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Distribution of ganglion cells in the retina of an amazon river dolphin *Inia geoffrensis*

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Introduction

The visual system of *Cetaceans* gives interesting examples of adaptation of sensory systems to different environmental conditions in which representatives of this order occur. Of particular interest is the family of river dolphins *Platanistidae* inhabiting tropical rivers of different regions of the world. Inhabitation in turbid water has markedly influenced the morphology of the visual system of *Platanistidae*. This influence manifests itself in the diminished size of the eyeball, visual nerve and oculomotor nerves, as well as in the alternation of the eye optics (Pilleri, 1977; Dral, 1981). These peculiarities are most pronounced in *Platanista*, least pronounced in *Lipotes* whereas an Amazon dolphin *Inia geoffrensis* occupies an intermediate position in this respect (Pilleri, 1977; Chen *et al.*, 1980).

The small eye size and other morphological peculiarities concerned with inhabitation in turbid water favoured the conception of the reduced vision in river dolphins (Pilleri, 1977; Zhou *et al.*, 1979; Chen *et al.*, 1980). However, all *Platanistidae* have laminar organization of the retina (Dral & Beumer, 1974; Pilleri, 1977; Li, 1983). The detailed study of the retinal ultrastructure (so far carried out only for *Inia geoffrensis*) showed no signs of regression; on the contrary, it appears that *Inia geoffrensis* retina has a well developed structure capable of visual perception (Waller, 1982).

To assess visual faculties of *Platanistidae*, it is important to acquire data on the topographic distribution of density of ganglion cells and photoreceptors over the surface of the retina. Special attention should be given to the zones of the maximum cell density and highest resolving power that are similar to the *area centralis* or visual streak in terrestrial mammals. The position of these zones on the retina, their size, shape and cell concentration are closely connected with the ecology of animals and serve as a basis for estimation of visual capabilities of the species (Hughes, 1977; Shkolnik-Jarros & Kalinina, 1986).

The present study is concerned with the topographic distribution of density and sizes of ganglion cells in the retina of an Amazon river dolphin, *Inia geoffrensis Blainville*.

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Material and methods

Seven total retinal preparations of an Amazon river dolphin have been analysed. The animals were caught in the basin of the middle stream of the Amazon tributary Ukajali. The animals were perfused with 10% formalin, and the retina was removed from the eyecup. Before the retina excision, the positions of dorsal, ventral, nasal and temporal poles of the eyeball were noted by the pattern of blood vessels; in later studies the preparation was oriented according to the vessel pattern. Radial cuts made near the periphery of the excised retina allowed reasonable flatting of the retina on the object slides. The preparation was glued to a slide with the ganglion cell layer upward, covered with filter paper and kept under a load for several hours. The preparation was stained according to method of Pishinger with a 0.06% solution of methylene blue and placed into Appaty syrup without dehydratation. This method makes it possible to stain selectively the retina ganglion cell layer under visual control. It is worthwhile to note that there is practically no staining of retinal elements located under the ganglion cell layer. Such a preparation allows regular examination of the whole retina surface and measurement of ganglion cell density in all its areas. This method and its modifications are widely used to study the density of ganglion cells in the retina of terrestrial (Hughes, 1977; Long & Fisher, 1983; Stone, 1983; Perry & Cowey, 1985) and water mammals (Dral, 1977, 1983; Li, 1983; Mass & Supin, 1986; Mass, 1987; Gao & Zhon, 1987).

