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THE RETINAL GANGLION CELLS OF *DELPHINUS DELPHIS* AND THEIR DISTRIBUTION

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Introduction

In Cetacea, studies of the density of the retinal ganglion cell population have only been done to some extent on *Tursiops truncatus* (PEERS, 1971; DRAL, 1975; 1977). In this species two areas with increased density of ganglion cells were observed, a distributional pattern known to exist in several representatives of the lower vertebrate classes, but which is exceptional in mammals. This paper primarily intends to contribute a comparable study on another cetacean species, viz. *Delphinus delphis.*

Besides, however, the collected data offer an opportunity to address the problems which have been raised because of the predominantly large size of the ganglion cell bodies in cetacean retinae. In many, if not all, mammalian retinae relatively large ganglion cells are found, but only in small numbers. In the cat for instance a few cells up to nearly 40 microns have been observed (FUKUDA and STONE, 1974). In Pinnipedia the ganglion cells range up to some 50 microns (NAGY and RONALD, 1970), but even these are moderate as compared with the really giant ones in Cetacea: up to 90 microns in Odontoceti (toothed whales) and even larger in Mysticeti (baleen whales). Ganglion cells exceeding 20 microns, being considered as "giant" in cats (HUGHES, 1975), are quite common in the cetacean retina.

Some morphological variants of cetacean ganglion cells and size groups have been described previously, including statements about their distribution (summarized by DRAL, 1977), probably in the hope to find any aspect which might be helpful in arriving at a functional interpretation. The material presented in the present paper enables an evaluation of these statements.

Material and methods

The eyes of *Delphinus delphis* were obtained from animals caught in a trawl east of Napier (New Zealand), January and February 1972. The specimens were collected and preserved in 10% formaldehyde by Mr. F.D. Robson, New Zealand. Judging from the condition, preservation must have taken place shortly after the death of the animals.

Whole mounts - By means of a circular cut through the limbus the cornea, iris and lens were removed, after which the bulbus was bisected vertically. In water, the vitreous body was removed from the rostral and temporal parts as much as possible, subsequently the retina was dissected. Radial cuts near the periphery allowed a reasonable flattening of the retinal fragments on gelatinised object slides. These were lifted from the water and covered with filter paper, wetted with a 4% formaldehyde solution. With another slide on the top the preparations were left under some pressure in the fixing fluid for half an hour. Subsequently the slides were placed upright in a glass of water, facilitating the filter paper to float off, leaving the retinal fragments fixed to the slides.

Shrinkage - In this stage the preparations were photographed, which was repeated after staining, dehydration and mounting. Comparison of distances between recognisable landmarks on both photographs revealed that overall shrinkage was negligible (less than 3%). However, an appreciable and variable shrinkage had taken place in a narrow zone along the edges. As far as

the retinal ora is concerned, where ganglion cell densities are very low, this is of no importance. More consequences can be expected along the radial cuts, especially where these pass through regions of high cell densities. Because this shrinkage is limited to one direction, only perpendicular to the edge, an artificial pattern results, showing a "regular orientation" of the ganglion cells. We will return to the effect of shrinkage at these locations in the presentation of the results.

Comparison of the two photographs mentioned above leaves out of account a shrinkage which may be caused by the preservation of the specimen. The fact, however, that the retinae used in this work were lying in the bulbi in an apparently natural way makes us trust that in this stage shrinkage did not play an important part.

Staining - From a number of methods tried, cresyl violet and gallocyanin yielded usable results; for quantitative purposes, however, acid fuchsin (Ziehl Neelsen) appeared to give the most reliable results. By using 2½% of the stain instead of 10% and adapting the staining and differentiation times accordingly, it was possible to stain the nuclei and Nissl substance of the ganglion cells, while nuclei from deeper layers remained almost unstained. As a rule the ganglion cells of type I (to be described later) remain much paler than type II cells. The type I cells, however, stand out clearly in interference contrast illumination. This, combined with a green filter, made both types of ganglion cells equally well discernable.

Counting - The ganglion cells were counted in both eyes of one animal. On a number of sites, systematically dispersed over the preparations of the right eye (Fig. 1), the numbers of ganglion cells were counted in 9 adjacent fields of 0.24 x 0.36 mm, together forming a rectangular field of 0.72 x 1.08 mm. This number, multiplied by 1.286, gave the number of cells per mm^2. In addition counts were made in fields of 0.38 x 0.55 mm at 0.5 mm intervals in those regions (Fig. 1) where changes in cell density occurred at small distances. In the left eye the counts were restricted to such trajectories only. The data collected permit isopleths to be drawn with reasonable accuracy.

