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VISUAL PIGMENTS OF THE TREE SHREW (*TUPAIA BELANGERI*) AND GREATER GALAGO (*GALAGO CRASSICAUDATUS*): A MICROSPECTROPHOTOMETRIC INVESTIGATION

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Abstract—Optical density, linear dichroism and bleaching difference spectra were measured in photoreceptors from the cone-dominated retina of the tree shrew (*Tupaia belangeri*) and from the rod-dominated retina of the greater galago (*Galago crassicaudatus*) using a single-beam, wavelength-scanning, dichroic microspectrophotometer. In *Tupaia*, we obtained spectral records from 272 cone receptors (from 10 eyes), of which 264 were long-wave sensitive ($\lambda_{max} = 555 \pm 6$ nm) and 8 were short-wave sensitive ($\lambda_{max} = 428 \pm 15$ nm). Also, one anatomically-recognizable rod receptor was encountered and showed a peak absorption at approx. 496 nm. No mid-wave sensitive cone pigment was found, as would be expected in deutan-type dichromats like the tree shrew. Pre-retinal absorption by the cornea and lens was maximal at 370 nm and negligible beyond 430 nm. In *Galago*, all outer segments measured were rod-like in appearance (λ_{max} near 501 nm). Measurements of pre-retinal absorption yielded a single-peaked function with a maximum at 363 nm.

Visual pigments Lens absorption *Tupaia* *Galago* Tree shrew Bushbaby Microspectrophotometry Color vision primate

INTRODUCTION

The visual systems of Tupaiidae and Galagidae have been studied rather extensively, in part because of the taxonomic relationship of these families to primates, and also because of their rather extreme retinal adaptations to respective diurnal and nocturnal environments. In a similar vein, the present study was undertaken (1) to examine the characteristics of the visual pigments of *Tupaia* and *Galago* with regard to those of man and other primates, and (2) to determine how the number and spectral position of these rod and/or cone pigments may relate to functional aspects of vision in these animals.

The visual system of Tupaiidae has played a major role in its controversial classification as a primate (LeGros Clark, 1934; Simpson, 1945) rather than insectivore (McKenna, 1966). It is now generally accepted, however, that the well-developed visual system of *Tupaia* is the result of convergent evolution, shaped by the visual demands of its arboreal lifestyle (Campbell, 1966; Raczkowski, 1975) and few taxonomists maintain its inclusion in the order Primates (see Luckett, 1980, for review). Most recently Tupaiidae has been classified in the order Scandentia (MacDonald, 1984). Nevertheless, the idea that *Tupaia* may represent a prototype of primitive primates has maintained much interest in this species. In general appearance the tree shrew resembles a common gray squirrel, including the lateral placement of its relatively large eyes. Like the gray squirrel, its central visual system displays especially well-developed retinogeniculostriate and retinotectal pathways (see Campbell, 1980: Norton, 1982), although it lacks specializations found exclusively in primates (Allman, 1977; Campbell, 1980). On the other hand, the family Galagidae (a prosimian primate in the sub-family Lorisidae), exhibits a central visual system with characteristics common to other primates (see Casagrande & DeBruyn, 1982; Weller & Kaas, 1982, for review).

The retinal organization in *Tupaia* and *Galago* is radically different and reflects the very different habitats of these animals. In the

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diurnal *Tupaia* a dense yellow lens filters light projected onto a highly cone-dominated retina that includes only a small population (less than 5%) of rod receptors, and exhibits a low ratio of convergence of receptors onto ganglion cells (Bunt & Klock, 1980; Immel, 1981; Kuhne, 1983; Muller & Perchl, 1989; Rohen & Castenholtz, 1967; Samorajski, Ordy & Keefe, 1966). The nocturnal habits of *Galago*, however, are reflected by its larger eyes and large crystalline lens, the presence of a tapetum, and by a high degree of convergence of primarily rod receptors onto ganglion cells (Rohen & Castenholtz, 1967; Wolin & Massopust, 1970). Although only rod photoreceptors have been anatomically identified in the *Galago* retina by light microscopic analysis (Dartnall, Arden, Ikeda, Luck, Rosenberg, Pedler & Tansley, 1965; Detwiler, 1939; Kolmer, 1930), other evidence (Dodt, 1967; Petry & Casagrande, 1983) suggests that cone pigment(s) may be present.

The fact that *Tupaia* has color vision of some sort was established by several psychophysical studies which required these animals to distinguish colored papers (Tigges, 1963) or colored lights (Shriver & Noback, 1967; Snyder, Killackey & Diamond, 1969) irrespective of brightness. Snyder et al. concluded that *Tupaia* must be a dichromat, with a neutral point lying somewhere between 450 and 530 nm. Polson's (1968) extensive psychophysical tests of spectral sensitivity, neutral point, saturation discrimination, wavelength discrimination and isochromatic confusion lines confirmed that *T. glis* is dichromatic and concluded that its dichromacy is of the deutan type, with a neutral point at about 505 nm. This conclusion has been confirmed by recent behavioral studies in *T. belangeri* (Jacobs & Neitz, 1986; Kelly & Petry, 1989). The present microspectrophotometric measurements were undertaken to characterize the visual pigments that underlie *Tupaia*'s dichromacy, as well as to explore the possibility that cones may accompany rods in the *Galago* retina.

METHODS

Retinal tissue was obtained from 16 eyes of 11 tree shrews (*Tupaia belangeri*) and from two eyes of one greater galago (*Galago crassicaudatus*). Due to extremely rapid and extensive deterioration of the receptor outer segments in *Tupaia*, a variety of Ringer's media and preparation techniques were tried before arriving upon a combination that resulted in reasonably consistent maintenance of outer segment structure and pigment in this species.* Hence the data presented here for *Tupaia* are from tissue restricted to the seven retinae of our adult males and one adult female. These animals had been colony-reared and ranged from 175 to 225 g in weight. The *Galago* was an adult male approx. 11 yr in age and 5 kg in weight. The problem of outer segment deterioration experienced with tree shrew retina was not observed in the *Galago* material.

All retinae were prepared as follows. Animals were dark-adapted for at least 1 hr then deeply anesthetized with a lethal dose of sodium pentobarbital (Nembutal) under deep red photographic safelight illumination. Also, a topical ophthalmic anesthetic (proparacaine hydrochloride) was applied to each eye. The eye was removed quickly and hemisected at the limbus to produce an eyecup preparation that was immediately placed in a cold Ringer's solution. This solution consisted of 120 mM NaCl, 5 mM KCl, 2 mM $MgSO_4$, 30 mM HEPES and 2 mM $CaCl_2$ and was adjusted with NaOH to a pH of 7.4, yielding an osmolarity of approx. 300 mOs. The retina was dissected from the eyecup under infra-red illumination using a dissecting microscope. For all *Tupaia* retinae and for one *Galago* retina it was then fixed for 15–20 sec in a cold, 2% glutaraldehyde/Ringers solution. (The other *Galago* retinea was prepared unfixed.) Small pieces of retina were mounted on a microscope coverslip, suspended in Ringer's and gently macerated with forceps. The preparation was then covered with a second coverslip and sealed.