The ganglion cells were counted over the whole surface of the retina regularly at 0.5 mm intervals in 0.084 mm^2 squares. The results of counting were used to calculate the ganglion cell density, i.e. the number of cells per 1 mm^2. These data served as the basis for

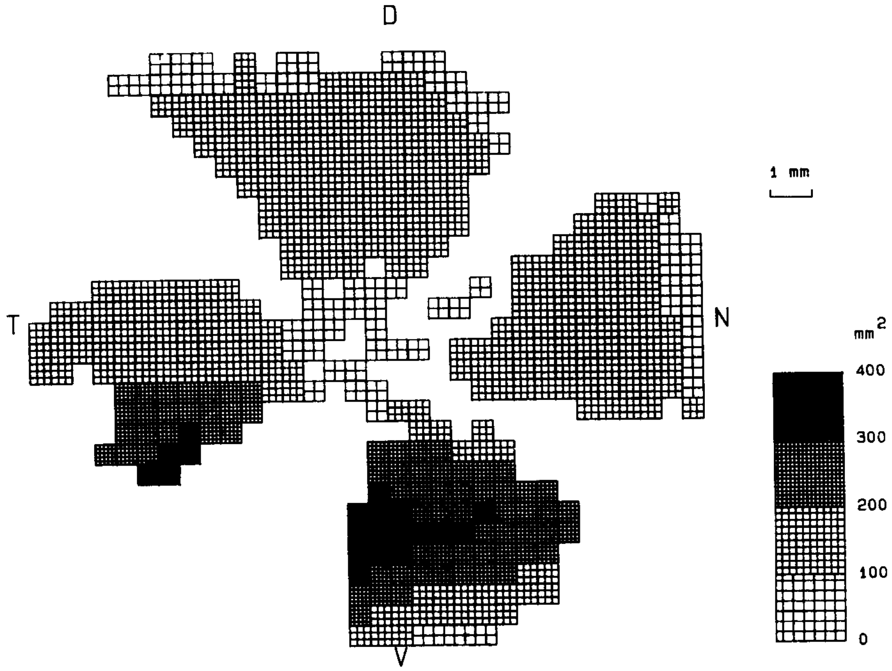

Figure 1. The map of ganglion cell density in the retina of *Inia geoffrensis*. The results of cell counting were subjected to computer smoothing as described in 'Methods'. The cell density is designated by shading intensity according to the scale on the right. Abbreviations: N, T, D, V—nasal, temporal, dorsal and ventral poles of the retina.

mapping ganglion cell density distribution over the surface of the retina with a 0.5 mm intervals. The computer smoothing of the maps, i.e. the calculation of the mean number in 1.5 × 1.5 mm squares, was carried out if necessary. The cell size was determined by measurlng the long and the short perpendicular diameters, and the mean of two values was considered to be the result.

Results

Identification of ganglion cells. To identify ganglion cells, the generally accepted criteria were used (Stone, 1965; Hughes, 1975; Provis, 1979). According to these criteria, the cells of the surface layer with intensive staining of Nissle substance in the cytoplasm and lighter nucleus with clear-cut nucleolus were considered to be ganglion cells. The nucleus might be located either in the centre of a cell or excentrically. Ganglion cells varied in shape but the majority of them had a typical polygonal shape with several (3–6) clearly visible processes. The processes were best distinguishable in large neurons.

Total number of ganglion cells. The overall area of *Inia's* retina ranged from 87 to 117 mm^2 with mean value for 6 preparations being 102 mm^2. The total number of ganglion cells amounted to 11,800–25,100 (the mean 14,900). The mean ganglion cell density was about 180 cells per mm^2.

Topographic distribution of ganglion cell density. All the analysed preparations revealed non-uniform distribution of ganglion cells over the surface of the retina. The maximum cell density was noted in the ventral section of the retina. Typical measurements of ganglion cell density made on one retinal preparation are presented in Fig. 1.

The highest cell density was recorded in the ventral section of the preparation where it exceeded 300 cells per mm^2. Maximum cell density for this preparation was 370 cells per mm^2, whereas in other preparations it ranged from 220 to 490 cells per mm^2. The mean value of maximum density for all preparations was 390 cells per mm^2. The high density area (more than 300 cells per mm^2) in the sample of Fig. 1 was about 5 mm^2. On the greater part of the ventral sector adjacent to this area the cell density exceeded 200 cells per mm^2. On the remaining retina surface (dorsal, nasal and temporal sectors) the ganglion cell density was considerably lower, usually less than 200 cells per mm^2.

In several preparations there were regions of increased cell density in other sectors of the retina.

