Aquatic Mammals 1986, **12.3**, 95–102

Topographic distribution of sizes and density of ganglion cells in the retina of a Porpoise, *Phocoena phocoena*

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Introduction

Dolphins are well-known for their developed hearing and echolocation systems. However, data on the organization of other sensory systems in these animals are limited. Namely, the organization of the visual system of dolphins and other cetaceans adapted for visual perception both in the water and air is still unclear.

To understand the mechanisms of visual perception it is important to obtain the information on the structural organization of the best vision zone of the retina with high density of ganglion cells, similar to the area centralis in the retina of some vertebrates. Such data were obtained by DRAL (1975, 1977, 1983) for two species of dolphins—*Tursiops truncatus* and *Delphinus delphis*. The dolphin's retina was shown to have two areas with increased density of ganglion cells. This fact is of utmost importance, indicating specific organization of vision in the dolphins.

The present paper is concerned with a topographic study on the density of ganglion cells in the retina of another species of dolphins—the porpoise *Phocoena phocoena L.* The aim of our study was to perform measurements enabling accurate mapping of ganglion cell distribution in the retina.

Material and methods

Four total retinal preparations of a porpoise *Phocoena phocoena* have been analysed.

The preparations were fixed in 10% formalin. Prior to the retina excision from the eyecup, the position of dorsal, ventral, nasal and temporal poles of the eyeball was noted with regard to the pattern of blood vessels; later the position of the poles on the retinal preparations was determined by the vessel pattern.

The retina was excised from the eyecup and separated from the vitreous body and coats. Radial cuts near periphery allowed a reasonable flattening of the retina on the object slides. The preparation was glued to a slide (with a ganglion cell layer upward), stained according to the method of Pishinger with a 0.06% solution of methylene blue and placed into Appaty syrup without dehydration. The latter prevented the preparation shrinkage that affects the accuracy of calculations. This method was initially suggested by Stone (1965) for cat retina, and its modifications were widely used for studying the density of ganglion cells in the retina of other mammals (Hughes, 1977; Hebel & Hollander, 1979; Peterson & Row, 1980; Rapaport *et al.*, 1981; Oyster *et al.*, 1981; Long & Fisher, 1983; Stone, 1983; Budnik *et al.*, 1984). It was experimentally established that there was practically no staining of retinal elements located under ganglion cell layer (Stone, 1965, 1966). Such a preparation allows regular examination of the whole retina surface and measurement of ganglion cell density in all its areas.

To identify ganglion cells the following generally accepted (Hughes, 1975; Stone, 1965; Provis, 1979) criteria were used: the presence of a clear-cut cytoplasm ring surrounding the nucleus with well-stained Nissle substance and the presence of a clear-cut nucleolus.

The ganglion cells were counted over the whole surface of the retina regularly at 1 mm intervals in 0.15 mm^2 squares. The results of counting were used to calculate the number of cells per 1 mm^2. These data served as the basis for mapping ganglion cell density distribution over the surface of the retina, with the overall number of ganglion cells in the retina calculated. The cell size was determined by measuring long and short perpendicular diameters, and the mean of the two values was considered to be the final result.

Results

Identification of ganglion cells. According to the criteria adopted, the cells of the surface layer with intensive staining of Nissle substance in the cytoplasm and lighter nucleus with stained nucleolus were considered to be ganglion cells. The presence of a clear-cut nucleolus helped to distinguish ganglion cells from the glial ones in cases where the cells were

