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VARIETIES OF GANGLION CELLS OF THE MARINE FISH RETINA PROJECTING TO THE OPTIC TECTUM: AN HRP STUDY

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ABSTRACT

The morphology of ganglion cells (GCs) projecting to the optic tectum (OT) was studied in three species of marine fishes: *Hexagrammos octogrammus*, *Myoxocephalus stellery* and *Pholidapus dybowskii*. The method of retrograde transport of HRP was used to label GCs. Morphological criteria such as cell body size, dendritic field size, dendritic branching pattern and the level of their spreading in the inner plexiform layer of the retina have been used to classify GCs. From their appearance in the retinal whole-mounts 6 morphological varieties of GCs were identified among heterogeneous population of cells labelled from the OT. Three varieties of large GCs were identified as α_a, α_{ab} and biplexiform cells, and 3 variety of medial GCs were termed ν_a, ν_b and ν_{ab} cells.

Key words: fish retina, ganglion cells, tectal projection, HRP-method

ANATOMSKE VARIJACIJE GANGLIJSKIH ĆELIJA KOJE SE PROJEKTUJU NA TECTUM OPTICUM MORSKIH RIBA: HRP STUDIJA

REZIME

Morfologija ganglijskih ćelija (GCs) koje se projektuju na tektum optikum (TO) izučavane su kod tri vrste morskih riba *Hexagrammos octogrammus, Myoxocephalus stellery i Pholidapus dybowskii.* Za markiranje GCs korišćen je metod retrogradnog transporta HRP. Morfološki kriterijumi kao što su veličina ćelija, veličina dendritnog polja, razgranatost dendrita i nivo njihovog prisustva u spoljašnjem pleksiformnom sloju retine uzimani su kao kriterijum za klasifikaciju GCs. Šest morfoloških varijacija GCs je identifikovano iz heterogene populacije markiranih ćelija u TO. Tri tipa velikih GCs identifikovano je kao α_a, α_{ab} i bipleksiformne ćelije i tri tipa srednjih GCs koje označene kao ν_a, ν_b and ν_{ab} ćelije.

Kjučne riječi: retina riba, ganglijske ćelije, tektalna projekcija, HRP – metod

INTRODUCTION

In fishes midbrain roof or the optic tectum (OT) is the major visual centre that receives direct inputs from the retina. This retinotectal projection is composed by small, medium and large retinal ganglion cells (GCs), and shows orderly topographic organization (Jacobson & Gaze, 1964; Saidel & Butler, 1997). GCs send their axons via the optic nerve and the optic tract to the contralateral OT where they terminate in an ordered manner and segregate into several sublayers depending on their calibre (Ito et al., 1984), but nothing is known about dendritic morphology of these cells so far.

In electrophysiological studies have been described different specialized types of retinal ganglion cells: direrectionally-selective cells, detectors of moving contrast, detectors of oriented lines, dimming detectors, colour-opponent as well as non-colour-coded cells and others (Jacobson & Gaze, 1964; Zenkin & Pigarev, 1969; Maximova & Maximov, 1981; Maximova et al., 1971). For understanding how GCs combine signals from photoreceptors and relay visual information to the OT, it is necessary know their morphology.

The aim of our work was to study anatomy of GC varieties sending their axons to the OT in marine fishes. We have used the method of retrograde filling of GCs after application of the HRP into the OT.

The study was carried out on three species of the marine fishes: greenling, Hexagrammos octogrammus, sculpin, Myoxocephalus stelleri, and *Pholidapus dybowskii*. All these fishes demonstrate high visually dependent behaviour. Their high visual acuity mediates by specialized zone of the retina – *area temporalis* where a peak of cell density is observed.

