

MORPHOLOGICAL TYPES OF BIPOLAR CELLS IDENTIFIED
 BY REGULAR GRIDS OF THEIR AXONAL EXPANSIONS IN THE
 INNER PLEXIFORM LAYER OF THE HERRING RETINA

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The morphological structure of the inner plexiform layer in the region of sharp vision was investigated under the light microscope in the retina of five species of herring. This layer is a three-dimensional regular grid formed by the club-shaped expansions (synaptic complexes) of the axons of the bipolar cells. These expansions, located at different levels of the inner plexiform layer, form mutually conforming periodic grids differing in their orientation and periods. Analysis of this structure shows that at least three types of bipolar cells participate in its organization.

INTRODUCTION

The receptors in the retina of most species of teleosts are arranged as a mosaic, forming a distinct geometric pattern [7, 12, 8]. In the study of the spatial organization of the retina a regular arrangement of the cells has also been found at the level of the horizontal and ganglion cells; the periods of the grids formed by these cells conform in a definite way to the receptor mosaic [15, 14, 5, 10, 16, 4]. Besides grids formed by cell bodies, regular grids are also known in the plexiform layers of the retina. In the outer plexiform layer the grid is formed by the bases of the cone pedicles and by dendrites of the horizontal and bipolar cells in contact with them. In the inner plexiform layer the grid is formed by axons of the bipolar cells [15].

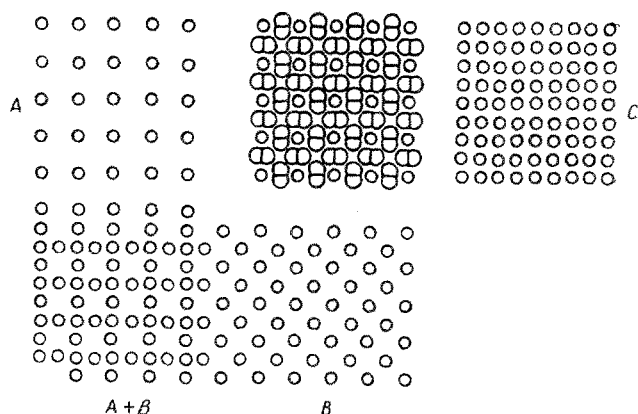


Fig. 1. Relations between different types of grids forming expansions of bipolar cell axons with the receptor mosaic. A, B, and C) Three types of elementary grids; A +B) overlapping grids of types A and B. Top center, diagram of cone mosaic formed by double and single cones.

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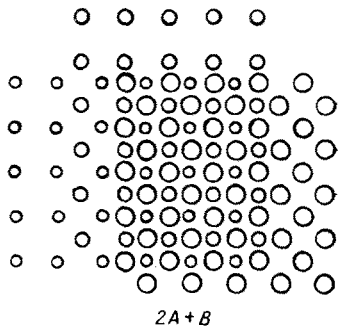


Fig. 2. Diagram showing overlapping of two type A grids and one type B grid to produce a heterogeneous type C grid.

The study of the organization of the inner plexiform layer (IPL) is very difficult for this layer is a complex interweaving of process of the amacrine cells, axons of bipolars, dendrites of ganglion cells, and Müller's fibers. To investigate the structure of the IPL, processes of cells of different types and subtypes [18] and their synapses must be identified. It is not sufficient to use only the fine structural features of the cytoplasm to distinguish (under the electron microscope) the processes of different types of cells. The geometric characteristics of the mutual arrangements of the cell structures can also be useful for this purpose.

Axons of bipolar cells pass through IPL in a radial direction and form expansions along their course. Axons of the rod bipolars have a club-shaped expansion at their end, much larger than the cell body [6, 11, 13]. Numerous synapses are located on the surface of these club-shaped endings [9, 17]. The axons of different types of bipolar cells in the retina of many species of teleosts are arranged in a strict geometric order; the mosaic of the bipolar axon is seen most clearly in the zone of sharp vision [15].

The structure of ILP was studied in the ventral zone of the retina, corresponding to the region of sharp vision [1], in five species of herring. An attempt was made to discover structural differences between axons of different types of bipolars that could help substantially with their identification.

