Electrical response of bat retina to spectral stimulation: comparison of four microchiropteran species[1]

G.M. Hope[2] and K.P. Bhatnagar

Departments of Ophthalmology and Anatomy, University of Louisville Schools of Medicine and Dentistry, 301 East Walnut Street, Louisville (Kentucky 40202, USA), 14 December 1978

Summary. Electrical responses of the retinas of 4 species of microchiropteran bats stimulated by spectrally restricted light flashes were found to diverge systematically from the rhodopsin absorption spectrum. The divergence was progressively greater across the 4 species. The results appeared explainable by assuming a second photoreceptor class and photopigment which was present in progressively greater numbers in the retinas of *Eptesicus fuscus, Desmodus rotundus, Artibeus jamaicensis* and *Carollia perspicillata.*

The retinas of 4 species of microchiropteran bats were found previously to saturate (become incapable of producing recordable electroretinograms (ERGs) to a constant intensity light stimulus) at different adaptation levels[3]. The ordering of the 4 species on the basis of the adaptation level producing retinal saturation correlated well with their order in terms of preference for ambient light levels suggested by their natural behavior[3]. Reports of cone receptors in bat retinas have occasionally appeared in the anatomical literature[4,5]. Chase applied a number of modern criteria for distinguishing among photoreceptor classes and concluded that most of the retinas of the over 20 species studied contained more than 1 class of photoreceptors[5]. The possible presence of cones in these retinas suggests that the order of the 4 species in terms of saturating adaptation level[3] might reflect increasing numbers of photoreceptors capable of functioning at high light levels. The present report describes differences among these species with respect to the retinal response to spectrally restricted stimuli which tend to support this possibility.

Methods. The species studied were *Eptesicus fuscus, Desmodus rotundus, Carollia perspicillata* and *Artibeus jamaicensis.* Collection, maintenance and preparation of the bats, as well as the recording and stimulating systems have been previously reported[3]. Stimuli in the present experiments consisted of 10-msec flashes of light at 20 nm intervals across the spectrum between 440 and 680 nm. Control of the spectral content of the stimuli was provided by narrow band (8–13 nm half-bandwidth) interference filters. Adaptation was empirically adjusted for each subject to yield ERGs of approximately 40% maximum. Stimuli were delivered at a rate of 1/sec and were equated for energy (2.2 log $\mu W/cm^2$).

Results. Figure 1 illustrates samples of the 2 extreme forms of the ERGs from the 4 species. The signals from *Artibeus* were of largest amplitude and most rapidly developing (shortest latencies and implicit times) while those from *Eptesicus* were smallest and slowest of the 4. The stimulus eliciting the signals was of the wavelength indicated opposite each pair of responses and onset was at the time indicated by the vertical line at the beginning of each trace. The amplitudes of the responses at each wavelength were averaged separately for each species and normalized to the amplitude of the response for that species at 500 nm. The relative response amplitudes expressed as percentage of the response at 500 nm are presented as a function of stimulus wavelength in figure 2,A. The mean relative response amplitude for all 4 species is plotted in a similar fashion in the lower portion of figure 2,B along with the absorption spectrum of a photopigment having peak absorption at 500 nm[6], approximating rhodopsin. The data in figure 2,A indicate that the retinas of *Desmodus, Artibeus* and *Carollia* were progressively more responsive to stimulus wavelengths between about 540 and 620 nm than that of *Eptesicus.* A similar differential responsiveness at the shorter wavelengths (440–480 nm) will not be considered because the measurements did not include wavelengths below 440 nm. The comparison in figure 2,B indicates that mean response amplitudes were accounted for by the rhodopsin absorption curve only for wavelengths around 500 nm. The response amplitudes diverged systematically from the absorption curve on either side of the peak.

For illustration, the rhodopsin absorption curve was subtracted from the normalized response amplitude curves. The relative residual response amplitudes, the portion of the amplitudes unaccounted for by the absorption of rhodopsin, are shown in figure 3,A. The progression in responsiveness of the 4 retinas, *Eptesicus, Desmodus, Artibeus* and *Carollia* in increasing order, is more evident. The residual amplitude curves appeared to have peaks at about 560–580 nm (figure 3,A). The latter point is illustrated in the lower portion of figure 3,B. The mean residual response amplitudes were calculated from the curves in the upper portion and re-normalized to the maximum at 580 nm. The mean relative residual response amplitudes were a reasonable approximation to the relative absorption of a photopigment having peak absorption at 580 nm[6].

