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GENERAL BIOLOGY

Ganglion Layer Topography and Retinal Resolution of the Caribbean Manatee *Trichechus manatus latirostris*

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The Caribbean manatee *Trichechus manatus latirostris* belongs to the order Sirenia, a relict group of unique, completely aquatic mammals adapted to a herbivorous life style. The study of these animals is of significant interest for evolutionary and ecological neuromorphology.

Little is known about the sensory systems of manatees, and estimates of their visual capacity are contradictory (see review [1]). Researchers' estimates range from poor vision [2] to the existence of binocular vision [3].

Only a few reports on the manatee retina have been published. Piggins *et al.* [1] showed that the retina of the Amazon manatee *T. inunguis* is adapted to night vision. It contains almost no retina cones and very few ganglion cells [1]. According to the only comprehensive study [4], the retina of the Caribbean manatee has a completely laminar structure and contains both rodlike and conelike photoreceptors. However, the visual acuity of manatees has not yet been assessed quantitatively.

To solve this problem, we used a method based on the examination of the ganglion cell distribution in retinal wholemounts. This method allows the areas of the maximum cell density to be detected, and the retinal resolution to be calculated. The retinal resolution of some terrestrial [5] and aquatic [6–9] mammals has been determined with this method.

The goal of this work was to determine the distribution of ganglion cells in wholemounts of the retina of the Caribbean manatee *T. manatus latirostris* and to calculate the retinal resolution.

The experimental material was obtained through an exchange with Sea World, Florida; the exchange was approved by the US Fish and Wildlife Service (Certificate PRT-68453) and by CITES (Certificate US-770760).

Experiments were carried out on wholemounts of the retina of adult animals. The retina was fixed in 10% buffered formalin. Wholemounts were prepared by the method described in [10] with slight modifications [8] and stained with 0.1% cresyl violet by the method of Nissl [8].

Although ganglion cells in wholemounts of the retina of terrestrial mammals have been identified, the problem of identification of ganglion cells in retinal wholemounts of aquatic mammals remains unsolved and is particularly urgent in the case of exotic species. In this work, we used the identification criteria developed earlier for terrestrial mammals [10, 11]. The number of ganglion cells identified in our experiments in the retinal preparations of some other aquatic mammals [8, 12] was consistent with the number of optic nerve fibers in these animal species, which is known from the literature [13]. Therefore, the identification criteria used in our work were adequate.

The majority of cells identified by us as ganglionic were represented in the manatee retina by neurons separated by wide intercellular spaces. The cells had abundant cytoplasm with well-stained Nissl granules. The neuron nucleus was clear with a clearly visible nucleolus. Only a few neurons were difficult to identify.

The ganglion cells were counted systematically over the wholemount of the retina in 0.067-mm^2 squares 1 mm apart. The total number of cells, their density, and distribution were determined from the results obtained. Upon topographic mapping, the results of the calculation were averaged over 3×3 mm^2 domains.

The macroscopic anatomy of the manatee eye is unusual for marine mammals. The cornea is circular or slightly vertically elongated. The shape of the pigmented margin of the cornea is rhombic (vertically elongated), which is not typical for either terrestrial or aquatic mammals. In contrast to the hemispherical flattened eyeball with a spherical lens that is characteristic of whales and pinnipeds, the manatee eyeball is virtually circular with a lenticular lens and a thin sclera. The pupil is slightly vertically elongated. The optic nerve is thin (1.5–2 mm).

The sagittal section of a frozen eye was studied. The contour of the section is shown in Fig. 1. The distance from the center of the lens to the retina was taken as the

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posteronodal distance. According to our measurements, this distance is 10–10.5 mm.

The retina of the Caribbean manatee, like the retina of other aquatic mammals, consists mainly of large neurons. The size of most cells ranged from 14 to 30 μm (average, 24.3 μm). None of the cells found was smaller than 10 μm or larger than 60 μm.

The total area of the preparation ranged from 446 to 501 mm^2 (average, 481 mm^2), and the total number of cells per preparation ranged from 62000 to 81800 (average, 66400).

