Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4002–4005, April 1995
Neurobiology

Spatial organization of retinal information about the direction of image motion

FRANKLIN R. AMTHOR*† AND CLYDE W. OYSTER‡

*Department of Psychology and Neurobiology Research Center and ‡Department of Physiological Optics, School of Optometry, University of Alabama at Birmingham, Birmingham, AL 35294-1170

Communicated by John E. Dowling, Harvard University, Cambridge, MA, January 3, 1995

ABSTRACT **The visual stimuli that elicit neural activity differ for different retinal ganglion cells and these cells have been categorized by the visual information that they transmit. If specific visual information is conveyed exclusively or primarily by a particular set of ganglion cells, one might expect the cells to be organized spatially so that their sampling of information from the visual field is complete but not redundant. In other words, the laterally spreading dendrites of the ganglion cells should completely cover the retinal plane without gaps or significant overlap. The first evidence for this sort of arrangement, which has been called a tiling or tessellation, was for the two types of "α" ganglion cells in cat retina. Other reports of tiling by ganglion cells have been made subsequently. We have found evidence of a particularly rigorous tiling for the four types of ganglion cells in rabbit retina that convey information about the direction of retinal image motion (the ON–OFF direction-selective cells). Although individual cells in the four groups are morphologically indistinguishable, they are organized as four overlaid tilings, each tiling consisting of like-type cells that respond preferentially to a particular direction of retinal image motion. These observations lend support to the hypothesis that tiling is a general feature of the organization of information outflow from the retina and clearly implicate mechanisms for recognition of like-type cells and establishment of mutually acceptable territories during retinal development.**

The four types of ON–OFF direction-selective ganglion cells in the rabbit retina are distinguished from one another by the direction of stimulus motion to which they respond maximally; the preferred directions are orthogonal and correspond roughly to up, down, anterior, and posterior in the visual field (1). And as a class, the ON–OFF direction-selective cells have a distinctive dendritic morphology; each cell has dendrites ramifying at two levels within the inner plexiform layer (IPL) and both ramifications form a mesh-like space-filling array of processes (2, 3). The four types of direction-selective cells, however, cannot be distinguished from one another by their morphology (2, 3).

Estimates of direction-selective cell density and the average size of their dendritic fields suggest that the direction-selective cells have a retinal coverage factor equal to four (2, 3). Since there are four functional cell types, the obvious possibility is unity coverage by each of them. Vaney (4) provided experimental support for the hypothesis that direction-selective cells, or some subset thereof, had unity coverage by showing that an injection of biocytin into a single ganglion cell soma could label an array of six or so cells, whose dendrites touched, but did not cross, and whose morphology was consistent with that of ON–OFF direction-selective cells.

The original demonstrations of the α (Y) cell tiling in cat retina (5–7) used a selective stain to label the α cells that were shown to map, one to one, with physiologically identified Y cells from earlier extracellular recordings. Here, we have used a direct method in which we physiologically identified neighboring ON–OFF direction-selective cells, thereby establishing their directional type, and injected the identified cells with horseradish peroxidase under visual control. In the anatomical analysis, it was readily apparent that some neighboring dendritic fields did not overlap while others overlapped extensively. The nonoverlapping cells were of the same physiological type, and the overlapping cells were of a different physiological type. These observations can be explained only by the existence of four spatially independent dendritic mosaics, one for each of the four cell types.

METHODS

Except for mounting and visualizing the retinas, the methods were similar to those we used earlier (8). The rabbits were deeply anesthetized by intravenous injection of urethane (1.5 g/kg), and under dim red illumination, one eye was removed and hemisected (the animals were then given a lethal dose of anesthetic). The vitreous was removed from the posterior segment of the eye and the retina was carefully removed from the eye cup. The retina was hemisected so that it could be flattened onto a porous tissue culture membrane and placed in a perfusion chamber mounted on a microscope stage. A small amount of methylene blue added to the perfusate was incorporated into the ganglion cell somata, allowing them to be seen with a ×20 or ×40 water-immersion objective.

Under visual control, an extracellular electrode was used to record the responses of ganglion cells to visual stimuli focused on the retina, thereby allowing the cells to be classified by their response properties. By recording from a number of cells in the field, we generated a map showing the relative locations of various types of cells. These same cells were then impaled with a micropipet, their identity was confirmed, and cells were injected with horseradish peroxidase to produce a dark reaction product throughout the cells' dendrites when the retina was reacted with diaminobenzidine. After the processed retina was mounted on a slide and covered with a coverslip, the labeled cells were drawn with the aid of a camera lucida using a ×100 objective on the microscope.