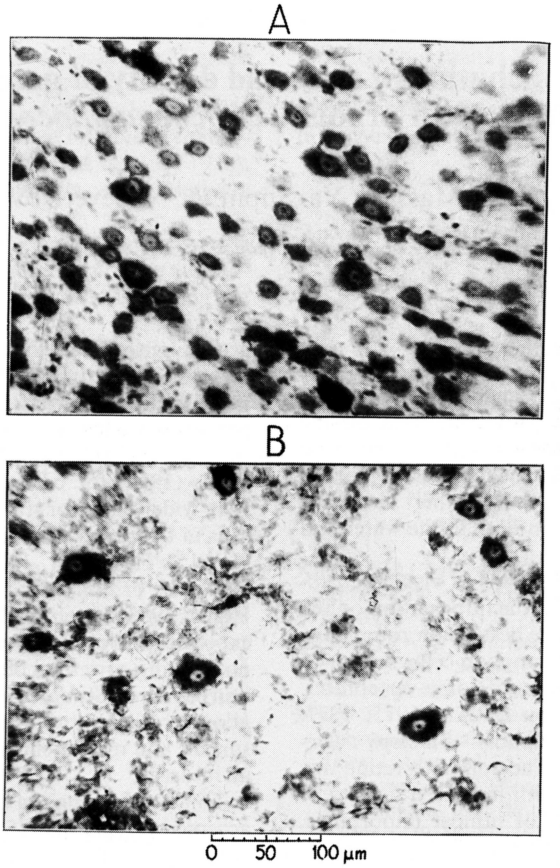

Figure 1. Ganglion cells in the retina of a porpoise. A—in the temporal high density zone, B—in the central low density zone.

very small. We found no reasons for separation type of cells with darkly stained nucleus, with scarcely discernible nucleolus and with coarser and dark Nissle substance (Type II cells by Dral, 1975, 1977).

The majority of stained cell bodies in the surface layer of the retina had typical polygonal shape indicative of the presence of several processes characteristic of ganglion cells. The cells with one process (amacrine cells) were rare.

Total number of ganglion cells. With an overall area of different retinal preparations being 675 to 747 mm^2, the total number of ganglion cells in the retina amounted to 96.5–133 thousands, respectively (mean 140–170 cells per mm^2).

Topographic distribution of ganglion cell density. All the preparations revealed considerable irregularity of ganglion cell distribution in the retina (Figure 1). Illustrative measurements made on one preparation are presented in Figure 2. In the temporal section of the retina there is area containing up to 700 ganglion cells per mm^2 and in the nasal section—area containing up to 500 cells per mm^2. In other retinal areas ganglion cell density is lower, with it being less than 100 cells per mm^2 in the periphery of the retina and near the optic disk.

The measurements served as a basis for mapping ganglion cell density in the retina (Figure 3). Distribution of cells on the maps is marked with lines of equal density, plotted according to the measurements with minimal smoothing.

The maps demonstrate the presence of two well delineated zones of high ganglion cell density in the temporal and nasal parts of the retina. The highest density (more than 700 cells per mm^2) is observed in the temporal area, with a maximum density in individual samples reaching 705 cells per mm^2. The