Discussion. The data presented suggest that the relative amplitude of the retinal response to spectral stimulation in these bats can be accounted for by assuming 2 photopigments; rhodopsin and a 2nd pigment absorbing at about 560–580 nm. It must be emphasized that this observation does not constitute a demonstration of the presence of either photopigment in these retinas. The relationship between the ERG amplitude at a given wavelength and the absorption of photopigments at that wavelength is not

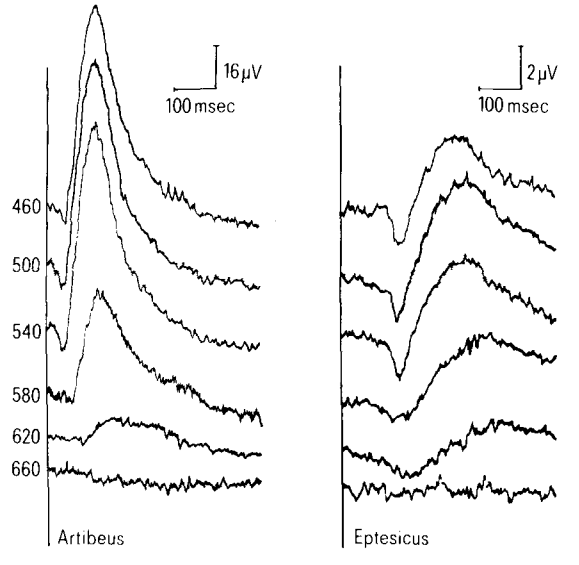

Fig. 1. Sample ERGs from 2 species of microchiropteran bats. Stimuli for each pair of records are given at the left (in nm). Records for *Artibeus* and *Eptesicus* at each wavelength tested are adjacent to the indicated wavelength.

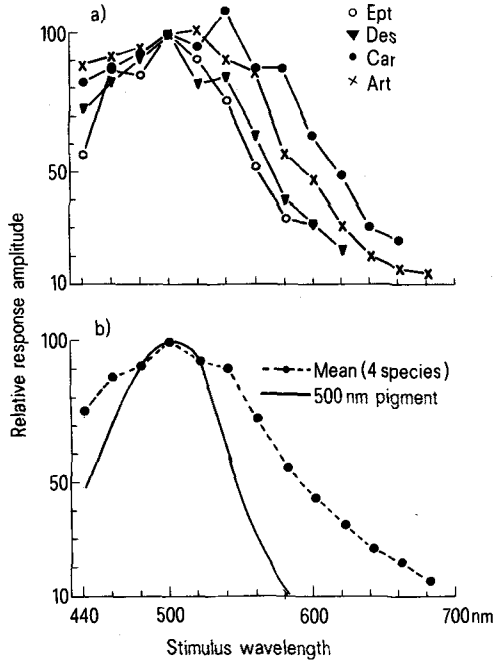

Fig. 2. *A* Relative ERG amplitudes of the 4 species for stimuli at 20 nm intervals between 440 and 680 nm. Data expressed as percent of the amplitude at 500 nm for each species (ordinate). Ept, *Eptesicus fuscus* (n=4); Des, *Desmodus rotundus* (n=2); Art, *Artibeus jamaicensis* (n=4); Car, *Carollia perspicillata* (n=3). *B* Relative mean amplitudes of ERGs from the 4 species at 20 nm intervals across the spectrum. Data are expressed as percent of the mean ERG amplitude at 500 nm. n=13. Solid curve is the relative absorption of a 500 nm photopigment.

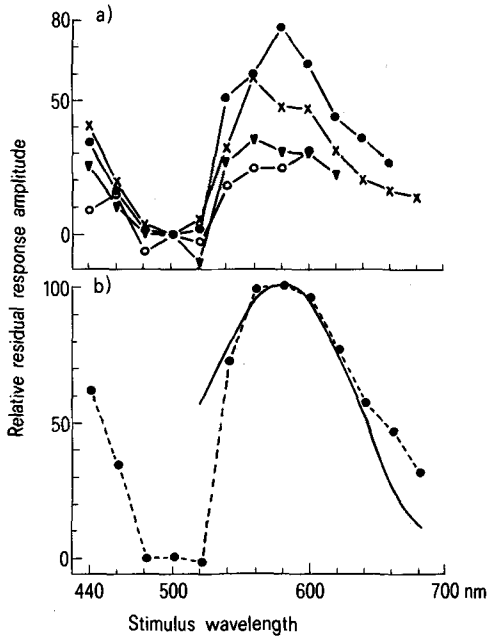

Fig. 3. *A* Relative residual response amplitudes for the 4 species obtained by subtracting the normalized photopigment curve from the data of figure 2, A; symbols as in figure 2, A. *B* Mean residual response amplitudes obtained by averaging the data in the upper portion of this figure. Mean residual responses are expressed as percent of the residual response at 580 nm. Solid line represents relative absorption of a photopigment with peak absorption at 580 nm.

