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PHYSIOLOGY

Localization of the High-Resolution Area in the Ganglion Cell Layer of the Baikal seal *Pusa sibirica* Gm.1788

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Abstract—The morphological and functional density of the retinal ganglion cells of the Baikal Lake endemic seal *Pusa sibirica* was studied using cresyl-violet-stained whole-mounts. An area of the highest concentration of ganglion cells has been identified by drawing up a density map. This was an ellipsoid spot in the upper temporal part of the retina 6–7 mm from the visual nerve output. The maximum cell density in this area was 3800 cells/mm^2. The retinal resolution estimated from the maximum density of ganglion cells and the posterior nodal distance (24 mm) was 2.4′ in the water and 3′ in the air, and this can be used as an estimation of the retina resolving power.

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Recent studies of the marine mammal retina revealed uncommon examples of the morphological and functional organization of their visual system as compared to that of terrestrial mammals, which were related to habitat conditions of the marine mammals and their taxonomic affiliation [1]. These findings have contributed to understanding the general organization of the visual system, visual orientation, and behavior. In this respect, of interest is the visual system of pinnipeds (Pinnipedia).

Pinnipeds retain many features of the morphological and functional specialization typical of terrestrial carnivores. However, the aquatic medium, in which pinnipeds spend much of their life, requires a certain specialization of the body systems, including the visual one. The visual system has gained a series of adaptive properties which enabled the terrestrial animals to get the eye ambivalence, i.e., visual perception in both water and air [2, 3]. Pinnipeds have a great variety of visual systems with different degrees of adaptation to the aquatic and air media. The adaptive mechanisms are provided by the eye optic system and by other structures, including the retina. The retina adaptive properties are mostly manifested in its topography (in particular, in organization of areas with the highest cell density in the ganglion layer) rather than in the laminar organization. The high-density areas have the maximum resolving power as compared with other retinal regions. They are known also in terrestrial mammals as the area centralis and visual strip.

Using the cross-section analysis, the researchers failed to locate the areas with high concentration of ganglion cells in the pinniped retina (the best vision areas) [4]; therefore, the question of their existence remained controversial for a long time. The method of retinal topography on whole-mounts made it possible to locate these areas in the pinniped retina and, in addition, to reveal differences in their organization depending on the habitat conditions and taxonomic position [1].

Few pinniped species have been studied so far [5]. Seals of freshwater lakes remain unexplored [5]. Because of this, the Baikal seal (Baikal phoca) *Pusa sibirica* Gm. 1788, an endemic species of Lake Baikal, is of special interest. The Baikal seal visual system operates under specific conditions: not only in two media (in water and air), but also at the extreme levels of illumination, from the glare on ice to complete darkness at great depths. The Baikal water is fresh, demineralized, and extremely clear. These peculiar habitat features have influenced the Baikal seal organs [6], though the visual system has never been studied.

Exploration of the retinal ganglion layer morphology, as well as the ganglion cell size and density, was the purpose of this study. With these topographic data, we aimed at locating the area of the highest ganglion cell concentration to assess the retinal resolution.

We used the method of retinal topography on whole-mounts, which was modified for the large eyes of the marine mammals [7]. This approach makes it possible to stain all ganglion cells with a 0.1% cresyl-violet solution under visual control in order to exam-

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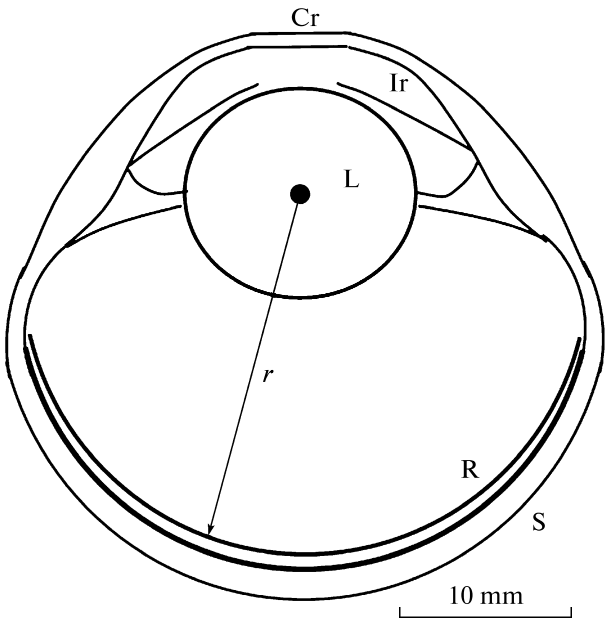

Fig. 1. A scheme of the Baikal seal eye (left) according to MRT data. Cr, cornea; Ir, iris; L, lens; R, retina; S, sclera; *r*, posterior nodal distance.

ine them under a light microscope. With these preparations the ganglion cell distribution over the retinal surface could be observed, as well as the maximum cell density in different areas, which, in turn, allowed us to estimate retinal resolution [8].

