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## **ANALYSIS OF CONE - HORIZONTAL CELL CONNECTIVITY PATTERNS IN THE JACK MACKEREL RETINA**

Tatyana A. PODUGOLNIKOVA\* and Vadim V. MAXIMOV

Institute for Information Transmission Problems,  
Russian Academy of Sciences, Moscow, Russia

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The morphology of horizontal cells was described in the retina of the Black Sea jack mackerel, *Trachurus mediterraneus ponticus*, using the Golgi method. On the basis of morphological differences and the cell body location we have classified them into three types of cone horizontal cells (EHC, MHC and IHC) and one type of rod horizontal cells (RH). The study was focused on the dendritic morphology and cone-horizontal cell connections. We found that EHC cells make contacts with both double (red-sensitive) and single (blue-sensitive) cones, MHC cells selectively with double cones, and IHC cells with single cones. A model of the functional organization of cone horizontal cells is discussed.

*Key words:* cones, horizontal cells, jack mackerel, retina

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\*Corresponding author: Tatyana Podugolnikova, Institute for Problems of Information Transmission, Russian Academy of Sciences, 19, Bolshoi Karetnyi per., GSP-4, Moscow 101447, Russia ; e-mail: [ochakovo@iitp.ru](mailto:ochakovo@iitp.ru)

## INTRODUCTION

In the vertebrate retina, horizontal cells are interneurons postsynaptic to the photoreceptors. There is a body of evidence that horizontal cells perform a major role during early stages of visual processing. They are implicated in colour processing, because they respond with different polarities to changes in the wavelength of the light stimulus (Stell and Lightfoot, 1975; Burkhardt and Hassin, 1983), form feedback onto cones (Byzov *et al.*, 1977) and are responsible for the surrounding mechanism of the receptive fields in cones (Baylor *et al.*, 1971), bipolar and ganglion cells (Maximova, 1969; Naka, 1976).

In the teleost retina there are up to four types of horizontal cells which are arranged in four layers: three of them are related to cones and one to rods (Parthe, 1972; Stell and Lightfoot, 1975). Horizontal cells of every layer differ from each another in both morphology and synaptic connectivity. Their processes terminate in synaptic endings of receptors where they are either central or lateral in ribbon synaptic triads, depending on cone and horizontal cell type (Stell *et al.*, 1975). Functional differences corresponding to the morphological type of horizontal cells were described in many fishes (Kaneko and Yamada, 1972; Stell and Lightfoot, 1975). It has been shown that most Cypriniformes possess 3 or 4 spectral types of cones (Stell and Harosi, 1976; Bowmaker, 1984), and connections of horizontal cells with cones are colour-specific in their retinas (Stell and Lightfoot, 1975). On the other hand, the dichromatic retina of a Perciform fish, *Nannacara anomala*, has been shown to have only one morphological variety of the cone horizontal cells synaptically connected with both types of cones (Wagner, 1978).

The retina of the jack mackerel also contains two morphologically different types of cones: equal double (twin) cones and single cones that are organized in a very orderly square mosaic (Podugolnikova and Maximov, 1973, 1984). It has been shown earlier that the chromatic identities of fish cones can be determined by morphological criteria (Scholes, 1976; Stell and Harosi, 1976). A microspectrophotometric study showed that in the retina of the jack mackerel twin cones contain long-wave sensitive pigment with  $\lambda_{\max} = 525$  nm, while single cones are short-wave sensitive with  $\lambda_{\max} = 433$  nm (Govardovsky and Zueva, 1988).

In this study we have examined the morphology of horizontal cells and their connectivity patterns with cones of different type in the jack mackerel retina. The experiments were carried out in the adult Black Sea jack mackerel (*Trachurus mediterraneus ponticus*), and were based on studies of radial and tangential serial sections stained with silver, according to Bielschowsky, and by means of the Golgi impregnation method reported earlier (Podugolnikova, 1985).

Using the light microscope, we were able to recognise in the jack mackerel retina four distinctive varieties of horizontal cells on the basis of their position, size, pattern of dendritic branching, and their synaptic connectivity with photoreceptors. They can be divided into three types contacting with cones: external (EHC), medial (MHC) and internal (IHC) cone horizontal cells and one type contacting with rods: the rod horizontal cells (RH). In Fig.1 all types of horizontal cells are shown as they appear in Golgi preparations.

