Evidence for spatial regularity among retinal ganglion cells that project to the accessory optic system in a frog, a reptile, a bird, and a mammal

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Abstract
The vertebrate retina contains only five major neuronal classes but these embrace a great diversity of discrete types, many of them hard to define by classical methods. Consideration of their spatial distributions (mosaics) has allowed new types, including large ganglion cells, to be resolved across a wide range of vertebrates. However, one category of large ganglion cells has seemed refractory to mosaic analysis: those that project to the accessory optic system (AOS) and serve vestibulocerebellar mechanisms of motion detection and image stabilization. Whenever AOS-projecting cells have been analyzed by nearest-neighbor methods, their distribution has appeared almost random. This is puzzling, because most aspects of visual processing require the visual scene to be sampled regularly. Here, spatial correlogram methods are applied to distributions of large ganglion cells, labeled retrogradely from the AOS in frogs, turtles, and rats, and to the AOS-projecting displaced ganglion cells of chickens. These methods reveal hidden spatial order among AOS-projecting populations, of a form that can be simulated either by superimposing a single regular mosaic on a random population or, more interestingly, by overlapping three or more regular, similar but spatially independent mosaics. The rabbit is known to have direction-selective ganglion cells (not, however, AOS projecting) that can be subdivided into functionally distinct, regular mosaics by their tracer-coupling patterns even though they are morphologically homogeneous. The present results imply that the direction-selective AOS-projecting ganglion cells of all vertebrates may, likewise, be subdivided into regular, independent mosaics.

Keywords: Retinal ganglion cells, Spatial order, Accessory optic system, Direction selectivity, Tetrapod vertebrates

Introduction
The vertebrate retina contains only five major classes of neurons but these classes embrace a great diversity of discrete neuronal types. To identify and characterize them all is not a trivial task.

Over many years, successes have been achieved through detailed studies of their morphology, physiology, histochemistry, and immunocytochemistry, often in combination; yet many types still remain uncharacterized (Vaney & Hughes, 1990; MacNeil & Masland, 1998).

The study of orderly neuronal distributions (mosaics) can make a special contribution to this task, and has been notably successful in allowing up to four large ganglion cell types to be resolved reliably across a wide range of vertebrate species including fish, amphibians, and mammals (Cook, 1998). In a physiological study using multielectrode arrays, DeVries and Baylor (1997) found 11 functional ganglion cell types in the rabbit, of which at least eight were shown to tile the retina as efficient mosaics.

Yet there is one grouping of morphologically homogeneous large ganglion cells that has seemed refractory to conventional mosaic analysis. These cells project to nuclei of the accessory optic system (AOS), chiefly the nucleus of the basal optic root (nBOR) in nonmammals and its homolog, the medial terminal nucleus (MTN), in mammals (Reiner et al., 1979; Fite et al., 1988). Unlike most retinal ganglion cells, they project neither to the tectum nor to the lateral geniculate nucleus. These ganglion cells, and also the AOS neurons to which they project, are sensitive to low rates of...
movement of large, textured stimuli in specific directions, and are thus very sensitive transducers of relative motion between the retina and visual field (Morgan & Frost, 1981; Britto, 1983; Cochran et al., 1984; Soodak & Simpson, 1988). The neurons of the AOS project, in turn, to the vestibulo-cerebellum and (in nonmammals at least) direct to the oculomotor complex, driving optokinetic reflexes that stabilize the retinal image against self-motion (Finger & Karten, 1978; Brecha et al., 1980; Fite et al., 1988; Simpson et al., 1988).

In published studies where the spatial distribution of AOS-projecting cells has been analyzed by standard methods based on nearest-neighbor distance (NND), this distribution has been found to be nonrandom but poorly ordered (Dann & Buhl, 1987; Podugolnikova et al., 1993; Zhang & Eldred, 1994). The apparent lack of mosaic order among these cells is puzzling because most aspects of visual processing require the visual scene to be sampled consistently over the whole visual field (Perry, 1989).