The morphological features distinguishing the structure of the region of sharp vision in these five species of fish at the receptor level have been described by Baburina [1, 2] and Baburina and Kovaleva [3]. The present writers found a mosaic of cells not only at the receptor level, but also at the level of the horizontal cells, in the layer of ganglion cells, and in IPL [4].

During investigation of the IPL in this way it was found to contain a set of periodic "square" grids, located at different levels and formed by approximately spherical structures in the corners of the grids from 1.5 to 4 μ in diameter. On staining the retina with azan by Heidenhain's method the inner part of these structures appeared less dense than the surrounding space of the IPL. In agreement with the observations of Vrabc [15] and Goodland [9], these structures must evidently be regarded as expansions and club-shaped endings of axons of bipolar cells.

The object of the present investigation was to study details of the geometric structure and mutual arrangements of these periodic grids formed by expansions of bipolar axons.

EXPERIMENTAL METHOD

The retina was studied in five species from the herring family (Clupeidae): the common kilka Clupeonella delicatula Nordmann, the bigeye kilka Clupeonella grimmii Kessler, the anchovy kilka Clupeonella engrauliformis Borodin, the Baltic herring Clupea harengus L., and the Caspian shad Alosa caspia caspia Eichwald.

The material was fixed in 10% formalin and cut into sections tangentially and radially. The sections were stained with azan by Heidenhain's method. All species except Clupea harengus were studied on the basis of specimens kindly provided by Baburina.

RESULTS

At different levels of IPL in the retinas of all the species of fish studied there are only three types of elementary grids formed by expansions of axons of the bipolar cells that conform in their structure with each other and with the receptor mosaic. These elementary grids were conventionally described as types A, B, and C grids (Fig. 1). The type A grid has a period and direction to match the cone mosaic. The type B grid has twice as many elements per unit area as the type A grid and it lies at an angle of 45° relative to the direction of the rows of the cone mosaic. The type C grid is four times as frequent as the type A grid and its direction is the same.

The various elementary grids may lie at different levels of IPL or they may overlap. An example of overlapping of grids of types A and B is shown in Fig. 1. The possibility is not ruled out that an externally

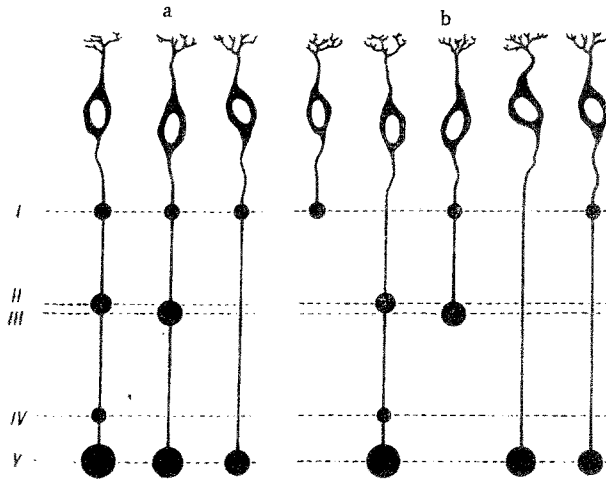


Fig. 3. The two most probable of 135 possible examples of the structure of the inner plexiform layer. Axons of 3 (a) and 5 (b) types of bipolar cells form 9 types of expansions at 5 levels of the inner plexiform layer.

homogeneous grid may be heterogeneous in its component elements. In particular, a heterogeneous type C grid may be formed from several matching but more widely spaced homogeneous grids (Fig. 2). They could not always be broken down into elementary components purely on the basis of their geometric characteristics (the period and direction of the grids). The following additional criteria were used: heterogeneity of size of the expansions forming the grid; possible omissions of one of the elementary grids in some zones of the retina; difference in levels of the components of the grids.

In the last case the superposed grids always differed in the level at which they lay and they could be differentiated in oblique tangential sections through the retina.

The IPL of the species of herring studied had a similar organization although each species showed individual differences.

