Mosaic Regularity of Horizontal Cells in the Mouse Retina Is Independent of Cone Photoreceptor Innervation

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PURPOSE. To determine whether the density and the mosaic regularity of the population of horizontal cells is dependent on innervation from the cone photoreceptors by comparing these features in wild-type and transgenic mice expressing an attenuated diphtheria toxin in the cones.

METHODS. Retinal wholemounts from coneless transgenic mice and their wild-type littermates were immunostained for calbindin or for cone opsins, and labeled cells and outer segments were counted to determine horizontal cell and photoreceptor density. The x-y positional coordinates of each horizontal cell were also determined, from which the geometrical properties of the horizontal cell mosaic were examined using nearest-neighbor and Voronoi domain analyses. Autocorrelation and density-recovery profile analyses were also conducted to identify the presence of exclusion zones within the population of horizontal cells. For each sampled field, random simulations of matched density, constrained by the physical size of the horizontal cells, were generated in parallel and analyzed with the real data.

RESULTS. Coneless mice were confirmed to contain only 3% of the normal cone photoreceptor population. Despite the loss of these afferents, horizontal cell density did not differ between the wild-type and coneless retinas. Mosaic regularity in wild-type and coneless retinas did not differ, but each differed significantly from random simulations of identical density. Horizontal cells in both the wild-type and coneless mouse retina exhibited exclusion zones extending beyond the physical size of the soma, suggested to reflect intercellular interactions during early development that drive tangential dispersion; these were slightly larger in the wild-type retina.

CONCLUSIONS. Cone innervation is not a necessary condition for horizontal cell survival during postnatal development. The resiliency of the regularity in the horizontal cell mosaic is consistent with the hypothesis that such global patterning is an emergent property of these cells as they engage in local interactions that are largely independent of their afferent innervation. (Invest Ophthalmol Vis Sci. 2003;44:965–973) DOI:10.1167/iovs.02-0831

The neural retina is composed of several distinct types of retinal nerve cells that are positioned within one of three different cellular layers. Within each layer, these retinal neurons occupy discrete strata, and, within each stratum, individual types of retinal neuron are typically distributed as orderly arrays, or mosaics, producing a uniform tiling of the retinal surface. The mechanisms generating such regularity in these mosaics are still uncertain, but accumulating evidence has implicated intercellular interactions occurring at the time of dendritic differentiation. These interactions are thought to be restricted to cells of the same type, driving two cells apart from one another to establish a minimal spacing between them. Through the engagement of such local interactions in the presence of sufficient densities of cells, an initial distribution of cells produced by fate determination events may acquire its characteristic regularity.

The evidence supporting this hypothesis now comes from diverse sources. First, the very nerve cells that form regular retinal mosaics have been shown to disperse tangentially during development. Consistent with this observation, other studies have shown that the geometrical properties of developing mosaics are maintained during the period of cellular arrival, implying that nerve cells must be actively shifting their positions as new cells enter the mosaic stratum. Mosaic regularity has been shown to decline in an orderly manner as a function of cell density, suggesting that it depends on local interactions with cells of the same type. Finally, modeling studies show how random distributions of nerve cells can be progressively transformed into more regular ones as the constituents elaborate their dendrites to repel neighboring cells apart from one another.

That such regular regular mosaic are generated by homotypic interactions is further supported by the fact that these mosaics appear to be indifferent to the densities or positioning of other cell types on the retina. For example, cross-correlation analysis has shown that the positioning of various types of retinal neurons that are assembled as orderly mosaics is independent of other mosaics in the retina (but see also Ref. 10), even those with which they share synaptic relationships (but see also Ref. 12). Furthermore, the mosaic regularity of one class of amacrine cell, the cholinergic amacrine cell, is unaltered by the complete elimination of one of their target neurons, the retinal ganglion cells, during early development. Horizontal cells in albino mice have also been found to establish regular retinal mosaics, although they migrate through a disordered neuroepithelium. Together, these results suggest that the intercellular interactions between like-cells driving tangential dispersion are sufficient for the genesis of global patterning characteristic of these mosaics.

