

E.J. Maier[1], **J.K. Bowmaker**[2]

[1] Universität Regensburg, Institut für Zoologie, Universitätsstr. 31, W-8400 Regensburg, Germany
[2] University of London, Department of Visual Science, Institute of Ophthalmology, Bath Street, London EC1V 9EL, UK

Accepted: 4 December 1992

Abstract. The visual receptors in the retina of the passeriform bird *Leiothrix lutea* were examined microspectrophotometrically. The rods had a maximum absorbance close to 500 nm. Four spectrally different classes of single cone were identified with typical combinations of photopigments and oil droplets: a long-wave sensitive cone with a photopigment P568 and a droplet with a cut-off wavelength at 564 nm, a middle-wave sensitive cone with a P499 and a droplet with a cut-off at 506 nm, a short-wave sensitive cone with a P454 and a droplet with maximum absorbance below 410 nm and an ultraviolet sensitive cone with a P355 and a transparent droplet. Double cones possessed a P568 in both the principal and accessory members. A pale droplet with variable absorbance (maximal at about 420 nm) was associated with the principal member whereas the ellipsoid region of the accessory member contained only low concentrations of carotenoid. The effective spectral sensitivities of the different cone classes were calculated from the characteristic combinations of oil droplets and photopigments and corrected for the absorbance of the ocular media. Comparison of these results with the behavioural spectral sensitivity function of *Leiothrix lutea* suggests that the increment threshold photopic spectral sensitivity of this avian species is mediated by the 4 single cone classes modified by neural opponent mechanisms.

Key words: *Leiothrix lutea* – Photopigments – Oil droplets – Spectral sensitivity – UV-cone

Introduction

Diurnal birds have a highly developed visual system that exhibits a number of distinctive features (see for example Meyer 1977; Martin 1985). The retina is dominated by cones (up to 80% of the photoreceptors) that contain coloured oil droplets within their inner segments. This complex array of cones not only gives these species sensitivity to our visible spectrum, but also extends their vision into the near ultraviolet (e.g. Goldsmith 1980; Chen et al. 1984). A behavioural spectral sensitivity function, extending into the UV, has recently been determined for the red-billed Leiothrix (Peking robin), *Leiothrix lutea* (Timalidae, Passeriformes) (Burkhardt and Maier 1989; Maier 1992) and shows 4 distinct sensitivity peaks with maxima at about 370, 460, 530 and 620 nm. Combined with results from behavioural tests under selective chromatic adaptation (Maier 1990), these data indicate 4 spectrally different cone mechanisms for this bird species. To explore further the photopic visual system of *Leiothrix lutea,* direct information about the spectrally different cone types and their sensitivities is crucial.

Microspectrophotometric and electrophysiological studies have shown that a number of avian species (Chen and Goldsmith 1986; Jane and Bowmaker 1988; Bowmaker 1991b) posses a set of at least 5 spectrally distinct cone types. In general, diurnal passeriform birds have 4 classes of single cone, comprising about 50% of the total cone population, together with a class of double cones that have a broad spectral sensitivity maximal at about 560–570 nm (Bowmaker 1991b). Each class of cone contains a different coloured oil droplet and, since the droplets are located in the ellipsoid region of the inner segment, incident light must pass through the droplets before it stimulates the photopigments in the outer segments. The effective spectral sensitivity of a cone is therefore determined, not only by the spectral sensitivity of the photopigment, but also by the absorption characteristics of the droplet (Donner 1960; Bowmaker 1977, 1980).

The present study describes a microspectrophotometric analysis of the visual pigments and oil droplets in the retina of *Leiothrix lutea,* including the identification of a cone class maximally sensitive in the UV, and compares the derived spectral sensitivities of the different cone

Abbreviations: LWS, long wave sensitive; MWS, middle wave sensitive, SWS, short wave sensitive (cones)

Correspondence to: J.K. Bowmaker

classes with the behaviourally determined spectral sensitivity of this species.

Materials and methods

Three individuals of each sex were purchased commercially. After dark adaptation, either overnight or for at least 1 h, a bird was killed and the eyes enucleated under dim red light. One eye was examined immediately, whereas the other was placed in a dark box and stored at 4 °C for later use. For microspectrophotometry, the eye was hemisected, the vitreous removed from the posterior half and the eye cup placed in ice cold calcium-free avian Ringer's solution (pH 7.1). A small piece of retina (approximately 1 mm^2) was taken and prepared for examination as described elsewhere (Mollon et al. 1984). Measurements were continued for up to 36 h post mortem.

Microspectrophotometric measurements. Measurements were made with a modified Liebman dual beam microspectrophotometer (Liebman and Entine 1964; Knowles and Dartnall 1977, pp 562–566) which has been placed under the control of a PC 386. Using an infra-red image converter for visualization, the outer segment or oil droplet of a receptor cell was aligned with the measuring beam, while the reference beam passed through a nearby area free of cellular material. The beams, typically 2 × 2 μm, could be adjusted to fit the dimensions of those structures being measured. All measurements were made transversely through the cells, with the dichroism of visual pigment molecules exploited by polarizing the beams so that the e-vector of the beams was perpendicular to the long axis of the outer segment. Spectra were recorded from 750 nm to either 370 or 360 nm in 2 nm steps and then on the return to long wavelengths at the interleaved odd-number wavelengths. Initially, a baseline spectrum was recorded with both beams in a clear area of the preparation, then the absorbance of the cell recorded, followed by a second baseline spectrum. Both baselines were subtracted individually from the absorbance spectrum to produce two spectra for each cell. To confirm the presence of a photosensitive pigment, presumptive outer segments were bleached by exposure to white light after their absorbance spectra had been recorded.