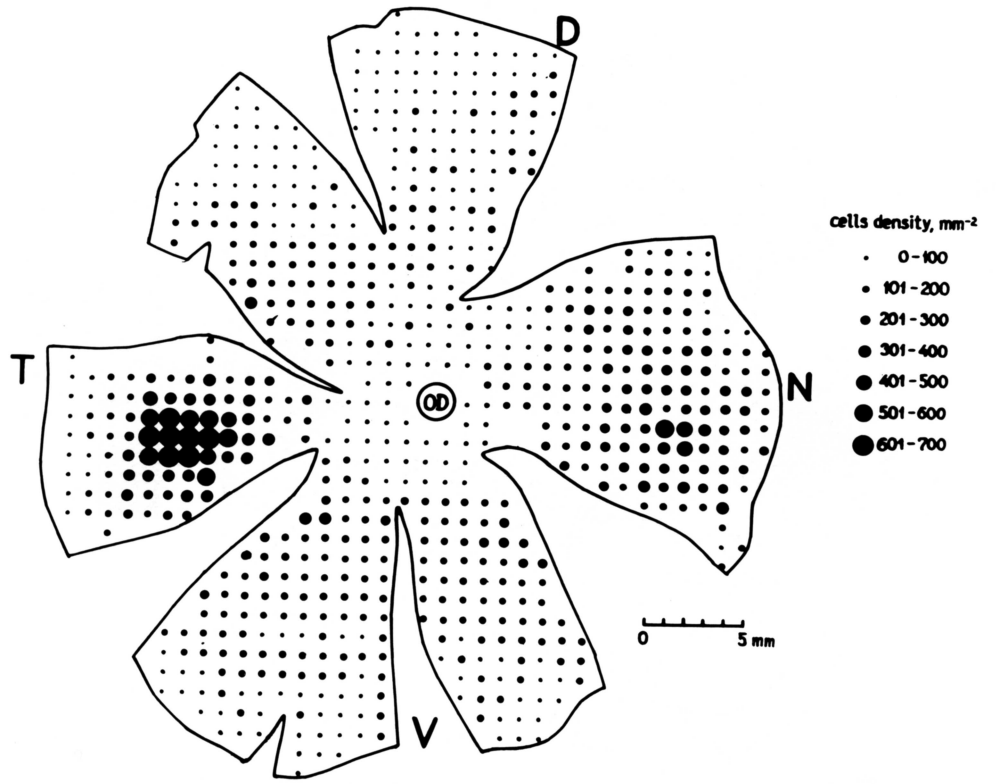

Figure 2. The results of ganglion cell density measurement on the total retinal preparation. The number of ganglion cells per mm^2 is designated by the diameter of the circles according to the scale presented on the right. D, V, N. T—dorsal, ventral, nasal and temporal poles of the retina.

density of cells in the nasal zone is somewhat lower, usually not exceeding 500 cells per mm^2.

In the central retinal part adjacent to the optic disc ganglion cell density is very low—less than 100 cells per mm^2. In the range of 2–2.5 mm from the optic disc ganglion cell density is about zero; only individual cells are observed. This central zone of low density separates high density zones in the temporal and nasal parts.

In the dorsal and ventral parts of the retina ganglion cell density is considerably lower than in the temporal and nasal ones. The lowest cell density was observed in the dorsal zone, where in most areas it did not exceed 200 cells per mm^2 and in some areas was less than 100 cells per mm^2. In the ventral part there is an area of somewhat higher cell density (up to 300 cells per mm^2); this narrow area connects the temporal and nasal zones of high density.

In high density zones (temporal and nasal) there is a tendency toward more orderly distribution of ganglion cell in radial rows, with the cell bodies stretched in the same direction (see Figure 1A). Low density zones showed no orderliness in cell distribution.

Continuous maps of ganglion cell density in the retina were reconstructed on the basis of the maps reflecting ganglion cell distribution in the preparation. In doing so, the shape of the retina was approximated to be a hemisphere, represented on the map in spheric coordinates. Though the true shape of the retina is not an exact hemisphere, such approximation is quite possible to show the basic regularities of ganglion cell distribution in the retina.

An example of the reconstructed map is present in Figure 4. The map shows that the temporal and nasal zones of high density are situated in the vicinity of the horizontal retinal diameter, 50–70° from the geometric centre of the retina.

With the radius of the retinal hemisphere being about 11.5 mm, a 1° distance corresponds to about 0.2 mm, i.e. 1 $degree^2$ corresponds to 0.04 mm^2. Thus, ganglion cell density in the temporal and nasal

