Peak Density and Distribution of Ganglion Cells in the Retinae of Microchiropteran Bats: Implications for Visual Acuity

J.D. Pettigrew\textsuperscript{a}, B. Dreher\textsuperscript{b}, Christine S. Hopkins\textsuperscript{c}, M.J. McCall\textsuperscript{b}, M. Brown\textsuperscript{a}

\textsuperscript{a} Neuroscience Laboratory, Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Qld;
\textsuperscript{b} Department of Anatomy and \textsuperscript{c} School of Biological Sciences, The University of Sydney, NSW, Australia

Key Words. Nissl stain · Retrograde labelling · Posterior nodal distance · Axial length · Nocturnal eyes · Diurnal eyes · Sampling theorem

Abstract. We have estimated the total number, distribution and peak density of retinal ganglion cells (RGCs) in retinal wholemounts of several species of microchiropteran (echolocating) bats. The estimates are based on counts of Nissl-stained, presumed RGCs. The total number of presumed RGCs varies among the species: from about 4,500 in \textit{Rhinolophus rouxi} to about 120,000 in \textit{Macropus gigas}. In addition, in two species (\textit{Nyctophilus gouldi} and \textit{M. gigas}), the estimates are based on counts of positively identified RGCs retrogradely labelled with the enzyme horseradish peroxidase injected into the retinorecipient nuclei. In these two species, the numbers and distributions of retrogradely labelled RGCs and Nissl-stained presumed RGCs are very similar. In all six species studied, the peak-density regions of presumed (or positively identified) RGCs are located in the inferotemporal retinae, and the RGC isodensity lines tend to be horizontally elongated. However, the RGC densities in the high-density regions are only 2–4 times greater than those in the low-density regions in the superior retinae. The somal sizes of RGCs vary from 5 to 16 \textmu m in diameter and are unimodally distributed. There is no indication of the existence of distinct morphological classes of RGCs.

The axial lengths of microchiropteran eyes vary from 1.8 mm in \textit{R. rouxi} to 7.0 mm in \textit{M. gigas}. For all species the posterior nodal distance (PND) was assumed to be 0.52 of the axial length of the eye. This assumption is based on the analysis of published data concerning schematic eyes of nocturnal vertebrates. These derived values of the PNDs allowed us to calculate the retinal magnification factors and the number of RGCs per degree of visual angle. From these, the upper limits of visual acuity were derived on the basis of the assumptions of the sampling theorem. The estimated upper limits of visual acuity of the six species of echolocating bats vary from about 0.35 cycles/degree in \textit{R. rouxi} to about 2 cycles/degree in \textit{M. gigas}. This range is quite similar to the range of visual acuities in murid rodents.

Introduction

The accuracy with which microchiropteran bats can create ultrasonic acoustic ‘images’ and assess the position and relative velocity of small moving objects has led to the assumption that their vision makes little or no contribution to telereception. Indeed, the eyes of many microchiropterans are small both in absolute terms and relative to body size, and, like those of the moles [Quilliam, 1966], are frequently considered to be ‘near vestigial’ [for reviews, see Walls, 1942; Suthers and Wallis, 1970; Chase, 1972; Lythgoe, 1979].

It is therefore not surprising that there are relatively few studies concerning the role of vision in the sensory ecology of microchiropterans. However, studies of optomotor responses of microchiropterans indicate that they clearly respond to visual stimuli and that visual spatial resolutions vary substantially among different microchiropteran families [Suthers, 1966; Suthers et al., 1969; Chase, 1972]. Furthermore,
Pentney and Cotter [1976] provided evidence that microchiropterans have well-developed retinofugal projections, which terminate in a number of contralateral nuclei homologous to those of other mammalian orders: (1) the external and, to a lesser extent, the internal layer of the ventral lateral geniculate nucleus; (2) the lateral and, to a lesser extent, the medial parts of the un laminated dorsal lateral geniculate nucleus; (3) the lateral and rostral parts of the superior colliculus; and (4) the nucleus of the optic tract and the pretectal olivary nucleus of the pretectal complex. In addition, some axons of retinal ganglion cells (RGCs) terminate in the contralateral and ipsilateral suprachiasmatic nuclei. Such widespread termination of the retinofugal pathway in microchiropterans contrasts sharply with the very limited area of termination of the retinofugal fibres observed in the other group of mammals with presumably nearly vestigial eyes, the moles, in which the retinofugal fibres terminate only in the contralateral pretectal region and the contralateral ventral lateral geniculate nucleus [Lund and Lund, 1965]. Indeed, in microchiropteran echolocating bats, vision appears to be important during migration over long distances [Griffin, 1970; Suthers and Wallis, 1970] and in the detection of small prey on surfaces such as foliage [Fiedler, 1979; Bell, 1985].

The process of visual resolution is a complex one and is limited by several factors including the optics, the coarseness of the mosaic of photoreceptors as well as the degree of convergence of the photoreceptors on the output cells of the eye - the RGCs. Since the axons of RGCs provide the only link between the eye and behavioural output, the spatial resolving power of the RGC mosaic places a limit (information bottleneck) on the spatial resolving power of the whole animal.

In the present study, we have examined the distribution of putative RGCs in Nissl-stained retinal wholemounts from six species of microchiropterans. In addition, in two of the species studied, RGCs were positively identified by retrograde labelling with the enzyme horseradish peroxidase (HRP) injected into the retinorecipient nuclei of the brain. For each species the retinal magnification factor (RMF; in micrometres per degree of the visual angle) and the number of RGCs per degree of the visual angle in the area of peak RGC density were determined. The visual acuity has been estimated from the RMFs on the basis of the assumptions of the sampling theorem. An appendix is provided which treats, in detail, the assumptions underlying the estimation of visual acuity.

Materials and Methods

Subjects
We have examined six microchiropteran species belonging to five families. Nine specimens of Gould's long-eared bat, Nyctophilus gouldi (small, 7–12 g, nocturnal, insectivorous; fig. 1A), family Vespertilionidae, were collected in Southeastern New South Wales using Harp traps [Tidemann and Woodside, 1978]. Two specimens of the Australian false vampire (ghost bat) Macroderma gigas (relatively large, 140–160 g, nocturnal, carnivorous; fig. 1B), family Megadermatidae, were taken from a colony kept at the University of Queensland. Two specimens of the Brown sheath-tailed bat Taphozous georgianus (medium-sized, 67–80 g, nocturnal, insectivorous; fig. 1C), family Emballonuridae, were collected from a cave entrance in Chillagoe, North Queensland, using a hand net. Single specimens of Greater false vampire bat Megaderma lyra (medium-sized, 40–60 g, nocturnal, carnivorous), family Megadermatidae, and Horseshoe bat Rhinolophus rouxi (small, 9–13 g nocturnal, insectivorous), family Rhinolophidae, were taken from the wild near Madurai, Southern India, by mist net. Finally, the single specimens of Gervais fruit eating bat Aristeus cinereus (small, about 12 g, nocturnal, insectivorous), family Phyllostomidae, was captured by mist net in Peru.

Whole Eye Examination
The eyes of three N. gouldi, one R. rouxi, one T. georgianus, one A. cinereus, one M. lyra and one M. gigas were excised and examined using a light microscope. The axial length of each eye was determined using an eyepiece micrometer. One eye of each species was sectioned at either 5 μm (after snap freezing) or at 1 μm (after fixation with Karnovsky’s fixative and plastic embedding) and stained with toluidine blue.