RESULTS

Unity coverage for each of the direction-selective cell types can be seen in the morphological relationships between pairs or triplets of neighboring direction-selective cells with the same or different preferred directions. Fig. 1 illustrates the key result. In this case, three neighboring ON–OFF direction-selective cells were injected with horseradish peroxidase; their dendritic branching in the outer part of the IPL is shown in Fig.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: IPL, inner plexiform layer.

†To whom reprint requests should be addressed at: Department of Psychology, University of Alabama at Birmingham, Birmingham, AL 35294-1170.

FIG. 1. Like and unlike-type ON–OFF direction-selective ganglion cells. The dendritic arbors of these cells are bistratified in the IPL and the two branching planes are shown separately. (*Upper*) The outer IPL level. (*Lower*) The inner IPL level. The two cells with the same preferred direction are labeled cell 1; the cell with the opposite preferred direction is labeled cell 2. The like-type cells (cells 1) have dendrites that touch but do not cross and the dendritic domains of the two cells do not overlap. Cell 2 does not respect the territories occupied by dendrites of the other two cells; there are numerous dendritic crossings and overlap of the dendritic fields. In places, the dendrites of cell 2 run parallel to or intertwine with those of the other cells (cells 1). This sort of fasciculation is characteristic of the relationship between unlike-type cells. The three cells here demonstrate the pattern we have found consistently; cells of the same type participate in tilings of the branching planes, but cells of unlike type belong to different tilings.

1 *Upper* and the inner IPL branching is in Fig. 1 *Lower*. The two cells labeled 1 had the same preferred direction (rightward in the figure or posterior on the retina), whereas the cell labeled 2 had a preferred direction opposite to the other two cells.

The dendrites of the like-type pair (cells 1) do not overlap in either of the IPL branching planes; there are places where the dendrites touch but there is no significant crossing of dendrites. The two cells have different ramification areas in the two branching planes—the upper cell 1 branches more extensively in the outer IPL, whereas the lower cell 1 branches more extensively in the inner IPL—but each cell establishes separate domains in the respective sublayer on which the other cell does not intrude. Thus, the dendritic ramifications of these two cells form independent tilings in each of the IPL branching planes.

The cell labeled 2 has a preferred direction opposite to the others and its dendrites do not respect the territories of the other two cells; crossing branches are quite apparent and there is considerable overlap of the dendritic fields. (This cell was lightly labeled and we have not drawn all of its dendrites; the overlap was actually more extensive than is shown here.) This result is very straightforward and the conclusion is obvious; cells with different preferred directions do not respect one another's spatial domains. The amount of overlap between unlike pairs varies, and the overlap shown here is neither the most nor the least extensive we have seen; the fact that there is variability in the amount of overlap suggests that the mosaics of unlike-type cells have considerable spatial independence.

Some details of the dendritic interactions between neighboring cells are shown in Fig. 2. The arrowheads in Fig. 2 *A* and *B* show sites of contact or near contact between the dendrites of two cells with the same preferred direction (like-type cells). In some instances, a small gap can be seen between adjacent dendrites, but in others one dendrite appears to abut the other (it is not shown here, but these dendritic terminals often have terminal swellings at the contact sites). These dendritic appositions between cells having the same preferred direction are like those of the biocytin-coupled cells reported by Vaney (4).

Where dendrites of the cell labeled 2 overlap those of cells labeled 1 in Fig. 1, the overlapping dendrites tend to run in parallel or fasiculate. The dendrites often intertwine very tightly, a feature best seen in Fig. 2 *C* and *D* (arrowheads). We do not know if this fasciculation results from an active interaction between cells or some passive constraint on the paths that developing dendrites can follow. It is, in any event, an unexpected observation that is characteristic of all the unlike-type cell pairs we have seen. It is interesting that the fasciculating dendrites of the unlike pairs, where opportunities for junctional coupling could be most numerous, do not appear to be those that are biocytin-coupled (4).

We have labeled all four possible like-type pairs and several like-type triplets with results identical to those illustrated in Figs. 1 and 2 (see Table 1); the dendrites of like-type neighbors always tile, abutting one another without gaps or overlap. Of the various possible unlike pairs, we have labeled orthogonal cases, where the cells had horizontal and vertical preferred directions, and pairs with opposite horizontal preferred directions (Table 1). We are missing only the case where cells had vertical but opposite preferred directions. With this modest caveat, we conclude that unlike pairs always invade one another's territory with crossing and fasciculating dendrites. Unlike-type cells are not members of the same tiling.