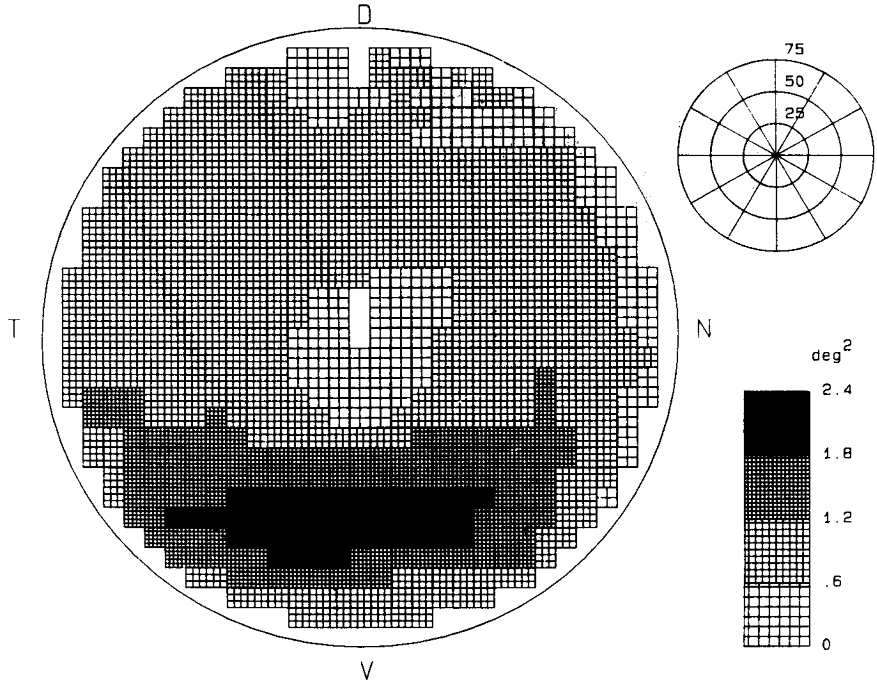

Figure 2. The reconstructed continuous map of ganglion cell density distribution in *Inia*'s retina. The preparation shown in Fig. 1 was used. The spheric coordinates in degrees are presented above on the right.

However, they were small and did not form a single zone like that observed in the ventral sector.

To assess the functional value of the obtained results it is important to estimate the ganglion cell density plotted on the visual field. The data on cell density were subjected to the computer transformation to be mapped on a hemisphere approximating the shape of the retina. According to our data, the distance between the lens centre and the retina in *Inia*'s eye was about 4 to 5 mm. With the radius of the retinal hemisphere taken to be 4.5 mm, a 1° corresponds to 0.08 mm. The transformed maps were used to calculate ganglion cell density as the number of cells per 1 degree^2.

An example of the continuous map constructed through transformation of the map in Fig. 1 is presented in Fig. 2. The map shows that the maximum ganglion cell density zone was situated 50° from the geometric centre of the retina towards its ventral part (i.e. towards dorsal part of the visual field). The density in this zone reached 2.3 cells per degree^2.

The data on ganglion cell density presented as a continuous map with spheric coordinates make it possible to integrate and average the results obtained on different retinal preparations. Fig. 3 presents a map showing the combined results obtained on 5 preparations studied. The highest cell density was noted in the ventral part of the retina (i.e. the upper part of the visual field) at a distance of 50° from the geometric centre. This zone with density exceeding 2 cells per degree^2 was surrounded by the area of lower density (more than 1.2 cells per degree^2), while in the remaining part of the retina the density was low, less than 1.2 cells per degree^2.

The data on cell density distribution were obtained with the overall number of ganglion cells counted. However, according to the histological criteria, two types of cells could be distinguished in the retina of *Inia geoffrensis* (Fig. 4). The major part was constituted of the lightly stained neurons with the size ranging from 10 to 40 μm. These cells had a wide rim of light cytoplasm surrounding even more light nucleus with dark nucleolus. Another group of cells was less numerous and consisted of large (more than 20 μm) darkly stained cells. In these cells dark cytoplasm with intensively stained Nissle substance surrounded the lighter nucleus with clearly discernible nucleolus. In different preparations the darkly stained cells constituted 9–21% of the total number of ganglion cells (mean 16%).