Retrograde Labelling of RGCs
Surgery. Three specimens of N. gouldi and one specimen of M. gigas were anesthetized with a gaseous mixture of 1.0–1.5% halothane in 65/35% N₂O/O₂, placed in a plaster mould [Lithgow and Barr, 1982] and immobilized with dental impression material (Palgix). The skin over the skull was cut sagitally and the several layers of thick muscle bands (those responsible for the rapid and precise movement of the pinnae) were dissected. After the area of the skull above the superior colliculi had been cleared, two small holes were drilled through the bone, one on either side of the sagittal crest (1 mm lateral to the midline and 1.5 mm anterior to the lambdoid suture) and the dura mater removed. The superior colliculi in adult microchiropterans, unlike those in other eutherian mammals, are easily accessible since they are not covered by the cortex (fig. 2A). Thus, injections of 0.5–1.0 μl of a 50% solution of HRP (grade 1, lyophilized, Boehringer-Mannheim) with 5% dimethyl sulfoxide (DMSO) in 0.1 M Tris-HCl buffer (pH 7.6) into the superior colliculi were made under visual guidance to a depth of 1.5 mm below the surface. The injections were made using a 1-μl microsyringe. Upon completion of the injections, the injection sites were covered with warm agar and the skin was sutured.

To facilitate recovery after surgery, 0.4 mg of the corticosteroid dexamethasone sodium phosphate (Decadron) in 0.9 ml of 5% dextrose solution was injected into the head muscles 24 h before surgery. After surgery, the bats were left for 12–48 h in a warm (27°C) and humid recovery box. During this time they would resume feeding and become moderately active.
Visual Acuity of Microchiropterans

Fig. 1. Photographs of three specimens of the microchiropteran bats used in the present study. Note the large mobile pinnae used in echolocation. Scale bars = 20 mm. A N. gouldi, Gould’s long-eared bat, family Vespertilionidae; weight 7–12 g, head and body length 55–65 mm. B M. gigas. Australian false vampire (ghost bat), family Megadermatidae (photograph: R. and A. Williams); weight 140–165 g, head and body length 100–130 mm. C T. georganus. Brown sheath-tailed bat, family Emballonuridae (photograph: R. and A. Williams); weight 17–27 g, head and body length 67–79 mm.

Perfusion, Wholemounting and HRP Reactions. The bats were anaesthetized with sodium pentobarbitone (Nembutal, 8 mg/kg) and perfused transcardially with 200 ml of warm (37°C) Hartmann’s (6 g sodium chloride, 3.22 g sodium lactate, 400 mg of potassium chloride and 270 mg of calcium chloride per litre at pH 6.5) solution (containing 0.5% sodium nitrite and 1,000 units of heparin) followed by 200 ml of fixative (0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4). The fixative was then flushed out with 100 ml of Hartmann’s solution. While still in situ, the eyes were lanced along the dorsal surface, deep enough to ensure the incision included the superior retina. The eyes were then excised and left in fixative for not more than 2 h. The need for this additional fixation is probably related to the lack of retinal circulation in microchiropterans (see fig. 2B and Results).

The eyes were then rinsed and the retinae dissected out in 0.1 M Tris-HCl buffer (pH 7.6). The entire retinal cup was dissected by first removing the lens and the vitreous and then ‘peeling’ the lanced sclera back over, and away from, the retina [cf. Stone, 1981]. Occasionally, the separation of very small retinae from the pigment epithelium was facilitated by placing the specimen overnight in distilled water after fixation and the removal of the anterior chamber, lens and vitreous. This ‘osmotic shock’ procedure often completely
eliminated the need for dissection, since it usually resulted in a retina which was hanging free except for the optic nerve head.

The retinæ were rinsed for 10 min in 0.1 M Tris-HCl buffer (pH 7.6) and treated according to the protocol developed by Leventhal [1982]. Briefly, they were incubated for 15 min in warm (37°C) 0.1 M Tris-HCl buffer (pH 7.6) containing 1% cobalt chloride [Adams, 1977] and 0.5% DMSO. The retinæ were rinsed for 5 min in Tris buffer, then in 0.1 M phosphate buffer (pH 7.4) and pre-reacted for 15 min in 37°C phosphate buffer containing 0.03% phenylenediamine dihydrochloride (PPD) and 0.06% pyrocatechol (PC) chromogen reagent [Hanker et al., 1977]. The retinæ were then reacted for 15 min in fresh warm (37°C) phosphate buffer containing PPD-PC reagent, 0.5% DMSO and 0.01% H2O2. After rinsing in fresh phosphate buffer for about 30 min, the retinæ were mounted onto gelatinized slides, dried overnight in water vapour and coverslipped.

Directly following perfusion, the brains were removed from the skulls and stored for 2–3 days at 4°C in a solution of 20% sucrose in 0.1 M phosphate buffer (pH 7.4) containing 1% DMSO. The brains were sectioned coronally at 50 μm on a freezing microtome. Sections were collected at room temperature and processed using PPD-PC reagent to demonstrate the distribution of the injected HRP solution. Examination of the sections has shown that in all specimens the HRP spread not only to the superficial retinoreceptor collicular laminae but also to the entire dorsal and ventral thalamus.

**Nissl Staining of RGCs**

After transcardial perfusion (as for the HRP labelling procedure), the eyes were fixed for at least 4 days with 10% formalin solution. The retinæ were excised as detailed in the previous section and the retinal wholemounts were stained with cresyl violet by conventional procedures [cf. Stone, 1981].

**Analysis of the Ganglion Cell Layer in the Wholemount**

The retinal areas were measured from outline drawings of flat-mounted retinæ made at a magnification of 10 or 20× with the aid of a camera lucida attached to a microscope. The camera lucida drawings were traced onto a graphics tablet interfaced with an Apple IIe microcomputer. We believe that in four of the species studied by us we had complete retinæ and consequently the values of the retinal areas obtained by us for these species are reliable. However, for two species, *M. lyra* and *A. cinereus*, we were unable to reliably estimate the retinal area, as significant portions of the retinæ were lost during dissection.

The total numbers of RGCs retrogradely labelled with HRP (fig. 2B, 3A) or presumed ganglion cells in the Nissl-stained retinæ (fig. 3B, 4) were estimated on the basis of counts made under a 100× oil immersion objective in 90–150 sampling areas (each of 6,400 μm2) distributed across the entire retinal surface. In this way, 10–25% of the area of each retina (depending on the size of the retina) was sampled.

In the case of the Nissl-stained retinæ, we have attempted to identify ganglion cells using morphological criteria [cf. Hughes, 1977, 1981a, 1985; Hughes and Wieniawa-Narkiewicz, 1980; Stone, 1978, 1981; Wong and Hughes, 1987] as well as criteria based on the appearance and somal sizes of cells labelled with HRP. As a result of these considerations, we have identified as RGCs the cells which had: (1) a nucleus (and occasionally nucleolus) and substantial cytoplasm with Nissl substance fairly evenly distributed around the
nucleus, and (2) a soma diameter of at least 5 μm. However, some cells which satisfied the above criteria were excluded from our counts of presumed RGCs on the basis of certain peculiarities in their morphology (see Results).

**Ganglion Cell Sizes**

The somal diameters were measured with a bright-field camera lucida and a graphics 'mouse' interfaced to an Apple Ile microcomputer [Halasz and Martin, 1984]. Sample areas were selected from regions of highest and lowest density. Somal sizes are expressed as the diameter of a circle whose area is equal to the area of the cell.

**Estimation of Visual Acuity**

To determine the posterior nodal distance (PND) of the eye, we multiplied the axial length of the eyeball by 0.52. The basis for the assumed relationship between axial length and PND of the nocturnal eye is set out in the Appendix. Using the PND, we calculated the distance on the retina which subtended 1° (RMF) according to the following formula: \[ 2\pi \times \text{PND}/360 \]. Assuming a square sample array of ganglion cells, this value was then used to calculate the number of ganglion cells per linear degree from the square root of the maximal density. Acuity was estimated in cycles per degree as half the number of ganglion cells per linear degree [cf. Hughes, 1977, 1981b, 1985, 1986; Snyder et al., 1986].

**Results**

**General Features of the Eyes and Retinae**

The eyes of all six microchiropteran species studied have relatively large corneas and lenses, features typical for nocturnal mammalian eyes. On the other hand, the ocular fundi lack the reflecting tapetum commonly found in the eyes of other nocturnal mammals [for review, see Walls, 1942]. Furthermore, in all six species the fundi are heavily pigmented, the retinae are avascular and the fibre layer is not prominent (fig. 2B, 3, 4) [cf. Walls, 1942; Chase, 1972].