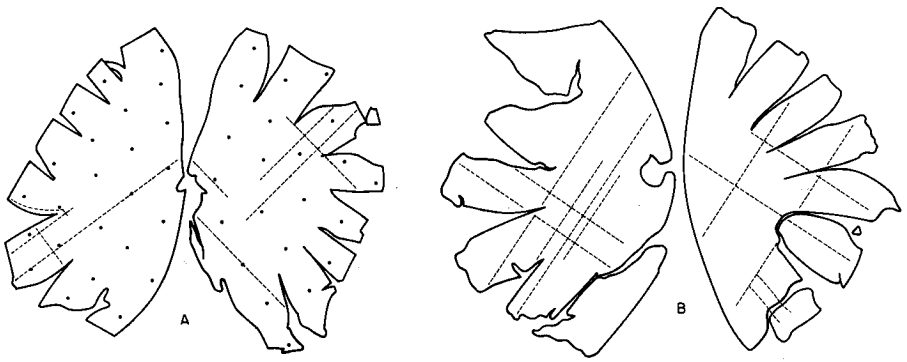

Fig. 1. *D. delphis.* Sketch of the flat mountings of the retinae of the right (A) and the left (B) eye. Indicated are the locations of counting and measuring as well as the trajectories of counting.

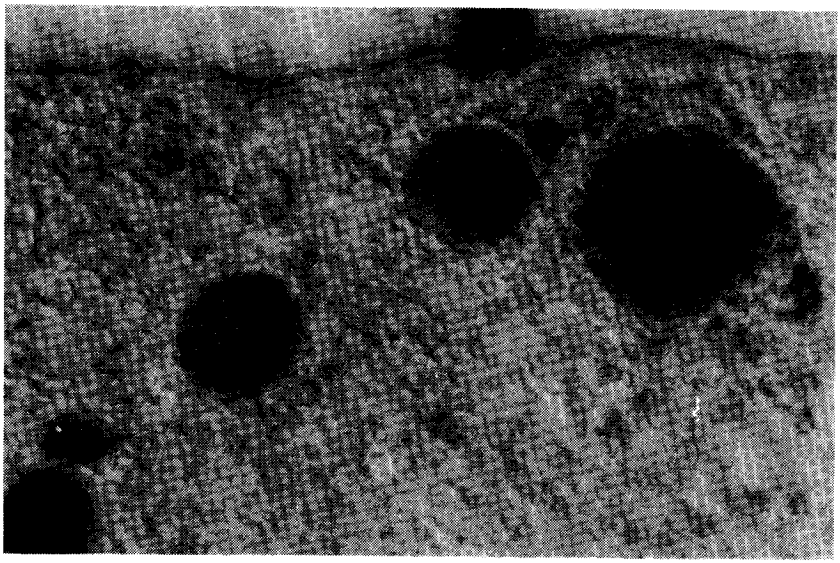

Fig. 2. *D. delphis*. Retinal ganglion cells of type I from the dorsal periphery. Acid fuchsin, interference contrast. Cell diameters 22, 23 and 36 microns.