How such cells interact through their dendrites at the time of differentiation is unclear, but one possibility is that competitive interactions for afferent input may drive dendritic differentiation in turn affecting the homotypic exercise of local exclusion zones and the consequent formation of mosaic regularity. The absence of cross-correlational patterning between synaptically related cell types does not necessarily render those synaptic relationships as ineffectual in influencing the establishment of mosaic order. Nor does the demonstration of a clear positional correlation between one class of bipolar cell

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and its cone photoreceptor input prove that afferents control the establishment of mosaic regularity in the latter population.\textsuperscript{14,15} It would be helpful to know whether the elimination of afferent inputs during early development has an effect on the mosaic regularity of another population of cells. 

To this end, in the present study we sought to determine the fate of the remaining horizontal cells in the population of cone photoreceptors during early development on the population of horizontal cells in the mature mouse retina. The mouse retina contains only one type of horizontal cell, the axon-bearing horizontal cell, receiving rod input to the axon terminal system and cone input to the dendrites.\textsuperscript{19,20} These cells differentiate their mature horizontal morphology toward the end of the first postnatal week,\textsuperscript{5,21,22} coincident with the formation of synaptic contacts from cone photoreceptors.\textsuperscript{23} In some species, the horizontal cells are known to establish cone-opsin specific connectivities at different locations within the dendritic arbor,\textsuperscript{5} suggesting that their dendritic differentiation is indeed influenced by these afferents. In the present study, cones were ablated by using transgenic mice that produce an attenuated diphtheria toxin under the control of an upstream sequence derived from the human L-cone opsin promoter.\textsuperscript{24} This same sequence has been shown to drive lacZ expression and the production of its reporter gene product in murine M- and S-cone opsin (1:50,000; both kindly provided by Jeremy Nathans, The Johns Hopkins University) was used to detect their respective and cone opsin (1:1000; Oncogene Research Products; La Jolla, CA), incubated with the HRP subsequently detected using a reaction kit (SG; Vector Laboratories, Inc.). In the sectioned material, the two cone opsin antibodies were used separately rather than being combined (L/M-cone opsin at 1:30,000 and S-cone opsin at 1:50,000). A mouse monoclonal antibody to cytochrome oxidase (1:100; Molecular Probes, Eugene, OR) was also used in sectioned retinas, to detect the presence of cone somata containing large, perinuclear mitochondria in the outermost stratum of the outer nuclear layer (ONL). A biotinylated goat anti-mouse IgG (1:200; Jackson ImmunoResearch) was used to detect the primary antibody, followed by streptavidin-HRP and the reaction kit (SG; Vector Laboratories, Inc.), as just described. Additional tissue from two litters on P5 was also immunostained for UV-cone opsin to confirm the early postnatal onset of this cone ablation. Images of the whole-mounts and sectioned retinas were obtained with a photomicroscope with fluorescence attachment (FXA; Nikon, Tokyo, Japan) and a digital camera (model DP11; Olympus, Tokyo, Japan).

Labeled horizontal cells were sampled in wholemounts near the optic nerve head (central samples) or near the retinal periphery (peripheral samples) along two to four retinal axes from 10 wild-type and 9 coneless retinas. Each sampled field occupied 0.225 mm\textsuperscript{2} of retinal area (aspect ratio of 1:1.25), so that approximately 15% of the retinal surface was analyzed. Average densities, in cells per square millimeter, were determined for the central and peripheral samples in each animal, and group averages (wild-type versus coneless) in the center and the periphery were then compared. Retinal area was also measured (Bioquant Nova Prime; R&M Biometrics, Nashville, TN), and retinal area was subsequently scaled to the mean area to ensure that differences in fixation did not increase the variance in our measures of mosaic geometry. All density measures were corrected for this scaling.