On the other hand, the sizes of the eyeballs of the six species studied vary substantially. Thus, as indicated in table 1, the axial length of the eyeball in *M. gigas*, at 7.0 mm, is the largest, while those of *R. rouxi* and *N. gouldi*, at 1.8 and 1.9 mm, respectively, are the smallest. The retinal areas vary consistently with the size of the eyeball, from about 4–5 mm² for *R. rouxi* and *N. gouldi* to about 80 mm² for *M. gigas* (table 1).

**Morphology of Neurons in the Ganglion Cell Layer**

In the Nissl-stained retinas of all six species examined, the majority of cells in the ganglion cell layer had a clear-cut nucleus and substantial cytoplasm with Nissl substance fairly evenly distributed around the nucleus (fig. 3B, 4). We presumed that these cells were RGCs. Small cells with somal diameters of less than 5 μm and round or oval darkly stained nuclei having very little (if any) discernable cytoplasm almost without Nissl substance (profiles labelled in fig. 3B, 4) were identified as microneurons or glia [Hughes and Weniawa-Narkiewicz, 1980; Hughes,
have excluded the above-mentioned presumed neurons from our counts of presumed RGCs.

As illustrated in figures 5B–E, in the microchiropterans studied by us, the somal sizes of presumed RGCs (in Nissl-stained material) vary in diameter from about 5 to 16 μm and are unimodally distributed. The mean somal sizes of the presumed RGCs and positively identified RGCs of microchiropterans are unusually small (8.5–10.5 μm for the species studied by us; fig. 5). The subdivision of ganglion cells on the basis of soma size grouping is not apparent, and somal sizes of RGCs located in the high RGC density regions (HD in fig. 5) in the inferotemporal retinae do not differ significantly from those in the low RGC density regions (LD in fig. 5) in the superior retinae. Similar trends are apparent in the distribution of the somal sizes of positively identified RGCs (cf. for N. gouldi fig. 5A and B).

In one of the species in which we retrogradely labelled RGCs with HRP (N. gouldi), we were able to visualize the primary dendrites of some of the cells with relatively large (10–16 μm in diameter) somata (fig. 3A). Each of these cells appears to have 3–7 fairly thin (0.4–0.8 μm) primary dendrites. However, since labelling of the dendrites never extended beyond 30–50 μm from the cell body, we do not have any information about the size of the dendritic trees. Furthermore, we were unable to visualize the dendrites of the RGCs with smaller somata.

Numbers and Distributions of RGCs

The total number of presumed RGCs in Nissl-stained retinae of the microchiropteran species studied by us varies over almost two orders of magnitude, from only about 4,500 in R. rouxi to about 120,000 in M. gigas (table 1). In the case of N. gouldi, the number of RGCs retrogradely labelled with HRP is about 14% lower than that of presumed RGCs in the Nissl-stained retinae (about 8,800 positively identified RGCs vs. 10,200 presumed RGCs; table 1). In the case of M. gigas, the number of positively identified RGCs is about 17% lower than that of presumed RGCs (about 100,000 positively identified RGCs vs. 120,000 presumed RGCs; table 1).

In all six species studied, the distribution maps of presumed RGCs reveal a consistent pattern (fig. 6, 7), with the following features. (1) An area of maximal density is located in the inferotemporal retina. The density of presumed RGCs in this high-density region is lowest in R. rouxi (about 2,000 cells/mm²; fig. 7A)
Fig. 5. Frequency histograms of RGC size (soma diameter) in four species of microchiropteran bats. Note that the majority of RGCs in all four species tend to be small, the range of RGC somal sizes is also small (5–16 μm), and they are unimodally distributed without any indication of distinct morphological classes. Note also that the histograms of the somal sizes of HRP-labelled RGCs (A) and those of presumed RGCs from the Nissl-stained retina (B) of N. gouldi are fairly similar. HD = High-density regions in the inferotemporal retina; LD = low-density regions in the superior retina. Mean somal sizes of HRP-labelled cells in N. gouldi (A) are 8.6 ± 1.5 μm (in HD region) and 9.4 ± 1.5 μm (in LD region). Mean somal sizes of Nissl-stained presumed RGCs are: in N. gouldi (B), 8.9 ± 1.9 μm (in HD region) and 9.0 ± 1.8 μm (in LD region); in A. cinereus (C), 10.4 ± 2.3 μm (in HD region) and 10.0 ± 2.8 μm (in LD region); in M. lyra (D), 8.6 ± 2.1 μm (in HD region) and 8.9 ± 2.1 μm (in LD region, and in M. gigas (E), 8.8 ± 2.6 μm (in HD region) and 8.8 ± 1.9 μm (in LD region).

Fig. 6. Isodensity contour maps for RGCs in N. gouldi. S = Superior; T = temporal; N = nasal; I = inferior; OD = optic disc. Numbers indicate density in thousands of ganglion cells per square millimeter. A Map constructed from the counts of RGCs positively identified by retrograde labelling with HRP injected 48 h before the perfusion. B Map constructed from the counts of presumed RGCs identified in Nissl stain. Note (1) the general similarity between the two maps despite the substantial shrinkage of the HRP labelled retina (area of 2.7 vs. 4.2 mm² for the Nissl-stained retina); (2) the presence in both maps of an area of maximal density in the inferotemporal retina (over 5,000 cells/mm²); (3) a generally higher RGC density in the inferior, as opposed to the superior, retina; (4) a tendency for the horizontal elongation of isodensity contours, and (5) a small RGC density ratio between areas of high RGC density and areas of low RGC density.

and highest in T. georganus and M. lyra (over 6,000 cells/mm², fig. 7B, D; table 1). (2) In all species, the inferior retina has, overall, a higher RGC density than the superior retina. (3) There is a relatively small gradient between the area of highest RGC density in the inferotemporal retina and the area of lowest RGC density in the superior retina. (4) Isodensity contours tend to be horizontally elongated. This tendency results in an apparent horizontal streak across the inferior retina.

It is apparent from the comparison of figures 6A and B that at least in N. gouldi the location of the peak RGC density as well as the overall density of positively identified RGCs are very similar to those of presumed RGCs. On the other hand, the density gradient of positively identified RGCs is substantially steeper than that for presumed RGCs, and the density of positively identified RGCs in the superior retina is substantially lower than that of presumed RGCs. It is also important to point out that the inclusion of the 'dubious' RGCs from the Nissl-stained material results in the case of N. gouldi and M. gigas in distribution maps similar to those constructed from the counts of positively identified RGCs.
Estimates of the Upper Limits of Visual Acuity

Our estimates of the upper limits of visual acuity, which are based on the peak density of presumed RGCs and the PND (see Appendix), vary from a very low value of 0.35 cycles/degree in the case of *R. rouxi* to a relatively high value of almost 2 cycles/degree in *M. gigas* (table 1). However, the species with the greatest peak RGC densities (*M. lyra* and *T. georgianus*) are not the species with the highest estimated visual acuity. Instead, the rank order of acuities is the same as the rank order of eye sizes, indicating that, at least in the six species studied, the variation in eye size has a greater effect on the visual acuity than the variation in RGC density (see Appendix).

Discussion

Identification of RGCs

It is commonly recognized that in the ganglion cell layer of mammalian retinæ, apart from RGCs, there are a number of neuronal elements that do not project to the retinorecipient nuclei (cf. Hughes and Wieniawa-Narkiewicz, 1980; Perry, 1981, 1982; Hughes, 1985; Wong and Hughes, 1987). These other neurons are not always easy to distinguish from RGCs, and some of them are included in the counts of presumed RGCs. The question then arises to what extent the numbers and distribution of presumed ganglion cells (identified on the basis of morphological criteria in Nissl-stained material) reflect the numbers and distribution of the positively identified RGCs. In two species of bats in which we positively identified RGCs by retrograde labelling with HRP, the correspondence is rather good. The numerical discrepancies between presumed and labelled RGCs were only about 15% (14% for *N. gouldi*, 17% for *M. gigas*). Furthermore, in both species, the topographic details, such as the position of the specialized area in the inferotemporal retinae, the horizontal elongation of the isodensity lines, and low density in the superior retinae, were very similar in the maps constructed from the distribution of positively identified retrogradely labelled cells and the maps constructed from the distribution of presumed ganglion cells.