The diameter of avian cone outer segments and especially those of *Leiothrix lutea* is extremely small, (about 1–2 μm), so that adjustment of the size of the measuring beams often reached practical limits, with the unavoidable problem of some light leakage around the outer segment. In addition, the dimensions of the outer segments of the different cone types varied. The long-wave sensitive (LWS) cones were the largest, followed by middle-wave (MWS) and short-wave sensitive (SWS) with the UV cones being the smallest. This led to significant differences in the quality of the recordings from the different cone types so that different selection criteria had to be applied to the different spectral classes (see below).

Analysis of individual visual pigment records. A standardized computer program was employed to estimate the wavelength of maximum absorbance (λ_{max}) of each outer segment. First the two spectra from a cell were averaged and then the absorbance values at pairs of adjacent wavelengths were averaged to obtain a mean curve from the outward and return records. Each of the 20 absorbance values on the long wavelength limb of the curve (corresponding to a 40 nm segment and to absorbances in the range of approximately 45–50% of the maximum of the cell) was then referred to a standard template curve in order to obtain and estimate of λ_{max}. This operation amounts to finding the spectral location of a standard curve that gives the percent absorbance value under consideration. A second estimate of λ_{max} was obtained from the top of the absorbance curve by fitting each of 50 consecutive absorbance points, centred on the highest point, to the template curve and averaging the resulting estimates. The template curve used in the analysis was the Dartnall standard curve for rhodopsin (Knowles and Dartnall 1977, p76) placed with its λ_{max} at 502 nm and expressed on an abscissal scale of log frequency, since absorbance curves of visual pigments have almost the same shape when expressed on such an abscissa (Mansfield 1985; Bowmaker et al. 1991).

Normally only records that passed rigid selection criteria were used for detailed analysis, the criteria for LWS cones and rods being i) a transverse density at the λ_{max} greater than 0.015, ii) a standard deviation from the right-hand limb estimate of λ_{max} of less than 8 nm, iii) a shortwave absorbance of less than 45% but greater than 10% and iv) the difference between the two estimates of λ_{max} less than 5 nm. For MWS cones the criteria were relaxed to i) a transverse density at the λ_{max} greater than 0.01, ii) a standard deviation from the right-hand limb estimate of λ_{max} of less than 9 nm, iii) a shortwave absorbance of less than 70% but greater than 10% and iv) the difference between the two estimates of λ_{max} less than 6 nm. For SWS cones the criteria had to be further relaxed to a standard deviation from the right-hand limb estimate of λ_{max} of less than 13 nm and the difference between the two estimates of λ_{max} less than 7 nm.

Recording from presumptive UV cones was most difficult since these cones were rare and had extremely small (about 1 μm in diameter) outer segments. Further, the sensitivity of the microspectrophotometer extended only to 360 nm with a relatively low signal to noise ratio at these short wavelengths. Presumptive UV cones were identified by having outer segments showing no absorbance above about 450 nm, but with rising absorbance to shorter wavelengths that could be reduced (bleached) by exposure to white light. The cones also contained a fully transparent oil droplet in their inner segment. Pooled data from 3 pre-bleach and 3 post-bleach spectra from each outer segment were used to compensate for the low signal to noise ratio of the individual recordings.

Oil droplet absorbance spectra. Because of the difficulty of recording reliable spectra from small oil droplets with very high concentrations of carotenoid, details of cut-off wavelengths and densities were taken from records with the highest absorbances. With droplets of diameter about 2 μm, considerable light leakage occurs around the droplet during measurement and this accounts for the 'flat-topped' spectra obtained for the R and Y type droplets. These droplets were classified by their 'cut-off' wavelengths (λ_{cut}) (Lipetz 1984). In the case of droplets with absorbances less than 0.3 that probably do not act as cut-off filters, the wavelengths of maximum absorbance are given.

Results

Visual pigments

Rods were identified by their typical rod-shaped outer segments and the lack of oil droplets in the inner segments. The mean absorbance spectrum from 9 cells is shown in Fig. 1 and has a λ_{max} at 500 ± 3 nm. The mean transverse density was 0.024.

Double cones and 4 classes of single cone were identified. The 4 classes of single cone contained visual pigments with λ_{max} at about 568, 499, 454 and 355 nm. The mean absorbance spectra and details of the analyses are shown in Fig. 1 and Table 1. Because of the limited spectrum available, the λ_{max} of the UV cones could not be determined from the computerized analysis program, but curve fitting 'by eye' suggests a λ_{max} close to the limits of the measured data, between 350 and 360 nm and probably close to 355 nm. The outer segments of both the principal and accessory members of the double cones contained a pigment spectrally similar to that of the long-wave single cones, λ_{max} at about 568 nm.