The prepared retinal tissue was viewed in a dichroic microspectrophotometer. This laboratory-built instrument is a modified version of an earlier instrument (Hárosi & MacNichol, 1974) and is described fully by Hárosi (1982, 1987). It was used to obtain spectral measurements of average and modulated light fluxes projected through single receptor outer segments which protruded from the edges of the retinal tissue. Typical preparations for *Tupaia* and *Galago*

*In *Tupaia*, the following unsuccessful preparations yielded cones with deteriorated or mising outer segments; fresh Ringer's (no glutaraldehyde); fresh Ringer's with taurine (25 mM); fresh Ringer's with ascorbate (1 mM), glutathione (2 mM), choline chloride (1 mM), inositol (30 mM), PMSF and quinacrine dihydrochloride. Fixation of retinae for more than 30 sec in 2% glutaraldehyde/Ringer's solution or in 2% glutaraldehyde/Ringer's plus 10% DMSO, resulted in good outer segment preservation, but no visual pigment.

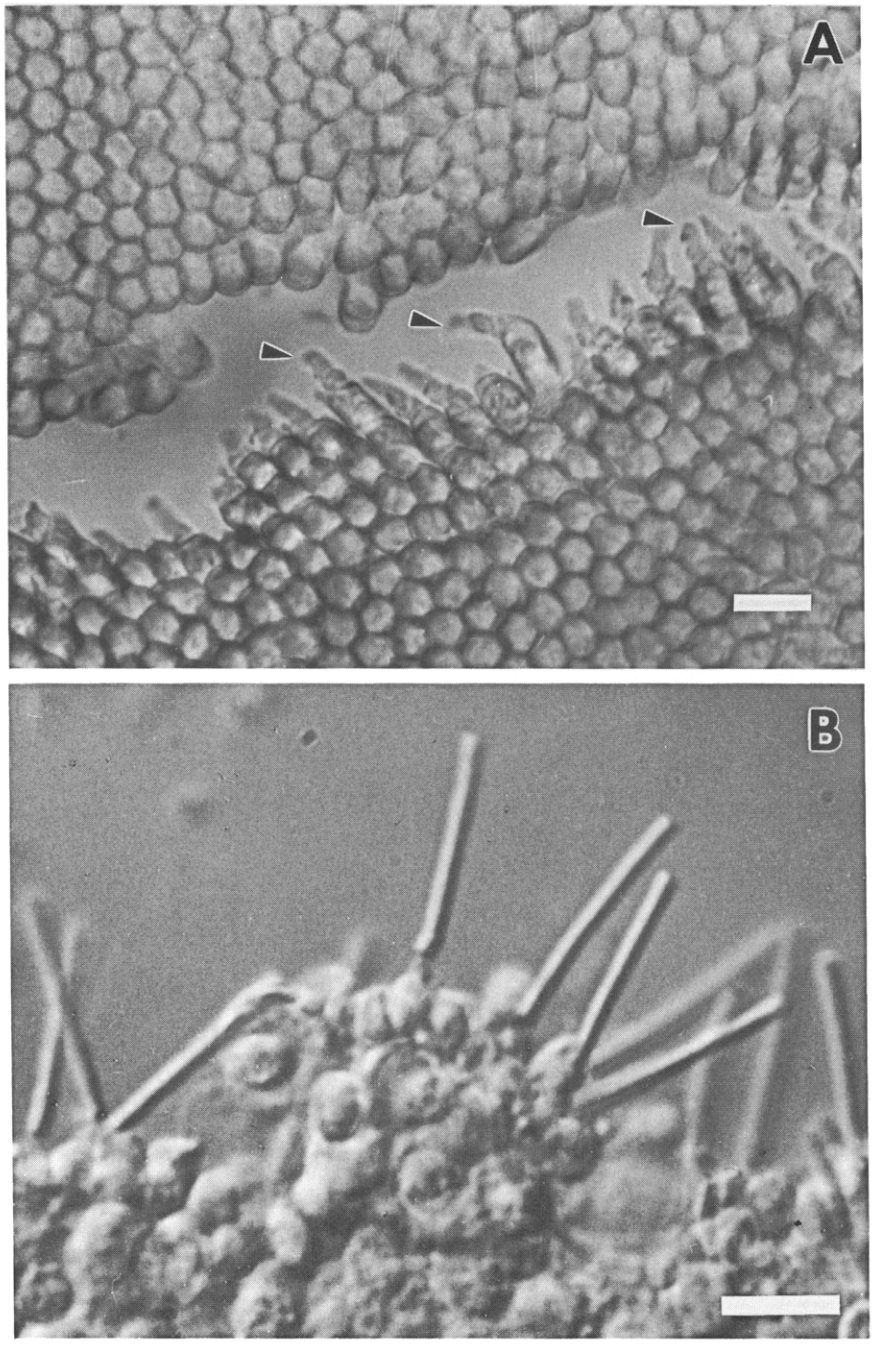

Fig. 1. Photomicrographs of unstained retina. (A) Retinal fragment from *Tupaia*. Arrows point to several of many cone outer segments shown protruding from the receptor mosaic. (B) *Galago* photoreceptors are shown detached from the retina. The individual photoreceptors shown in (A) and (B) were not among those actually measured (bar = 10 μm for (A) and (B); photomicrograph in (B) taken by Barbara Ann Collins, MBL).

retinae are shown in Fig. 1. Under dim red background illumination (tungsten source with 650 nm peak, 15 nm half-bandwidth filter), the preparation was searched for transversely-oriented photoreceptors. The measuring beam was adjusted in size to about $1 \times 3\,\mu m$ in the object plane and oriented on the long axis of the receptor outer segment. Each measurement typically consisted of eight 1-sec scans of the spectrum from 325 to 695 nm. A dedicated digital computer calculated the average optical density (OD) and linear dichroism (LD) of the outer segment using measurements taken through the sample and through a nearby cell-free reference area. Bleaching (optical density) difference specra (BD) were obtained by measuring absorption in the same outer segment before and after actinic light exposure that bleached its visual pigment. The bleaching was accomplished using an auxiliary beam of a photographers' flashgun (1–3 flashes), or, by the measuring beam with the monochromator set manually to the appropriate wavelength (2-min exposure). The OD, LD, and BD spectra were used to characterize the visual pigments and to distinguish visual pigment absorption from that of late photoproducts.