On the other hand, in view of the fact that the retinae which contained retrogradely labelled cells were substantially shrunken (compare the retinal areas in
Table I. Data base for visual acuity of microchiropteran bats

<table>
<thead>
<tr>
<th>Species</th>
<th>Maximal retinal area, mm²</th>
<th>AL mm</th>
<th>Estimated PND, mm</th>
<th>RMF μm/degree</th>
<th>Total number of RGCs</th>
<th>Peak RGC density/mm²</th>
<th>Estimated visual acuity cycles/degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinolophidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. rouxi</td>
<td>4.0</td>
<td>1.8</td>
<td>0.94</td>
<td>16.4</td>
<td>4,500</td>
<td>2,000</td>
<td>0.35</td>
</tr>
<tr>
<td>Vesperilionidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.2a</td>
</tr>
<tr>
<td>N. goudi</td>
<td>5.0</td>
<td>1.9</td>
<td>0.99</td>
<td>17.3</td>
<td>Nissl</td>
<td>10,200a</td>
<td>5,200</td>
</tr>
<tr>
<td>Emballonuridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>T. georgianus</td>
<td>25</td>
<td>3.7</td>
<td>1.92</td>
<td>33.5</td>
<td>75,000</td>
<td>6,100</td>
<td>0.7-1.0b</td>
</tr>
<tr>
<td>Phyllostomidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. cinctus</td>
<td>NA</td>
<td>4.4</td>
<td>2.29</td>
<td>40.0</td>
<td>NA</td>
<td>4,500</td>
<td>1.3</td>
</tr>
<tr>
<td>Megadermatidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7-1.0b</td>
</tr>
<tr>
<td>M. lyra</td>
<td>NA</td>
<td>4.2</td>
<td>2.18</td>
<td>38.0</td>
<td>NA</td>
<td>6,400</td>
<td>1.5</td>
</tr>
<tr>
<td>M. gigas</td>
<td>80</td>
<td>7.0</td>
<td>3.64</td>
<td>63.5</td>
<td>Nissl</td>
<td>120,000</td>
<td>3,600</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PND was calculated by multiplying the axial lengths by 0.52 (cf. table III). AL = Axial length of the eye; NA = data not available as parts of the retinae were lost during dissection; PND = posterior nodal distance; RMF = retinal magnification factor.

a The range of the numbers of presumed RGCs was for Nissl-stained, retinae, 9,800-10,650, and for HRP-labelled retinae, 7,500-9,850.

b Acuity of optomotor response [from Suthers, 1966].

fig. 6A and B), we cannot conclude that the peak density of positively identified RGCs is the same as that of the presumed RGCs. The substantially lower density of positively identified RGCs in the superior retina suggests that the proportion of real RGCs in the ganglion cell layer in this part of the retina is lower than that in the region of high density. In other words, in the superior retina, the neurons which are not RGCs (displaced amacrines) seem to represent a higher proportion of neurons in the ganglion cell layer. The good agreement between the numbers of presumed RGCs and the numbers of positively identified RGCs observed in the present study seems to be related to the fact that in both N. gouldi and M. gigas we have excluded from our counts of presumed RGCs the neurons with unusual morphology. It appears that in the microchiropteran bats, like in marsupials [Beazley and Dunlop, 1983; Kolb and Wang, 1985; Wong et al., 1986], rodents [Perry, 1981, 1982], lagomorphs [Hughes, 1985], or carnivores [Hughes and Wiernitz-Narkiewicz, 1980; Wong and Hughes, 1987], a substantial proportion of neurons in the ganglion cell layer are not RGCs.

The overall number of RGCs in the smallest of the microchiropterans studied by us, e.g. about 4,500 in R. rouxi, seems to be much lower than that of any other mammalian species studied, with the possible exception of the common mole Talpa europaea [Quilliam, 1966]. On the other hand, the number of RGCs in medium-sized microchiropteran species such as T. georgianus is comparable to that in the house mouse [Dräger and Olsen, 1981] or in a monotreme, echidna (Tachyglossus aculeatus) [Stone, 1983a], while the number of RGCs in the larger microchiropteran species, such as M. gigas, is quite large (100,000-120,000) and comparable to that in rats, hamsters, North and South American opossums or cats [for references, see Appendix].

Somal Sizes of RGCs

Judging from the unusually small mean somal size and unusually narrow range of somal sizes of microchiropteran RGCs, one could argue that microchiropteran RGCs, unlike those of other mammalian orders, are not differentiated into distinct morphological and functional classes (cf. for recent reviews of morphological and functional classes of RGCs in carnivores: Leventhal [1982], Rowe and Dreher [1982a], Wässle

There is another important difference in relation to somal sizes of RGCs between microchiropterans and many other mammalian orders. In many mammalian species other than microchiropterans, the RGCs of any morphological class located in the high-density region tend to have smaller somata than their counterparts located in the low-density regions [for recent reviews, see Stone, 1983b; Dreher et al., 1984, 1985]. By contrast, in microchiropterans, such a correlation is not apparent. It has been argued that the perikaryal sizes of RGCs are partially determined by the interactions among the neighbouring cells, and the increase in somal sizes of RGCs located in low-density regions is related to the 'decrowding effect'—presumably the reduction of inhibitory influences from neighbouring cells [cf. Linden and Perry, 1982; Rapaport and Stone, 1983; Dreher et al., 1984]. This apparent lack of the decrowing effect in microchiroptans is probably related to the unusually low (2-3:1) density ratio between the regions of low and high RGC density (cf. centre-periphery RGC density ratio in cats, 80:1 [Stone, 1978]; rabbits, 45:1 [Robinson et al., 1986]).

Comparison with Megachiropteran Bats

There are a number of points of difference between the common features we have described in the eyes of microchiropteran bats (which have highly developed ultrasonic echolocating systems) and those reported for megachiropteran bats (which do not have ultrasonic echolocating systems). These include the absence of a tapetum lucidum (widespread distributed in megachiropterans [Walls, 1942]), a horizontal visual streak which passes below the optic disc (the corresponding specialization passes above the optic disc of megachiropterans [Pettigrew, 1986]; note, however, that in that paper the retinæ of microchiropteran bats are drawn upside down), and a simple, avascular retina which contrasts with the more complex papillations and choroidal capillaries of the much thicker megachiropteran retina [cf. Walls, 1942]. Furthermore, the upper limits of the visual acuities of microchiropterans seem to be substantially lower than those of megachiropterans (see below).

Visual Acuity and Distribution of RGCs in Microchiropterans

In the six species of microchiropteran bats studied by us, the estimated upper limits of the visual acuities (based on the peak density of presumed RGCs) vary from 0.35 cycles/degree (R. rouxi) to almost 2 cycles/degree (M. gigas; table I). One has to remember, however, that behaviourally determined upper limits of visual acuities tend to be lower than those determined anatomically (see Appendix). Indeed, while in N. Gouldi the upper limit of visual acuity determined on the basis of peak RGC density is 0.6 cycles/degree (table I), the behaviourally (optomotor responses consisting of movements of head and body in response to visual stimulation) determined upper limit of visual acuity of another vespertilionid bat is only 0.2 cycles/degree [Suthers, 1966] (cf. table I). Similarly, anatomically determined upper limits of visual acuities of T. georganus and A. cinereus are about 1.35 cycles/degree (table I) while the behaviourally determined upper limits of the acuities of bats belonging to families Emballonuridae (cf. T. georganus) and Phyllostomidae (cf. A. cinereus) are about 0.7-1.0 cycles/degree [Suthers, 1966] (table I).