Clupeonella delicatula. The regular grids of expansions of the bipolar cell axons in IPL were arranged at five levels. The uppermost (close to the amacrine cells) grid belonged to type C. The size of the expansions forming it was uniform. At the next level was a heterogeneous grid of type A + B (Fig. 1). Expansions forming the type A grid were appreciably larger than those forming the type B grid. The next, third level, at which a type A grid was observed, was immediately adjacent to the previous level. In oblique tangential sections including both these levels the smaller expansions forming the type B grid could be seen to disappear gradually with a change in depth of the IPL, leaving expansions forming a type A grid. The type A grid, located at the third level, was thus formed by the same expansions of bipolar axons as one of the elementary grids at the previous level. At the next level there was a type B grid. The orientation and arrangement of the expansions of this grid relative to the squares of the third-level grid matched the arrangement of the smaller expansions forming the type B grid at the second level. On the grounds that expansions in the type B grid (at the second and fourth levels) were placed exactly one beneath the other, they can be considered to be formed by two expansions of the same axons at different levels of IPL. A type C grid (Fig. 2), formed by very large (up to 3-4 μ in diameter) expansions, heterogeneous in size, of bipolar cell axons were found at the fifth level (near the ganglion cells).

Clupeonella grimmi. Regular grids of bipolar cell expansions could be observed at four levels of the IPL. A closely spaced type C grid was found close to the amacrine cells. The different sizes and the mutual arrangement of the expansions composing it indicate that at least one type B grid was a component of this heterogeneous grid. At the next level of IPL there was a type A grid, followed by a type B grid, and, finally, a type C grid near the ganglion cells. The grid at the last level was formed by the largest expansions, but no differences in the size of these expansions could be found.

Clupeonella engrauliformis. Regular grids of bipolar cell expansions could be observed at two levels of IPL. A type A grid was present near the amacrine cells, and a type C grid, consisting of large

heterogeneous expansions near the ganglion cells. It was probably formed by three elementary grids (two A + B), for in some of its zones one or even two type A grids were missing.

Clupea harengus. Regular grids of bipolar cell expansions could be observed at three levels of IPL. The layer of amacrine cells was followed by a type C grid. At the next level there was a type B grid, near the ganglion cells there was a type B grid, and examination of oblique tangential sections showed that the expansions of the second and third grids lay with one immediately above the other. Above the third grid a regular grid formed by the interweaving of cell processes could be seen. Its period and direction coincided with the period and direction of the third grid.

Alosa caspia caspia. Regular grids of expansions of bipolar cell axons could be observed at three levels of IPL. The layer of amacrine cells was followed by a type C grid. It can be concluded from the different sizes and the mutual arrangement of the expansions composing it that this grid contained at least one type B grid. The second grid had the type A structure. The third type B grid was immediately adjacent to the ganglion cells and had an exceptionally clear pattern.

DISCUSSION OF RESULTS

The IPL in the region of sharp vision of the herring retina was similar in structure although the degree of its development differed in the different species. It was most highly developed in C. delicatula and least developed in C. harengus. This similarity suggests a single scheme of structure of IPL in the Clupeidae. The species differences observed can be explained by variations on this single scheme taking place, for example, through a disturbance of the regularity of arrangement of the individual grids of expansions (so much so that the grids could no longer be observed); through the fact that the expansions are too small to allow identification in the light microscope; through the possible absence of some types of bipolars, and so on. In the IPL of the Clupeidae nine elementary grids located at five different levels were described. The uppermost grid, close to the layer of amacrine cells, has the type C structure. On the grounds that in some species (C. grunni and A. caspia caspia) it is evident that the expansions of this grid are different in size, and that omission of expansions of a particular size is found in different zones of the retina, it can be concluded that this grid in all these fish is evidently a combination of three different grids: two type A grids and one type B grid. At the subsequent levels the fourth is a type B grid, the fifth a type A, and the sixth a type B. The grid close to the ganglion cells has the type C structure. Like the grid located near the amacrine cells, it consists of three superposed elementary grids of type A and B. Presumably, therefore, the basic elements of all the grids are grids of type A and B, and the type C grid does not exist as an independent entity.

It must not, however, be supposed that each of these nine grids must necessarily be formed by expansions of axons of a special type of bipolars. Vrabc [15], who described the region of sharp vision in the greater dragonet, showed that bipolar cell axons may have several expansions along their length, and these form grids with the same direction and period in different sublayers of IPL. On the basis of this feature the nine types of expansions discovered by the present writers can be distributed in different ways (two of these are illustrated in Fig. 3) on the bipolar cell axons (not less than 3 and not more than 9 different types of bipolars are needed for this purpose). Which of these schemes is actually found in the herring retina cannot at present be stated more definitely on the basis of these data alone.