Measurements of pre-retinal absorption were made in six *Tupaia* eyes and in two *Galago* eyes. Preparations included squashed lenses, lens slices and intact cornea-and-lens combinations. All lens preparations were initially placed in the cold Ringer's solution described above. The squashed preparations were produced by compressing the sphere shaped lens between two coverslips to a thickness of approx. $400\,\mu m$. Lens slices were approx. $200\,\mu m$ in thickness and were also sandwiched in Ringer's between two coverslips. In those cases where the anterior half of the eye remained intact after hemisection, the absorption of lens, aqueous humor and cornea were measured by placing the unit cornea-side-down in Ringer's on a coverslip, and advancing the preparation until the lens made contact with the microscope objective in the microspectrophotometer. Measurements of optical density for all of the lens preparations were made as described above for visual pigment, but the optics of the microspectrophotometer were changed as needed. For lens measurements, various Zeiss Ultrafluar objectives combined with a $10\times/0.2$ Ultrafluar as condenser (because of its longer working distance) were used, whereas for photoreceptor absorption a $100\times/1.25$ Ultrafluar was used as objective with a $32\times/0.4$ Ultrafluar as condenser.

RESULTS

Individual spectra were evaluated based on two criteria: (1) that the width of the α-band was similar to that of other visual pigments; and (2) that the absolute absorbance on the long-wave limb of the α-band (i.e. baseline) did not exceed a value of about 10% of the peak. Usable data were filtered digitally by a method involving Fourier transformation of spectral data to generate smooth curves. (See Hárosi, 1987, for a detailed description of this procedure.)

Tree shrew (Tupaia belangeri)

Spectral records were obtained from 272 cone receptors, of which 264 were maximally sensitive in the long wavelength region of the spectrum (long-wave or red-sensitive cones) and 8 were maximally sensitive in the short wavelength region (short-wave or blue-sensitive cones). No cones containing a mid-wavelength sensitive pigment were found. There was no gross difference in the appearance of the cones containing different pigments. However, one anatomically recognizable rod receptor was encountered and measured.

Absorption characteristics for the population of long-wave-sensitive (LWS) cones were obtained by statistically averaging the data from the best spectral records (i.e. 12 OD, 5 BD and 2 LD spectra), using the 2 criteria specified above. In 3 cases acceptable OD and BD, or OD and LD, spectra were obtained from the same cells. Mean peak absorption was 555.1 nm ($s = 5.6$) and the median value was 555.0 nm (range = 543.5–564.0 nm). The mean half-bandwidth (spectral width at 50% of peak absorbance) was $4240\,cm^{-1}$ ($s = 480$), which is only slightly larger than values characteristic of LWS cone specra in macaque monkey (Hárosi, 1987). Examples of OD, BD, and LD spectra are shown in Fig. 2.

Of the 8 short-wavelength-sensitive (SWS) cones encountered, 5 spectra were suitable for analysis. These included 4 absorbance (OD) spectra that displayed peaks at 410.0, 413.5, 439.0, and 441.7 nm, as well as one bleaching difference spectrum with a peak at 435.0 nm. Mean peak absorbance for these five records was 427.8 nm ($s = 14.9$). One SWS cone absorbance spectrum and the SWS cone bleaching difference spectrum are shown in Fig. 3.

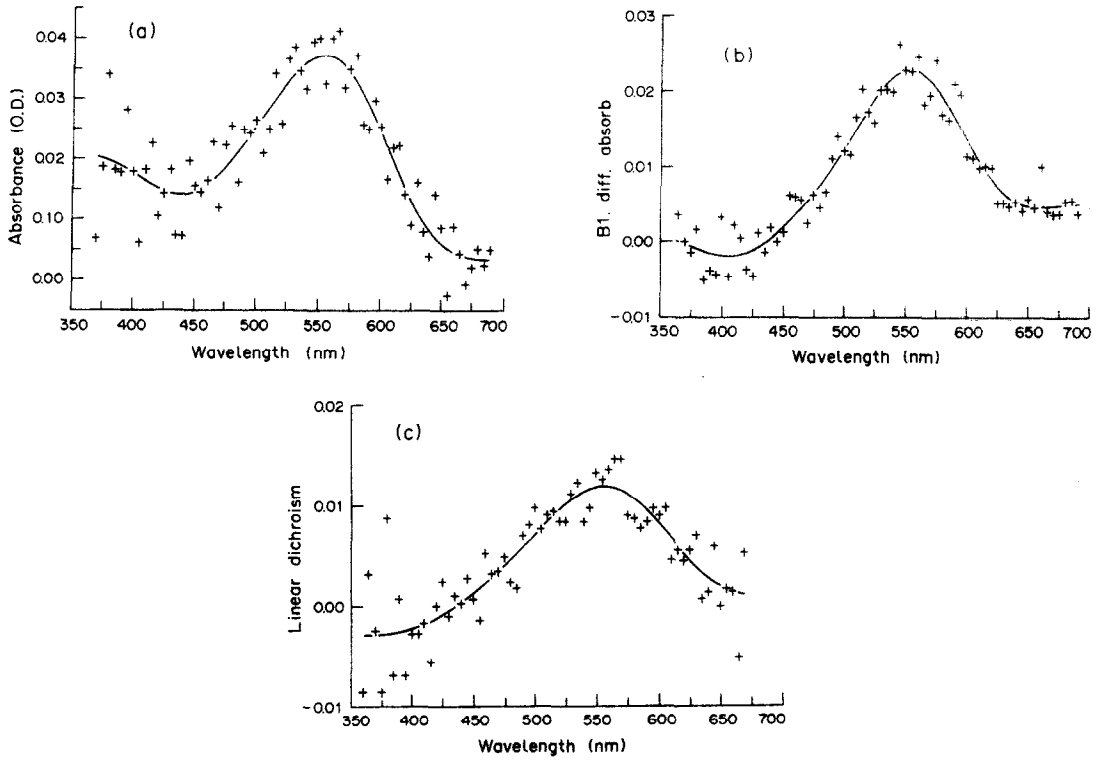

Fig. 2. *Tupaia* LWS cone spectra. (a) Absorbance (OD) spectrum of a single cone outer segment. The data points (crosses) were obtained in 8 spectral scans. The continuous curve is the result of Fourier filtering of the data and shows a peak absorption at 554.5 nm. (b) Bleaching difference (BD) spectrum of another single cone outer segment. Peak absorption is at 554.5 nm; 16 spectral scans. (c) Linear dichroism (LD) spectrum of another single cone outer segment. Peak dichroism is at 556.3 nm; 24 spectral scans.