To determine whether horizontal cell soma size differed between the coneless and wild-type retinas, the retinas from three wild-type and four coneless animals were processed for calbindin immunocytochemistry as wholemounts, with 0.005% diaminobenzidine (Sigma, St. Louis, MO) used to detect the streptavidin-HRP. The areas of horizontal cells were imaged from central retinal samples using a ×100 oil-immersion objective and video camera attached to the microscope, measured on computer (Bioquant Nova Prime; R&M Biometrics), and subsequently converted to diameters for circular profiles of identical area. A minimum of 100 cells was measured from each retina. Because these retinas had been dehydrated and cleared before being coverslipped, but none of the fluorescent specimens used for mosaic analysis had undergone dehydration, we corrected the measurements for shrinkage by adjusting them by a factor determined by the difference in each retinal area to the mean of those areas for the wild-type. To determine whether horizontal cell mosaic geometry, as evidenced in wholemount preparations stained for both UV- and M-cone opsin, (C, D) The near-complete elimination of the M cones was confirmed in sectioned retinas. One of the rare surviving M cones in dorsal retina is shown in the center of (D). (E, F) A greater number of UV cones survived into adulthood, contributing most of the surviving cones in these coneless mice. (F, large arrow) Row of immunoreactive outer segments typical of the wild-type retina. (F, small arrows) A few of the individual or clustered outer segments surviving in the coneless retina. (G, H) A row of cone somata in the outermost parts of the ONL, evidenced by their signature perinuclear mitochondria immunoreactive for cytochrome oxidase (G, arrows) was no longer detectable in these coneless mice. A row of immunoreactive inner segments was present in both. (I, J) The reduction in cones was well advanced by P5. (I, large arrow) A row of UV-cone photoreceptor cells and their terminals extending to the future site of the OPL (their outer segments had yet to differentiate). (J, small arrows) Some of the few remaining UV cones in this P5 retina, one of the more severely reduced specimens at this age. Scale bar: (A, B) 80 μm; (C, D) 52.5 μm; (E, F, I, J) 500 μm; (G, H) 35 μm.

MATERIALS AND METHODS

Consecutive transgenic mice were obtained from Jeremy Nathans and Yanshu Wang (The Johns Hopkins University, Baltimore, MD) and subsequently bred in the Central Vivarium at the University of California, Santa Barbara (UCSB). Heterozygotes were mated with C57BL/6 mice from Charles River Laboratories (Cambridge, MA), generating mixed litters of wild-type and heterozygous transgenic offspring, hereafter referred to as “wild-type” and “coneless.” Littermates at 5 weeks of age were heavily anesthetized with 120 mg/kg sodium pentobarbital (intraperitoneally) and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2 at 20°C). All research with animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and was conducted under authorization by the Institutional Animal Care and Use Committee at UCSB.

Left and right whole retinas were dissected from the eyecups immediately, or the anterior chamber and lens were removed and the eyecup was embedded and cryosectioned at 16 μm in the plane passing radially through the optic nerve head. Whole retinas were prepared for immunofluorescence, as previously described.\textsuperscript{5} A rabbit polyclonal antiserum to calbindin was used to detect the horizontal cells in one retina (1:1000; Oncogene Research Products, La Jolla, CA), and a cocktail of rabbit polyclonal antiserum to L/M-cone opsin (1:3000) and S-cone opsin (1:50,000; both kindly provided by Jeremy Nathans, The Johns Hopkins University) was used to detect their respective cone photoreceptors in the opposite retina. Primary antibodies were detected in wholemounted retinas with either Cy2- or Texas red-conjugated goat anti-rabbit IgG (1:200; Jackson ImmunoResearch, West Grove, PA). Sectioned retinas were prepared for immunohistochemistry using biotinylated goat anti-rabbit IgG and streptavidin-horseradish peroxidase (HRP) at the recommended dilutions (Elite Vectastain ABC Kit) and visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories Inc., Burlingame, CA).
Consequently, greater regularity is required of the real mosaic to reliably differentiate it from random. An average size was calculated for each animal, from which group averages and standard errors were determined.