In Kaneko's experiments [11] responses of bipolar cells in the goldfish retina were recorded. Kaneko described two electrophysiological types of bipolar cells: with an on- and off-center. A surprising feature of his paper is the absence of morphological correlation. The staining of the cells with Procion yellow showed that both types of response were recorded in cells of the same morphological type — the so-called rod bipolars. These cells ramify widely in the outer plexiform layer and form contacts chiefly with the rods, whereas in IPL they form club-shaped endings in the immediate vicinity of the layer of ganglion cells [6, 17, 11]. The results of the present investigation show that at this level of IPL in the fish retina there are large club-shaped endings of at least three types; consequently, the rod bipolars are heterogeneous and, in turn, they can be divided into a number of subtypes.

The use of geometric criteria of identification can thus provide a basis for classification of cell subtypes and for a more detailed analysis of the architectonics of a structure so complex as the inner plexiform layer of the retina.

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LITERATURE CITED

1. E. A. Baburina, "Structure of the eye and its retina in the common kilka," Dokl. Akad. Nauk SSSR, 102, 625 (1955).
2. E. A. Baburina, "The eye and its retina in the Caspian shad," Dokl. Akad. Nauk SSSR, 100, 1167 (1955).
3. E. A. Baburina and N. D. Kovaleva, "Structure of the retina of the Caspian kilka," Dokl. Akad. Nauk SSSR, 125, 1349 (1959).
4. T. A. Podugol'nikova and V. V. Maksimov, "Regularity of the spatial structure of the receptor and neuronal layers of the teleost retina: light microscopy," Zool. Zh., 52, 541 (1973).
5. I. -B. Ahlbert, "The organization of the cone cells in the retina of four teleosts with different feeding habits (Perca fluviatilis L., Lucioperca lucioperca L., Acerina cernua L. and Coregonus albula L.)," Arkiv fur Zoologi, 22, 445 (1968).
6. S. R. Cajal, "La retina des vertébrés," Cellula, 9, 119 (1892).
7. C. H. Eigenmann and G. D. Shafer, "The mosaic of single and twin cones in the retina of fishes," Amer. Nat., 34, 109 (1900).
8. K. Engström, "Cone types and cone arrangements in teleost retina," Acta Zoologica (Stockholm), 44, 179 (1963).
9. H. Goodland, "The ultrastructure of the inner plexiform layer of the retina of Cottus bubalis," Exp. Eye Res., 5, 198 (1966).
10. E. Hibbard, "Grid patterns in the retinal organization of the cichlid fish Astronotus ocellatus," Exp. Eye Res., 12, 175 (1971).
11. A. Kaneko, "Physiological and morphological identification of horizontal, bipolar and amacrine cells in the goldfish retina," J. Physiol. (London), 207, 623 (1970).
12. A. H. Lyall, "Cone arrangements in teleost retinae," Quart. J. Micr. Sci., 98, 198 (1957).
13. V. Parthe, "Horizontal, bipolar and oligopolar cells in the teleost retina," Vision Res., 12, 395 (1972).
14. A. S. de Testa, "Morphological studies on the horizontal and amacrine cells of the teleost retina," Vision Res., 6, 51 (1966).
15. Fr. Vrabc, "A new finding in the retina of a marine teleost, Callionymus lyra L.," Folia Morphol., 14, 143 (1966).
16. H. I. Wagner, "Vergleichende Untersuchungen über das Muster der Sehzellen und Horizontalen in der Teleostier — Retina (Pisces)," Z. Morphol. Tiere, 72, 77 (1972).
17. P. Witkovsky and J. E. Dowling, "Synaptic relationships in the plexiform layers of carp retina," Z. Zellforsch., 100, 60 (1969).
18. P. Witkovsky and W. K. Stell, "Retinal structure in the smooth dogfish, Mustelus canis: light microscopy of bipolar cells," J. Comp. Neurol., 148, 47 (1973).