A newer statistical tool, the spatial correlogram, has been used here to analyze several distribution patterns of large ganglion cells that were labeled retrogradely from the AOS, either in previous published studies in the case of the rat (Dann & Buhl, 1987) and turtle (Zhang & Eldred, 1994), or in new, unpublished studies of similar material from the frog Rana temporaria. In the case of the chicken, we have analyzed the distribution of displaced (inner nuclear layer) ganglion cells in retinas that were labeled retrogradely in vitro from the optic nerve. Although this labeling method is not, in itself, selective for AOS-projecting cells, the large displaced ganglion cells of the chicken are known to be exclusively AOS projecting (Reiner et al., 1979; Yang et al., 1989).

Comparison of the results with various simulations reveals that the low level of spatial order in each AOS-projecting population can most plausibly be explained by the overlap of several similar, spatially independent mosaics, containing cells with different preferred directions of motion. In this paper, such a combination of overlaid, morphologically indistinguishable mosaics is described as a polymosaic.

Methods

Analysis of observed ganglion cell distributions

Amphibian AOS-projecting ganglion cells were studied directly in retinal flatmounts of the frog Rana temporaria, kindly made available by Dr. Vladimir Bastakov. In adult frogs anesthetized by intraperitoneal injection of urethane, horseradish peroxidase (HRP) was introduced into the left nBOR (previously located by anterograde labeling from the optic nerve in frogs of the same size and species) with a sharp, fine needle, through a flap reflected in the mouth just posterior to the optic chiasm. The flap was then closed, and after 12–15 days survival each animal was killed under deep anesthesia. The right retina was isolated, fixed for 20 min in 2.5% glutaraldehyde in 0.1 M phosphate buffer, processed to reveal HRP in vitro with 2.5% glutaraldehyde for 10 min, processed by a variant of the coxalnickel-intensified diaminobenzidine method of Adams (1981), dehydrated with alcohols and xylene, and mounted in DPX (Aldrich, Milwaukee, WI). The coordinates of labeled ganglion cells were plotted across large regions of three retinas (E18, E20, and E21) in which these cells were well preserved and labeled, and autocorrelograms and density profiles were prepared as for the frog. Because of the limited HRP transport time, labeling was typically confined within 4–5 mm of the choroid fissure. However, this included all of the central retina and parts of the ventral and ventrotemporal periphery. As no significant spatial gradients were detected for these cells at these ages, no attempt was made to define an area centralis or visual streak. The density profile analyses presented here were based on pooled samples from all three retinae (n = 2381). However, each of the three samples gave an essentially similar distribution profile when tested in isolation.

Mammalian AOS-projecting ganglion cells were studied by Dann and Buhl (1987) in retinal flatmounts from albino rats, labeled by retrograde transport of horseradish peroxidase from injections into the MTN. For the present analysis, the spatial coordinates of these cells were determined from enlarged copies of the published figures (their Fig. 4) because the original specimens and cell plots were no longer available. The main drawback of working from published figures was an inability to resolve close neighbors that were represented by oversized, overlapping dots. (This difficulty was also found, but to a smaller degree, with the original cell plots from the turtle retina). Any possible systematic bias from this source was excluded by ignoring the most central bins of each spatial correlogram, where these neighbors would...
have been represented. The cells shown in Fig. 4 of Dann and Buhl were more uniformly distributed across the retina than those of *Rana or Pseudemys*, falling off in density generally towards the retinal margin but lacking an area of consistently high density that could justifiably be excluded; thus, the analysis was performed on the entire population, as for the chicken. The two retinas presented by Dann and Buhl (1987) gave similar density profiles, so their data were pooled to increase the sample size and reduce the bin-to-bin variation.

**Construction of simulated polymosaics**

Two different methods of simulation were used. To simulate a three-fold polymosaic, three independent large ganglion cell mosaics from the same catfish retina (the alpha-a, alpha-b, and alpha-c mosaics) were analyzed separately and in pairs, as for Figs. 9 and 10 of Cook and Sharma (1995), and then overlaid for an autocorrelation analysis of the combined population. This analysis simulates the result that would have been obtained if the three mosaic-forming types had not been morphologically distinguishable. Catfish mosaics were chosen because all three are similar in spacing, unlike the equivalent mosaics of other nonmammals (Cook & Noden, 1998).