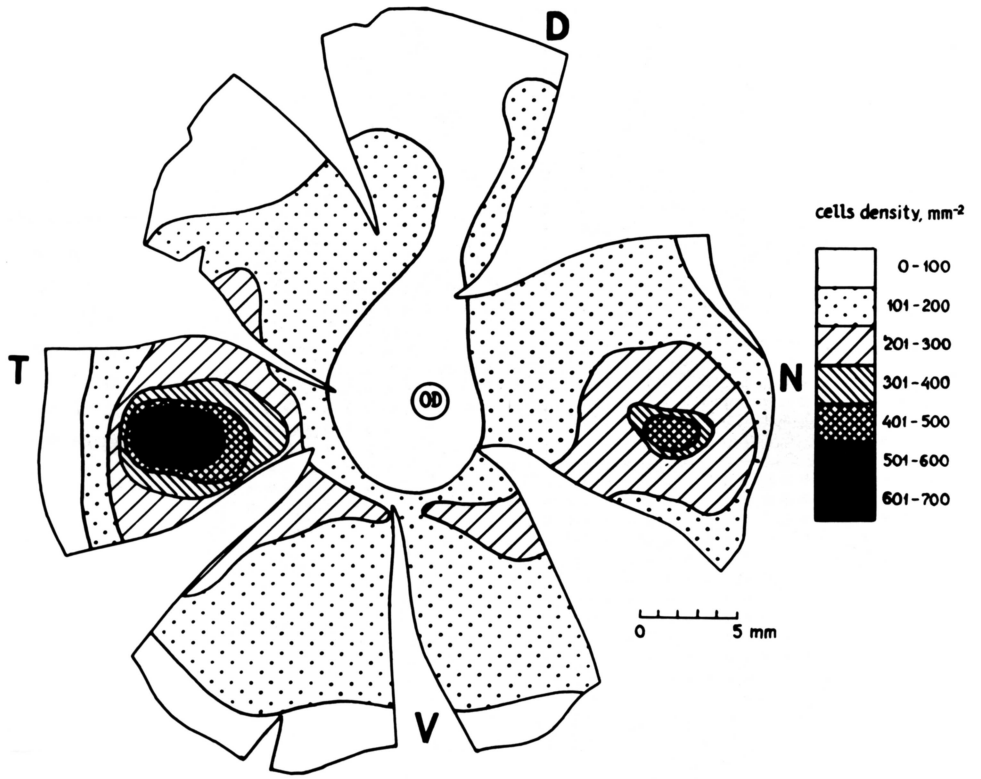

Figure 3. Distribution of ganglion cell density in the retinal preparation. Cell density is designated by isodensity lines and by shading according to the scale presented on the right. The preparation is the same as in Figure 2. For abbreviations see Figure 2.

zones—700 and 500 cells per mm^2—corresponds to 28 and 20 cells per degree^2. In low density areas it amounts to 8 cells per degree^2 in the ventral zone and part of dorsal zone and less than 4 cells per degree^2 in part of the dorsal zone and near the optic disk.

The data on ganglion cell density presented as a continuous map with spheric coordinates allows a direct comparison of the results obtained on different retinal preparations. Figure 5 presents a map showing combined results obtained on the four preparations studied. To avoid map overloading, only two characteristic isodensity lines are depicted here: 12 and 24 cells per degree^2. It is evident that the position of high density zones is similar in all the preparation. The highest density is noted in the temporal zone in the vicinity of a horizontal diameter; zones with the density exceeding 24 cells per degree^2 for all the preparations cover the area from 50 to 70° from the centre of the retina. A less marked increase of density in the nasal section covers a greater area: zones with the density exceeding 12 cells per degree^2 spread from 35 to 90° in individual preparations, covering an area from 55 to 70° in all the preparations.

Ganglion cell sizes in different parts of the retina. Ganglion cell size was measured on three preparations in the most characteristic retinal areas: temporal and nasal high density zones and central and peripheral low density zones.

Figure 6 presents histograms of ganglion cell size distribution in different areas of retinal preparation. The histograms show that ganglion cell sizes range from 8 to 50 μ, with the bulk of cells having a diameter of 20–30 μ. Small cells, 8–12 μ in diameter, are not numerous and occur mainly in the nasal high density zone. Large cells, over 35 μ in diameter, are present mainly in low density zones, mostly in the peripheral areas and sometimes in the centre of the retina; in high density zones these cells are rare.

Mean cell diameter slightly vary from one area to another; they are somewhat shorter in high density zones (Figure 5: 23 μ in the temporal zone and 21.1 μ in the nasal zone) and longer in low density zones