It is of interest that appreciable linear dichroism was measurable from only two *Tupaia* cones, both of them LWS type. The first of these cones was measured three times and their average linear dichroism spectrum is depicted in Fig. 2c. It shows a peak at 556.3 nm and a half-bandwidth of 4099 cm^{-1}. The dichroic ratio (computed from the peak OD and LD values, see Methods in Hárosi, 1987) was determined to be 2.2. For the second cone, spectral scanning resulted in shifted baselines for both the OD and the LD spectra. Since rescanning during the experiment was not possible due to technical reasons, the baselines of the records were manually corrected during data analysis and a dichroic ratio of 2.1 was computed (λ_{max} was 543.5 nm, half-bandwidth = 4201 cm^{-1}). The dichroic ratios obtained from both these cones are somewhat larger than those obtained previously from monkey cones, where the ratios

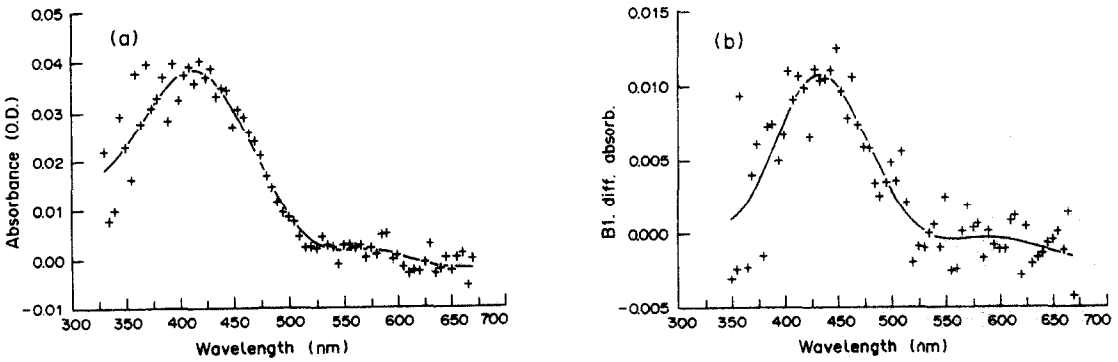

Fig. 3. *Tupaia* SWS cone spectra. (a) Absorbance spectrum of a single cone outer segment. Peak absorption at 413.5 nm; 8 spectral scans. (b) Bleaching difference spectrum of another single cone outer segment. Peak absorption at 435.0 nm; 16 spectral scans (conventions as in Fig. 2).

were always less than 2.0 (Hárosi, 1987). It is to be noted, however, that while measurement of appreciable cone dichroism was a rare event in *Tupaia* as well as in primates, the monkey measurements were done using unfixed tissue, whereas the *Tupaia* tissue was lightly fixed. This difference alone may account for the slight improvement in experimentally determined dichroic ratio values for *Tupaia* cones.

The one rod photoreceptor encountered in *Tupaia* retinae had a cylindrical outer segment of *ca* 1.4 μm in diameter and 4 μm in length (determined using photomicrographs taken with a Zeiss microscope with Nomarski differential interference contrast optics). The outer segment was still attached to a slightly thicker, yet relatively slender, inner segment wedged between two cone ellipsoids. Using a rectangular beam about 1 × 2 μm in cross section, the rod outer segment was measured four times, each measurement consisting of eight violet-to-red spectral scans. It was then exposed for 2 min to 500 nm light for the measuring beam, following which post-bleach spectral scans were made. Unfortunately, the obtained BD spectra were unacceptable due to large baseline shifts, but nevertheless a clear loss of the major band near 500 nm was observed.

The spectra resulting from the third pre-bleach measurement of the rod are shown in Fig. 4. The Fourier-filtered absorbance spectrum (Fig. 4a) was found to peak at 488.3 nm, with a half-bandwidth of 4775 cm^{-1}. On the other hand, the linear dichroism spectrum (Fig. 4b), processed in a similar manner, yielded a λ_{max} = 503.0 nm and 3661 cm^{-1} for half-bandwidth. Because the α-band peak in absorbance spectra can occasionally appear blue-shifted, whereas in linear dichroism spectra can often appear red-shifted, a numerical average of the two peaks (i.e. 496 nm) may be closer to the actual value of the *Tupaia* rod pigment. If the λ_{max} shift between the OD and LD spectra in Fig. 4 is disregarded, the dichroic ratio may be calculated from the peak amplitudes. The derived dichroic ratio for this rod was 3.0. Although this value is less than that of most amphibian rods, it compares favorably with the dichroic ratios obtained from small diameter rods in general, and from monkey rods in particular (Hárosi, 1987). To determine the transverse specific density ($S_\perp$) the peak transversely-polarized absorbance ($A_\perp$, the optical density for light polarized perpendicular to the length of the rod) was first calculated from the average peak absorbance (A_{av}) and the dichroic ratio (R) using equation (1):

$$A_\perp = A_{av}/f; \quad (1)$$

where $f = (1 + R)/2R$.

For this rod $A_\perp = 0.0149$. The transverse specific density was then calculated using equation (2):

$$S_\perp = A_\perp/d; \quad (2)$$

where d = outer segment diameter.

Based on the outer segment diameter of 1.4 μm, $S_\perp = 0.0107\ \mu m^{-1}$. This value is smaller than expected for a rhodopsin-containing rod (i.e. the transverse specific density of *Bufo marinus* red rods was found to be 0.0161 μm^{-1}; Hárosi, 1975), but given the small cell size and the uncertainty about its actual dimensions, it is an acceptable value.

Pre-retinal absorption by the lens and cornea was found to peak at about 370 nm, with negligible absorbance beyond 430 nm. These findings are illustrated by the absorbance spectrum shown in Fig. 5, which was the result of 16 spectral scans through an intact lens-and-cornea combination. The Fourier-filtered smooth curve

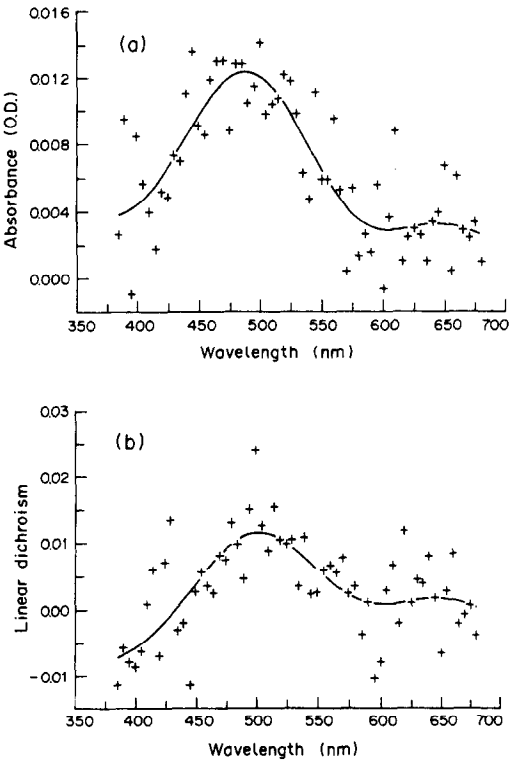

Fig. 4. *Tupaia* rod visual piogment. (a) Absorbance spectrum of a single rod outer segment. Peak absorption at 488.3 nm; 8 spectral scans. (b) Linear dichroism spectrum of the same structure. Peak dichroism at 503.0 nm; 8 spectral scans (conventions as in Fig. 2).

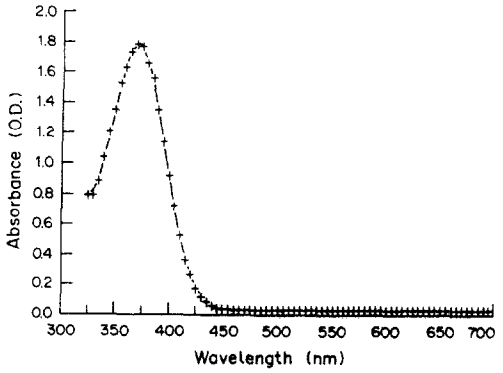

Fig. 5. *Tupaia* pre-retinal absorbance spetrum. Optical density of a lens/cornea combination measuraed in 16 spectral scans. Peak density at 371 nm (note that absorption appears negligible beyond 430 nm). (Conventions as in Fig. 2.)

shows a peak at 371.0 nm. Characteristically similar density curves were observed in measurements of the lens material alone.