To simulate a four-fold polymosaic in the absence of suitable data from real mosaics, four copies of a single large ganglion cell mosaic were combined in such a way that cells from different retinal regions were overlaid. The *Xenopus* alpha-ab mosaic of Shamim et al. (1997a) was chosen because it is extensive and regular and lacks strong density gradients. The inclusion of large, apparently cell-free zones (such as the relieving cuts of the flat-mount) would have led to lower multiples of overlap in some parts of the combined mosaic, so the sample was trimmed to a rectangle that lacked major gaps. To ensure that very few of the cells could have any copies of themselves as neighbors, and that no cell could have more than one copy of itself as a neighbor, each copy of the mosaic was inverted about a different combination of axes and given a different offset displacement, greater than the mean nearest-neighbor distance.

**Results**

**Ganglion cells labeled from the AOS in the frog**

A population of up to about 1500 ganglion cells, distributed throughout the retina, can be labeled retrogradely from the nBOR in *Rana temporaria*. These cells were previously described by Podugolnikova et al. (1992) and their spatial distribution was shown, by nearest-neighbor analysis (Podugolnikova et al., 1993), to lack the degree of order that usually characterizes a single cell type. Although Montgomery et al. (1981) divided the AOS-projecting cells of the frog into “at least six types” on the basis of their size and displacement, they probably represent a spectrum of regional and local variants within a single morphological group (Podugolnikova et al., 1992) and may constitute a subclass as defined here (see footnote to Introduction).

In the new specimens used for this study, as in previous studies, most of the large- or medium-sized nBOR-projecting ganglion cells were found in the ganglion cell layer, but rare examples were displaced. Two, three, or four primary dendrites ran from each soma to the most scleral sublamina of the inner plexiform layer, where they formed an extensive and highly planar dendritic tree (Fig. 1). Some trees showed strong asymmetries, but they lacked any obvious regional bias. Dendritic fields tended to be largest in the periphery and smallest in the visual streak where the cell density was highest. For the mid-peripheral zone that dominates the present spatial analysis, Podugolnikova et al. (1993) recorded a mean dendritic-field area of 0.120 mm², which corresponds to a mean equivalent radius of 195 μm. The somata of neighboring cells were often clustered, and the major dendrites of one cell often bypassed the somata of others to continue onwards: these are not normal properties for ganglion cells of a single type.

A notable feature of these cells, not previously reported, is that their dendrites often showed intimate co-fasciculation with the dendrites of labeled near neighbors, in some cases both running in the same direction, away from both somata (Fig. 1), and in others running countercurrent, each towards the soma of the other. Similar behavior has been observed among the dendrites of other direction-selective ganglion cells (see below and Discussion).

Fig. 2A shows the population of labeled frog cells on which the following spatial analysis is based. Only 1001 labeled cells were found in this specimen, suggesting that there may have been significant undersampling. However, density profile analysis is known to be remarkably resistant to undersampling (Cook, 1996), so it is unlikely to have been influenced.

**Ganglion cells labeled from the AOS in the turtle**

Zhang and Eldred (1994) were able to label about 1500 ganglion cells in each retina by injection of rhodamine B into the nBOR, of
of Lucifer Yellow was necessary to reveal isolated cells because the rhodamine-filled dendrites were too densely intertwined with those of neighboring cells to be resolved (Fig. 2 of Zhang & Eldred, 1994). This intertwining may have included co-fasciculation like that described above for the frog, although it was not specifically mentioned. Fig. 6C of Zhang and Eldred (1994) shows the population on which the following spatial analysis is based.