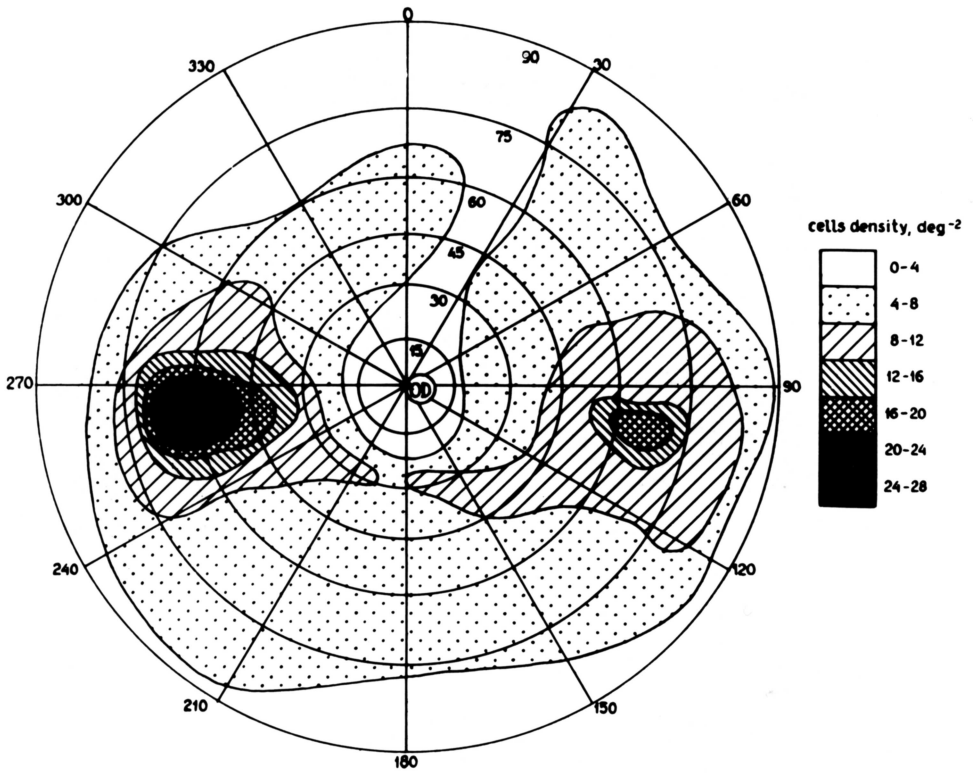

Figure 4. The reconstructed continuous map of ganglion cell density distribution in the retina. The map was obtained on the preparation described in Figures 2–3. Ganglion cell density is marked according to the scale presented on the right.

(25.1 μ and 27.2 μ in the central and peripheral zones, respectively).

Data on cell size distribution obtained on 3 preparations were similar. Figure 7 presents combined data obtained on 3 preparations in the form of summarized histograms. Though histogram dispersion is somewhat increased, the same regularity as in Figure 6 is observed: mean cell diameter is minimal in the nasal high density zone (21.5 μ), a little longer in the temporal high density zone (23.2 μ), still longer in the central low density zone (24.9 μ) and maximal in the peripheral low density zone (26.6 μ). The differences for each pair are small but statistically significant ($p < 0.001$).

Discussion

Comparison with other mammals has revealed a relatively large size of ganglion cells in the retina of a porpoise–20–30 μ according to our data. Large ganglion cells are also reported in other cetaceans (Pilleri & Wandeler, 1964; Shibkova, 1969; Perez *et al.*, 1972; Dawson & Perez, 1973; Andreev, 1974; Dral, 1977, 1983; Waller, 1982; Dawson *et al.*, 1982).

The presence of two high density ganglion cell zones in the dolphin's retina is of particular interest. These zones seem to be the areas of the best vision. They are located at some distance from the geometric centre of the retina, with ganglion cell density in the central area being very low. Higher ganglion cell density in the temporal zone as compared to the nasal one is indicative of the greater functional role of this area ensuring frontal vision.

The presence of two high density ganglion cell zones was earlier described for other dolphin species as well (Dral, 1975, 1977, 1983). These works reported higher ganglion cell density in the nasal area, while our data designate the temporal area as a zone of highest ganglion cell density. Further studies are necessary to elucidate if this discrepancy is due to inter-species differences or to counting inaccuracy.

The dolphin's retina with two zones of best vision differs from that of other mammals having one zone of best vision either in the form of a spot (in