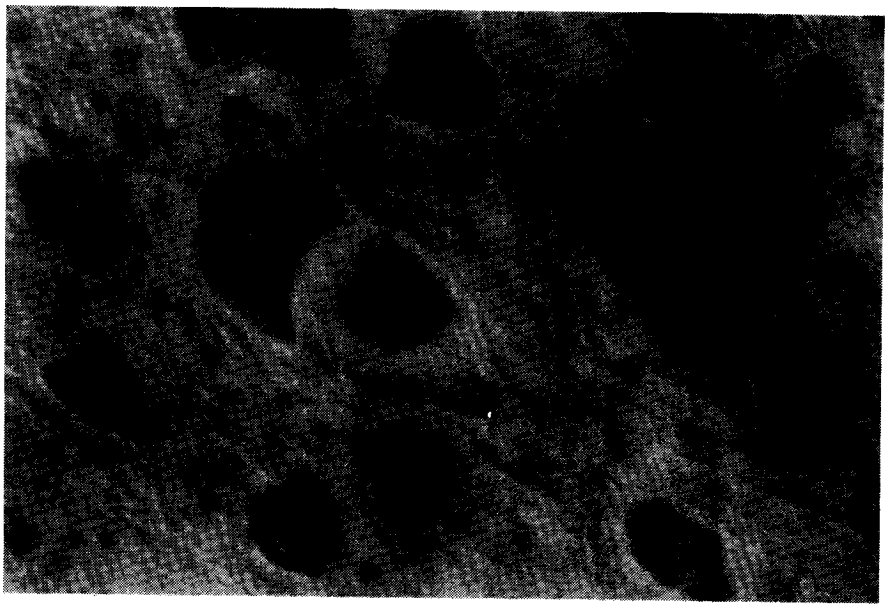

Fig. 3. *D. delphis* Retinal ganglion cells from the temporal periphery. Gallocyanin, interference contrast. A large, crescent shaped type II cell "embraces" a type I cell with centrally concentrated Nissl substance. Magnification as in Fig. 1.

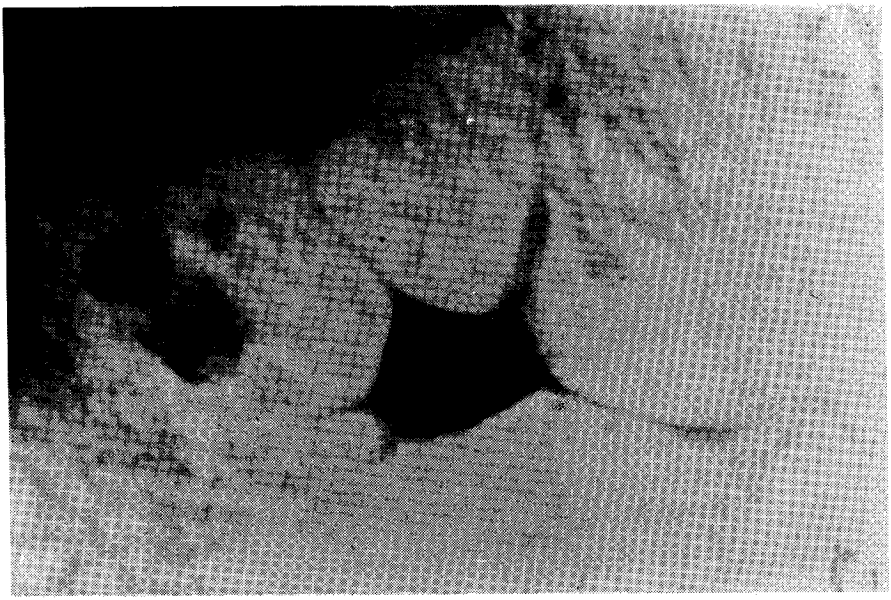

Fig. 4. *D. delphis.* Retinal ganglion cell of intermediate type (type I nucleus plus coarse Nissl substance) with axon and four dendrites. Cresyl violet. Cell diameter 42 microns.

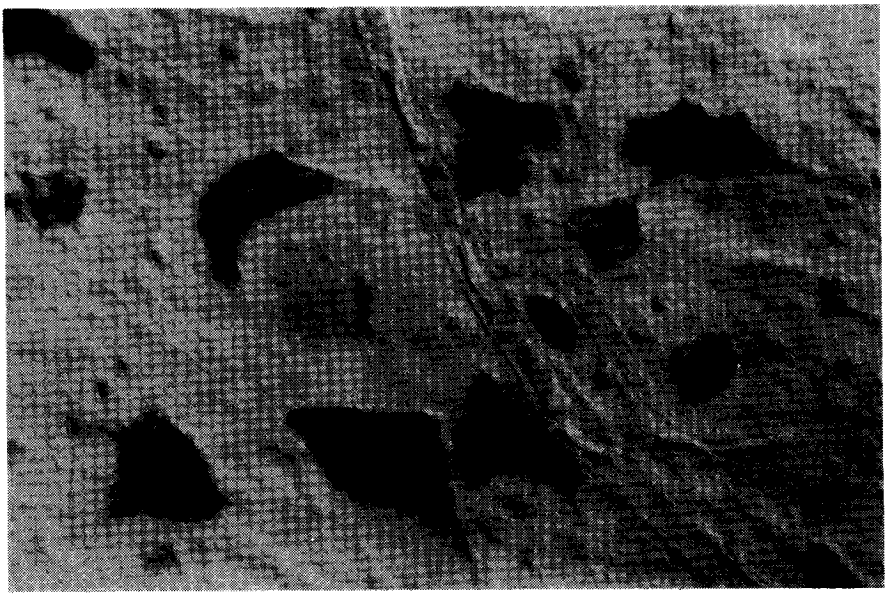

Fig. 5. *D. delphis.* Retinal ganglion cells of various types and partly with heterogeneously dispersed Nissl substance. Acid fuchsin, interference contrast. Cell diameters 20 - 25 microns; the "embraced" type I cell measures 36 microns.