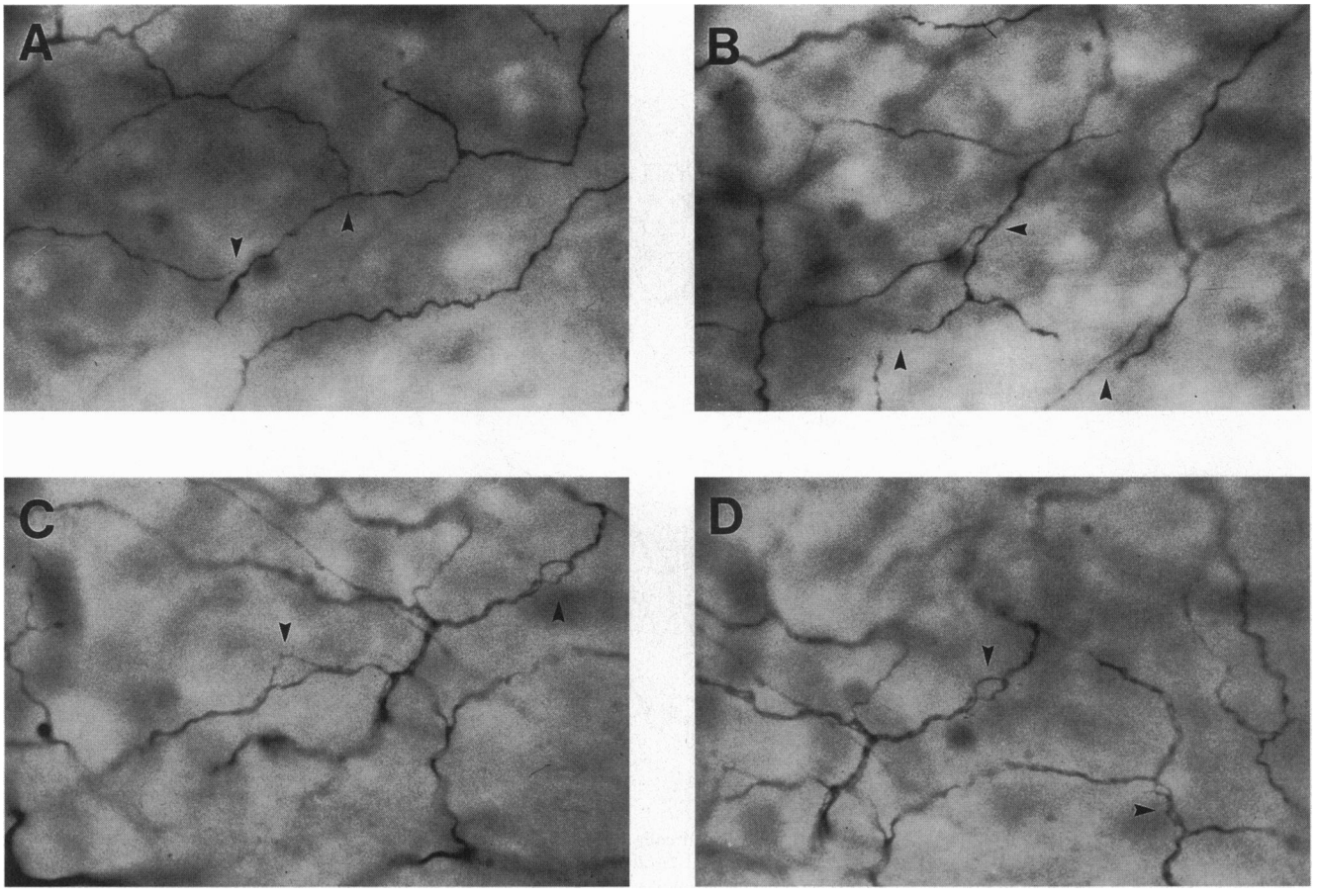

FIG. 2. Dendritic appositions and fasciculations between like and unlike-type cells. These photomicrographs are from a set of labeled cells in which there were three like-type cells and one other cell with a different preferred direction. (*A* and *B*) Dendrites from like-type neighbors come close to or abut one another without crossing (arrows). (*C* and *D*) Displaced but overlapping pictures at slightly different planes of focus that show the dendrities of unlike-type neighbors often run parallel to one another with considerable intertwining of the two cells' dendrites (arrowheads).