Displaced ganglion cells in the chicken

As reported previously after retrograde labeling from the nBOR (Reiner et al., 1979; Keyser et al., 1988; Yang et al., 1989) and confirmed here after retrograde labeling from the optic nerve, the large displaced ganglion cells of the chicken have large somata (typically 20 µm in diameter) that lie at the inner margin of the inner nuclear layer, and 2–5 relatively thick primary dendrites that ramify extensively in stratum 1 of Ramón y Cajal (1892) like those of the turtle. The axon passes through the inner plexiform layer to reach the ganglion cell and optic fiber layers. Cells with this morphology that project to the nBOR are known to stain selectively with antibodies to nicotinic acetylcholine receptors (Keyser et al., 1988) and their distal dendrites co-stratify with the dendrites of type 1 cholinergic amacrine cells (Yang et al., 1989). In a significant minority of cases, the dendrites of neighboring cells in our specimens showed the same appearance of intimate co-fasciculation as those in the frog, although the phenomenon was not as common.

Fig. 2B shows a population of 987 labeled displaced ganglion cells in one of three specimens pooled for spatial analysis. The sampled area covers 33% of this retina and contains 27% of the maximum number of displaced ganglion cells (3600) found in hatching chickens by Reiner et al. (1979). Slight undersampling may be present, or this small discrepancy may be explained by the observation that these cells (in contrast to most other retinal cell types) are more sparsely distributed in central retina than in the periphery (Reiner et al., 1979; Fite et al., 1981).

Ganglion cells labeled from the AOS in the rat

Dann and Buhl (1987) were able to label just over 1700 ganglion cells in each of two rat retinae from injections into the MTN. All were in the ganglion cell layer. Among members of this population that were then injected with Lucifer Yellow to reveal the full dendritic tree, those from ventral retina were predominantly monostratified in the vitread half of the inner plexiform layer, while those from dorsal retina were more extensively bistratified, with a minority of dendrites in the sclerad half. Their dendritic fields were, however, rather uniform in size, with a mean area of 0.14 mm² and a mean radius of 260 µm. The authors remarked on the frequency with which these cells were found in pairs with intricately interwoven dendrites, and this is evident in several of their figures. Individual cells of this type are shown in their Figs. 9–12, while their Fig. 4 shows the two specimens whose AOS-projecting populations were used for the following spatial analysis.

Detection of polymosasics by correlation analysis

The diagrams of Figs. 3A–3C illustrate the principles of this analysis. Fig. 3A represents a typical probability density profile, derived by autocorrelation from a single, moderately regular mosaic population. Segment a represents the “well” of the profile, which is the zone around each mosaic member from which other
members are reliably excluded. Segment \( b \) is a transition zone that exists because cells vary in their spacing; its steepness depends on the regularity of the mosaic and any systematic variation in its density across the sample. The probability density profile reaches its peak at the point where first-order neighbors are closest: from here, segment \( c \) then extends outwards. In theory, rings of higher-order neighbors should show as damped oscillations in segment \( c \), but these are often lost in noise. Also in theory, the trend of segment \( c \) should be flat, but samples of finite size show a variable fall-off with distance (unless a buffer zone is used) because increasingly distant neighbors are increasingly likely to lie outside the sample boundary (Cook, 1996).

Fig. 3B shows a typical profile derived by cross-correlation between two overlapping mosaic populations (Rodieck, 1991). Members of one mosaic typically have no positional influence on members of the other at distances beyond their soma diameter, so the profile is flat apart from any fall-off towards the edges caused by boundary effects.

An exception commonly arises in the central bin, because short-range interactions are inevitable where both populations have their somata in the same layer (Rodieck, 1991; Cook & Sharma, 1995; Rockhill et al., 2000). Partly for this reason, but mainly because very close neighbors cannot be resolved in the cell plots from which our turtle and rat data are derived, the central bin is masked throughout Fig. 3.

Fig. 3C illustrates the key principle of this paper. It depicts a profile (derived by the usual autocorrelation procedure) from a sample that contains four overlapping, regular, independent mosaics of similar mean spacing, representing a polymosaic. As each cell is placed at the reference point of the correlogram, other members of the same mosaic fall into the “well-like” pattern of Fig. 3A around it, while members of the other three mosaics fall into the “flat” pattern of Fig. 3B. The total probability density is the weighted sum of these two distinct patterns. Although each mosaic is as regular as ever, their exclusion zones interfere to give a dish-like depression, nominally of one-quarter of the usual depth.