The x-y coordinates of each cell in a field were also determined (Metamorph, Downingtown, PA), from which nearest-neighbor and Voronoi domain analyses were then conducted with software programs designed for this purpose. The Voronoi domain of a cell is the area in the plane of the retina that contains all points closer to that cell than to any other cell. Voronoi domain borders define the population of near neighbors for a given cell, from which the distance to the nearest neighbor is determined. Regularity indices for each field were subsequently determined, by dividing the average nearest-neighbor distance or Voronoi domain area by the SD. Autocorrelograms were also prepared from every sampled field, from which density-recovery profiles were constructed. The autocorrelogram is the plot of the position of all cells in the field with respect to every other cell, generated by sequentially moving each cell to the origin of the autocorrelogram and then plotting the position of all other cells relative to the origin. The density-recovery profile is a graphic depiction of these data, showing how density varies from the origin. The autocorrelogram and density-recovery profile reveal the presence of “exclusion zones,” regions surrounding individual cells in which other cells of like type are less frequently or never positioned. The “effective radius,” an indirect measure of the size of such exclusion zones, was then calculated from the density-recovery profile.

For every sampled field (37 vs. 35 central samples and 38 vs. 35 peripheral samples, from wild-type versus coneless retinas, respectively), a random simulation of identical density was also generated, in which the positioning of cells was constrained by the soma size parameters (mean ± SD) for the real horizontal cell population (hereafter referred to as “random” simulations). (Because the horizontal cell population in the wild-type and coneless mice did not differ in soma size, they were combined to generate this estimate of soma size constraining those random simulations). Consequently, for every nearest-neighbor, Voronoi domain, or autocorrelation analysis conducted on the real data, a simultaneous random simulation was performed.

The density of cone photoreceptors was also estimated in these wild-type and coneless retinas by sampling each of the opposite retinas at a location midway between the optic nerve head and the retinal margin along each of the four retinal axes, in fields 0.225 mm², thereby sampling roughly 7% of the retinal surface in each retina. These were averaged to generate within-animal averages, which were subsequently plotted as group averages and standard errors.

A Student’s t-test was conducted to compare retinal area size, cone density, and horizontal cell size, and a two-way mixed ANOVA was conducted to compare horizontal cell density in the wild-type and coneless mice in both the center and the periphery. One-way independent ANOVAs were conducted to compare regularity indices and effective radii in the wild-type and coneless retinas and in the random simulations, followed by post hoc Scheffé test comparisons between groups. Asterisks indicate significant differences in the figures: Double asterisks indicate differences between the biological data and the random simulations, whereas single asterisks indicate differences between the wild-type and coneless retinas.

**RESULTS**

All the coneless retinas were readily distinguished from the wild-type retinas by virtue of their near-complete elimination of the cone photoreceptor population (Figs. 1A, 1B), as described in the literature. Measurements of cone density in wild-type retinas were consistent with previous estimates, whereas the reduction in the coneless retinas, a 97% decrease (Fig. 2A; P < 0.001), was consistent with previous estimates in these transgenic mice. Those few surviving cones should be mainly UV-sensitive cones, as previous studies have indicated that virtually all the M cones are eliminated in these mice, whereas approximately 5% of the UV cones survive into adulthood. Examination of retinal sections stained for either the population of M cones or the UV cones in these coneless mice confirmed the near-complete loss of the M cones (Figs. 1C, 1D) from the dorsal retina, where they are normally situated, whereas few or no UV cones were present, most commonly within the ventral retina, where they are most frequent in the wild-type retina (Figs. 1E, 1F). Examination of sections that had been stained for cytochrome oxidase also permitted the faithful discrimination of coneless from wild-type retinas. The ONL of a normal adult mouse displays large, perinuclear mitochondria in the outermost stratum (within three to four soma diameters from the outer limiting membrane), indicative of cone somata. This portion of the ONL in the coneless mice was immunonegative (Figs. 1G, 1H), confirming the loss of the cone photoreceptor cells. This cone loss was well under way by P5 (Figs. 1I, 1J), before the transdifferentiation of horizontal cells from their immature radial or stellate appearance into their mature horizontal morphology and before cone photoreceptor innervation of those horizontal cells.