MATERIAL AND METHODS

Three species of adult marine fishes: *Hexagrammos octogrammus* (body length – 13-15 cm; n = 4), *Myoxocephalus stelleri* (body length – 12-14 cm; n = 3) and *Pholidapus dybowskii* (body length – 12-15 cm, n = 7) were used in this study. Fishes were captured in the Bay of Peter the Great (See of Japan) and maintained pre- and post-surgically in the seawater in aerated aquarium at 15-17°C. Ganglion cells were labelled after application of the HRP into the OT. For this fishes were anaesthetized by immersion in a weak (0.01%) MS 222 solution. In order to expose the OT the skin, bone and *dura mater* were removed, and superficial injury was made by a very thin tungsten need in the dorsomedial tectal area. The crystals of HRP (Sigma VI) were placed at the site of injury. After 15 minutes the bone and skin were replaced, and the incision was closed by adhesion with the cyanacrylic glue. A period of 5-6 days was the optimal postoperative survival time. Then fishes were dark-adapted for an hour,

deeply anaesthetized and decapitated. The eyes were enucleated and detached from the cornea, lens and vitreous body. The retina was gently removed from the sclera in 0.1M phosphate buffer (pH 7.4) and whole-mounted receptor side down on a gelatinised slide. The visualization of the HRP was carried out according to Adams method (1981) using 3,3'- diaminobenzidine. Then slides were washed in the phosphate buffer (pH 7.4), dehydrated in increasing grades of ethanol, cleared in xylene, and mounted in DPX.

Retinas were examined as whole-mounts by the light Leica microscopy, and cells were drawn with the aid of a camera lucida, using a Zeiss Plan x40/0.85 objective. Cell body and dendritic field sizes were calculated by measuring the drawings. The level of dendritic stratification in the IPL was determined by registration their focal depth.

RESULTS AND DISCUSSION

Morphological criteria such as cell body size, dendritic field size, dendritic branching pattern and the level of their spreading in the inner plexiform layer (IPL) have been used to classify GCs. From their appearance in the retinal whole-mounts three large- and three medium-sized varieties of GCs were identified.

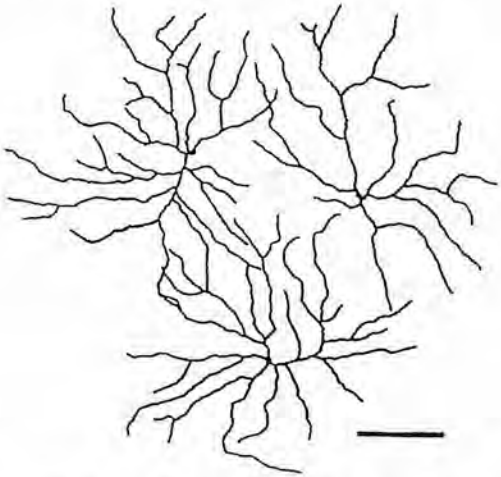

Figure 1. Large monostratified ganglion cells of the greenling retina. Scale bar 100 mkm.

Large ganglion cells

Large GCs were easily identified in whole-mounts accordingly to the large sizes of their cell bodies (maximal diameter of cell body > 20 mkm), thick

primary dendrites, pattern of dendritic arborisation and the level of branching in the IPL. In recent years J. Cook with co-authors have revealed four distinct types of large GCs in the series of fishes (Cook & Becker, 1991; Cook *et al.*, 1992; Cook & Sharma, 1995, Cook *et al.*, 1996, 1999; Podugolnikova *et al.*, 1998a, b) and termed them α_a, α_{ab}, α_c and biplexiform (BCs) cells. These large GC types demonstrate striking morphological similarities among different species of fishes. It was shown that cell bodies of large GCs form regular independent mosaics, and their dendritic arbors cover the entire retina. In our material we have described 3 types of large GCs sending their axons to the OT.

Monostratified large GCs had cell bodies in orthotopic or displaced positions. Each cell had 2-3 thick primary dendrites bifurcated 2-4 times along their course and forming sparsely branching dendritic tree narrowly stratified in scleral (*a*) sublamina of the IPL. They had the largest dendritic fields among retinal GCs. Axons of these cells were thick (about 2 mkm in diameter), and arise from the cell body or primary dendrite. In the figure 1 three neighbouring monostratified cells of the greenling retina are shown. Maximal diameters of their dendritic fields are equal to 395, 380 and 385 mkm (retinal area = 31.4 mm^2). The comparison of large monostratified GCs with α_a cells allows to suggest that they belong to the same GC type.

Bistratified large GCs also had large cell bodies and 2-4 thick primary dendrites that form clearly bistratified dendritic arbor in both scleral (a) and middle (*b*) sublaminae of the IPL. These dendrtic arborisation were about 2-3 times smaller and more densely branched than those of monostratified large GCs. Axons were thick and originated from the cell body. In figure 2, the group of four neighbouring bistratified GCs of the sculpin retina is shown. Dendritic fields of these cells are oriented toward the periphery of the retina. Maximal diameters of their dendritic arborisation were equal to 189, 145, 158 and 152 mkm (retinal area 29.3 $mm^{2)}$. The morphological features of bistratified OT-projecting GCs are exactly coincide to those of α_{ab} cells.