Galago (Galago crassicaudatus)

Six retinal preparations, 4 histological slides and 3 lens (or lens/cornea) preparations from the two eyes were studied. The 6 retinal preparations (3 made of fresh tissue and 3 of lightly-fixed pieces of retina) yielded a total of 26 spectral records. The 3 lens (or lens/cornea) preparations yielded 10 records.

Most of the visual pigment spectra were recorded from single rods ($n = 16$), some from multiple rods ($n = 6$) and some from edge-folded bunches ($n = 4$). All these records showed the presence of a "typical rhodopsin" with peak absorbance near 500 nm. One such record is depicted in Fig. 6a. The Fourier-smoothed curve has a peak at 501.3 nm; its half-bandwidth is 4236 cm^{-1}. The dichroic ratio was determined in 12 instances, with values ranging from 1.4 to 4.3. The linear dichroism spectra depicted in Fig. 6b corresponds to the absorbance spectrum in Fig. 6a. In this case the dichroic ratio at 500 nm was determined to be 2.3. Commonly, well-aligned bunches yielded higher values than single rods, presumably because the rod outer segments were so very thin (slightly more than 1 μ in dia.) that light could "leak around" them by diffraction, or if the measuring beam was defocused.

In a fortuitous situation, three perfectly straight, long rods were encountered directly adjacent to one another. Measurement of the central rod with a long and thin rectangular light beam (1 × 8 μm) aligned parallel to its long axis, yielded 0.01563 for the average peak absorbance (A_{∞}) of the α-band and a dichroic ratio (R) of 2.3. From these values the peak transversely-polarized absorbance was calculated from equation (1) to be $A_{\perp} = 0.02178$. In order to obtain the transverse specific density using equation (2), the pathlength of the measuring light within the rhodopsin-containing structure would need to be known. Assuming, however, the *Galago* rhodopsin has the same molar extinction and packing density (cellular concentration) as amphibian rhodopsins in

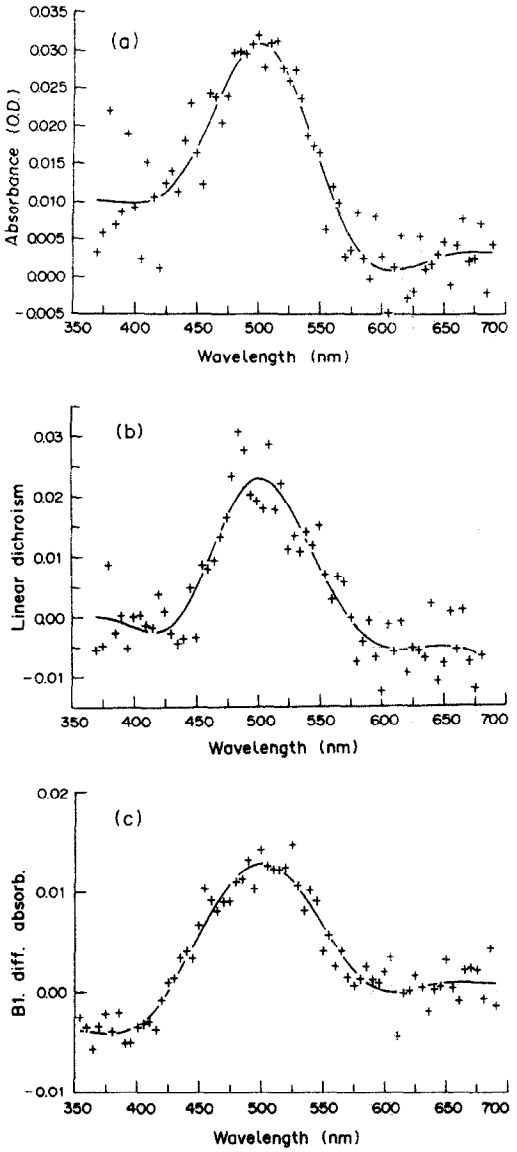

Fig. 6. *Galago* rod visual pigment. (a) Absorbance spectrum of a bunch of 6 or 7 rod outer segments. Peak absorption at 501.3 nm; 8 spectral scans. (b) Linear dichroism spectrum of the same bunch of cells. Peak dichroism at 502.3 nm; 8 spectral scans. (c) Bleaching difference spectrum obtained from a bunch of about 20 rod outer segments. Peak absorption at 501.8 nm; 16 spectral scans (conventions as in Fig. 2).

larger rods (such as the "red rods" in the adult (land phase) *Ambystoma tigrinum* for which $S_\perp = 0.0177\,\mu m^{-1}$, Hárosi, 1975), the effective cell diameter may be estimated: $A_\perp/S_\perp = 0.02178/0.0177 = 1.23\,\mu m$. Alternately, the best estimate of the diameter of *Galago* rod outer segments that was obtainable from measurements made from photomicrographs of fresh tissue fragments of *Galago* retina (such as shown in Fig. 1B), was $1.2\,\mu m$, which is consistent with the calculated value. The conclusion is that within the accuracy of these determinations, *Galago* rods contain a similar rhodopsin, packed at similar concentration, as the larger amphibian rods.

Cornea and lens together yielded spectra like those obtained from the lens substance alone. Thus, the cornea must have a much broader transparency range than the lens. Due to total lens dimensions (10 mm dia., 7 mm thickness in the center), it was not possible to focus a microbeam through an intact lens without compression. During an attempt to slice the lens, it was found to consist of a capsule filled with a gelatinous substance. After removing some of the gel, the sample was made thinner and could be measured. Figure 7 shows an absorbance spectrum obtained from such a preparation, which displays a maximum at 363 nm. Characteristically similar absorbance curves were obtained from measurements of the gel alone, albeit with much smaller amplitudes. The spectrum depicted in Fig. 7 was obtained about 20 hr following enucleation. Although the tissue was kept refrigerated and in the dark during this time, the apparent light scattering could have been caused fully or partially by postmortem degradations. Another point to be made about this spectrum is that the peak density would certainly be larger had we succeeded in recording through the full 7 mm thickness of the central lens.

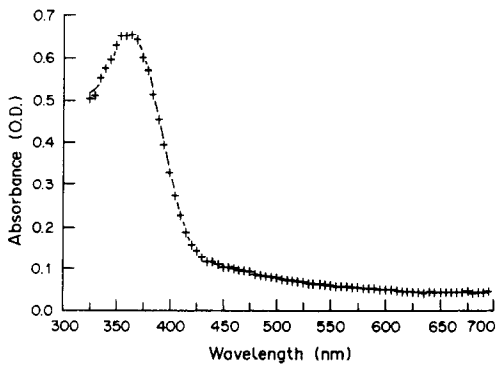

Fig. 7. *Galago* lens absorbance spectrum. Peak density at 363 nm; 16 spectral scans (conventions as in Fig. 2).