Since most ganglion cells were of the light type, the data on cell density presented here refers, in fact, only to the lightly stained cells. The distribution of large dark neurons was studied separately. The results are

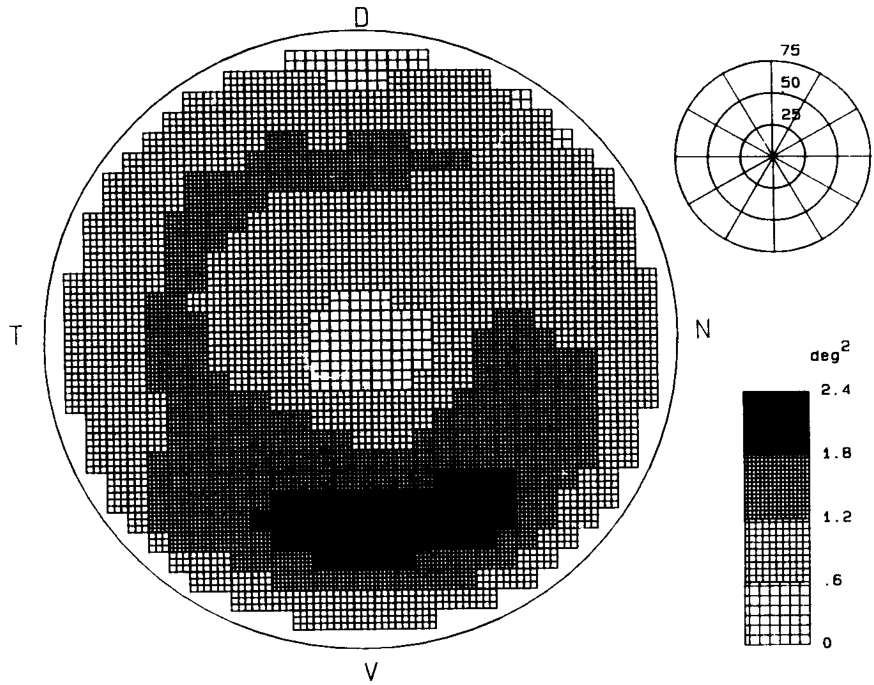

Figure 3. Averaged ganglion cell density continuous map obtained on five preparations.

presented as a map in Fig. 5. The density of this type of cells proved to be low and never exceeded 45 cells per mm^2. However, as in the case of the light cells, the highest density of dark neurons was registered in a relatively small area of the retina, in its ventral part, i.e. the same area as for light cells. Beyond this zone the density of dark cells was less than 30 cells per mm^2; in the other parts of the retina it was even lower, less than 15 cells per mm^2.

Ganglion cell size. Ganglion cell size was measured on 4 preparations in areas of different density. More than 1500 cells were analysed. Fig. 6 presents histograms of ganglion cells size distribution in the areas of high and low cell density. The histograms show that large size (from 10 to 38 µm) is characteristic of ganglion cells of *Inia geoffrensis*. The bulk of cells have a diameter of 15–30 µm. Small cells (10–16 µm in diameter) are not numerous. Large neurons with a diameter up to 40–42 µm occur in some individual samples.

No correlation between the size and the density of cells has been revealed. Cells of all the sizes within the indicated limits occur both in the zones of high and low density, with the average cell size being 23–26 µm in all the areas of the retina. The darkly stained cells were larger but approximately of one and the same size (mean 29.5 µm) in all the areas of retinal preparations.

Though the total population of ganglion cells consisted of two histologically distinguishable cell types, the aggregated histograms of ganglion cell size distributed virtually had a monomodal form (Fig. 6).