The material was two eyes of Baikal seals. Fixation of the material in a buffered 10% formalin solution was the most appropriate for the large whole-mount retinal preparations. One of the eyes was used for studying the macromorphology and measuring the geometrical parameters, including the posterior nodal distance. Measurements were conducted using a BioSpec 70/30 magnetic resonance tomograph (Bruker Corp., Germany) in the Center of Magnetic Tomography and Spectroscopy of Moscow State University. The other eye was used to prepare a whole-mount for studying the ganglion layer morphology and the ganglion cell topography.

Morphological examination of the Baikal seal eye showed that the eyeball was spherical (Fig. 1), with almost equal external vertical and horizontal diameters of 38–39 mm. A large part of the eyeball has the shape of a spherical segment with a horizontal arc of about 150°; the lens within it is almost spherical and slightly flattened in the axial direction; the lens transversal diameter and axial length are of 14 and 13 mm, respectively. The distance from the lens center to the fundus is 24 mm. On the formalin-fixed eye, the pupil is drop-shaped, the pointed end down. The sclera is not uniform in thickness: mostly in the ventral part and to the least extent along the horizontal diameter of the eyeball. A thickening at the junction of sclera and cornea forms a 2-mm-wide platen (limbus). The ciliary process is well defined.

Topographic distribution of the ganglion cells was examined on a retina whole-mount. The microscope objective was focused on the ganglion layer depth, and the ganglion cells were counted within this layer in 0.25-mm^2 squares systematically at intervals of 0.5 or 1 mm of the of high cell density area and 2 mm on the rest of retinal surface. The values obtained were converted into the number of cells per 1 mm^2 in order to map the ganglion cell density on the retinal surface. On the basis of LabVIEW software (National Instruments, United States) we have developed our own software to design a map.

The ganglion cells were identified according to criteria defined earlier [7, 8]. These were multipolar neurons of the surface layer of retina with a large amount of cytoplasm containing the Nissl-substance clumps and clearly discernible light nucleus with a nucleolus (Fig. 2). The cell body size was at least of 10 μm. Cells smaller than 10 μm which contained a large bright nucleus with nucleolus and a narrow rim of poorly stainable cytoplasm with sparse Nissl corpuscles were assumed to be non-ganglion displaced amacrine cells. These cells were located at the same levels as the ganglion neurons, but they were not taken into account. There were also the small dark regularly shaped cells. These were identified as neuroglial cells [8] and were not counted too.

Figure 3 represents a topographic map developed on calculations of the ganglion cell density. The total number of cells in this preparation was 396 000. Cell density on the map is shown as the multilevel lines. The ganglion cells were unevenly distributed over the retinal surface. An ellipsoid spot of the maximum cell concentration was clearly discernible; it was slightly elongated in the nasotemporal direction and located in the temporal section of the retina, somewhat above its horizontal diameter, at a distance of 6–7 mm from the optical disc. The tightly packed ganglion cells constituted the spot of high cell concentration with the maximum of 3800 cells/mm^2. The cell density across this area formed a steep gradient, so that, at a distance of 2.5 mm from the maximum point, it was already as low as 1000 cells/mm^2. The retinal area around the spot with the cell density from 500 to 1000 cells/mm^2 was horizontally elongated.

According to the maximum density of ganglion cells, the average angular distance between adjacent cells was estimated in the corresponding areas as $\alpha = 180°/\pi r\sqrt{d}$, where d is the cell density (the number of cells per mm^2) and r is the posterior nodal distance in millimeters. Since the refraction of light on cornea is virtually absent under water and the spherical lens is the main light-refracting structure, the lens center was assumed to be the nodal point of the optical eye system

