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

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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

these experiments. Of more general interest is the rank order of the 4 species and the range of saturation levels, almost 2 orders of magnitude. The rank order of the 4 species was *Eptesicus, Desmodus, Carollia* and *Artibeus,* from lowest to highest saturation.

Discussion. The data presented indicated that the retinas of the 4 species saturated, that is, became incapable of producing criterion ERGs, at progressively higher adaptation luminances. It seems reasonable to interpret these results as suggesting that the retinas of *Eptesicus, Desmodus, Carollia* and *Artibeus,* in order, are progressively more light tolerant. This order compares reasonably well with the natural behavior of the 4 species. *Eptesicus* tends to roost in very dim light and emerges 30–40 min after sunset[12]. *Desmodus* roosts in more exposed locations, as in well-lit caves and hollow trees[13] but emerges only after dark[14] or after moonset[16] and peak activity occurs in the absence of moonlight and in the darkest part of the night[15–17], although they have been reported to forage early in the night[14]. Roosting sites of *Carollia* overlap those of *Desmodus* but they can be found under palm leaves as well[13]. *Carollia* emerges shortly after dusk[13] and peak activity occurs later in the evening[18]. *Artibeus* prefers to roost under palm leaves, in the foliage of trees and occasionally in well-lit caves[13], emerges before dark[13], appears at the food sites before sunset[19] and peak activity occurs early, 18.00–18.30 h[19] or shortly after dark[18]. These progressive tendencies to emerge and forage earlier and roost in more exposed locations suggest a trend in light tolerance in the same order as that seen in the present results. In addition, several anatomical features of the eyes of these species, taken as indices of visual capacity, suggest this ordering[8] as do the visual acuities[7,8].

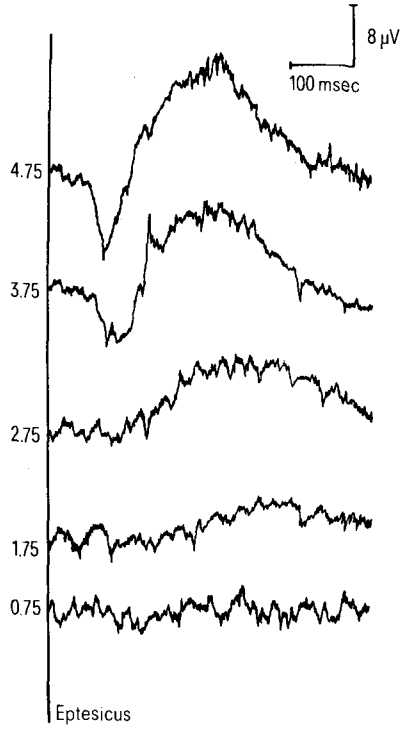

Fig. 1. Sample set of ERG's from *Eptesicus fuscus.* Stimulus luminance in log mL indicated at left of each trace. Adaptation luminance, −2.25/log mL. Voltage and time calibration indicated.

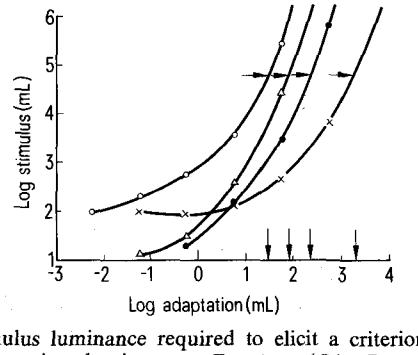

Fig. 2. Stimulus luminance required to elicit a criterion ERG at several adaptation luminances. *Eptesicus* (○), *Desmodus* (△), *Carollia* (●), *Artibeus* (×). Arrows indicate position on curves where 4.75 log mL elicits criterion ERGs and corresponding adaptation levels on abscissa.

There have been previous reports of ERG recordings from only 2 species of microchiropteran bats, *Myotis myotis* and *Eptesicus serotinus serotinus*[21–23]. The results of the present and previous reports are not easily compared due to technical differences but 2 observations from the prior work suggest that the retinas of these 2 species are less light tolerant than those of the 4 species in the present study. First, extensive dark adaptation was required for ERG production and second, the ERG reached maximum amplitude at stimulus intensities within a few log units of threshold in both *Myotis* and *Eptesicus serotinus*[21–23]. These limitations were not encountered in recordings from the species in the present work.