The population of horizontal cells within these wholemounts was identified with antibodies to the calcium-binding protein calbindin (Figs. 3A, 3B). The area of the wholemounts themselves did not differ between the wild-type and coneless retinas (Fig. 2B). The elimination of the cone photoreceptors had no obvious effect on horizontal cell size (Fig. 2C) or on horizontal cell density (Fig. 2D), although horizontal cell density was significantly lower in the peripheral samples (P < 0.01). These calbindin-immunoreactive horizontal cells in wholemount preparations looked essentially normal (Figs. 3A, 3B).

**Nearest-Neighbor and Voronoi Domain Analyses**

The patterning of horizontal cells in coneless retinas was qualitatively indistinguishable from that observed in wild-type retinas (Figs. 3C, 3D), but both were readily distinguished from random simulations constrained by soma size (Fig. 3E). The distribution of the nearest-neighbor distances derived from each of the wild-type and coneless samples showed that, although both differed conspicuously from nearest-neighbor distributions derived from random simulations constrained by soma size, they were essentially indistinguishable from one another (Figs. 4A, 4B). Whereas the nearest-neighbor distances in the coneless population tended to slightly smaller values (Figs. 4A, 4B), the overall distribution of nearest-neighbor distances contained in these histograms for the wild-type and coneless retinas did not differ in their general shapes, suggesting that there was no greater variability in one than in the other population. Independent confirmation of this was found by comparing the regularity indices derived for each of the central or peripheral samples (Figs. 4C, 4D). Although there was the slightest tendency for regularity indices to be lower in the periphery than in the center, there was no difference between those derived from the wild-type and coneless samples. Notice that the random simulations generated regularity indices as high as 3.5 (Figs. 4C, 4D). This was a direct consequence of the high density of cells and our conservative assumption that no horizontal cells could overlap one another physically. The average soma size (Fig. 2C) thus constrained cell positioning, prohibiting nearest-neighbor distances less than this distance (e.g., Figs. 4A, 4B) and reducing the variability that would be achieved from a truly random simulation. If this constraint was eliminated, the regularity indices for the random simulations decreased to approximately 2.0. Regardless, the regularity indices for the random simulations were still significantly
smaller than were those from the wild-type or coneless mosaics ($P < 0.001$).

The same conclusions are to be drawn from a comparison of the distribution of Voronoi domain areas derived from each of the sampled and simulated fields (e.g., Figs. 3C–E). The Voronoi domain analysis is considered a more efficient means of describing the uniformity by which a population of cells is distributed across (or “tiles”) the retina. Although the Voronoi domain distributions of the wild-type and coneless samples were more narrowly tuned than were those derived from random simulations of matched density, they did not differ from one another, at either central or peripheral locations (Figs. 5A, 5B). By calculating a regularity index for these Voronoi domain data (the average Voronoi domain area in a sample divided by the standard deviation from that sample), we found a similar lack of difference between the wild-type and coneless samples, yet significant differences from the random simulations (Figs. 5C, 5D; $P < 0.001$).

**Autorcorrelation and Density-Recovery Profile Analyses**

Autocorrelation analysis of each of these sampled fields revealed a region in the center of the autorcorrelograms in which horizontal cells were less likely to be positioned (Figs. 3F, 3G). The size of this exclusion zone was greater than would be expected by the physical size of two horizontal cells preventing one another from occupying the same location on the retina, evidenced from the autocorrelograms generated from such random simulations (Fig. 3H). The density-recovery profile derived from these autocorrelograms (actually, the average density-recovery profile, derived from the individual density-recovery profiles after their individual bins had been averaged) permits a graphic representation of these data (Figs. 6A, 6B), showing that the size of the central region of lower density in the autocorrelograms derived from the wild-type and coneless data were conspicuously larger than that produced by the soma size alone (compare with continuous and broken lines in Figs. 6A, 6B). The size of this exclusion zone in these density-recovery profiles can be determined by integrating the unoccupied space in these histograms beneath the average density and then calculating the radius of a cylinder of equivalent size, which is the effective radius (Figs. 6C, 6D). This short-hand confirmed that the exclusion zones of the wild-type and coneless samples were significantly different from those achieved by random simulations ($P < 0.001$). In addition however, they revealed a slight but significant difference between the wild-type and coneless retinas ($P < 0.05$), with the effective radius being marginally smaller in the coneless retinas.