It is worthwhile to point out in this context that the upper limits of visual acuities determined in two other species of microchiropterans Eptesicus sp. (small, nocturnal and insectivorous, family Vespertilionidae) and Saccopteryx sp. (medium-sized, crepuscular, insectivorous, family Emballonuridae), on the basis of the peak densities of presumed RGCs at, respectively, 0.7 and 1.0 cycles/degree [Marks, 1980] are within the range of acuities determined for the species in the present study.

On the other hand, the upper limit of visual acuity of a megachiropteran bat, Rousettus sp. (family Pteropodidae), which echolocates with lingually produced clicks (rather than with the ultrasonic echolocating system), at about 3.0 cycles/degree [Marks, 1980] appears to be substantially higher than that of any microchiropteran. Similarly, the upper limits of the visual acuities of other megachiropterans-fruits bats or 'flying foxes' (which do not have an echolocating system), seem to be substantially higher than those of any microchiropteran bat. Thus, behaviourally estimated upper limits of the visual acuity (3.5 cycles/degree) were consistent among the three tested individuals of Pteropus giganteus [Neuweiler, 1962]. Similarly, one of us estimates, on the basis of the peak density of presumed [Pettigrew, 1986] or positively identified
Table II. Visual acuity estimated from RGC or cone mosaic compared to behaviourally determined acuity (or acuity determined from extrapolation of visual-evoked potentials (VEP) from the visual cortex)

<table>
<thead>
<tr>
<th>Species</th>
<th>Peak RGC density/mm²</th>
<th>RFM µm/degree</th>
<th>Estimated acuity cycles/degree</th>
<th>Behavioural acuity cycles/degree</th>
<th>VEP acuity cycles/degree</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>House mouse (Mus musculus)</td>
<td>8,000</td>
<td>30.8</td>
<td>1.4</td>
<td>0.5b</td>
<td></td>
<td>1–4</td>
</tr>
<tr>
<td></td>
<td>4,000a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South American opossum (Didelphis marsupialis aurita)</td>
<td>2,900</td>
<td>89</td>
<td>2.4</td>
<td>–</td>
<td>1.3</td>
<td>5–7</td>
</tr>
<tr>
<td>Pigmented laboratory rat (Rattus norvegicus)</td>
<td>2,500–3,000</td>
<td>59.2</td>
<td>1.5–1.65</td>
<td>–</td>
<td>–</td>
<td>8–12</td>
</tr>
<tr>
<td></td>
<td>2,000a</td>
<td></td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Golden hamster (Mesocricetus auratus)</td>
<td>5,300c</td>
<td>47.6</td>
<td>1.75</td>
<td>–</td>
<td>–</td>
<td>13, 14</td>
</tr>
<tr>
<td></td>
<td>3,200c</td>
<td></td>
<td>1.35</td>
<td>0.7</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>European rabbit (Oryctolagus cuniculus)</td>
<td>4,800</td>
<td>172</td>
<td>6.0</td>
<td>–</td>
<td>–</td>
<td>15–19</td>
</tr>
<tr>
<td></td>
<td>2,500c</td>
<td></td>
<td>4.3</td>
<td>3.4</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Domestic cat (Felis domesticus)</td>
<td>7,000–8,000d</td>
<td>218</td>
<td>9.1–9.8</td>
<td>7.0–9.0</td>
<td>8.5</td>
<td>20–26</td>
</tr>
<tr>
<td>Native cat (Dasyurus hallucatus)</td>
<td>2,600</td>
<td>105</td>
<td>2.6</td>
<td>2.3</td>
<td>–</td>
<td>27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Peak cone density/mm²</th>
<th>Cone separation, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wedge-tailed eagle (Aquila audax)</td>
<td>550,000</td>
<td>394</td>
</tr>
<tr>
<td>Rhesus monkey (Macaca mulatta)</td>
<td>140,500</td>
<td>223</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
<td>147,000</td>
<td>294</td>
</tr>
</tbody>
</table>


a Peak RGC density of the binocular fixation area.

b Optokinetic nystagmus in response to vertical square wave gratings.

c This is likely to be an overestimate since the retinae shrank substantially during processing [ref. 13].

d Refers only to the density of X-type RGCs.

RGCs [H. Cooper and J.D. Pettigrew, unpubl. data], the upper limit of the visual acuity of Pteropus scapulatus (little red flying fox, family Pteropidae) at about 4.0 cycles/degree and that of Pteropus poliocephalus (family Pteropidae) at about 5.5 cycles/degree. Clearly, the estimates based on the peak density of RGCs are in reasonable agreement with the behavioural estimates obtained by Neuweiler [1962]. The most important factor resulting in the superior acuities of megachiropterans is the size of the eyeball.
(axial length of the eyeball in *P. scapulatus* is 11 mm, that in *P. poliocephalus* is 13.3 mm) rather than the peak density of RGCs (see Appendix).

The location of the presumed high-acuity area (peak RGC density region) in the inferotemporal part of the retina is consistent with the idea that microchiropterans use their vision for the tele-reception of objects located at the distances at which echolocation becomes ineffective [Suthers and Wallis, 1970; Chase, 1972]. There is also some evidence indicating that at least some microchiropteran bats use vision in capturing their prey [cf. also Fiedler, 1979; Kulzer et al., 1984; Bell, 1985].

The presence of the 'streak-like' specialization in the RGC mosaic of microchiropterans, with their nocturnal habitat in forests (rather than in open country), suggests to us that the visual streak does not necessarily represent an adaptation to scan the horizon for predators or prey (the 'terrain theory' of Hughes [1977]). More likely, the presence of a weak visual streak in microchiropterans suggests it to be an inherited characteristic from ancestral species [Stone, 1983b]. It is important to note in this context that the visual streak in megachiropterans is located above, rather than below, the optic disc [Pettigrew, 1986]. This, in turn, is consistent with the idea of the different ancestry of micro- and megachiropterans [Pettigrew, 1986].

In view of the very low centroperipheral RGC density gradient, it is unlikely that microchiropterans have exploratory eye movements. More likely, the eye movements are triggered by optomotor (movement of the whole or large part of the visual field) and/or vestibular stimuli.

Overall, the upper limits of the visual acuities of microchiropterans seem to be fairly similar to those determined by a variety of techniques in another group of nocturnal mammals, the murid and cricetid rodents, and only slightly lower than the visual acuities of such polyprotodont marsupials as the Australian native cat (*Dasyurus hallucatus*) or the American didelphid opossums (table II). In view of the fact that the Muridae and Cricetidae are widely and justifiably used as experimental subjects in studies of the visual system [see for reviews, Finlay and Sengelaub, 1981; Sefton and Dreher, 1985; Finlay and Berian, 1985], there seems little basis for the common practice of dismissing the visual capabilities of microchiropterans ('near vestigial eyes' [Lythgoe, 1979]; 'blind as a bat'). On the other hand, the upper limits of the visual acuities of microchiropterans are not only well below those of many groups of birds or such highly visual mammalian orders as carnivores or primates, they are substantially lower than those of lagomorphs or megachiropteran bats as well (compare tables I and II).

**Acknowledgements**

This work was supported by grants to J.D.P. and B.D. from the National Health and Medical Research Council and the Australian Research Grants Scheme. The work on *N. gouldi* was carried out as part of a BSc honours project of C.S.H. in the Zoology Department of the School of Biological Sciences, The University of Sydney. We are grateful to Rita Collins for her expert technical assistance and to Dr. Gerhard Neuweiler for providing the eyes of *M. lyra, R. rouxi* and *A. cinereus*. The visit of J.D.P. to the Zoology Department of the University of Munich was supported by the 'Deutsche Forschungsgemeinschaft'.