Measurements - The diameters of the ganglion cells were measured at the same sites as indicated above, only in the right eye and not along the trajectories. We measured at a magnification of 320x in units of 3.03 microns. Generally the cell bodies are irregular in shape, the diameter depending on the direction of the axis which is measured. Considering that any average may be used as long as the size groups remain comparable, the cells were measured along the axis which happened to coincide with the (fixed) direction of the micrometer. In measuring thousands of cells, it is difficult and - considering the variation to be expected - also not necessary to maintain utmost accuracy. Therefore any cell, assigned to a certain size class, could equally well belong to either the left or right neighbouring size class. For this reason moving averages over two consecutive size classes were calculated.

Qualitative observations - Morphological studies were carried out mainly on the flat mountings described above. Sections of paraffin embedded material, stained with H.E., were used for additional observations.

Results

Morphology of the ganglion cells

As was the case in *Tursiops* (DRAL, 1975; 1977), also in *Delphinus* the ganglion cells were of two types. Their characteristics are the same in both species. The cells of type I (Fig. 2, 3) have a round or oval body, a pale staining nucleus up to 15 microns in diameter with a clear membrane and nucleolus and a fine Nissl substance in the cytoplasm. Type II cells (Fig. 5) have an angular outline, a darkly staining and more oval nucleus with a scarcely discernable nucleolus, no observable membrane and a much coarser and darker Nissl substance. The main difference between the types lies in the nucleus. With regard to cell body shape and content of Nissl substance a full range of intermediate cells has been found (Fig. 4, 5). Frequently cells contained a Nissl substance which partly had the characteristics of one type and partly of the other. Nearly all cells with intermediate appearances contained a type I nucleus and therefore have been considered to belong to that type.

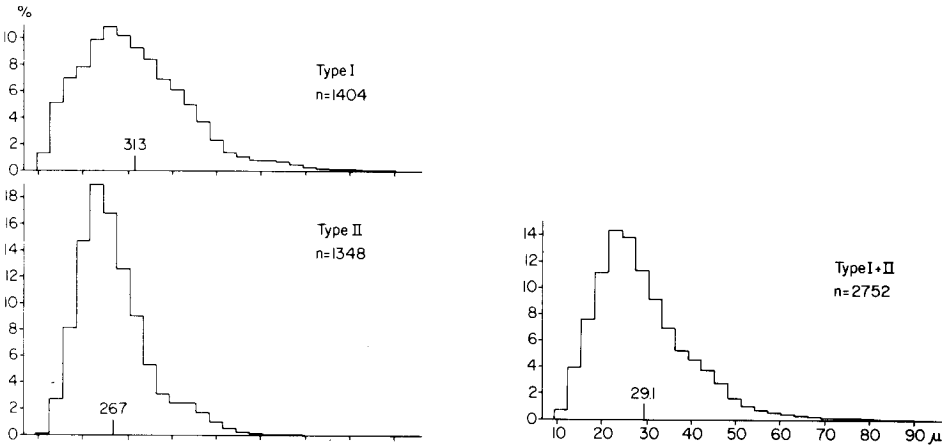

Fig. 6. *D. delphis*. Size frequency distribution of retinal ganglion cells.

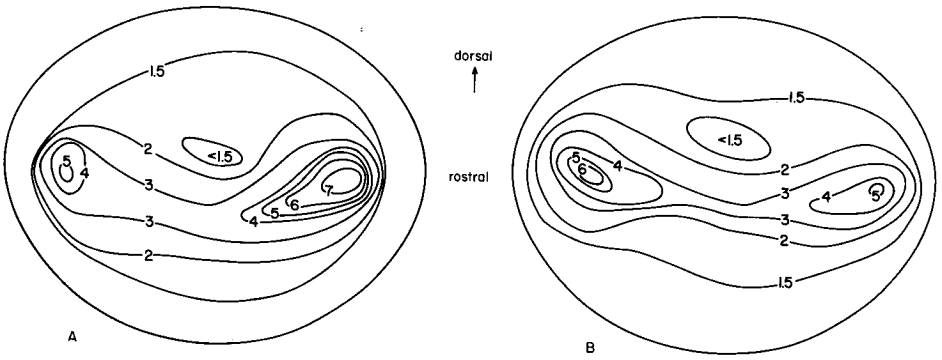

Fig. 7. *D. delphis.* Flat projection of the retina of the right (A) and left (B) eye, showing the numbers (in hundreds) of ganglion cells per mm^2.