**DISCUSSION**

The present study demonstrates that neither horizontal cell density nor mosaic regularity is perturbed in mice in which virtually all the cone photoreceptor population is missing. The loss of this cone photoreceptor population should be occurring during the first postnatal week, because this same upstream sequence, derived from the human L cone opsin promoter, drives $\text{lacZ}$ transgene expression in both types of mouse cones as early as P3.26 Although the developmental time
FIGURE 3. (A, B) The population of calbindin-immunoreactive horizontal cells, in two peripheral retinal fields, appeared qualitatively similar in wild-type and coneless retinas. (C–E) Typical distributions of horizontal cells and their Voronoi domains from central retinal fields of a wild-type and a coneless retina, and a random simulation of identical density. Broken lines exclude all cells with incomplete Voronoi domains (i.e., those intersecting the boundary of the sampled field). (F–H) Autocorrelograms for central fields from a wild-type and a coneless retina and from a random simulation, yielding a plot of the relative density of cells at increasing distance from each cell. Successive annuli are at 5-μm increments from the origin, extending to a radius of 50 μm. By averaging across the area within each annulus, a density-recovery profile (shown in Fig. 6) can be generated. Scale bar: (A, B) 80 μm.

FIGURE 4. (A, B) Bar histograms showing the average (±SE) nearest-neighbor distributions for wild-type and coneless central (A) and peripheral (B) samples. Line histograms show the nearest-neighbor distributions for random simulations matched in density to the real wild-type and coneless data and constrained by horizontal cell soma size. (C, D) Regularity indices derived from the individual samples that contributed the data shown in (A) and (B). Data are the mean ± SE. The two populations of random simulations (those matched to the wild-type versus those matched to the coneless samples) have been combined.
FIGURE 5. (A, B) Average Voronoi domain distributions for wild-type and coneless samples derived from central and peripheral retina and associated random simulations. (C, D) Regularity indices derived from the samples that contributed to (A) and (B).

FIGURE 6. (A, B) Average density-recovery profiles for wild-type and coneless central (A) and peripheral (B) retinas. The density-recovery profiles for the wild-type and coneless data were nearly identical, each defining a region of reduced density surrounding the horizontal cells that is greater in area than the exclusion zone to be expected by soma size alone (evidenced by the density-recovery profiles generated from the random simulations constrained by soma size). (C, D) Effective radii derived from the individual samples that contributed the data shown in (A) and (B). Effective radii were slightly but significantly smaller in the coneless retinas.
course for the loss of photoreceptors has not been described, immunostaining P5 retinas with antibodies to human S-cone opsin reveals that a 40% reduction in the density of the UV cones has already occurred, consistent with this expectation. Consequently, cone photoreceptor loss occurs contemporaneously with horizontal cell differentiation, when these cells change from a radial or stellate morphology into their mature horizontal morphology, and when they would normally be receiving innervation from the cones.

Density of Horizontal Cells

The present results imply that the survival of horizontal cells into adulthood is in no way dependent on the presence of an innervating population of cone photoreceptors. In light of various studies showing afferent control of cell survival during development (see Refs. 32, 33 for review), and given the exclusive presynaptic rights held by the cone photoreceptors for the dendritic field of the horizontal cells, a severe postnatal loss of horizontal cells might have been expected. Whether some other process substitutes for the cone photoreceptor terminals in sustaining the horizontal cells—for example, the rod spherules or the bipolar cell dendrites—is unknown. Horizontal cells are also known to be coupled by gap junctions at their dendrites, and gap junctional signaling has been shown to modulate cell survival in the developing retina, but there is currently no evidence for such a role in the control of the number of horizontal cells.