**Appendix**

**Estimation of Visual Acuity from Ganglion Cell Topography**

Given an estimate of the focal length of the eye, it is possible, using the assumptions of the sampling theorem [cf. Gabor, 1946; Shannon and Weaver, 1949] and reasonable assumptions about the behaviour of RGCs when they are a challenged with a spatially periodic stimulus [cf. Hughes, 1977, 1981a, 1985, 1986], to derive the limits of visual acuity from the density in the ganglion cell array. Furthermore, in the species in which there is the high-density regions a one-to-one correspondence between cones and ganglion cells, the upper limits of visual acuity could be derived from the cone mosaic [e.g. Reymond, 1985, Perry and Cowey, 1985]. Although counting cones is frequently easier than counting RGCs, especially in those retinae in which the RGCs are distributed in a number of sublayers, in some nocturnal mammalian species (e.g. domestic cats or laboratory rats [Snyder et al., 1986]), the cut-off acuity estimated from the cone mosaic substantially exceeds the estimates of cut-off limits of acuity determined from the ganglion cell mosaic. This difference indicates that in these species, even in the peak cone density region, there is a significant degree of convergence of cones onto ganglion cells (rather than the postulated one-to-one relationship).

The validity of the estimation of visual acuity from the RGC (or cone) mosaic becomes evident when one compares the limits of visual acuity determined in behavioural tests and/or by recording the cortical visual-evoked potentials to the contrast-reversing gratings (table II). Thus, for example in the domestic cat (the species in which the most detailed studies of visual acuity have been carried out), there is remarkable agreement among four different visual acuity estimates derived from: (1) the RGC mosaic at the peak RGC density region [Hughes, 1981a, 1985, 1986]; (2) the behavioural cut-off spatial frequency based on the animal's performance on the jumping stand [Mitchell et al., 1977] or avoidance-conditioning apparatus [Jacobson et al., 1976]; (3) the visual potentials evoked from the occipital cortex by phase-alternating gratings [Harris,
Visual Acuity of Microchiropterans

1978], and (4) the visual resolution of individual X cells located in the peak RGC density region, the area centralis [Cleland et al., 1979]. The question is now being asked as to whether a single subclass (i.e. the on-centre subclass or the off-centre subclass) of cat ganglion cells, cells specialized for high acuity, the X cells, can, by itself, account for the acuity demonstrated by domestic cats in behavioural experiments. Since at any part of the retina the on-centre and off-centre subclasses of X cells are present in equal numbers, that is, they have the same overall density [for reviews, see Wässle, 1982, 1986; Hughes, 1985, 1986], the difference between the alternative predictions is about 40% (the ratio of $\sqrt{2}$). This is an extremely small difference compared with interindividual variation in the acuity estimates, so the question is presently unanswerable. It is clear, however, that generally the upper limits of the visual acuity calculated from the retinal mosaic give a reasonable approximation of the behavioural limits. The process of calculating the upper limits of visual acuity from the RGC mosaic has, however, three elements, each of which has its own assumptions and errors, and which we will consider in turn.

A word of caution has to be inserted here, however, as in some cases the upper limits of visual acuity determined from the peak density of RGCs exceed the behaviourally determined limits of acuity by a wide margin. For example, in albino rats, the upper limits of visual acuity determined from the RGC mosaic are very similar to those of pigmented laboratory rats (the peak RGC densities of pigmented and albino rats are very similar [cf. Perry, 1981; McCull et al., 1987]). On the other hand, the behaviourally determined upper limits of visual acuity of albino laboratory rats at 0.35-0.45 cycles/degree [Birch and Jacobs, 1979] are substantially lower than the behaviourally determined limits of visual acuity of pigmented rats (1.2 cycles/degree [Birch and Jacobs, 1979; table II]). A similar discrepancy is apparent in the acuity estimates derived from the visual-evoked potentials recorded from area 17 of awake laboratory rats (0.35-0.45 cycles/degree for albino laboratory rats and 1.2 cycles/degree for pigmented laboratory rats [Boyds and Dyer, 1983]). Most likely, the poor visual acuity of albino animals is related to the excessive scatter of light and the resulting poor optics, rather than the information 'bottle-neck' of the ganglion cell mosaic.

Identification of the RGCs. The definitive identification of RGCs is based on the retrograde labelling from the retinorecipient nuclei in the brain. However, for such identification to be possible the animal has to be studied for at least a couple of days in the laboratory rather than in the field. Since in wild species this condition is frequently difficult to fulfill, we have tried to validate our ability to recognize bona fide RGCs in conventionally fixed, Nissl-stained material by cross-reference to HRP material in the same species. Indeed, a number of studies indicate reasonably close correlations between the numbers of presumed RGCs identified in Nissl-stained material and the numbers of RGCs positively identified by retrograde labelling from retinorecipient nuclei. First, as already discussed, in the case of microchiropterans the number of presumed RGCs exceeded positively identified RGCs only by about 15%. Second, in other mammalian orders, such as rodents (laboratory rats [Fukuda, 1977; Schober and Gruschka, 1977; Potts et al., 1982; Perry et al., 1983; Sefton and Lam, 1984; Crespo et al., 1985); golden hamsters [Tiao and Blakemore, 1976; Rhoades et al., 1979, 1982; Tay et al., 1986]; house mice [Gyllensten et al., 1966; Dräger and Olsen, 1980, 1981]; guinea pigs [Reynolds, 1986]; carnivores (domestic cats [cf. Stone, 1978; Hughes, 1981b, 1985; Wässle, 1982; Ng and Stone, 1982; Chalupa et al., 1984; Williams et al., 1986]; lagomorphs (rabbits [Provis, 1979; Vanev, 1980b; Stone et al., 1985; Robinson et al., 1987]) and primates (macaques [Rakic and Riley, 1983; Perry and Cowey, 1985]) and in a number of marsupials (South American opossums [Hokc and Oswaldo-Cruz, 1978; North American opossums [Rapaport et al., 1981; Kirby et al., 1982; Kob and Wang, 1985]; wallabies - quokka - Setonix brachyurus [Braeker et al., 1986]), the number of putative RGCs identified in Nissl-stained wholemounts also corresponds fairly closely to the number of positively identified RGCs (retrogradely labelled with HRP) and/or the number of axons in the optic nerve.

Furthermore, in all mammalian species studied so far, the distribution of putative RGCs is similar to that of positively identified RGCs (domestic cats [Hughes, 1977; Wässle, 1982; Stone, 1983]; laboratory rats [Fukuda, 1977; Hughes, 1977; Dreher et al., 1984; McCull et al., 1987]; house mice [Dräger and Olsen, 1981]; golden hamsters [Tiao and Blakemore, 1976; Rhoades et al., 1982]; guinea pigs [Hughes, 1977; Reynolds, 1986]; rhesus macaques [Stone and Johnston, 1981; Perry and Cowey, 1985]).

The overcounting of the putative RGCs in Nissl-stained material, in relation to the number of positively identified RGCs, amounts to not more than about 30% of neurons in the ganglion cell layer (e.g. house mice, 31% [Dräger and Olsen, 1981]; guinea pigs, 33% [Reynolds, 1986]). Since acuity is expressed as a linear measure (and therefore related to the square root of the cell density), the overcounting of RGCs by 30% will contribute no more than about 6% to the final estimate of the upper limits of acuity, an acceptable figure when one takes into account that even in the best-controlled conditions interindividually variation contributes as much as 40% to behavioural variation.

Judgement Concerning the Strategy to Be Used by the Retinal Mosaic in Achieving Maximal Spatial Resolution. First of all, we should also keep in mind the fact that different visual tasks might rely on different subpopulations of ganglion cells. To take just one example, the visual resolution revealed by the optomotor response (optokinetic nystagmus) depends to a large extent, especially in the so-called 'lower vertebrates' as well as in birds, rodents and lagomorphs, upon the RGCs that project to the accessory optic system, a subclass of RGCs that have substantially lower overall density and different properties than the majority of the RGCs projecting to the tectum and/or to the lateral geniculate nucleus [for recent reviews, see Colleli, 1981; Simpson, 1984; McKenna and Wallman, 1985]. We might therefore have some grounds to expect that visual behaviour that depends upon the retinotectal pathway or the retinohalamocortical pathway would have a higher spatial resolution than a behaviour that depends exclusively on those RGCs that project directly into the accessory optic system. Indeed, in house mice, the spatial resolution of the optokinetic nystagmus to vertically oriented square wave gratings is only 0.5 cycles/degree [Sine et al., 1979] (table II), substantially lower than the upper limits of visual acuity determined from the total population of RGCs (table II). On the other hand, however, in at least three families of microchiropterans the upper limits of visual acuity of the optomotor responses appear to be only slightly lower than those estimated from the mosaic of the total population of RGCs (table I).