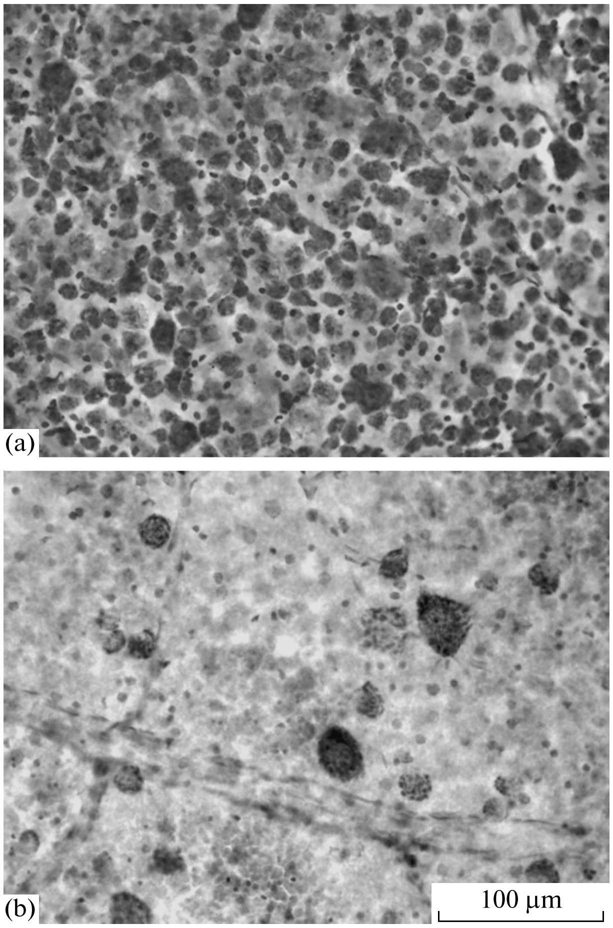

Fig. 2. Micrographs of the retina areas with different ganglion cell density on a whole-mount. At the top, the maximum density area; at the bottom, sparse cell distribution over the periphery of retina.

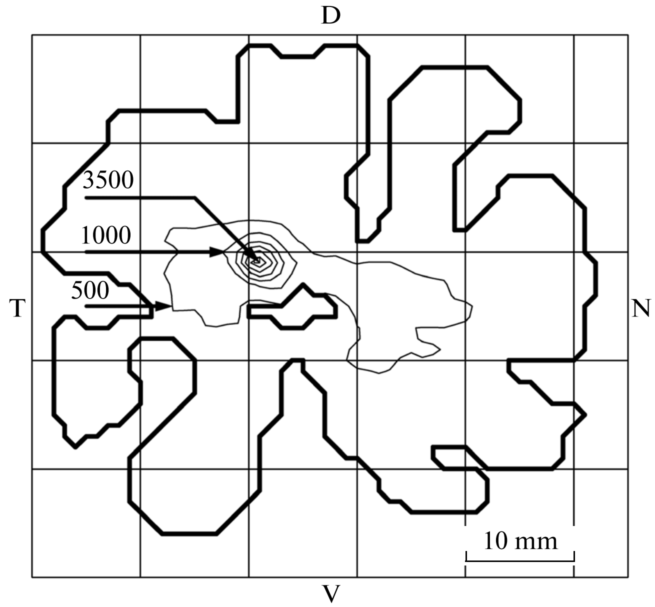

Fig. 3. A map of the ganglion cell density over the whole-mount of the retina (the right eye) of a Baikal seal. Cell density is represented by the multilevel lines after each 500 cells/mm^2; the arrows mark 500, 1000, and 3500 cells/mm^2. D, V, N, and T indicate the dorsal, ventral, nasal, and temporal poles of the right eye retina.

for the underwater vision, and the distance from the lens center to a retinal spot of the maximum cell density was the posterior nodal distance. This distance was of 24 mm. With $d = 3800$ cells/mm^2 and $r = 24$ mm, the $\alpha = 0.038°$ (2.4′). This value can be considered as the retina resolution. Upon the assumption that the retinal resolution and the optical eye parameters are functionally interdependent, the same value can be considered as the underwater visual acuity of the Baikal seal.

In the air, the nodal point is shifted because of refraction on the cornea surface; since the cornea curvature was not measured exactly, it is difficult to assess the nodal point position. As a first approximation, one can assume that the image size on retina in the case of the aerial vision is 1.33 times smaller than under water (the ratio of refraction indices in air and water). Then, in the air, $\alpha = 3.0'$.