Table 1. Sample of like and unlike cell pairs or triplets

	Like type, both				Unlike type		
Preferred direction	←	→	↑	↓	H	V	O
Number in sample	12	3	1	1	10	0	5

Triplets with two like-type and one unlike-type cells have been counted twice, once for the like-type pairing and once for the like–unlike pairing. H, horizontal; V, vertical; O, orthogonal.

DISCUSSION

The class of ON–OFF direction-selective cells is composed of four cell types, each with a different preferred direction. Since like-type cells have nonoverlapping dendritic domains but overlap with unlike-type neighbors, the spatial organization of each cell type can be described as a tiling that provides unity coverage of the retinal plane. In fact, each cell type participates in two tilings with its dendrites, one in the inner IPL and one in the outer IPL. Thus several isomorphic ganglion cell types from a single cell class can form different independent tilings of the retinal plane and, since the size of the excitatory component of the cells' receptive fields matches the size of the dendritic fields (9), independent tilings of visual information space.

ON–OFF direction-selective cells have been encountered at numerous retinal locations from center to periphery (10, 11) and we have observed tiling at a variety of retinal locations in both superior and inferior retina. It is therefore likely that each of the four tilings extends completely across the retina (although, because peripheral direction-selective cells tend to be larger, the spatial grain of the tiling will vary from center to periphery).

When compared to other species and other cell types, these direction-selective cell tilings are more precise than the mosaics of α and β ganglion cells that have been studied in cat retina. Both the α and β cells in cat have dendrites from neighboring cells that overlap; calculated coverage factors are on the order of 1.5 for the α cells and about 3.0 for the β cells (6). The α cells in other species have similar coverage factors (12, 13). In human retina, however, midget ganglion cells injected with neurobiotin have recently been reported to have very stringent tilings with coverage factors no greater than 1 for both cell types, one of which ramifies in the inner IPL and the other in the outer IPL (14).

An obvious question is whether or not tiling by ganglion cell types is a general principle of retinal organization. At present, it is fair to say that all the results in hand are consistent with the notion of tiling by ganglion cell types. Tiling not only tells us how the retina organizes its multiple streams of information outflow but also has implications for the developmental strategies used to organize the retina. The direction-selective cells, for example, must be able to distinguish between their like and unlike-type neighbors during development and to interact appropriately to join in a tiling with their like-type neighbors. Whatever the mechanism for such recognition may be, it may also allow the different types of ganglion cells to recognize and select the particular inputs that are appropriate for the cell type.

We thank L. DeAngelis, M. Villa, W. Belser, Dr. J. Tootle, and Dr. J. Peduzzi for discussions, technical assistance, and computer programming. This work was supported by grants from the National Eye Institute, the U.S. Public Health Service, and the U.S. Office of Naval Research.

1. Oyster, C. W. & Barlow, H. B. (1967) *Science* **155,** 841–842.
2. Amthor, F. R., Takahashi, E. S. & Oyster, C. W. (1989) *J. Comp. Neurol.* **280,** 97–121.
3. Oyster, C. W., Amthor, F. R. & Takahashi, E. S. (1993) *Vision Res.* **33,** 579–608.
4. Vaney, D. I. (1991) *Neurosci. Lett.* **125,** 187–190.
5. Wässle, H., Peichl, L. & Boycott, B. B. (1981) *Nature (London)* **292,** 344–345.
6. Wässle, H., Peichl, L. & Boycott, B. B. (1981) *Proc. R. Soc. London B* **212,** 157–175.
7. Wässle, H., Boycott, B. B. & Illing, R.-B. (1981) *Proc. R. Soc. London B* **212,** 177–195.
8. Amthor, F. R., Takahashi, E. S. & Oyster, C. W. (1989) *J. Comp. Neurol.* **280,** 72–96.
9. Yang, G. & Masland, R. H. (1992) *Science* **258,** 1949–1952.
10. Levick, W. R. (1967) *J. Physiol. (London)* **188,** 285–307.
11. Oyster, C. W. (1968) *J. Physiol. (London)* **199,** 613–635.
12. Peichl, L., Ott, H. & Boycott, B. B. (1987) *Proc. R. Soc. London B* **231,** 169–197.
13. Peichl, L., Buhl, E. H. & Boycott, B. B. (1987) *J. Comp. Neurol.* **263,** 25–41.
14. Dacey, D. M. (1993) *J. Neurosci.* **13,** 5334–5355.