The results of this and previous reports indicate significant differences in the abilities of the retinas of several species of microchiropteran bats to function at increasing light levels. The relatively good correlation of this ability with the natural behaviors of some species suggests that light tolerance of the retina may be an important factor governing these behaviors. In addition, this report appears to be the first in which electroretinography has been utilized specifically to compare the functional capacity of the retinas of several species of bats. The demonstration of the utility of this approach may stimulate its further application in the investigation of the importance of vision as a determinant of bat behavior.

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Superoxide dismutase and life span of *Drosophila melanogaster*[1]

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Summary. Comparison of superoxide dismutase activity in homogenates of wild and vestigial strains of *D. melanogaster* revealed a lower enzyme activity in the short-living vestigial strain.

The discovery of superoxide dismutase (SOD), the enzyme dealing with superoxide free radicals formed upon oxygen metabolism[3] induced a wide interest in the possible effects of this enzyme upon free-radical related biological processes. Some theories of aging assume that this process is due to side reactions of active free radicals with biologically active macromolecules[4]. Taking it for granted, one can expect possible relations between superoxide dismutase and aging phenomena. Indeed, age-related alterations in physicochemical properties of SOD itself were proved in rat liver[5] and we demonstrated a decrease in SOD activity on erythrocyte aging[6]. Some differences were found in SOD activity in brains and lungs of long- and short-lived mice of certain age[7]. This prompted us to resume studies on possible correlations between animal life span and SOD activity in animal tissues. In this communication, we report a lowered SOD activity in homogenates of a Drosophila strain of significantly reduced life-span.

Material and methods. Wild-type *D. melanogaster* and its vestigial mutant were obtained from the Department of Cytology, University of Łódź, and were grown as recommended by Lewis[8], at a photoperiod of 12 h light/12 h dark and at a temperature of 25±1 °C. Killing and homogenization of the insects was performed according to Fernandez-Sousa and Michelson[9]. SOD activity was determined in the homogenates by the adrenalin method[10] and converted into the most widely used McCord and Fridovich' units[3] by calibration with a commercial Sigma preparation of known activity. The assay medium for mitochondrial SOD included 2 mM KCN. Protein was estimated according to the method of Lowry et al.[11] as modified by Lees and Paxman[12]. All the results represent mean±SD from at least 3 parallel experiments.

Results and discussion. SOD activity was determined in homogenates of *D. melanogaster* imagines 1–10 days after emergence (table). The rather high scatter of data is partly conditioned by the method of SOD estimation applied which is very reproducible but, due to the low value of the reference rate of absorbance change (0.025/min), yields discrete values of per cent inhibition of adrenalin autoxidation when absorbance is measured with an accuracy of 0.001 (VSU-2P spectrophotometer, GDR).

The total SOD activity seems to be constant in the imagines within the time period studied. The increase observed in 10-day-old imagines is devoid of statistical significance ($p > 0.05$ when estimated using the Student's t-test). On the other hand, total SOD activity was always lower in the vestigial strain, the difference being statistically significant at 10 days after emergence ($p < 0.05$). This difference seems to be conditioned by cytosolic SOD.

The vestigial strain of *D. melanogaster* is characterized by a considerably shortened life-span in comparison with the wild strain[13,14]. It was reported impossible to find a correlation between the rate of accumulation of fluorescent pigment and the life span of different Drosophila strains[13]. On the other hand, the present results demonstrate that such a possibility may exist with respect to SOD activity and possibly to concentrations of other inhibitors of free-radical reactions.

SOD activity in homogenates of 2 strains of *Drosophila melanogaster* at different intervals after emergence (units/g protein)

Days after emergence	Wild C+M	Wild M	Vestigial C+M	Vestigial M
1	864±143	91± 9	590± 81	203±66
3	860±220	128± 7	725±224	209±52
4	839±188	183±60	519±136	168±49
10	1039± 11	174± 7	775±114	135±25

C: cytosol SOD, M: mitochondrial SOD.

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