Thus, we have identified an area of the highest density of ganglion cells with the maximum resolution in the retina of the Baikal seal. The location of this area in the temporal retinal section and its configuration and dimensions are similar to these parameters of other carnivores, both marine (the northern fur seal, harp seal, and the largha [7, 9, 10]), semi-aquatic (the sea otter [11]), and terrestrial (the cat [12]). Unlike the ellipsoid shape of the area centralis, which is characteristic of the aforementioned pinnipeds, the high-density area of a strip shape elongated horizontally has been found in four species, namely, the Steller sea lion (*Eumetopias jubatus*) [13], harbor seal (*Phoca vitulina*) [8], Caspian seal (*Pusa caspica*) [5], and walrus (*Odobenus rosmarus*) [9]; within this strip, there is a spot of the maximum cells density similar to the area centralis.

In the Baikal seal, the absolute value of the ganglion cell density (3800 cells/mm^2) is noticeably higher than in other pinniped species (from 1200 to 2000 cells/mm^2). However, all of these values are lower than those of the terrestrial carnivorous (from 7000 to 10 000 cells/mm^2) [12]. The retinal resolution and, hence, visual acuity, which were determined in this study, were also higher than visual acuity of other species of seals as judged from both the behavioral explorations and the retinal resolution (table). It is natural to assume that the high visual acuity is a result of the Baikal seal adaptation to the clear water of Lake Baikal. At a low ambient transparency, the objects would be seen only at a short distance, and the high visual acuity would be functionally useless. However, Estimates of the retina resolution of several seals in angular minutes

Species	Water	Air	Measurement method	References
Common seal *Phoca vitulina*	8.3		B	[15]
	–	5.6	B	[14]
	2.6	–	T	[8]
Caspian seal *Pusa caspica*	3.5	4.7	T	[5]
Harp seal *Pagophilus groenlandicus*	3.1	4.1	T	[7]
Largha *Phoca largha*	3.5	4.7	T	[10]
Baikal seal *Pusa sibirica*	2.4	3.0	T	This study

Measurement method: B, behavioral; T, method of retinal topography.

the transparency of the upper layers of Lake Baikal reaches 40 m according to the Secchi disc. Under these conditions, the visual acuity of 2.4′ enables an animal to discern 2.7-cm details at a distance of 40 m, which can be considered a good acuity allowing the animal to distinguish ambient objects, in particular, the objects of hunting. It is believed that the period of independent evolution of this species after its isolation in the lake (0.5–2 million years according to different estimates) proved to be sufficient at least for the quantitative (if not qualitative) changes in the seal visual system which are manifested in increasing of visual acuity.

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REFERENCES

1. Supin, A.Ya., Popov, V.V., and Mass, A.M., *The sensory physiology of Aquatic Ammals,* Boston: Kluwer Akademic, 2001.
2. Mass, A.M. and Supin, A.Ya., *Anat. Rec.*, 2007, vol. 290, pp. 701–715.
3. Hanke, F.D., Hanke, W., Scholtyssek, C., and Dehnhardt, G., *Exp. Brain Res.*, 2009, vol. 199, pp. 299–311.
4. Landau, D. and Dawson, W.W., *Vision Res.*, 1970, vol. 10, pp. 691–702.
5. Mass, A.M. and Supin, A.Ya., *Brain Behav. Evol.*, 2010, vol. 76, pp. 144–153.
6. Bogdanov, L.V., Pastukhov, V.D., Ivanov, M.K., et al., in *Morfofiziologicheskie i ekologicheskie issledovaniya baikal'skoi nerpy* (Morphophysiological and Ecological Studies on the Baikal Seal), Novosibirsk: Nauka, 1982.
7. Mass, A.M. and Supin, A.Ya., *Brain Behav. Evol.*, 2003, vol. 62, pp. 212–222.
8. Hanke, F.D., Peichl, L., and Dehnhardt, G., *Brain Behav. Evol.*, 2009, vol. 74, pp. 102–109.
9. Mass, A.M., in *Marine Mammal Sensory Systems*, New York: Plenum, 1992.
10. Mass, A.M., *Sens. Sist.*, 2015, vol. 29, pp. 131–141.
11. Mass, A.M. and Supin, A.Ya., *Brain Behav. Evol.*, 2000, vol. 55, pp. 111–119.
12. Stone, J., *Paralell Processing in the Visual System*, New York: Plenum, 1983.
13. Mass, A.M. and Supin, A.Ya., *Aquat. Mammals*, 2005, vol. 31, pp. 393–402.
14. Hanke, F.D. and Dehnhardt, G., *J. Comp. Physiol. A*, 2009, vol. 195, pp. 643–650.
15. Schusterman, R.J. and Balliet, R.F., *Nature*, 1970, vol. 226, pp. 563–564.

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