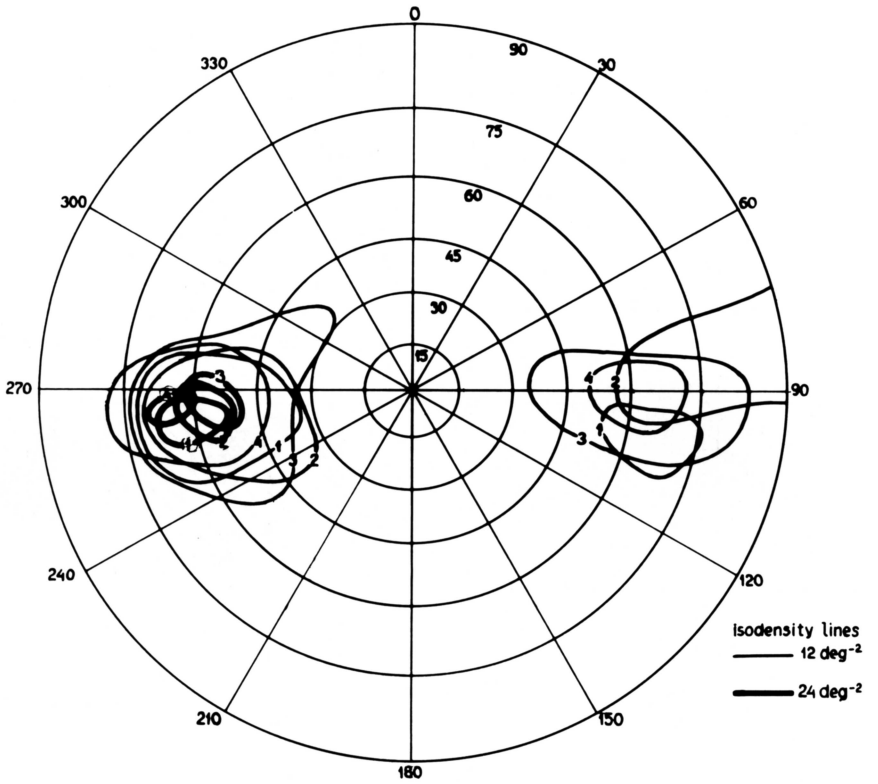

Figure 5. Combination of ganglion cell density maps obtained on 4 retinal preparations. Isodensity lines correspond to 12 cells per degree^2 and 24 cells per degree^2. Figures 1–4 on the isodensity lines indicate the number of a preparation.

carnivores and primates, etc.) or in the form of a streak (in rodents and lagomorphs) Hughes, 1977).

The position of high density ganglion cell zones on the maps (Figures 4, 5) is presented in spheric coordinates with regard of the centre of the hemisphere, approximating the retina, and not in visual field coordinates. However, the difference between the two coordinate systems seems to be minimal: the data on the structure of the dolphin's eye indicate that the optic centre of the eye is situated near the centre of the retinal hemisphere (Rivamonte, 1976). Thus, the spheric coordinates used can be roughly considered to be also visual field coordinates.

This suggests that high density ganglion cell zones correspond to visual field areas located 50–70° from the eye optic axis and that cell density with regard to visual field angle reaches 28 cells per degree^2 in the nasal area of the visual field and 20 cells per degree^2 in the temporal area of the visual field. If the mean distance between ganglion cells is considered to be about 1/D, where D is cell density, this value reaches 11–12′ in the nasal area of the visual field and 13–14′ in the temporal area. If the visual acuity is determined by the distance between the adjacent ganglion cells, the given values characterize the visual acuity of dolphin in areas of best vision. Note for comparison that measurements of ganglion cell density in *Tursiops*' retina gave visual acuity values of 10′ and 9.5′ for temporal and nasal areas, respectively (Dral, 1975, 1977). For *Delphinus* analogous data are 9.5′ and 8′ (Dral, 1983). Behavioural measurements of *Tursiops*' acuity gave values of 18′ (Pepper & Simmons, 1973).

It should be noted that ganglion cell density in high density zones of the dolphin's retina is not too great as compared to other animal species. For instance, in cats (Stone, 1965, 1966) and rabbits (Provis, 1979; Oyster *et al.*, 1981) ganglion cell density in best vision zone reaches 5000 cells per mm^2, in squirrels it is more than 20 000 (Long & Fisher, 1983), in monkeys—about 10 000 (Webb & Kaas, 1982), in some birds—tens of thousands of cells per mm^2 (Budnik *et al.*, 1984).

In dolphins this value, according to our data, does not exceed 700–705 cells per mm^2; in other dolphin species maximum ganglion cell densities were estimated to be 500–800 cells per mm^2. However, even at low ganglion cell concentration, differentiation of