## MORPHOLOGY OF HORIZONTAL CELLS

**EHC cells**, the external cone horizontal cells, are located below the outer plexiform layer (OPL) and form the tightly packed layer. Their cell bodies have an irregular oval form, the maximal diameter of the cell body amounting to 13-16  $\mu\text{m}$ . EHC cells had many short dendritic processes which protrude from the scleral and the lateral surface of the cell body (Fig.1 a, b). These dendrites end in a terminal structure,

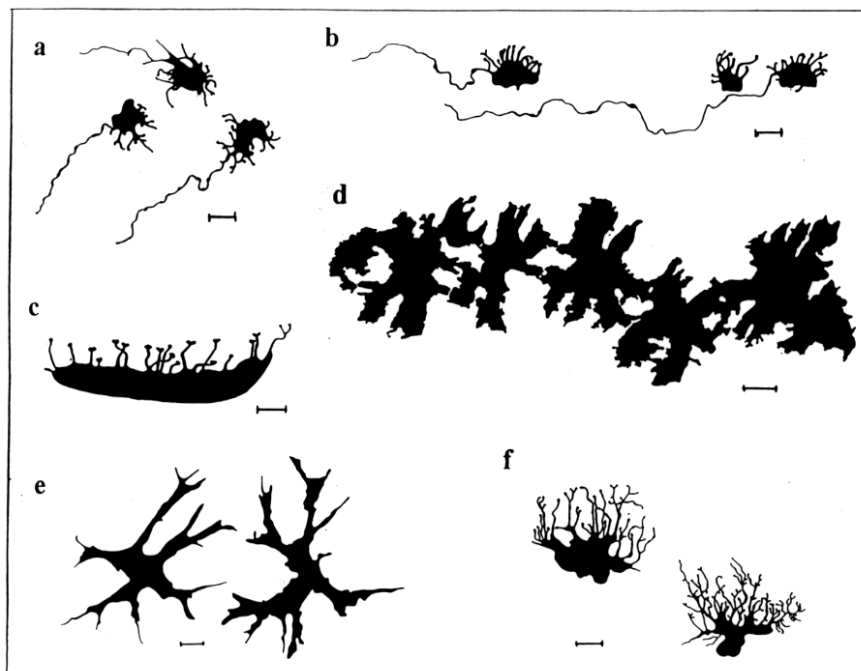


Fig.1. Camera lucida drawings of jack mackerel horizontal cells of different types from Golgi preparations. **a** and **b** - outer EHC cells as they are seen in tangential (**a**) and radial (**b**) sections; medial MHC cell in radial section (**c**) and 5 neighbor cells in tangential view; IHC cells in tangential section (**d**) and two rod horizontal cells in oblique sections. Scale bars are equal to 10  $\mu\text{m}$ .

composed of 3-4 terminal knobs at the level of the cone pedicles in the OPL. The diameter of the dendritic field of EHC cells was about 25-35  $\mu\text{m}$ . The axon (0.5 - 1.0  $\mu\text{m}$  in diameter) generally arises from the main dendrite and ends abruptly without a terminal expansion, thus appearing to be incompletely impregnated. In favorable preparations, the axon had a total length of 230  $\mu\text{m}$ . EHC was the only horizontal cell type that had an axon.

**MHC cells**, the medial cone horizontal cells are situated below the external EHC cells and are the largest of the three types of cone horizontal cells. In radial sections the cell bodies were twice as large as the external horizontal cells (the diameter of the cell body was about 40-60  $\mu\text{m}$ ), and were almost rectangular in form. In tangential sections they appear like indented polygons with 5-7  $\mu\text{m}$  thick extensions. When a large number

of these cells are stained, they form a continuous membrane, perforated by openings through which ascending processes of bipolar cells and Muller cells pass. Fig.1d shows five neighboring MHC cells stained side by side. MHC cells have thick ascending dendrites which may divide into 2 or 3 short and smooth final branches. Their large terminal structure (5  $\mu\text{m}$  in diameter) (Fig.1c) is composed of a bunch of 8-9 terminal knobs. Dendrites of MHC cells course vertically through the EHC cell layer to end at the level of the cone pedicles.

**IHC cells**, the internal cone horizontal cells lie in the inner nuclear layer below MHC cells. In radial sections they are fusiform and more flat than the medial MHC cells. In tangential sections the internal IHC cells have the form of an irregular star with 5-6 stout lateral dendrites, each branching into two thinner ascending processes. These were further subdivided into thinner smooth ascending terminal dendrites, reaching the OPL and forming sparse terminal structures at the level of the base of the cone pedicles.

**RH cells**, rod horizontal cells, form a layer below the internal cone horizontal cells. The main dendrites arise from the ovoid cell body and bifurcate to form a dense array of secondary dendrites. The secondary dendrites subdivide into several thin terminal processes. The processes reach the outer zone of the OPL where they make profuse terminal tufts ending in numerous, isolated terminal knobs at the level of rod spherules. Two RH cells are shown in Fig.1f.