The second problem concerns the dramatic variation in the overall density and in the relative proportions of the different morphological and functional subtypes of RGCs in a given species. A logical choice of the retinal region relevant to the upper limit of visual acuity is the area of highest ganglion cell density, commonly,
Table III. Relationship between axial length (AL) and PND in vertebrate eyes

<table>
<thead>
<tr>
<th>Species</th>
<th>AL, mm</th>
<th>PND, mm</th>
<th>PND/AL, ratio</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog (<em>Rana esculenta</em>)</td>
<td>7.9</td>
<td>4.1</td>
<td>0.52</td>
<td>1</td>
</tr>
<tr>
<td>House mouse (<em>Mus musculus</em>)</td>
<td>3.39</td>
<td>1.76</td>
<td>0.52</td>
<td>2</td>
</tr>
<tr>
<td>South American opossum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(<em>Didelphis marsupialis aurita</em>)</td>
<td>9.98</td>
<td>5.1</td>
<td>0.51</td>
<td>3</td>
</tr>
<tr>
<td>Laboratory rat (<em>Rattus norvegicus</em>)</td>
<td>6.3</td>
<td>3.39</td>
<td>0.54</td>
<td>4</td>
</tr>
<tr>
<td>Mean for nocturnal eyes</td>
<td></td>
<td></td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Tawny owl (<em>Strix aluco L.</em>)</td>
<td>28.5</td>
<td>17.24</td>
<td>0.60</td>
<td>5</td>
</tr>
<tr>
<td>Domestic cat (<em>Felis domesticus</em>)</td>
<td>22.3</td>
<td>12.5</td>
<td>0.56</td>
<td>6, 7</td>
</tr>
<tr>
<td>European rabbit (<em>Oryctolagus cuniculus</em>)</td>
<td>18.1</td>
<td>9.8</td>
<td>0.54</td>
<td>8</td>
</tr>
<tr>
<td>Mean for arrhythmic and crepuscular eyes</td>
<td></td>
<td></td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Goldfish (<em>Carassius auratus</em>)</td>
<td>4.26</td>
<td>2.86</td>
<td>0.67</td>
<td>9</td>
</tr>
<tr>
<td>Pigeon (<em>Columba livia</em>)</td>
<td>11.45</td>
<td>7.9</td>
<td>0.69</td>
<td>10</td>
</tr>
<tr>
<td>Wedge-tailed eagle (<em>Aquila audax</em>)</td>
<td>34.7</td>
<td>22.6</td>
<td>0.65</td>
<td>11</td>
</tr>
<tr>
<td>Squirrel monkey (<em>Saimiri sciureus</em>)</td>
<td>14.5</td>
<td>10.2</td>
<td>0.70</td>
<td>12</td>
</tr>
<tr>
<td>Cynomolgous macaque (<em>Macaca fascicularis</em>)</td>
<td>18.8</td>
<td>11.6</td>
<td>0.62</td>
<td>13</td>
</tr>
<tr>
<td>Rhesus macaque (<em>Macaca mulatta</em>)</td>
<td>18.0</td>
<td>12.06</td>
<td>0.67</td>
<td>14</td>
</tr>
<tr>
<td>Human (<em>Homo sapiens</em>)</td>
<td>24.1</td>
<td>17.05</td>
<td>0.71</td>
<td>15</td>
</tr>
<tr>
<td>Mean for diurnal eyes</td>
<td></td>
<td></td>
<td>0.67</td>
<td></td>
</tr>
</tbody>
</table>


albeit controversially, called the area centralis [see for reviews, Walls, 1942; Fite and Rosenfield-Wessels, 1975; Hughes, 1977; Rowe and Dreher, 1982b; Stone, 1983b; Rapaport and Stone, 1984]. Indeed, a very good agreement between the upper limits of acuity calculated from the mosaic of RGCs in the area of peak RGC density and behavioural acuity has been obtained for the domestic cat and the marsupial native cat (table II).

Problems can arise, however, when there is more than one area of specialization, such as the two foveas of some avian retinae [Walls, 1942; Fite and Rosenfield-Wessels, 1975; Reymond, 1985; Moroney and Pettigrew, 1987]. It appears for example that, in the case of the wedge-tailed eagle, the binocular fovea, with its lower spatial resolution, is not used for more difficult discriminations. Indeed, there is a very good correspondence between the calculated acuity (based on the cone mosaic) for the eccentric fovea and the upper limits of acuity achieved behaviourally (table II) [Reymond, 1985]. Similarly, in a number of mammalian species the area of the retina used in the behavioural task does not seem to correspond to the area of highest ganglion cell density. Thus, it appears that in rabbits the behavioural tests applied so far rely on the spatial resolution of the retinal fixation area for the frontal binocular field. This area does not correspond to the area of peak ganglion cell density [Van Hof and Lagers-van Haselen, 1973]. Indeed, only if one bases the estimate of rabbit visual acuity on the peak density of RGCs in the retinal fixation area for the frontal binocular field (about 2,500 cells/mm²; table II [Robinson et al., 1986]) does the agreement between the anatomically determined upper limits of visual acuity (4.3 cycles/degree) and the behaviourally determined upper limits of acuity (3.4 cycles/degree) become quite reasonable. The upper limits of visual acuity determined from the peak RGC density region (4,800 cells/mm²) at 6 cycles/degree are much higher. Similarly, rodents have a retinal specialization for frontal binocular vision that is displaced temporally from the region of highest ganglion cell density (golden hamsters [Tiao and Blake- more, 1976; Rhoades et al., 1982]; house mice [Dräger and Olsen, 1980]; laboratory rats [for review, see Dreher et al., 1985]). In laboratory rats, as in rabbits, anatomically determined upper limits of about 1.3 cycles/degree (table II) of visual acuity based on the RGC density (about 2,000 cells/mm² [McCull et al., 1987]) in the retinal fixation area used in binocular vision agree well with the upper limits of visual acuity determined behaviourally or extrapolated from the visual potentials evoked from the visual cortex by pattern reversal (1.2 cycles/degree; table II). Similarly, in golden hamsters, the anatomically determined upper limits of acuity based...
on the peak RGC densities in the retinal area specialized for binocular vision are in better agreement with behaviourally determined upper limits of visual acuity (note, however, that in golden hamsters the upper limits of visual acuity determined from the peak RGC density, due to excessive shrinkage of the retinae, are probably a substantial overestimate; table II).

**Extrapolation from Retinal Mosaic to Visual Space via Visual Optics**

The eyeball sizes in the vertebrates cover a range of almost two orders of magnitude (from under 1 mm axial length in the smallest shrews [Walls, 1942] or the common mole [Quilliam, 1966], and less than 2 mm in the smallest microchiropterans, table I, to around 28–36 nm in owls and eagles, table III [Shlaer, 1972], 54 mm in the baleen whale [Hughes, 1977] or 107 mm in the great blue whale *Balaenoptera musculus* [Walls, 1942]). Naturally, the visual acuities (expressed in the usual way with angular subtense in the denominator) could cover the same range even if there were no variations in the density of RGCs. The potential source of error in failing to take full account of size scaling is therefore of greater significance than the small errors incurred by possible ganglion cell misidentification. The crucial values for the determination of the RMF are the locations of cardinal points of the complex optic systems of vertebrate eyes which uniquely determine the behaviour of paraxial rays. The most important value is the distance, called the PND in the terminology of schematic eyes and visual optics, separating the posterior nodal point from the posterior foci point [see Westheimer, 1972; Hughes, 1977; Martin, 1983, and references in table III].