DISCUSSION

Tupaia visual pigments

Our findings of only two types of cone pigments, one long-wave sensitive (LWS) and one short-wave sensitive (SWS), in the *Tupaia* retina is consistent with behavioral studies of *T. glis* (Polson, 1968) and *T. belangeri* (Jacobs & Neitz, 1986; Kelly & Petry, 1989) that classify the tree shrew as a deutan-type dichromat. It is also consistent with the long-held hypothesis that dichromacy results from the presence of only two, rather than three cone pigments. In addition, our measurement of a rod with a rhodopsin type pigment in an anatomically-recognizable rod receptor confirms the presence of a sub-population of rod photoreceptors in *Tupaia*, previously suggested by several anatomical (Foelix, Kretz & Ragar, 1987; Immel, 1981; Kuhne, 1983; Muller & Peichl, 1989; Samorajski et al., 1966) and electrophysiological (Petry, Wooten, Kelly & Agarwala, 1987) studies. Our estimate of about 496 nm as the peak for this rod pigment is close to the 500 nm values reported for gray squirrel (*Sciurus carolinesis*) by Loew (1975) using microspectrophotometry and for ground squirrel (*Citellus lateralis*) by Kraft (1988) using suction electrode techniques.

Our determination of 555.1 ± 5.6 nm as the spectral peak of *Tupaia's* LWS cone pigment is in good agreement with the 556 nm peak estimate by Jacobs and Neitz (1986) using ERG flicker photometry. Also, measurements of ERG *b*-wave amplitude to flashes of monochromatic light by Tigges, Brooks and Klee (1967) in *T. glis*, and by Petry et al. (1987) in *T. belangeri*, have produced spectral sensitivity functions that display a major peak in this region (i.e. at 552 and 550 nm, respectively). Our measurements of the SWS cone pigment, however, suggest that it peaks at a wavelength shorter than Jacobs and Neitz' ERG data predicts. That is, the λ_{max} values for each of the five SWS cones we measured (i.e. 410.0, 413.5, 435.0, 439.0 and 441.7 nm) peaked at a value shorter than the 444 nm value reported by Jacobs and Neitz. Several factors may contribute to this difference. The most likely is Jacobs and Neitz' use of Tigges et al. (1967) density curve for the *Tupaia* lens to correct their ERG data for pre-retinal absorption. Whereas

our microspectrophotometric measurement of pre-retinal absorption in *T. belangeri* showed negligible absorption beyond 430 nm, the density curve published by Tigges et al. shows about 0.5 log units of density at 430 nm and as much as 0.2 log units at about 440 nm. Furthermore, at 410 nm the Tigges et al. estimate is nearly 1.2 log units greater. Whether these discrepancies are attributable to species differences (Tigges et al. studied *T. glis*) or to methodological variations (Tigges et al. did not publish the source of their lens estimation) is unclear. However, our measurements of pre-retinal absorption for several types of preparations (e.g. squashed lenses, sliced lenses and lens-and-cornea combinations) were very repeatable, and we think they represent the best data for *T. belangeri*. That being the case, Jacobs and Neitz' estimate of 444 nm for the spectral peak of the SWS cone probably reflects an overcorrection of their data for lens absorption, resulting in an erroneously long estimate of λ_{max}. Of course, our small sample size of SWS cones, the inherent differences of action spectra vs absorbance spectra, and the ever-preseent difficulties associated with making microspectrophotometric measurements (e.g. movement artifacts, defocussing, increased optical scatter in the short wavelength region of the spectrum) preclude us from ruling out other possibilities for the differences observed in the λ_{max} of SWS cones. It is of interest, however, that the peak sensitivity of 435 nm obtained for ground squirrel short-wave-sensitive cones by Kraft (1988) using suction electrode techniques, is closer to our estimate.

The fact that we measured 264 LWS cones and only 8 SWS cones deserves comment. We do not imply that these numbers reflect the actual ratio of LWS cones to SWS cones in the *Tupaia* retina. Estimates of the SWS cone population obtained from anatomical studies (Foelix et al., 1987; Immel, 1981), immunohistochemistry (Muller & Peichl, 1989) and selective staining (Petry, 1982) suggest that SWS cones constitute about 4% of the cone population across the retina, although a regional maximum of about 10% was observed in inferior retina (Muller & Peichl, 1989). Because of the small size of the *Tupaia* eye, we cannot guarantee that this area was represented in any of the successful preparations in which we found SWS cones. Furthermore, regardless of the fact that the SWS cones are well in the minority, we suspect that our encounter rates were also strongly influenced by the success of the preparation. It is well documented from the experimental (DeMonasterio, Schein & McCrane, 1981; Sperling, Johnson & Harwerth, 1980) as well as the clinical (Marre & Marre, 1982; Hood, Benimoff & Greenstein, 1984; Zrenner, 1983) literature that SWS cones in primates are especially vulnerable to damage. Since we encountered SWS cones only in the best of preparations, it is possible that the SWS cone outer segments were among the first to deteriorate also in *Tupaia*.

In light of the apparent similarities of the dichromatic color vision of *Tupaia* to that of human deuternopes that is evident from psychophysical and electrophysiological studies (Polson, 1968; Jacobs & Neitz, 1986), it is of interest to compare the spectral locations of their visual pigments. Measurements of human visual pigments (Dartnall, Bowmaker & Mollon, 1983) showed spectral peaks at 419 ± 3.6 nm and 558.4 ± 5.2 nm for the SWS and LWS cone pigments, respectively, and Mollon, Bowmaker, Dartnall and Bird (1984) reported a λ_{max} of 558 nm for the LWS cone pigment of a psychophysically-documented deuteranopic human observer. (No SWS pigment as measurable.) These LWS values may correspond to the λ_{max} of 555.1 ± 5.6 nm that we found in *Tupaia*. In fact, Dartnall et al. suggested that their LWS distribution was bimodal, with one sub-population maximum at 554.2 ± 2.3 nm, and the other at 563.2 ± 3.1 nm. The spectral locations of the SWS cone pigments for humans and *Tupaia* (419 ± 3.6 and 428 ± 15 nm, respectively), may also correspond, but the considerable variability in the SWS cone data sets precludes any conclusive comparison. Our measurement of a *Tupaia* rod showing a peak at about 496 nm is also consistent with the spectral peak of 496.3 ± 2.3 nm for human rods (Dartnall et al., 1983) and 499–502 nm for the rod photopigment in macaque monkeys (Bowmaker, Dartnall, Lythgoe & Mollon, 1978; Bowmaker, Dartnall & Mollon, 1980; Hárosi, 1987*).

With regard to LWS cone pigments, that of *Tupaia* peaks at a considerably shorter wavelength than the 565–567 nm peak of the LWS cone pigment measured in macaque monkeys

*The measurements of macaque monkey visual pigments by Hárosi (1987) were obtained using the same apparatus and analyzed with the same Fourier smoothing techniques as those described here.