So called "embracing" ganglion cells (Fig. 3, 5) have a crescent shaped cell body, closely adhering to another ganglion cell body. The latter was always a type I cell, the embracing one usually a type II. In those regions where exclusively type I cells were found (the periphery), the embracing variant showed intermediate characteristics. There were no indications that embracing cells were more abundant in the regions of the areae, as has been reported for *Tursiops* (DRAL, 1977).

The ganglion cells varied in size between 10 and 88 microns in a continuous range (Fig. 6). The smaller ones were barely more than a nucleus, surrounded by a narrow edge of cytoplasm, containing Nissl substance. Cells of more than 75 microns were only found in the periphery of the retina, in the regions where only cells of type I were present. Cells of about 70 microns could be found all over the retina, but they were rare. Less than 1% of the total ganglion cell population measured over 65 microns, of which there were 3-4 times as many of type I than of type II. On the average type I cells were larger: 31.3 microns against 26.7 microns for type II cells. Because there were slightly more type I than type II cells (51 against 49%), the average size of all ganglion cells was found to be 29.1 microns.

Density of the ganglion cell population

Fig. 7 presents the density distribution of the ganglion cells in flat projections of the retina. One should keep in mind that projection of the cup shaped retina on a flat plane brings about distortions, especially in the outer parts.

There were two distinct maxima in each eye, both located slightly ventral from the geometrical median of the bulbus, one at the temporal and the other at the rostral side.

As in *Tursiops* (DRAL, 1975; 1977), in *Delphinus* a higher density was found at the rostrally located, so-called "central" area, as compared to the temporal area. The former also occupied a larger area than the latter in both species.

A few remarks should be made on the central area of both eyes. In the right eye a maximum of 800 cells per mm^2 was counted. At that spot, however, the cells seemed to be orientated in neat rows, an indication that local shrinkage had taken place. At 5 mm from that spot the next highest value of 791 cells per mm^2 was found. In the left eye a radial cut in the tissue, passing through the central area, prevented the determination of its core. It is however likely that the maximum cell density exceeded a value of 666 cells per mm^2, which was found at a nearby undistorted spot. In some whole mounts of retinae from other *Delphinus* eyes, which were not

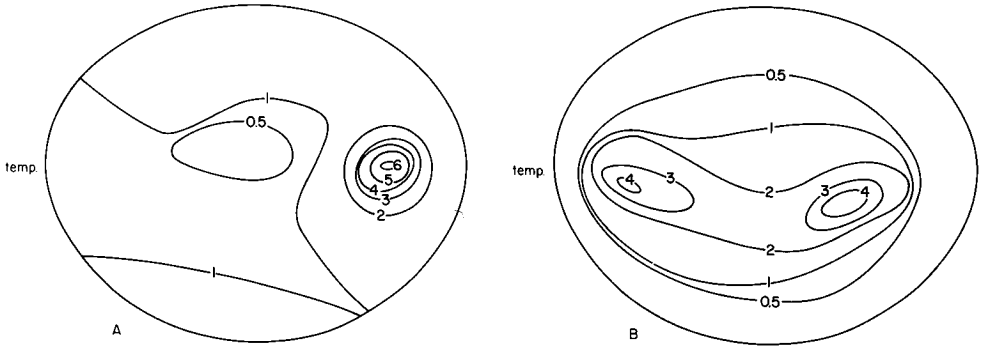

Fig. 8. *D. delphis.* Flat projection of the retina of the right eye, showing the numbers (in hundreds) per mm^2 of ganglion cells of type I (A) and type II (B).

subjected to systematical counting, the locations of the areas were sought by microscopical observation. In these preparations we found as maxima in the central area 647 and 754 ganglion cells per mm^2. From the total of four eyes it may be concluded that the central area in *Delphinus* contains 650-800 ganglion cells per mm^2.