A typical topographic map of the ganglion cell distribution is shown in Fig. 2. The ganglion cell distribution was not uniform across the retina. The highest cell density is observed in ventral sectors. In the wholemount shown in Fig. 2, the maximum cell density exceeds 250 cells/mm^2. The values of the maximum cell density in different wholemounts ranged from 200 to 337 cells/mm^2 (average, 269 cells/mm^2). However, the gradient of the density distribution was rather low. For example, the maximum cell density in the wholemount described above was 280 cells/mm^2, and regions with a density greater than 150 cells/mm^2 occupied about half of the total area of the retina. The density distribution can be approximated by a bell-shaped curve. In other words, the ganglion cell density is higher in a large central zone of the retina (except for the area immediately adjacent to the optic disk) and gradually decreases toward the periphery.

The distribution pattern is distinctly seen in the curves representing the horizontal and the vertical sectional views of the density distribution map. Such curves, obtained by averaging the distribution patterns of five wholemounts, are shown in Fig. 3. It is seen from Fig. 3 that the distribution pattern does not contain the areas with a clearly increased ganglion cell density, although the distribution is definitely nonuniform and the cell density in the ventral sector is slightly higher than in the other regions of the retina.

It is well known that the retinal resolution can be assessed with a sufficient accuracy by the maximum density of ganglion cells [5]. The results of this work allowed us to calculate the retinal resolution of the Caribbean manatee retina. The intercellular distance was calculated by the formula

$$L = 1/\sqrt{D},$$

where D is the cell density per mm^2. Therefore, the maximum mean density of 269 cells/mm^2 in the ventral sector corresponds to the intercellular distance of 61 μm. We considered the distance from the center of the eye lens to the center of the retina to be the posteronodal distance, because in water, the light refraction at the cornea surface is minimal. According to our measurements, this distance is 10.5 mm (Fig. 1). The retinal resolution was calculated by the formula

$$V = 180L/\pi N,$$

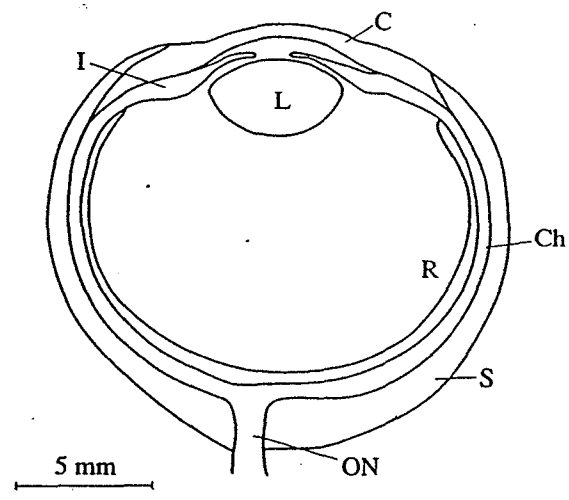

Fig. 1. Sagittal section of a frozen eye of the Caribbean manatee (sketch of a photograph): (C) cornea; (I) iris; (L) lens; (Ch) chorion; (S) sclera; (ON) optic nerve.

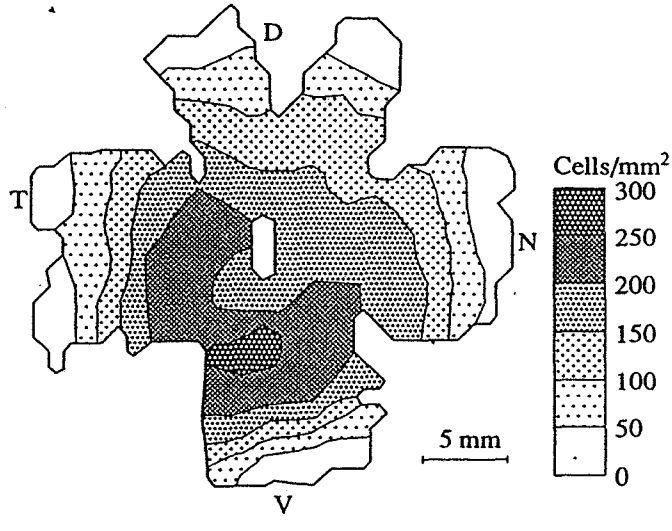

Fig. 2. The ganglion cell density in a wholemount of the Caribbean manatee retina: D, V, N, and T are the dorsal, ventral, nasal, and temporal poles, respectively. The scale to the right shows the cell density.

where V is the retinal resolution, deg; L is the intercellular distance, mm; N is the posteronodal distance, mm. According to our measurements, this value is 0.33° (20′).