known for microchiropteran retina. Consequently, the data can only suggest the possibility of a 2nd photopigment underlying the portion of the relative response amplitudes unaccounted for by the rhodopsin absorption curve. Given the anatomical evidence for a 2nd class of photoreceptor in microchiropteran retinas[4,5], and the existence of mammalian cone photopigments absorbing in the 560–580 range[7,8], this suggestion seems reasonable. If this proves to be the case, the present data also suggest that the photoreceptors carrying the 2nd pigment might be present in increasing numbers in *Eptesicus, Desmodus, Artibeus* and *Carollia,* in order. This order differs only in the reversal of the positions of *Carollia* and *Artibeus* from those obtained when these species are ranked on the basis of a number of other functional or anatomical characteristics suggesting visual capacity[3]. It is tempting to speculate that previously discussed differences in the ability of the 4 retinas to function at progressively higher light levels[3] might be related to the progressively greater responsiveness at longer wavelengths seen in the present data.

Dieterich and Dodt[9] found no evidence for the presence of a photopigment other than rhodopsin in their evaluation of the spectral sensitivity of the ERG of the dark adapted retina of *Myotis myotis. Myotis* retina also required extensive dark adaptation before ERGs could be recorded and the ERG reached maximum amplitude at stimulation intensities within a few log units of threshold[9]. Neither the requirement for dark-adaptation nor the limited dynamic range was encountered in the 4 species studied in the present work[3]. This suggests that *Myotis* retina may be more rod dominated than those of the *Eptesicus, Desmodus, Artibeus* and *Carollia.* Comparison of the species on the basis of retinal response to spectral stimulation tends to agree with this suggestion.

Electroretinographic estimates of retinal function have apparently been reported for only 6 species of microchiropteran bats. Of these, 3 have been insectivores (*Eptesicus fuscus*[3], *Myotis myotis*[9] and *Eptesicus serotinus serotinus*[10,11]), 1 a sanguivore (*Desmodus rotundus*[3]) and 2 have been frugivores (*Carollia perspicillata* and *Artibeus jamaicensis*[3]). In general, the retinas of the insectivorous species appear to be less capable ERG producers than those of the other 2 groups, the ERGs being generally smaller, slower and appearing under a more limited range of adaptation and stimulation conditions[3,9–11]. The insectivorous species appear to compensate by having a more highly developed capability to echolocate than the sanguivorous and frugivorous microchiropteran bats, based on relative energy of ultrasonic emissions, ability to avoid nets and extent of use of echolocation[12]. Chase[5] and Bhatnagar[13] have suggested a reciprocity between the relative development of the visual and echolocating systems of bats. Previous[3,9–11] and the present electroretinographic estimates of retinal function tend to support this contention since the more extensive functional ranges have been found in the retinas of the less effective echolocators. However, the electroretinographic data further indicate that the reduction in retinal function in the insectivores is most noticable at stimulation and adaptation levels[3,9–11], or regions of the spectrum in the present case, which usually suggest cone or photopic function.

1 This work was made possible by equipment provided by grant No. EY 01228 from the U.S.P.H.S., Bob Hope Fight for Sight award No. G 522 and a grant from Research to Prevent Blindness, Inc. Portions of this work were presented at the Fifth International Bat Research Conference, Albuquerque, New Mexico, August 11, 1978.

2 The authors would like to express appreciation to Drs A.M. Potts, J.B. Longley, R.S. Crockett and T.G. Wheeler for helpful suggestions and to Drs J.H. Richardson, J.G. Cooper, L. Kundrotas, Mr B. Spoonamore and Mr W. Lopez-Forment for facilitating collection and importation of the Mexican bats.
3 G.M. Hope and K.P. Bhatnagar, Experientia *35*, 1191 (1979).
4 R.A. Suthers, in: Biology of Bats, p.265. Ed. W.A. Wimsatt. Academic Press, New York 1970.
5 J. Chase, Thesis, Indiana University, Bloomington 1972.
6 T.G. Ebrey and B. Honig, Vision Res. *17*, 147 (1977).
7 T. Goldsmith, in: Comparative Animal Physiology, p.577. Ed. C.L. Prosser. Saunders, Philadelphia 1973.
8 J.J. Wolken, Vision, Biophysics and Biochemistry of the Retinal Photoreceptors. C.C. Thomas, Springfield 1966.
9 C.E. Dieterich and E. Dodt, in: Symposium on Electroretinography, p.120. Ed. A. Wirth. Pacini, Pisa 1970.
10 H. Bornschein, in: Das visuelle System, p.74. Ed. R. Jung and H. Kornhuber. Springer, Berlin 1961.
11 L. Wündsch and H. Bornschein, Experientia *28*, 409 (1972).
12 D.R. Girffin, Listening in the Dark. Yale University Press, New Haven 1958.
13 K.P. Bhatnagar, Experientia *31*, 856 (1975).