Unlike the other populations of retinal neurons, the horizontal cells may be unique in that they do not undergo a normal overproduction followed by naturally occurring cell loss. The number of horizontal cells can be increased by overexpression of the cell survival protein bcl-2, but this does not necessarily mean that the normal retina overproduces a population of cells committed to this horizontal cell fate. Our own counts of horizontal cells in the mouse retina labeled at various developmental stages also failed to find greater numbers on the day of birth (Raven MA, Reese BE, unpublished observations, 2002), well after they have all been generated and have migrated into the neuroblast layer, but before their transdifferentiation and innervation by the cones. Whether this lack of naturally occurring horizontal cell death is due to a modulation of their dendrites, or but before their transdifferentiation and innervation by the cones, remains worth keeping in mind.

The size of the population of horizontal cells should not be presumed to be rigidly determined in the mouse retina, however. The density of horizontal cells has been shown to differ tremendously between different strains of mice. For example, A/J mice contain roughly half the number of horizontal cells found in the retinas of C57BL/6 mice. These differences are more likely to arise from the variable control of horizontal cell fate specification, rather than being due to a modulation of cell survival among an initially overproduced population. This control of fate specification may be mediated through the regulation of Prox1 expression controlling cell-cycle exit decisions by progenitor cells, because both progenitor cells and horizontal cells express Prox1 during early development, and altered expression of Prox1 modulates the production of horizontal cells (Dyer MA, ARVO Abstract 830, 2002).

Despite this preserved density of horizontal cells, the horizontal cells are not oblivious to the loss of the cone afferents. Indeed, horizontal cells are notorious for displaying morphologic plasticity after insults that occur during early development or late in life. The present insult is no exception: Whereas the plexus of horizontal cell processes within the outer plexiform layer (OPL) looks largely unaffected, a number of neurofilament-positive processes have sprouted to course radially within the ONL (Raven MA, Reese BE, manuscript in preparation). Whether this sprouting relates to the slight but significant decrease in the size of the exclusion zone is unclear at this stage.

Regularity of Horizontal Cells

The present results demonstrate that the population of horizontal cells in the coneless mouse retina achieves its normal mosaic regularity, evidenced with either nearest-neighbor or Voronoi domain analyses, despite the early loss of the population of cone photoreceptors. These results are consistent with recent hypotheses suggesting that mosaic regularity is a consequence of intercellular interactions between homotypic cells, indifferent to the densities of other cell types or to their positioning on the retina. Horizontal cells have been shown here and elsewhere to exhibit exclusion zones, regions surrounding individual cells within which other horizontal cells are less likely to be positioned. The sizes of these exclusion zones are greater than the physical size of the horizontal cell somas, suggesting that some other constraint is keeping these cells apart. It has been suggested that dendritic interactions occurring at the time of morphologic differentiation mediate an intercellular repulsion that contributes to the establishment of mosaic regularity, supported by recent demonstrations that differentiating cells restrict their contacts to like-type cells and by modeling studies showing how dendritic interactions driving tangential dispersion can transform a random distribution of cells into a more regular one. Consistent with this view, disrupting the cytoskeleton of developing cholinergic amacrine cells alters the regularity of this mosaic. Still other studies indicate that diffusible factors are unlikely to mediate this intercellular interaction. Together, these studies suggest that like cells interact with one another in a contact-mediated manner to generate a spacing mechanism at the local level, establishing a regularity in the distribution of horizontal cells.

The present results suggest that these homotypic interactions, although for the most part independent of afferent input, are in fact subtly modulated in the presence of cone afferents. The size of this effect is very small, less than 1 μm so small, in fact, as to have no impact on the regularity indices associated with either the Voronoi domain or the nearest-neighbor analysis. Cone afferents may affect the intercellular spacing of the horizontal cells by driving dendritic differentiation directly or by mediating a competition for afferent input. Whether the sprouting of horizontal cell processes into the ONL is another manifestation of this altered local environment within the OPL or is a response to some other loss of trophic support or release of glutamate remains to be seen. Whatever the mechanism that leads to the reduced exclusion zone associated with the horizontal cells in the coneless retina, the reduction is not so severe that it alters the patterning of the horizontal cells, as assessed by either of the two spatial measures used in the current study. The regularity in these retinal mosaics is an emergent property of the population as it undergoes homotypic interactions that are largely independent of other cells, including their afferents, in the immediate vicinity.

References