There is a second way in which such a density profile could be generated: by combining a single population of mosaic-forming cells with a population of cells that are truly random in their distribution (that is, not themselves a polymosaic). During the autocorrelation of such a combination, whenever the cell at the reference point is from the mosaic-forming population, other members of its mosaic fall into the “well-like” pattern but randomly distributed cells fall into the “flat” pattern, tending to obscure the “well”. Whenever the reference cell is from the randomly distributed population, all of its neighbors fall into the “flat” pattern, filling the “well” even more. Correlation analysis alone cannot distinguish between these two ways of generating a “dish”, but it should be noted that at least one regular mosaic must be present in either case.

Figs. 3H–3O demonstrate these principles in action, through computed density profiles based on real ganglion cell distributions whose mosaic properties are already known. Fig. 3H shows an autocorrelation profile from a mosaic of large ganglion cells that was identified in the catfish by Cook and Sharma (1995). The catfish has three independent mosaics of large ganglion cells, with similar spacings but distinctive patterns of dendritic stratification. Fig. 3I shows a cross-correlation analysis of two of these distinct mosaics of unlike cells, which has the “flat” pattern because a cross-correlation considers only the spacing of unlike cells and the mosaics are spatially independent. Fig. 3J shows an autocorrelation analysis of the same two mosaics, overlaid as a single mixed population to simulate a hypothetical situation in which the two cell types had been plotted together because they could not be distinguished morphologically; this leads to a deep version of the dish-like pattern. Fig. 3K shows an equivalent analysis in which the dish is made shallower by including the third mosaic as well.

Fig. 3L shows the autocorrelation profile from a larger mosaic sample, of bistratified ganglion cells (alpha-ab cells) in the frog Xenopus laevis (Shamim et al., 1997a). In Fig. 3M, every cell in the same sample has been displaced in a random direction by a random distance in the range 0–250 \( \mu m \), to simulate a random population with the same mean density as the original. The autocorrelation profile of this “jittered” mosaic has the “flat” pattern of a random population. In Fig. 3N, a dish-like pattern has been created by overlaying the real mosaic of Fig. 3L with the simulated random population of Fig. 3M. Finally, in Fig. 3O, a similar dish-like pattern has been created by overlaying four separate copies of the mosaic population of Fig. 3L in different orientations and with different displacements (see Methods), to simulate a four-fold polymosaic.

**Evidence that AOS-projecting cells form mosaics**

The remaining four parts of Fig. 3 (Figs. 3D–3G) show density profiles based on autocorrelation analyses of samples from the AOS-projecting ganglion cell populations of the frog, turtle, chicken, and rat, as described above. All four profiles clearly show the dish-like pattern that indicates the presence of at least one regular mosaic.

In the frog profile (Fig. 3D) the margins of the dish lie at about 200 \( \mu m \), implying that the underlying individual mosaic(s) may have a mean distance to nearest neighbor of that order outside the visual streak. This would be consistent with the mean equivalent dendritic radius of 195 \( \mu m \) reported by Podugolnikova et al. (1993), because the longest dendrites of other large ganglion cells in fish and frogs are commonly found to extend as far as the somata of their immediate neighbors in the same mosaic (Shamim et al., 1997b).

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**Fig. 3.** (facing page) Diagrammatic, observed, and simulated probability density profiles showing how spatial regularity can be detected by correlation methods even when multiple mosaics are combined. (A–C) illustrate the principles of analysis described in the text. (D–G) show spatial density profiles obtained by autocorrelation from samples of AOS-projecting retinal ganglion cells from an amphibian, a reptile, a bird, and a mammal. (H–K) show profiles for other large retinal ganglion cells in the catfish, demonstrating how closely the combined profile for three independent mosaics matches these profiles for AOS-projecting cells. (L–O) show profiles for samples and simulated samples from Xenopus laevis, demonstrating how either the combination of a regular mosaic with a random distribution or the combination of four overlaid regular mosaics can approximately match these profiles for AOS-projecting cells. To avoid redundancy, each part is described fully only in the text.
In the turtle (Fig. 3E) and chicken (Fig. 3F), the dish is slightly wider, suggesting a mean mosaic spacing of about 250 μm. The turtle has about 1500 nBOR-projecting ganglion cells in all (Reiner, 1981; Zhang & Eldred, 1994), just as the frog does (Podogolnikova et al., 1992), but in the turtle their density falls off more sharply outside the streak, in the region where the sample was taken. The chicken has many more nBOR-projecting cells (at least 3600: Reiner et al., 1979) but its eye is much larger, even before hatching. Overall, a spacing of about 250 μm within each directional component of the polymosaic would be fully consistent with the numbers and typical dendritic-field sizes of these cells, as indicated in Fig. 3 of Zhang and Eldred (1994).