Biplexiform GCs had large round cell bodies located in ventral sublayer of the inner nuclear layer (INL) and dendritic arborisation spreading in both outer and inner plexiform layers. Three or four primary dendrites arise from the vitreal pole of the cell body in radial directions, branch at a short distance, and form large sparse inner dendritic field spreading in the most scleral sublayer of the IPL, along amacrine cell layer. Outer dendritic tree is formed by processes arise from the cell body or inner dendrites. These processes cross the INL in scleral direction and form large sparse terminal dendritic arbour in the by processes arise from the cell body or inner dendrites. These processes cross the INL in scleral direction and form large sparse terminal dendritic arbour in the outer plexiform layer (OPL) at the level of the photoreceptor terminals. Axon originates from a cell body, cross IPL and direct to the optic disc.

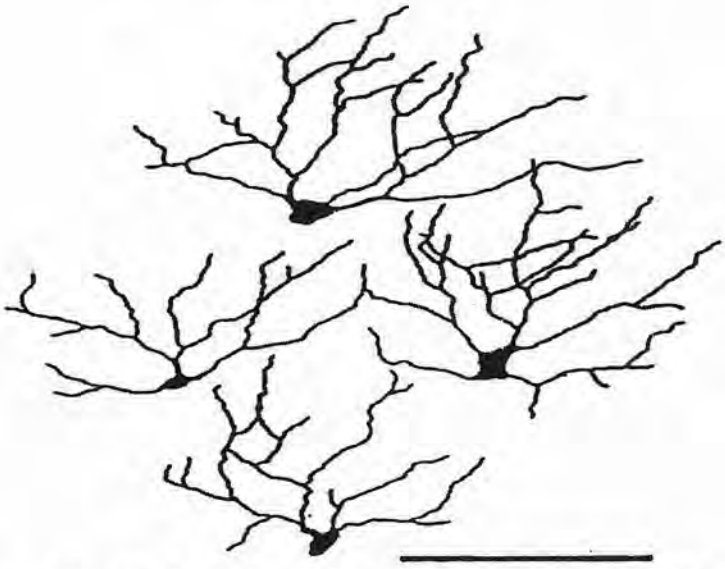

Figure 2. Large bistratified ganglion cells of the sculpin retina. Scale bar 100 mkm.

In our previous papers it was shown that there are only one type of BCs cells in the marine fish retina (Cook *et al.*, 1996, 1999; Podugolnikova *et al.*, 1998a). Although in our tectal specimens BCs ganglion cells were not filled complete, all large GCs with α_c cells cell bodies located in the INL, and having at least one process lying more scleral than cell body were considered to be the BCs cell. In figure 3A one incomplete BCs cell from the sculpin retina is shown. Outer dendrites are drawn by the dotted line. We didn't fined α_c in our specimens. It is possible that α_c cells form an exrtatectal projection.

Medium ganglion cells

We have identified three varieties of medium GCs projecting to the OT. Morphology of all these cells is very similar, but their dendritic arborisations are spreading in different sublayers of the IPL. We have termed them ν-cells.

Medium GCs had ovoid or round cell bodies of medium size (maximal diameter 12-18 mkm) located in the ganglion cell layer. Typically 2-4 thin and smooth primary dendrites leave the cell body, go in vertical direction and form dens terminal arborisation in a thin sublayer of the IPL. The secondary and next order dendrites are oriented in radial directions and form the compact dendritic field. For example, the diameter of the outer monostratified medium GC in middle peripheral zone of *Pholidapus* retina was equal to 58 mkm, and the

diameter of dendritic field of neighbour α_a cell was 460 mkm (retinal area = 37.2 mm^2). Terminal dendrites are very fine and bear small regularly spaced varicosities. The camera lucida drawings of medium cells are demonstrated in figure 4.