Discussion

The data presented here together with the results of other studies point to the large size of ganglion cells in the retina of *Inia geoffrensis* (Waller, 1982). Large ganglion cells are also reported in the total retinal preparation of *Lipotes* (Li, 1983) and some *Delphinidae* (Drall, 1983; Mass & Supin, 1986). Thus, large ganglion cells seem to be characteristic of the retina of many cetaceans (Pilleri & Wandeler, 1964; Shibkova, 1969; Dral, 1975; Dawson, 1980; Dawson *et al.*, 1982). We failed to detect small cells, less than 10 µm in diameter, in *Inia*'s retina. Such cells were not found in the total retinal preparations in other species of Cetacea (Dral, 1983; Mass & Supin, 1986) and in Goldgi preparations either (Shibkova, 1969).

The existence of two cell types different in size, staining intensity and ultrastructure is probably another specific feature of the retina of *Delphinidae* (Dral, 1975, 1977, 1983) and *Platanistidae* including *Inia geoffrensis* (Waller, 1982); it was confirmed by this study.

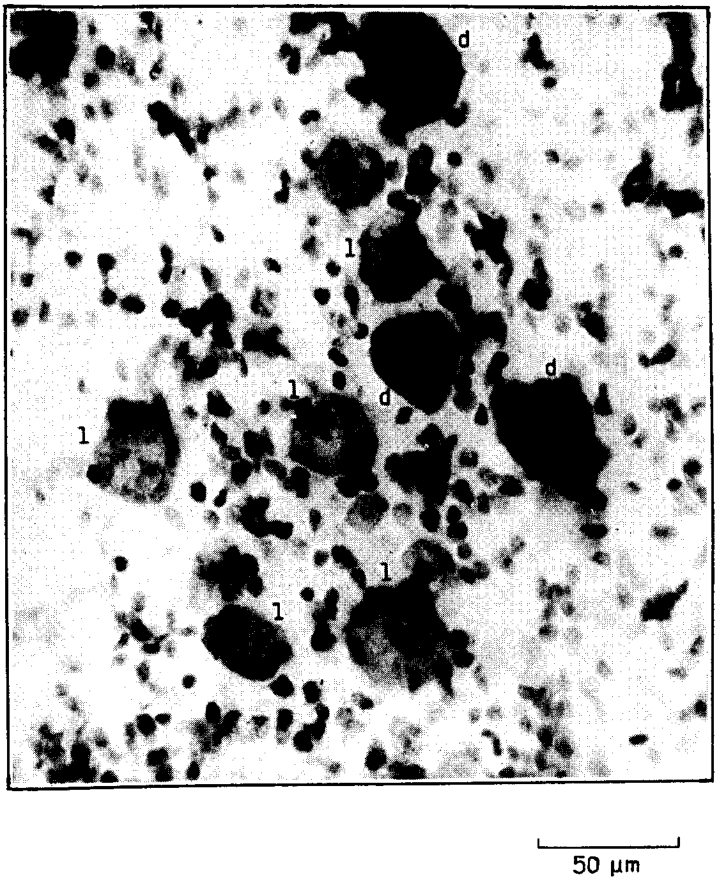

Figure 4. Retinal ganglion cells of various types; 1—light cells, d—dark cells.

However, in spite of the difference between two cell types, the histogram of ganglion cell size distribution in *Inia*'s retina has a monomodal form. This may be explained by the fact that the darkly stained cell group is not numerous. Besides, there are no distinct size bounds for different cell groups. The monomodal character of the ganglion cell size distribution was also described for other species of *Cetacea* (Dral, 1983; Mass & Supin, 1986).

The results obtained show the presence of a high ganglion cell density zone in the ventral part of *Inia*'s retina. Until recently it has not been clear if such zones actually exist in the retina of dolphins. The visual detection of such zones was impossible due to the absence of avascular areas in the sites of high density of ganglion cells and photoreceptors in the dolphin's retina (Dawson, 1987). It was only ganglion cell density study of total retinal preparations that allowed the identification of high density zones in the retina of dolphins. Recent studies (Dral, 1977, 1983; Mass & Supin, 1986) reported the presence of two high density zones in the retina of three dolphin species of *Delphinidae.* These zones were shown to be located in the nasal and temporal parts of the retina near the horizontal meridian. However, the results of similar studies on a Chinese river dolphin *Lipotes vexillifer* was undefined: LI (1983) failed to reveal the high cell density zones in its retina, while Gao & Zhou (1987) described the two zones of high density in the nasal and temporal parts of the retina.