The maximum of the temporal areas could be established without the above difficulties. We found 527 and 556 ganglion cells per mm^2 in the right and left eye respectively. The two additional eyes showed maxima of 512 and 540 cells per mm^2 at the temporal area. The conclusion from these four eyes may be that the temporal area in *Delphinus* contains 500-550 cells per mm^2.

Both areas of *Delphinus* were interconnected by a "visual streak" with a density of 300-400 cells per mm^2. Such a streak is not nearly as pronounced in *Tursiops* (DRAL, 1975; 1977). As in the latter a region with relatively low cell densities is found around and dorsally from the optic disc.

Distribution of cell types and size groups

If in the right eye the ganglion cells of type I and type II are regarded separately (Fig. 8), the picture for the type I cells is not nearly as clear as that found in *Tursiops* (DRAL, 1975; 1977). The ratios of the numbers of both cell types, although gradually changing from locality to

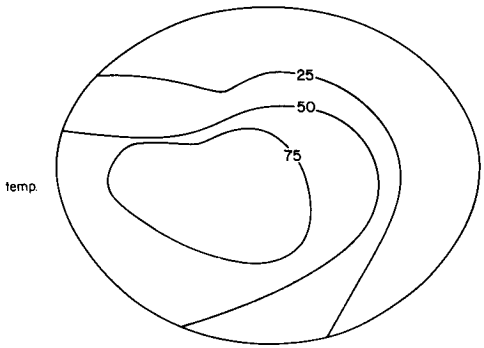

Fig. 9. *D. delphis.* Flat projection of the retina of the right eye, showing the share of type II ganglion cells, expressed in percentage of the total ganglion cell population.

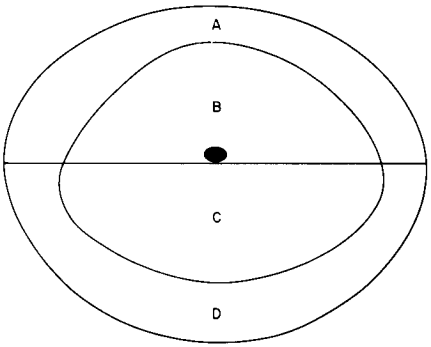

Fig. 10. *D. delphis.* The different regions of the retina as characterised by the size frequency distribution of the ganglion cells.

locality, reveal an asymmetric picture (Fig. 9). In *Tursiops* a quite different pattern of concentric rings was found. In counting the ganglion cells of the left eye, both types were noted separately only on a resticted number of locations, insufficient to make the relevant isocount drawing. From the available data, however, it can already be inferred that such a drawing would not conform to either the pattern in the right eye or to that found in the eye of *Tursiops.*

A survey of the size frequency diagrams for all measuring sites separately made clear that, according to their shape as well as retinal locality, the diagrams could be divided into three groups. The dorsal periphery (Fig. 10, A) was characterised by very broadly shaped diagrams, the dorsal eye fundus (Fig. 10, B) by narrower ones, peaking at about 30 microns. There was no clear difference between the central part and the periphery of the ventral half of the eye (regions C and D in Fig. 10), both being characterised by diagrams with a relatively sharp peak below 25 microns. They are presented separately here as region D contains cells larger than 75 microns - a peripheral characteristic - while in all other respects this region sharply contrasts with the dorsal periphery. Fig. 11 shows the cumulative size frequency distribution for each region. The average cell size decreases from dorsal to ventral, from 31.6 via 31.1 and 27.3 to 27.2 microns.
Both RITTER (1864) and SIRENA (1872), studying Mysticete eyes, suggested that the size of the ganglion cells may depend upon their local density. In such a case one might expect that the number of ganglion cells per unit of surface is inversely related with the square of the diameter of the cells. In the ganglion cells of *Delphinus* such a correlation is not found ($r = 0.4$). Apparently the cell sizes are not limited by the available space. Indeed, even at the highest densities (800 cells per mm^2) cells of all sizes, up to 60 microns, are present and find sufficient space to remain distributed at one level.