(i.e. 565 ± 2.5 nm, Bowmaker et al., 1978; 567 ± 6.1 nm, Bowmaker et al., 1980; 565.9 ± 2.8 nm, Hárosi, 1987). Furthermore, recent data by Travis, Bowmaker and Mollon (1988) shows two clusters of LWS pigment, near 559 and 567 nm, in a New World monkey, the common marmoset (*Callitrix jacchus jacchus*). These observations are in line with the hypothesis advanced by Dartnall and Lythgoe (1965) that the spectral peaks of visual pigments in the retinal series are limited by chemical factors to cluster at intervals of 6.5–8 nm.

Galago pigments

Our measurement of a λ_{max} near 501 nm for the rod photopigment in this species is consistent with Dartnall et al.'s (1965) estimate of a 501 nm peak in *Galago* from their measurements of photopigment extracts, and also with microspectrophotometric estimates of rod pigments in other primates (see above). However, our measurement of the absorption of the *Galago* lens showing a spectral peak at 363 nm is considerably shorter than the 380 nm peak reported by Dartnall et al. This difference we cannot readily explain. According to Shibata (1959), the opal glass transmission method approximates the value of the "semi-integral attenuance" for parallel light illumination (for nomenclature, see Shibata, 1958 or 1959). Incidentally, the use of an integrating sphere also leads to the semi-integral attenuance and, while this may be the most desirable way to collect diffuse light, there are associated errors which are dependent upon sample area and detector size as well as on the design of the sphere. We contend that a microspectrophotometer with a small numerical aperture condenser and a large numerical aperture objective also approximates the semi-integral attenuance: the former ensures near-collimated illumination, while the latter collects most, if not all, forward-scattered (diffuse) light. Thus the opal glass method used by Dartnall et al. (1965) and our microspectrophotometric method should be equivalent in principle. The fact that Dartnall et al. measured a freshly-excised whole lens is in their favor. The advantage in our method is its directness. The light scattering contribution, which is apparent in our spectrum (cf. Fig. 7), might cause a shift of the peak density toward shorter wavelengths, however, we would not expect this shift to be as large as the discrepancy between our measurements. We therefore lack a rational explanation for this difference in the *Galago* lens density spectrum, and defer its resolution to future redeterminations.

Although only rod photoreceptors have been anatomically identified in the *Galago* retina by light microscopic analysis (Dartnall et al., 1965; Detwiler, 1939; Kolmer, 1930), other evidence suggests that a cone pigment may be present. Selective staining of outer segments by Procion yellow dye has indicated the presence of a small population of cone-type photoreceptors (Petry & Casagrande, 1983), and Dodt (1967) has reported a Purkinje-shift, indicative of a second visual pigment peaking near 552 nm. Cone receptors have been documented in another nocturnal primate, the owl monkey, *Aotus*, (Hamasaki, 1967), and behavioral studies have shown *Aotus* to possess weak trichromatic color vision (Jacobs, 1977). Since no studies of *Galago* color vision are available, it was hoped that microspectrophotometry might reveal the presence of one or more cone types. The present findings of only rhodopsin-containing rod receptors in the *Galago* retina are consistent with its classification as an "all-rod" retina, but obviously these results cannot disprove the possibility that cone photoreceptors in small numbers are also present.

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REFERENCES

Allman, J. (1977). Evolution of the visual system in the early primates. In Sprague, J. M. & Epstein, A. N. (Eds.), *Progress in psychobiology and physiological psychology* (Vol. 7, pp. 1–53). New York: Academic Press.

Bowmaker, J. K., Dartnall, H. J. A., Lythgoe, J. N. & Mollon, J. D. (1978). The visual pigments of rods and cones in the rhesus monkey (*Macaca mulatta*). *Journal of Physiology, London, 274*, 329–348.

Bowmaker, J. K., Dartnall, H. J. A. & Mollon, J. D. (1980). Microspectrophotometric demonstration of four classes of photoreceptors in an old world primate, *Macaca fascicularis*. *Journal of Physiology, London, 298*, 131–143.

Bunt, A. H. & Klock, I. B. (1980). Comparative study of 3H-fucose incorporation into vertebrate photoreceptor outer segments. *Vision Research, 20*, 739–747.

Campbell, C. B. G. (1966). Taxonomic status of tree shrews. *Science, New York, 153*, 436.

Campbell, C. B. G. (1980). The nervous system of the tupaiidae: Its bearing on phyletic relationships. In Luckett, W. P. (Ed.) *Comparative biology and evolutionary relationships of tree shrews* (pp. 219–242). New York: Plenum Press.

Casagrande, V. A. & DeBruyn, E. J. (1982). The *galago* visual system: Aspects of normal organization and developmental plasticity. In Haines, D. E. (Ed.), *The lesser bush baby as a laboratory animal* (pp. 137–168). Boca Raton: CRC Press.

Dartnall, H. J. A. & Lythgoe, J. N. (1965). The spectral clustering of visual pigments. *Vision Research, 5*, 81–106.

Dartnall, H. J. A., Arden, G. B., Ikeda, H., Luck, C. P., Rosenberg, M. E., Pedler, C. M. H. and Tansley, K. (1965). Anatomical, electrophysiological and pigmentary aspects of vision in the bush baby: An interpretative study. *Vision Research, 5*, 399–424.

Dartnall, H. J. A., Bowmaker, J. K. & Mollon, J. D. (1983). Human visual pigments: Microspectrophotometric results from the eyes of seven persons. *Proceedings of the Royal Society, London B, 220*, 115–130.

DeBruyn, E. & Casagrande, V. A. (1978). Density and central projections of retinal ganglion cells in the tree shrew. *Neuroscience Abstracts. 4*, 624.

DeMonasterio, F. M., Schein, S. J. & McCrane, E. P. (1981). Staining of blue sensitive cones of the macaque retina by a fluorescent dye. *Science, New York, 213*, 1278–1281.

Detwiler, S. R. (1939). Comparative studies upon the eyes of nocturnal lemuroids, monkeys and man. *Anatomical Record, 74*, 129–145.

Dodt, E. (1967). Purkinje-shift in the rod eye of the bush baby, *Galago crassicaudatus*. *Vision Research, 7*, 509–517.

Foelix, R. F., Kretz, R. & Ragar, G. (1987). Structure and postnatal development of photoreceptors and their synapses in the retina of the tree shrew (*Tupaia belangeri*). *Cell and Tissue Research, 247*, 287–297.

Hamasaki, D. I. (1967). An anatomical and electrophysiological study of the retina of the owl monkey, *Aotus trivirgatus*. *Journal of Comparative Neurology, 130*, 163–174.

Hárosi, F. I. (1975). Absorption spectra and linear dichroism of some amphibian photoreceptors. *Journal of General Physiology, 66*, 357–382.

Hárosi, F. I. (1982). Recent results from single-cell microspectrophotometry: Cone pigments in frog, fish and monkey. *Color Research and Application, 7*, 135–141.