Our data indicate that in distinction to other dolphins, the zone of highest ganglion cell density in *Inia*'s retina localized in its ventral part. Besides, even in this zone the ganglion cell density is relatively low and does not exceed 300–400 cells per mm, which corresponds to 2 cells per degree^2. Note for comparison that the highest cell density in the retina of dolphins *Delphinus delphis* (Dral, 1983), *Tursiops truncatus* (Dral, 1977) and *Phocoena phocoena* (Mass & Supin, 1986) amounts to 700 cells per mm^2 (it corresponds to 28 cells per degree^2 in *Phocoena phocoena* (Mass & Supin, 1986); in the retina of fur-seal *Callorhinus ursinus* it reaches 1200–1400 cells per mm^2 (Mass, 1987), while in most terrestrial mammals it

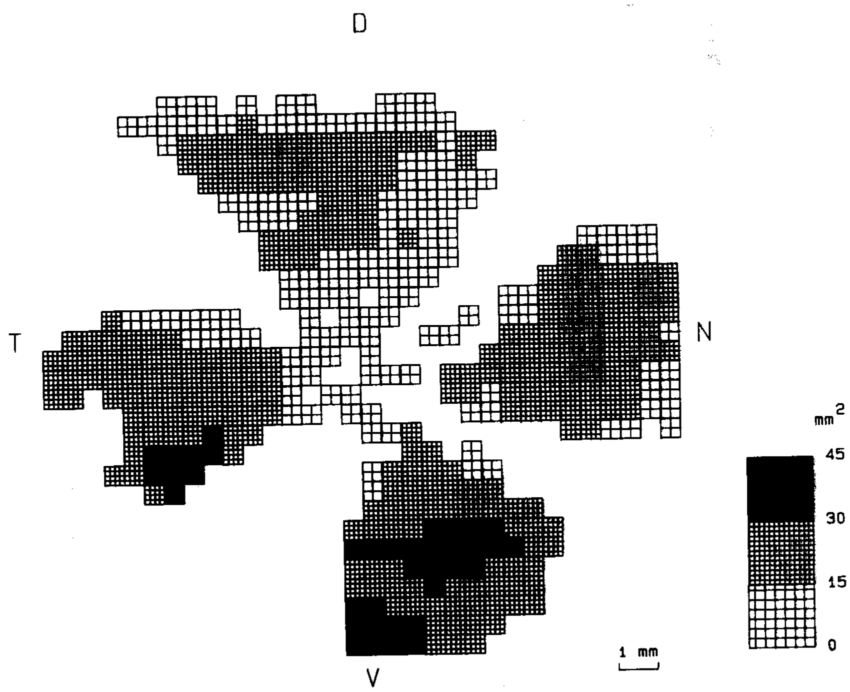

Figure 5. The map of dark ganglion cell density distribution in the retina of *Inia geoffrensis*. Abbreviations used are as in Fig. 1.