Discussion

The histologist should always be aware of the possibility of artifacts. As in *Tursiops* (DRAL, 1977), this danger was felt again in discriminating the two types of ganglion cells in the present material. The manyfold intermediate appearances and especially the distribution of both cell types, which differend in the material thus far (two eyes of *Delphinus* and one of *Tursiops*), evokes strong doubts. However, the same arguments as have been given for the *Tursiops* retina (DRAL, 1977) can be used against this suspicion. Especially the mixed presence of the types,

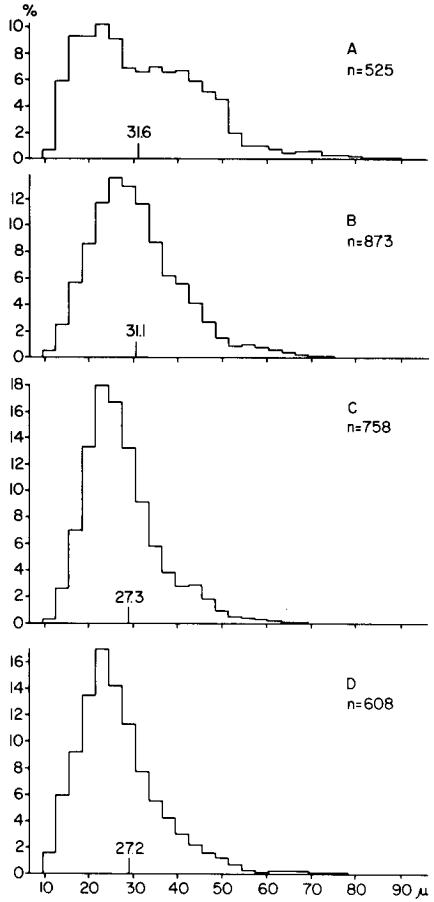

Fig. 11. *D. delphis.* Size frequency diagrams of the ganglion cells in the regions as indicated in Fig. 10.

many times in close contact with each other ("embracing"), suggests strongly that differences between the two types must exist. Two aspects seem beyond doubt: (1) both types must be considered to be functional ganglion cells (DRAL, 1975) and (2) we observed similar types in several terrestrial mammals (pig, chamois, elephant, Malayan bear, fox), hence, they are not characteristic for Cetacea.

Probably we missed a lot of smaller ganglion cells in our study. The presence of Nissl substance in the cytoplasm was used to distinguish these small ganglion cells from a host of other cells of similar sizes. By this criterion the number of ganglion cells smaller than 20 microns remained below 25% of the total population. This is in contrast to the findings of DAWSON (1980), who found that the smaller ganglion cells constituted a majority in three Odontocete species (*Tursiops, Kogia* and *Inia*). Only in *Balaenoptera*, a Mysticete, the reversed relation was found. The lack of smaller cells in our preparations may be explained by DAWSON's (1980, p 83)

remark that with some stains the small cells are not seen. Because, contrary to DAWSON, we did not find the smaller cells in our cresyl violet stained material either, we may add that also the fixation probably plays an important part. In this respect glutaraldehyde should be recommended. The different staining reactions imply that these cells differ in composition and thus probably also in function. SHIBKOVA (1969) observed in the retina of *Delphinus delphis* groups of small neurons, often surrounding a larger ganglion cell. These neurons did not send an axon to the optic nerve, but interconnected "real" ganglion cells (in the sense that real ganglion cells contribute an axon to the optic nerve). Such "association ganglion cells" were reported by DAWSON (1980) for *Tursiops* and *Kogia* (not for *Inia*), and also for some terrestrial species (DAWSON and LIEBERMAN, 1979). They apparently constitute another level of integration, supplementary to that of the horizontal and amacrin cells. We never saw any evidence of these neurons, probably because we did not apply the required preparational techniques. The above reasoning, however, implies that we did not necessarily miss any "real" ganglion cells.

In *Delphinus*, as in *Tursiops*, two regions were found where the ganglion cells reached a maximum density. Functionally these areas must represent the locations of best resolution of the visual image. The presence of a temporal area indicates the possibility of binocular vision in a field in front of the animal, which, of course, is enabled by the wide field of vision of each eye, combined with the fact that the geometrical eye axes make an angle of approximately 75° with the body axis (SCHENKKAN, pers. comm.) and by the ability of convergent movements of the eye. In *Tursiops* the temporal area was found to be located somewhat dorsally from the geometrical median of the eye. Therefore the corresponding field of vision must be directed a bit downward with respect to the body axis. The behaviour of *Tursiops* supports this conclusion (DRAL, 1977). In *Delphinus* the temporal area is located at or slightly ventrally from the median, which implies that the field of best vision lies straight ahead or slightly upwards. If, as seems to be the case in *Tursiops* (DAWSON, 1980), also in *Delphinus* the eye is unable to make cyclotorsional movements, this upward direction of view must be a rather fixed one and consequently it should be observable in the animal's behaviour. We are not aware of any observation in this respect.