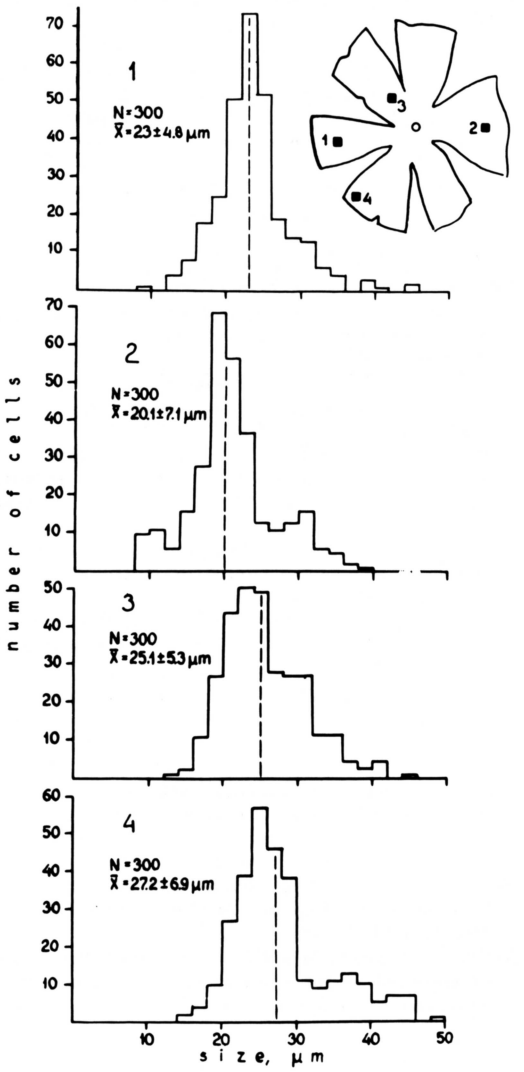

Figure 6. Histograms of ganglion cell size distribution in different zones of the retina. Measurements are made on one preparation. Measurement area of each histogram is marked by a square bearing the same number on the scheme of a retinal preparation: 1—temporal high density zone, 2—nasal high density zone, 3—central low density zone, 4—peripheral low density zone. Vertical intermittent lines on histograms indicate mean size values in each cell population.

the dolphin's retina is clearly manifested, indicating a specific organization of vision in these animals.

Summary

The distribution of size and density of ganglion cells was studied on total retinal preparations of a porpoise dolphin. Two high density zones were detected: temporal (density—up to 700 cells per mm^2 or 28 cells per degree^2) and nasal (density—up to 500 cells per mm^2 or 20 cells per degree^2). Both zones were located near the horizontal diameter, 50–70° from the geometric centre of the retina. In the central zone of the retina (near the optic disc) and in the peripheral zone ganglion cell density was low—up to 100–200 cells per mm^2 (4–8 cells per degree^2). The bulk of ganglion cells have diameters ranging from 20 to 30 μm. Diameters of some ganglion cells range from 8 to 15 μm. There were slight but significant differences in mean diameters of ganglion cells in different areas of the retina.

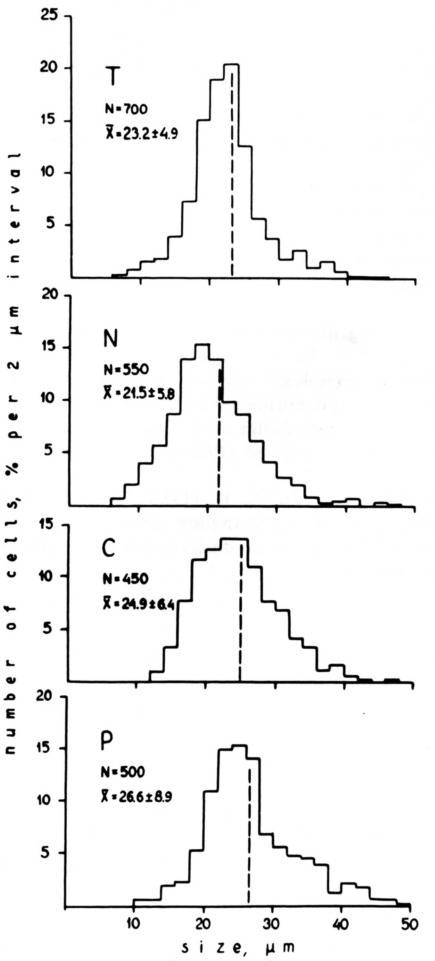

Figure 7. Histograms of ganglion cell size distribution in different areas of the retina. The data are combined results of measurements on 3 preparations. T—temporal high density zone, N—nasal high density zone, C—central low density zone, P—peripheral low density zone. Vertical intermittent lines indicate mean cell size.

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