### CONE-HORIZONTAL CELL CONNECTIONS

Our previous studies have shown that the jack mackerel retina has a regular structural organization (Podugolnikova and Maximov, 1973; Podugolnikova, 1985). Cones are organized in a very orderly square shaped mosaic, which tiles all the retina. In this pattern twin cones occupy the sides of the square. Such a regular distribution of cones facilitates the analysis of synaptic contacts with second-order neurones using the light microscope. Tangential sections showed that cell bodies of horizontal cells form three distinct mosaics in the jack mackerel retina. EHC cells are arranged in a regular square lattice which coincides in direction and period with the cone mosaic, and their cell bodies lie just under the pedicles of single cones. The two mosaics of MHC and IHC cells differed in spatial scale: they showed lower cell density than the EHC cells.

In general, cone pedicles can be seen in the OPL even if they are not stained, and show the regular square distribution as well. The relationship between cone pedicles and terminal knobs of horizontal cells enable the study of their synaptic contacts under light microscopy. In cases when we had a complete horizontal cell in the tangential view, we made drawings of this cell with its terminal knobs and a cone mosaic in the same retinal area. Such drawings were superimposed upon each other and the connectivity pattern of the given cell with cones was ascertained. An example of such analysis of EHC cell synaptic contacts with cones is shown in Fig.2 a.

The foregoing procedure enabled an easy mapping of the lateral dendritic contacts of the EHCs with double cones. But a dendrite directed to the central single cone was usually invisible, being covered by the horizontal cell body cone when examining the preparation in transmitted light. To study the connection between EHC and central single cone the tangential sections were investigated in reflected light. In this case, synaptic terminals of dendrites are seen as bright points on the background of the dark cell body.