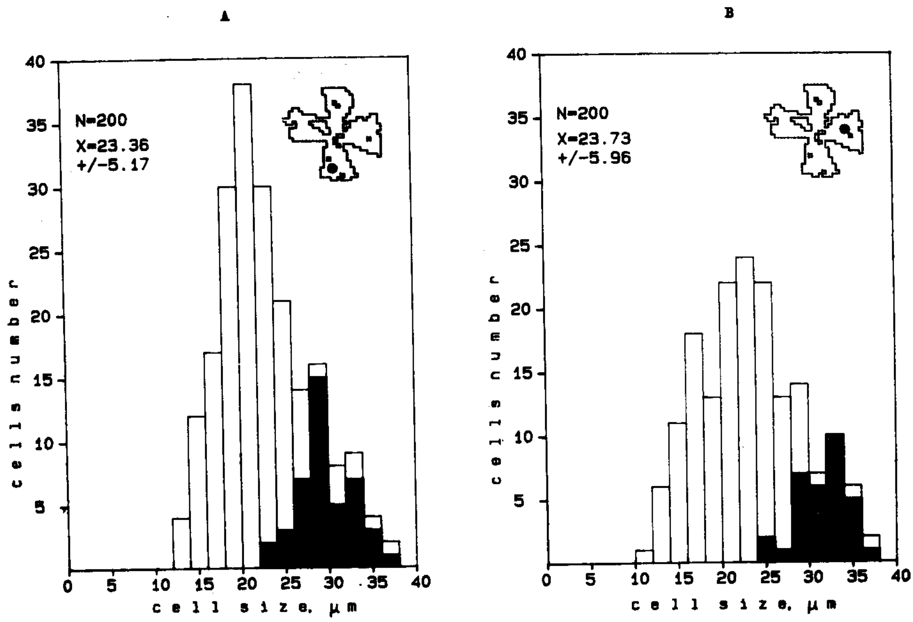

Figure 6. Distribution of ganglion cell size in the zones of different cell density of *Inia*'s retina. The light histogram was constructed with all cells taken into account; the dark histogram reflects size distribution only of the darkly stained cells.

ranges from thousands to tens of thousands of cells per mm^2 (Huges, 1977; Provis, 1979; Long & Fisher, 1983; Perry & Cowey, 1985; Wong *et al.*, 1986).

The data on ganglion cell density in the retina may serve as the basis for characterization of the visual acuity of animal species. Measurements of ganglion cell density in *Tursiop's* retina (Dral, 1975, 1977) gave the visual acuity values of 9.5′ and 10′ for nasal and temporal zones respectively; for *Delphinus delphis* the similar data were 8′ and 9.5′ (Dral, 1983) and for *Phocoena phocoena* 14′ and 11′ (Mass & Supin, 1986). For *Inia*'s retina with the highest ganglion cell density being about 2 cells per $degree^2$ these values calculated as $1/\sqrt{D}$ (where D is the cell density), are approximately 0.7° (40–45′). Thus, the visual acuity of *Inia geoffrensis* proved to be relatively low. However, the presence of the special zone of increased cell density in its retina suggests the possible involvement of this zone in the process of visual perception. Behavioural observations on dolphins in captivity also suggest that river dolphins are capable of visual perception (Phillips & McCain, 1964; Layne & Caldwell, 1964; Caldwell *et al.*, 1966; Pilleri *et al.*, 1980). The low visual acuity of river dolphins is possibly sufficient for vision in turbid water where only large objects and at a short distance are distinguished.

The unusual location of the high cell density zone in *Inia*'s retina is of particular interest. The similar zones in mammals with lateral localization of eyes are known to be situated near the horizontal diameter either in the form of a streak (Provis, 1979; Long & Fisher, 1983; Wong *et al.*, 1986) or in the form of two separate zones (in the other dolphins studied earlier—Dral, 1975, 1977; Mass & Supin, 1986). In mammals with frontal localization of eyes and acute binocular vision the high density zones are situated in the central or in the dorso-temporal parts of the retina (Stone, 1965; Stone & Johnson, 1981; Perry & Cowey, 1985). The high ganglion cell density zone in the ventral part of *Inia*'s retina is an example of specific organization of the retina in these animals. For the present the accumulated information is not sufficient to interpret this phenomenon unambiguously.

Summary

The topographic distribution of density and sizes of ganglion cells has been studied on the total retinal preparation of an Amazon river dolphin, *Inia geoffrensis*. The highest cell density was detected in the lower part of the retina where it reached 300–400 cells per mm^2 or 2 cells per $degree^2$. Cell density measurements gave the visual acuity value of 0.7°. The size of ganglion cells ranged from 10 to 40 μm with the mean diameter being 23–26 μm. The histogram of cell size distribution has a monomodal form.

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