The other, "central" area of best resolution is located in the ventro-rostral quadrant of the retina. Owing to the positioning of the eye in the animal's head, the geometrical axis aiming about 15° downward, this area will receive light from lateral directions.

In the last paragraph the logical assumption has been made that the resolution of the visual image will be better as the ganglion cell density is higher. One step further leads to the assumption that the minimum angle of resolution is determined by the extent of the image which is served by one ganglion cell. However oversimplified as this assumption may be, it has appeared that indeed a fair idea about an animal's visual acuity can be obtained by calculation of such a "morphologischer Sehwinkel" (von BUDDENBROCK, 1952). To this end one should know, apart from the density of ganglion cells, the dioptrics of the eye. Nothing is known about the latter in *Delphinus* but, as the dioptrics are related to the size of the bulbus, it is possible to deduce some speculative thoughts.

From measurements on seven *Delphinus* eye bulbi an average horizontal and vertical diameter of 35.1 and 31.1 mm respectively are found. For fourteen *Tursiops* eyes these values were 34.9 and 32.2 mm. Apart from the *Delphinus* eye being somewhat more ovally shaped than the *Tursiops* eye (which can be neglected in this context), the dimensions of the bulbi of both species do not differ significantly. It seems justified therefore to accept that in *Delphinus* the focal length equals that of *Tursiops*, being 16 mm with the eye in water (DRAL, 1975). This

means that one mm along the retina comprises 215 minutes of arc. In the temporal area of *Delphinus* per stretching mm, $\sqrt{500}$ to $\sqrt{550}$ ganglion cells are present, each serving 9.6 - 9.2, say 9.5 minutes of arc. In a similar way we arrive at 8.4 - 7.6, say 8 minutes of arc as the minimum resolving angle at the rostral area. These values compare well with *Tursiops,* where respectively 10.0 and 9.5 minutes were found (DRAL, 1975).

For two Mysticetes (*Eubalaena,* SIRENA, 1872; *Balaenoptera,* PILLERI and WANDELER, 1964) and one Odontocete (*Tursiops,* PEERS, 1971) it has been reported that the ganglion cell population falls apart in two separate size classes. In the present study of *Delphinus* we encountered a few peripheral sites, where the size frequency diagram showed two more or less separate peaks. This, however, was also found in the periphery of the cat's retina by FUKUDA and STONE (1974), who in addition demonstrated an extended "tail" of the largest cells in peripheral regions, similar to our findings as illustrated f.i. in Fig. 11, A. Therefore, these phenomena cannot be regarded as typical for Cetacea.
As a whole we found the ganglion cell sizes in *Delphinus* in a continuous range, in accordance with *Tursiops* as noted by PEREZ *et al.* (1972) and DRAL (1977). Not in accordance with *Tursiops* is the distribution of the larger cells, being predominantly present in central regions in this species (DAWSON and PEREZ, 1973; DRAL, 1977). In *Delphinus* we found them primarily in peripheral regions, as did RITTER (1864) in *Balaena* and SIRENA (1872) in *Eubalaena.* There seems to be no uniformity in various cetacean species in this respect.

Finally, the finding that embracing ganglion cells are found in a higher percentage at and around the areas in *Tursiops* (DRAL, 1977), is not confirmed for *Delphinus.*

We elaborated on these matters because they were raised in the literature in hopes of finding a clue to a functional interpretation. Now we may ask: What is, apart from their large size, so special about the cetacean retinal ganglion cells? Apparently nothing remains to justify the above hopes.

Acknowledgements.

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Summary

In the retina of *Delphinus delphis* two types of retinal ganglion cells were described. Both types together varied in size between 10 and 88 microns in a continuous range, the average being 29.1 microns. The ganglion cells of the dorsal part of the retina were slightly larger than those of the ventral part; the largest cells were predominantly found in peripheral regions. No relation between cell size and density could be demonstrated. Two areas with higher densities of ganglion cells were present, one at the temporal side with a maximum of 500 - 550 cells per mm^2 and one at the rostral side, with maximally 650 - 800 cells per mm^2, accounting for a calculated minimum resolving angle of 9.5 and 8 minutes of arc respectively.

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