The determination of the PND is rather difficult in the case of microchiropterans, who have tiny eyes and featureless ocular fundi. To overcome this problem, in the present study we have attempted to determine the PND from the axial length of the eyeball. (The determination of the PND in turn allowed us to determine the RMF in a given species.) The validity of our procedure can be assessed by reference to table III which shows the axial length and PND for those species for which information about both is available in the literature. It turns out that for the nocturnal species, such as frogs (*Rana esculenta*), house mouse (*Mus musculus*), South American opossum (*Didelphis marsupialis aurita*), and laboratory rat (*Rattus norvegicus*), the ratio of PND to axial length varies from 0.51 to 0.54. In the crepuscular species (e.g. European rabbits, *Oryctolagus cuniculus*) or nocturnal species that are partially diurnal in their activity patterns and therefore operate in a wider range of luminances than purely nocturnal species (e.g. domestic cats or owls [Martin, 1982, 1983]), the ratio of PND to axial length varies from 0.54 to 0.6. Finally, in the diurnal species, the PND to axial length ratio varies from 0.62 to 0.7. For four nocturnal species (listed in table III), the mean ratio of PND to axial length is 0.52, which is the value we have used for calculating the PND of microchiropterans.

The relation between the PND (that is, the focal length of the eye) and the axial length of the eye in nocturnal, arrhythmic and diurnal species (table III) conforms quite well with predictions concerning the general design of the vertebrate eye [Martin, 1983]. Thus, it appears that optimal spatial resolution at high light levels (diurnal birds of prey, diurnal primates) and over a wide range of light levels (arrhythmic species, e.g. domestic cats and owls) necessitates a long focal length. The optimal spatial resolution at low light levels (nocturnal species) and adequate but not optimal spatial resolution at high light levels (goldfish) can be achieved with a short focal length.

**References**


Chase, J.: The role of vision in echolocating bats; PhD thesis Indiana University, Bloomington, Ind. (1972).


Marks, J.M.: Retinal ganglion cell topography in bats; MA thesis Indiana University, Bloomington, Ind. (1980).


Moroney, M.; Pettigrew, J.D.: Some observations on the visual op-
Neuweiler, G.: Bau und Leistung des Flughundauges (Pteropus gig-
Ng, A.Y.K.; Stone, J.: The optic nerve of the cat: appearance and 
loss of axons during normal development. Devl Brain Res. 
Österberg, G.: Topography of the layer of rods and cones in the hu-
Oswaldo-Cruz, E.; Hokono, J.N.; Sousa, A.P.B.: A schematic eye for 
Pentney, R.P.; Cotter, J.R.: Retinal fugal projections in an echolo-
Perry, V.H.: Evidence for an amacrine cell system in the ganglion 
Perry, V.H.: The ganglion cell layer of the mammalian retina; in 
Osborne, Chader, Progress in retinal research, vol. 1, pp. 53–80 
Perry, V.H.; Cowey, A.: The ganglion cell and cone distributions in 
the monkey’s retina: implications for central magnification fac-
Perry, V.H.; Henderson, Z.; Linden, R.: Postnatal changes in reti-
nal ganglion cell and optic axon populations in the pigmented 
Petitgrew, J.D.: Flying primates? Mega-bats have the advanced 
Polyak, S.L.: The retina (University of Chicago Press, Chicago 
1941).
Potts, R.A.; Dreher, B.; Bennett, M.R.: The loss of ganglion cells in 
the developing retina of the rat. Devl Brain Res. 3: 481–486 
(1982).
Provis, J.M.: The distribution and size of ganglion cells in the retina 
of the pigmented rabbit: a quantitative analysis. J. comp. Neu-
Quilliam, T.A.: The problem of vision in the ecology of Talpa eu-
Rakic, P.; Riley, K.P.: Overproduction and elimination of retinal 
axons in the fetal rhesus monkey. Science 219: 1441–1444 
(1983).
Rapaport, D.H.; Stone, J.: Time course of morphological differen-
tiation of cat retinal ganglion cells: influences on soma sizes. J. 
Rapaport, D.H.; Stone, J.: The area centralis of the retina in the cat 
and other mammals: a focal point for function and develop-
Rapaport, D.H.; Wilson, P.D.; Rowe, M.H.: The distribution of 
ganglion cells in the retina of the North American opossum 
Remullia, S.; Hallett, P.E.: A schematic eye for the mouse, and 
Reymond, L.: Spatial visual acuity of the eagle Aquila audax: a 
behavioral, optical and anatomical investigation. Vision Res. 
Reymond, L.; Cook, M.: Relation between simultaneous spatial-
discrimination thresholds and luminance in man. Behav. Brain 
Reynolds, D.D.: The organization of the retinal ganglion cells and 
the pigment epithelial layer in the adult guinea pig; BSc thesis 
University of NSW (1986).
Rhoades, R.W.; Hsu, L.; Parfett, G.: An electron microscopic anal-
Rhoades, R.W.; Kuo, D.C.; Polcer, J.D.: Effects of neonatal corti-
cal lesions upon retino-collricular projections in the hamster. 
Robinson, S.R.; Dreher, B.; Horsburgh, G.M.; McCall, M.J.: De-
velopment of the ganglion cell density gradient in the rabbit re-
Robinson, S.R.; Horsburgh, G.M.; Dreher, B.; McCall, M.J.: Changes in the numbers of retinal ganglion cells and optic 
nerve axons in the developing albino rabbit. Devl Brain Res. 
Rodeck, R.W.; Brening, R.K.: Retinal ganglion cells: properties, 
types, genera, pathways and trans-species comparisons. Brain 
Rowe, M.H.; Dreher, B.: Retinal W-cell projections to the medial 
interlaminar nucleus in the cat: implications for ganglion cell 
Rowe, M.H.; Dreher, B.: Functional morphology of beta cells in the 
area centralis of the cat’s retina: a model for the evolution of 
central retinal specializations. Brain Behav. Evol. 21: 1–23 
(1982b).
Schober, W.; Gruscha, H.: Die Ganglienzenellen der Retina der 
Albinoratte: eine qualitative und quantitative Studie. Z. mikro-
Sefton, A.J.; Dreher, B.: Visual system in Paxinos, The rat nervous 
system, vol. 1, Forebrain and midbrain, pp. 169–221 (Academic 
Sefton, A.J.; Lam, K.: Quantitative and morphological studies on 
developing optic axons in normal and enucleated albino rats. 
Shannon, C.E.; Weaver, W.: The mathematical theory of communi-
cation (University of Illinois Press, Urbana 1949).
Shapley, R.; Perry, V.H.: Cat and monkey retinal ganglion cells and 
their visual functional roles. Trends Neurosci. 9: 229–235 
(1986).
Shlaer, R.: An eagle’s eye: quality of the retinal image. Science 
Shlaer, S.: The relation between visual acuity and illumination. J. 
Silveira, L.C.L.; Picano-Diniz, C.W.; Oswaldo-Cruz, E.: Contrast 
sensitivity function and visual acuity of the opossum. Vision 
Sinex, D.G.; Burdette, L.J.; Pearlman, A.L.: A psychophysical in-
vestigation of spatial vision in the normal and reeler mutant 
Snyder, A.W.; Bosso, T.R.J.; Hughes, A.: Optical image quality 
Stone, J.: The number and distribution of ganglion cells in the cat’s 
and analysis of retinal wholemounts (Maitland, Sydney 1981).
Stone, J.: Topographical organization of the retina in a monotreme: 
Australian spiny anteater Tachyglossus aculeatus. Brain Behav. 
Stone, J.: Parallel processing in the visual system. The classification 
of retinal ganglion cells and its impact on the neurobiology of 


Vakkur, G.J.: Studies on optics and neurophysiology of vision; MD thesis University of Sydney (1967).


Dr. B. Dreher
Department of Anatomy
The University of Sydney
NSW 2006 (Australia)