Effect of light adaptation on electrical responses of the retinas of four species of bats[1]

G.M. Hope and K.P. Bhatnagar

Departments of Ophthalmology and Anatomy, University of Louisville School of Medicine, 301 E. Walnut Street, Louisville (Kentucky 40202, USA), 13 November 1978

Summary. The levels of light adaptation at which the retinas of 4 species of microchiropteran bats became unable to generate electroretinograms were progressively ordered. The order correlated well with light preferences based on activity patterns of the 4 species. These results suggest that the ability of the retina to function in ambient light may govern some natural behaviors of these bats.

Vision has been implicated in orientation and homing in a number of species of microchiropteran bats[2-5] and behavioral investigations suggest that vision in some species, at least, is comparable to that of other nocturnal animals[6-11]. Natural behaviors (roosting, emergence, foraging) of microchiroptera suggest differences among species in preference for ambient light levels[12-19]. In spite of the presumed importance of ambient light as a limitation on vision in these nocturnal animals, few laboratory studies actively investigating light adaptation have been reported[6,11,20]. Only one of these, showing that obstacle avoidance by *Myotis lucifugus* was impaired by increasing illumination from 1 to 377 lux, clearly demonstrated a detrimental effect of ambient light on visual behavior[20]. The evidence for use of vision by bats, differential ambient light preferences among species and the paucity of controlled experimentation on adaptational limitations on bat vision prompted the present investigation of effects of light adaptation on electrical responses of the retinas of microchiropteran bats.

Methods. 3 of the 4 species studied, *Desmodus rotundus, Carollia perspicillata* and *Artibeus jamaicensis* were collected near Tlapacoyan, Puebla, Mexico, and maintained in captivity for 2 years under restricted light prior to use in these experiments. Specimens of *Eptesicus fuscus,* were collected around Louisville, Kentucky, immediately prior to use. The bats were anesthetized by i.p. injection of sodium pentobarbital (0.07 mg/1.0 g b.wt) and positioned in a small animal head-holder. Electroretinograms (ERGs) were differentially recorded between nickel-chromium corneal electrodes and lid retractors, amplified (1000×, 0.01-300 Hz) and summed on a signal averaging computer.

Stimulation and adaptation were provided by positioning the 3 mm diameter combination end of a randomized, bifurcated fibre optics bundle approximately 3 mm from the cornea. Each channel of a dual channel optical device imaged the filament of a tungsten-halogen source on one of the bifurcations of the fibre optics bundle. Independent control of intensity by insertion of neutral density filters and timing by an electronic chopper at an intermediate image plane were available in each channel. Stimuli consisted of 10-msec pulses of light delivered at rates determined empirically to allow complete ERG recovery between stimuli. The luminance of the output end of the bundle was approximately 4.75 log millilamberts (mL) for either adaptation or stimulation unless attenuated by neutral density filters. Each bat was adapted to a low luminance (−2.25−−0.25 log ml) and a series of ERGs recorded at each of 4 stimulus luminances (4.75-0.75 log mL). This procedure was repeated at progressively higher adaptation levels until no ERGs could be recorded.

Results. Figure 1 presents a sample set of ERGs from *Eptesicus* at 1 adaptation luminance (−2.25 log mL) in response to a series of stimulus luminances from 4.75 log mL (upper trace) to 0.75 log mL (lower trace). The responses from *Eptesicus* were the smallest of those from the 4 species studied. ERGs from *Desmodus, Carollia* and *Artibeus* were of greater amplitude and developed more rapidly than those illustrated. Extensive dark adaptation was not necessary prior to recording and signals could be recorded in response to stimulus rates in excess of 1/sec in all 4 species. Maximal ERG amplitudes from dark adapted eyes ranged from about 30 μV to over 100 μV depending on species.

ERG amplitudes from sets of signals similar to the samples in figure 1 were plotted versus stimulus luminance for each adaptation level for each species. The stimulus luminance required to evoke a barely detectable ERG was then determined from these curves for each species and was arbitrarily defined as the threshold stimulus for ERG production. Curves illustrating the change in threshold stimulus due to increasing adaptation luminance for representatives of each of the 4 species are shown in figure 2. It can be seen in this figure that the curves for the 4 species are similar but are progressively displaced along the abscissa. If the term saturation is used to refer to the adaptation luminance above which the maximum stimulus luminance available (4.75 log mL) was no longer capable of eliciting a criterion ERG, the saturation level can be estimated from these curves by determining the intercept on each curve corresponding to 4.75 log mL on the ordinate (arrows on curves), then reading the adaptation level corresponding to this intercept (arrows on abscissa). The absolute adaptation levels at which the 4 retinas saturated are of little interest, since they would be valid only for the specific conditions of