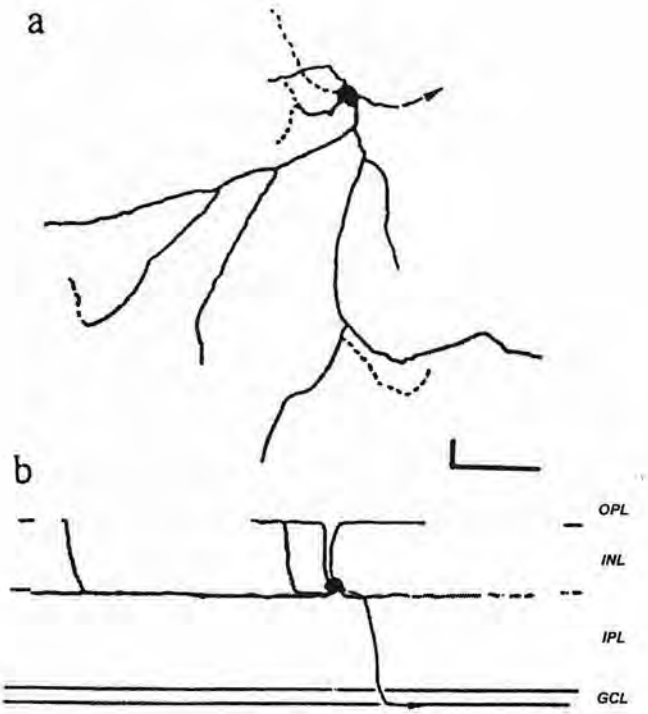

Figure 3. Biplexiform cell from the sculpin retina in plan view (a) and in profile view (b). Horizontal bar 100 mkm, vertical bar 10 mkm.

There were outer and inner monostratified varieties of medium GCs, which are spreading in scleral (a) and middle (b) subluminae of the IPL, correspondingly. By analogy with classification scheme of Cook & Sharma (1995), we have termed them ν_a (Fig. 4a) and ν_b (Fig. 4b) GCs.

Third variety had bistratified dendritic tree spreading in both *a* and *b* sublaminae of the IPL, and we call it ν_{ab} cell (Figure 4c). Depth comparison of dendritic arborisation of all ν cells shows that they stratified in different levels of the IPL.

The presence of mono- and bistratified medium-sized GCs has been reported earlier in the retina of mackerel (Podugolnikova, 1985), and their

dendritic morphology revealed by Golgi impregnation is remarkably similar to our ν-cells.

The retrograde filling of small GCs was usually incomplete and their terminal branches were not successful labelled.

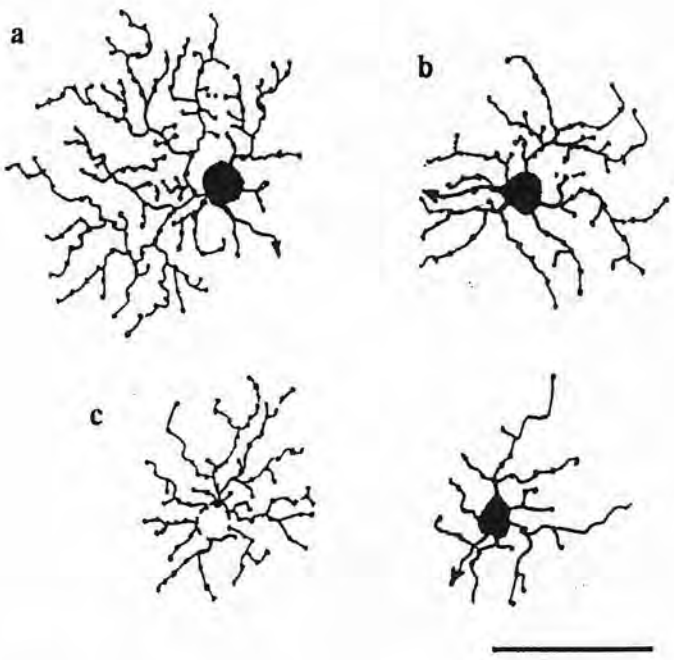

Figure 4. Medium-sized ganglion cells of the *Pholidapus* retina, projecting to the optic tectum: monostratified ν_a (a) and ν_b (b) cells and outer (left) and inner (right) arbores of bistratified ν_{ab} cell (c). Scale bar 50 mkm.

Previously it was shown that anatomy of GCs is correlated with their physiological properties. Our data may be another step toward understanding the functional significance of GC types projecting to the OT.

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