In the rat (Fig. 3G), where about 1700 MTN-projecting ganglion cells populate a relatively small eye, the breadth of the dish suggests that the spacing within each component of the polymosaic should be of the order of 150 μm, which is considerably less than the reported mean dendritic-field radius of 260 μm (Dann & Buhl, 1987). The rim of the dish in this case was not well defined, so the estimate of spacing might be in error. Alternatively, the trees of neighboring mosaic members might overlap more in the rat than they do in the other species. It was particularly hard to resolve points that represented very close neighbors in the published plots from the rat, so the low probability of finding neighbors in the second bin from the center of the density profile may either be an artefact of unresolved points or a reflection of real interactions between the proximal processes of the members of such pairs, as discussed above. Even when the most central bins are ignored, however, the dish appears to be deeper in the rat than in the nonmammalian examples, and this may reflect the distinctive organization of the AOS in mammals (see Discussion).

Discussion

These observations on retinal flatmounts confirm that large ganglion cells that were labeled retrogradely from the AOS in the frog, turtle, and rat, and also displaced ganglion cells in the chicken that have previously been shown to project exclusively to the AOS (Reiner et al., 1979), all share a common pattern of spatial distribution that is neither fully random nor yet of the right form for a single mosaic. Specifically, they show that a zone exists around each labeled cell, broadly matching the radius of its dendritic tree, in which the probability of finding another labeled cell is distinctly reduced, although much higher than in a single mosaic. The simulations demonstrate that a “dish-like” probability profile of this nature can be created in either of two ways: by superimposing a single orderly mosaic on an entirely random population; or by superimposing several (typically three or more) mosaics upon each other, each one having a similar minimum spacing but being spatially independent of the others.

An origin of the first kind seems biologically implausible. Even non-tessellating neurons might be expected to retain a limited degree of spatial order from their developmental origins (Rodieck & Marshak, 1992) and there is no reason to expect one subset of these morphologically similar cells to differ so radically in spatial organization from the rest. Moreover, the second interpretation, which regards the labeled population as a mixture of distinct, independently mosaic-forming types, is in full agreement with what is already known about the organization of other retinal neurons, most of which belong to regular, independent, type-specific mosaics. DeVries and Baylor (1997) identified eight functionally distinct ganglion cell types in the rabbit with generalized Gaussian receptive fields, and demonstrated that all eight were spaced so as to tile the retina with near-optimal efficiency. (Sadly, this demonstration did not extend to the ON-center, direction-selective, AOS-projecting cells because not enough recordings were obtained from each directional type to allow their mosaic spacing to be determined.) More recently, Rockhill et al. (2000) used spatial cross-correlation to show that six different types of retinal interneuron in the rabbit form mosaics that are spatially independent even though some of them are synaptic partners.

The AOS is phylogenetically ancient and in many respects independent of the retinotectal and retinohalamic visual pathways (Fite, 1985). It is directly related to the control of eye movements, and its nuclei project to the vestibulo-cerebellum and are closely related to the mesencephalic nuclei of the cranial nerves that control those movements (Simpson, 1984). Although it mediates optokinetic nystagmus, and may contribute to the perception of self-motion and optical flow in higher neural centers, it seems not to contribute to the perception of form (Wylie et al., 1998). The receptive fields of its neurons are very large, and their projection fields are not organized as retinotopic maps (Rosenberg & Ariel, 1990).