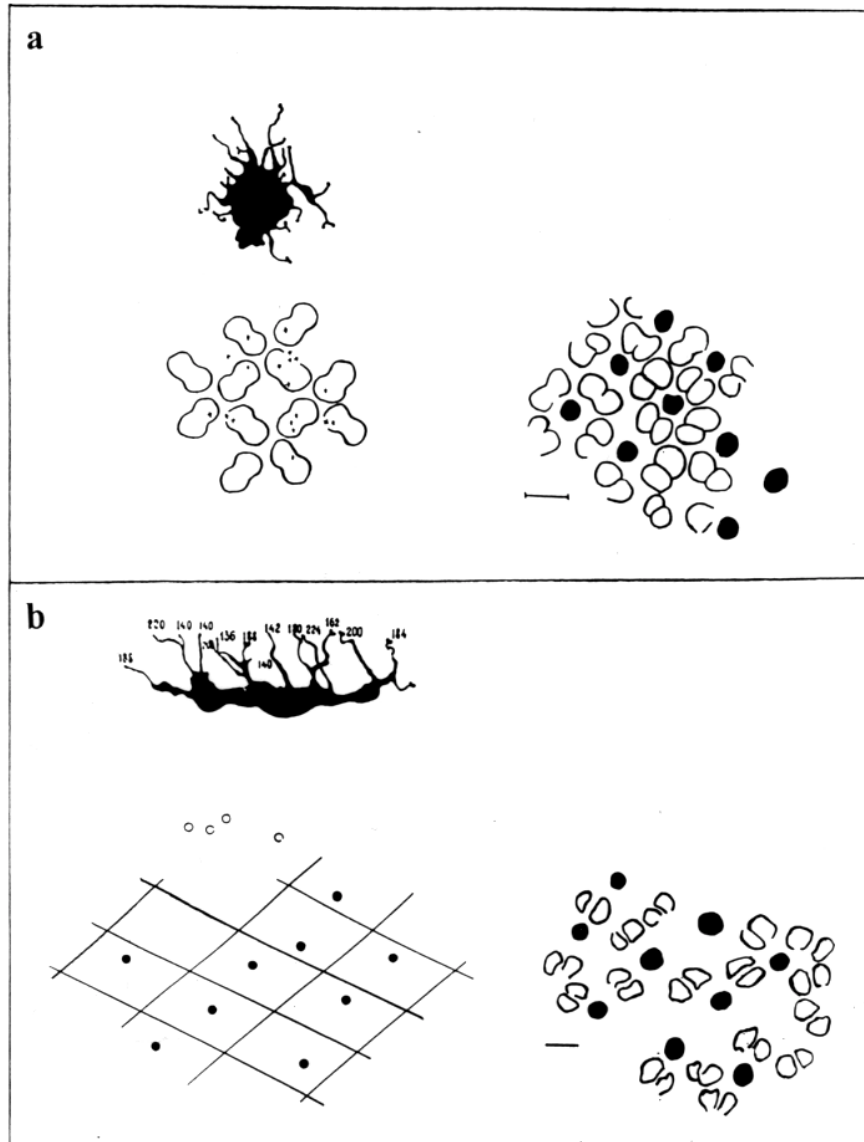


Fig.2. Jack mackerel outer (a) and inner (b) horizontal cells and their cone contacts. Dendritic terminals are represented by points. The real cone mosaic pattern from the same area of the retina is shown. Scale bar = 10  $\mu$ m.

The same procedure was used to study synaptic connections of MHCs, which have massive cell bodies, obscuring dendritic terminals in the tangential sections. When a good complete horizontal cell was found in radial sections, we measured the 3-dimensional positions of its dendritic terminals under the light microscope. Figure 2b shows a theoretical reconstruction of the distribution of the IHC terminal dendrites.

The analysis of synaptic connections of cone horizontal cells shows that EHC cell dendritic terminals non-selectively innervate both the overlying single and double cone pedicles. It was found that every EHC cell makes about 25 contacts (Fig.1 a). It sends one dendrite to the single cone situated just under its cell body, two dendrites to every member of the four overlying double cones, and one to one member of the more distant double cones. This scheme of contacts exactly coincides with the pattern of contacts of the cone horizontal cells in the *Nannacara anomala* retina described by Wagner (1978).

MHC cells have a large number of bunch terminals. Our reconstruction of six MHC cells shows that they make selective contacts with red-sensitive double cones only. The MHC cells send one dendrite to every element of the double cones located in its receptive field.

IHC cell dendritic terminals form a sparse pattern which coincides in period with the cone mosaic (Fig.2 b). We may speculate that IHC cells are connected with all blue-sensitive single cones situated in their dendritic field. In favorable cases it can be seen under the light microscope that the terminal processes of the IHC cell make contact with central single cone pedicles. Stell and Lightfoot (1975) have also described selective contacts with the blue-sensitive single cones for the third type of cone horizontal cells (H3) in the goldfish retina. In contrast, however, to the goldfish retina, in the jack mackerel retina there are two varieties of cone horizontal cells, forming selective contacts with separate types of cones.

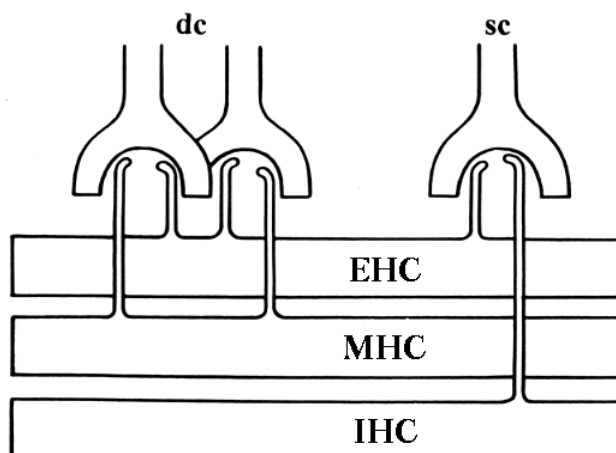


Fig.3. The scheme summarizing cone-horizontal cell contacts determined by light microscopy in the jack mackerel retina. dc - double cones, sc - single cones; EHC, MHC and IHC - external, medial and inner cone horizontal cells, correspondingly.

Our results showed that the morphology of EHC, MHC and IHC cells reveals the pattern of their contacts with cones of different types, which provided an anatomical basis for their different responses. A scheme of cone-horizontal cell contacts is shown in Fig.3. An important difference from what can be seen in the retina of *Cypriniformes*, is that here the variety of horizontal cells exceeds the variety of receptors. It is reasonable to assume that the mechanisms of cell interactions in the jack mackerel

retina are the same as in the goldfish, and EHCs connected with both types of cones serve interneurons which realize color opponency of signals by means of a feed-back to cones. In particular, MHC synaptically connected only to long-wave-sensitive cones, will receive a signal of the opposite sign from short-wave-sensitive cones via interneurons. Physiologically this corresponds to color-opponent cells responding with depolarization to short-wave stimulation, and with hyperpolarization to long-wave stimulation. It is noteworthy that the type of cells with such physiological properties was described in the retina of another representative of Perciform fishes - the river perch *Perca fluviatilis* (Maximova and Maximov, 1969).

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