Thus, the results of this work show that the manatee retina does not contain a distinctly localized area similar to the area centralis or the visual streak of terrestrial animals. There is only an area of slightly increased cell density in the ventral sector of the retina. The study of the cross sections of the Caribbean manatee retina [4] also showed that the number of ganglion cells in the ventral sector is higher than in other regions.

The pattern of the ganglion cell distribution in the Caribbean manatee differs substantially from the pat-

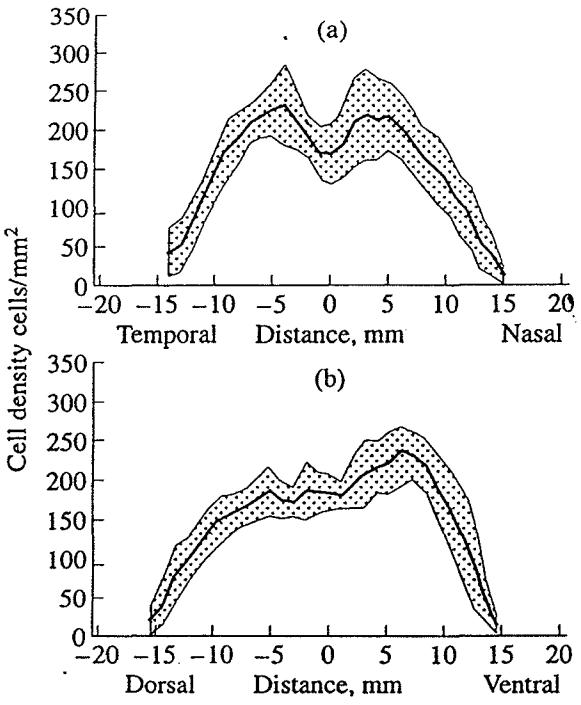

Fig. 3. The ganglion cell density (averaged over five wholemounts): (a) along the nasotemporal line drawn 2 mm below the optic disk; (b) along the dorsoventral line drawn 2 mm from the optic disk toward the temporal pole. The shaded area shows the standard deviation.

terns observed in the majority of other marine mammals. In pinnipeds, the area with a high cell density is represented by the area centralis (northern fur seal) or a visual streak (walrus) [14]. In whales, there are two areas of high cell density [6–9]. There are only certain similarities between the topography of the retina in the Caribbean manatee and in the Amazon river dolphin, which has a streak in the ventral sector of the retina [12]. This type of retinal structure is probably inherent in aquatic mammals living under conditions of low illuminance, because it may be well adapted to eyes tilted toward the upper water layers, where the illuminance is higher. However, the area of high cell density is outlined more clearly in the Amazon river dolphin than in the Caribbean manatee.

Thus, the manatee is characterized by the lowest degree of retina differentiation among all marine mammals studied. Among terrestrial mammals studied, echidna is the only species that has a retina with no particular area of high cell density [15].

The retinal resolution value measured in this work (20′) shows that the visual acuity of the Caribbean manatee is much lower than the visual acuity measured by the same method in whales and dolphins (retinal resolution, 9′–14′) [6–9] or in pinnipeds (retinal resolution, 5′–8′) [14]. However, the resolution of the Caribbean manatee retina is slightly higher than that measured by the same method in the Amazon river dolphin (40′–45′) [12].

The conclusion on the low retinal resolution of the manatee visual system is supported by the results of the only experimental study of a closely related species, the Amazon manatee *T. inunguis* [1]. The Amazon manatee eye was shown to be able to fix and trace only underwater objects located at a distance of no more than 1 m. Therefore, we can suggest that such a low retinal resolution of the visual system allows the manatee to distinguish only nearby objects.

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