AOS-projecting retinal ganglion cells are known to be direction selective, and to drive at least three functional types of AOS neuron with different preferred directions of motion. In mammals, these directions seem to vary between species when expressed in eye-centered coordinates because they correspond closely to the rotational sensitivities of the semicircular canals of the inner ear (Simpson et al., 1988). The two planes of rotation that have a vertical component are represented in MTN and the third, horizontal plane in the nearby dorsal terminal nucleus (Soodak & Simpson, 1988). In birds, four or more functional types may be represented within the nBOR complex (Wylie & Frost, 1990; Wylie et al., 1994). As there are no known morphological markers for these directional preferences, morphological studies will inevitably have confounded these functional types together. If each functional type does form an independent mosaic, it is clear that their overall distribution would be of the kind reported here.

The apparently greater depth of the correlogram “dish” in the rat than in the other species, together with the predominance of close cell pairs (rather than clusters of three or more) in the published cell distributions, suggests that the labeled cells in this species may represent only, or mainly, two functional types. This would be consistent with what is known about the MTN-projecting neurons of the rabbit, which represent only two of the three preferred directions of motion (Soodak & Simpson, 1988).

The interpretation of the autocorrelogram data in this study as evidence for a set of independent, orderly mosaics among AOS-projecting cells is given extra weight by extensive experimental observations on another directionally selective ganglion cell type, the ON–OFF BiS1 cells of the rabbit. These constitute an entirely independent set of motion detectors, not projecting to the AOS and showing clear differences from AOS-projecting cells, particularly of size, dendritic stratification, and preferred velocity (Buhl & Peichl, 1986; Famiglietti, 1992; He & Masland, 1998). Thus, any similarities between them and the AOS-projecting cells are very likely to reflect general functional requirements of motion-detecting systems.

And, indeed, there are several such similarities. First, BiS1 cells have also been shown to comprise distinct functional types with distinct preferred directions of motion, and yet these remain morphologically indistinguishable even when functionally identified and contrasted (Oyster et al., 1993). Second, the patterns of local dendritic interaction of AOS-projecting and BiS1 cells are
unusual and similar. Among the AOS-projecting cells of the rabbit (Buhl & Peichl, 1986; He & Masland, 1998), as in the frog and to a smaller extent in the chicken (this paper), dendrites co-fasciculate with those of their immediate neighbors, in some cases running in such intimate contact that the light microscope cannot resolve them apart except where they briefly diverge. Similar behavior, when seen by Amthor and Oyster (1995) among BiS1 cells, was reported to occur exclusively between cells of unlike directional preference; and He and Masland (1998) have argued convincingly, on grounds of spacing and coverage, that co-fascilating AOS-projecting cells in the rabbit should also be of unlike preference. Third, the AOS-projecting and BiS1 cells of the rabbit—although differing from each other in size, receptive-field diameter, and stratification pattern—share quantitative features of dendritic branching that ganglion cells which are insensitivity to motion do not share (Famiglietti, 1992). Fourth, and most important for the present discussion, the overall distribution of both AOS-projecting cells and BiS1 cells shows only a low level of order, yet BiS1 cells with the same directional preference are now known to form regular, independent, and accurately tessellated mosaics, distinguishable after intracellular injection by their patterns of tracer coupling (Vaney, 1994; Amthor & Oyster, 1995).

Thus, the BiS1 cells represent an experimentally confirmed example of exactly the type of spatial organization proposed here for AOS-projecting cells: a polysomatic, or superimposition of independent mosaics that cannot be distinguished by morphology alone.

In future studies, direct evidence that the AOS-projecting population is indeed a polysoma might be obtained either by the intracellular injection of several neighboring cells of the same electrophysiologically identified direction preference, or by the demonstration of type-specific tracer coupling similar to that of the BiS1 cells, or possibly by the selective retrograde labeling of its individual components, at least in those species where cells with different directional preferences have been reported to project to different regions of the AOS (birds: Wylie et al., 1994; mammals: Soodak & Simpson, 1988).

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