Hárosi, F. I. (1987). Cynomolgus and rhesus monkey visual pigments: Application of Fourier transform smoothing and statistical techniques to the determination of spectral parameters. *Journal of General Physiology, 89*, 717–743.

Hárosi, F. I. & MacNichol, E. F. Jr (1974). Dichroic microspectrophotometer: A computer-assisted, rapid, wavelength-scanning photometer for measuring linear dichroism in single cells. *Journal of the Optical Society of America, 64*, 903–918.

Hood, D. C., Benimoff, N. I. & Greenstein, V. C. (1984). The response range of the blue-cone pathways: A source of vulnerability to disease. *Investigative Ophthalmology and Visual Science, 25*, 864–867.

Immel, J. H. Jr (1981). The tree shrew retina: Photoreceptors and retinal pigment epithelium. Doctoral dissertation. University of California, Santa Barbara.

Jacobs, G. H. (1977) Visual capacities of the owl monkey (*Aotus trivirgatus*): I. Spectral sensitivity and color vision. *Vision Research, 17*, 811–820.

Jacobs, G. H. & Neitz, J. (1986). Spectral mechanisms and color vision in the tree shrew (*Tupaia belangeri*). *Vision Research, 26*, 291–298.

Kelly, J. P. & Petry, H. M. (1989). The effects of red-light-rearing on color vision in the tree shrew. *Investigative Ophthalmology and Visual Science* (Suppl.), *30*, 126.

Kolmer, W. (1930). Zur Kenntnis des Auges der Primaten. *Zeitschrift für Anatomie und Entwickelungsgeschichte, 93*, 679–722.

Kraft, T. W. (1988). Photocurrents of cone photoreceptors of the golden-mantled ground squirrel. *Journal of Physiology, London, 404*, 199–213.

Kuhne, J.-H. (1983). Rod receptors in the retina of *Tupaia belangeri*. *Anatomy and Embryology, 167*, 95–102.

Loew, E. R. (1975). The visual pigments of the grey squirrel, *Sciurus carolinensis leucotis*. *Journal of Physiology, London, 251*, 47P–49P.

LeGros Clark, W. E. (1934). *Early forerunners of man*. London: Bailliere, Tyndall & Cox.

Luckett, W. P. (Ed.) (1980). *Comparative biology and evolutionary relationships of tree shrews*. New York: Plenum.

MacDonald, D. (Ed.) (1984) *The encyclopedia of mammals*. New York: Facts on File.

Marre, M. & Marre, E. (1982). The blue cone mechanism in diseased eyes with eccentric fixation. *Documenta Ophthalmologica Proceedings Series, 33*, 133–138.

McKenna, M. (1966) Paleontology and the origin of the primates. *Folia Primatologia, 4*, 1–25.

Mollon, J. D., Bowmaker, J. K., Dartnall, H. J. A. & Bird, A. C. (1984). Microspectrophotometric and psychophysical results from the same deuteranopic observer. *Documenta Ophthalmologica Proceedings Series, 39*, 303–310.

Muller, B. & Peichl, L. (1989) Topography of cones and rods in the tree shrew retina. *Journal of Comparative Neurology, 282*, 581–594.

Norton, T. T. (1982). Geniculate and extra geniculate visual systems in the tree shrew. In Morrison A. R. & Strick, P. L. (Eds.), *Changing concepts of the nervous system* (pp. 377–409). New York: Academic.

Petry, H. M. (1982). Selective staining of cells in tree shrew and gray squirrel retinae following intravitreal injection of Procion yellow dye. *Neuroscience Abstracts, 8*, 131.

Petry, H. M. & Casagrande, V. A. (1983). Evidence for cone receptors in the galago retina. *Investigative Ophthalmology and Visual Science* (Suppl.), *24*, 258.

Petry, H. M. & Hárosi, F. I. (1987). Tree shrew visual pigments by microspectrophotometry. *Annals of the New York Academy of Sciences, 494*, 250–252.

Petry, H. M., Wooten, B. R., Kelly, J. P. & Agarwala, S. (1987). Effects of adaptation on tree shrew ERG spectral sensitivity. *Neuroscience Abstracts, 13*, 1301.

Polson, M. C. (1968). Spectral sensitivity and color vision in *Tupaia glis*. Doctoral dissertation, Indiana University.

Raczkowski, D. (1975). Primate evolution: Were traits selected for arboreal locomotion or visually directed predation? *Science, New York 187*, 455–456.

Rohen, J. W. & Castenholtz, A. (1967) Uber die zentralisation der retina bei primaten. *Folia primatologia, 5*, 92–147.

Samorajski, T., Ordy, J. M. & Keefe, J. R. (1966). Structural organization of the retina in the tree shrew (*Tupaia glis*). *Journal of Cell Biology, 28*, 489–504.

Shibata, K. (1958). Spectrophotometry of intact biological materials. Absolute and relative measurements of their transmission, reflection and absorption spectra. *Journal of Biochemistry*, *45*, 509–623.

Shibata, K. (1959). Spectrophotometry of translucent biological materials—Opal glass transmission method. *Methods in Biochemical Analysis*, *7*, 77–109.

Shriver, J. E. & Noback, C. R. (1967). Color vision in the tree shrew (*Tupaia glis*). *Folia primatologia*, *6*, 161–169.

Silver, P. H. (1966) Spectral sensitivity of a trained bush baby. *Vision Research*, *8*, 153–162.

Simpson, G. G. (1945) The principles of classification and a classification of mammals. *Bulletin of the American Museum of Natural History*, *85*, 1–350.

Snyder, M., Killackey, H. & Diamond, I. (1969). Color vision in the tree shrew after removal of posterior neocortex. *Journal of Neurophysiology*, *32*, 554–563.

Sperling, H. G., Johnson, C. & Harwerth, R. S. (1980) Differential spectral photic damage to primate cones. *Vision Research*, *20*, 1117–1125.

Tigges, J. (1963). Untersuchungen uber den Farbensinn von *Tupaia glis* (Diard 1820). *Zeitschrift für Morphologie und Anthropoligie*, *53*, 109–123.

Tigges, J., Brooks, B. A. & Klee, M. R. (1967) ERG recordings of a primate pure cone retina (*Tupaia glis*). *Vision Research*, *7*, 553–563.

Travis, D. S., Bowmaker, J. K. & Mollon, J. D. (1988). Polymorphism of visual pigments in a callitrichid monkey. *Vision Research*, *28*, 481–490.

Weller, R. E. & Kaas, J. H. (1982). The organization of the visual system in *galago*: Comparisons with monkeys. In Haines D. E., (Ed.), *The lesser bush baby as a laboratory animal* (pp. 107–135). Boca Raton: CRC Press.

Wolin, L. R. & Massopust, L. C. (1970). Morphology of the primate retina. *Advances in primatology*, Vol. 1: *The primate brain*. New York: Appleton, Century-Crofts.

Zrenner, E. (1983). *Neurophysiological aspects of color vision in primates*. Berlin: Springer.