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Visual Stimulation Is Required for Refinement of ON and OFF Pathways in Postnatal Retina

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Summary

ON and OFF pathways separately relay increment and decrement luminance signals from retinal bipolar cells to cortex. ON-OFF retinal ganglion cells (RGCs) are activated via synaptic inputs onto bistratified dendrites localized in the ON and OFF regions of the inner plexiform layer. Postnatal maturational processes convert bistratifying ON-OFF RGCs to monostratifying ON and OFF RGCs. Although visual deprivation influences refinement of higher visual centers, no previous studies suggest that light regulates either the development of the visual-evoked signaling in retinal ON and OFF pathways, nor pruning of bistratified RGC dendrites. We find that dark rearing blocks both the maturational loss of ON-OFF responsive RGCs and the pruning of dendrites. Thus, in retina, there is a previously unrecognized, pathway-specific maturation that is profoundly affected by visual deprivation.

Introduction

An elemental feature of vertebrate visual systems is the functional separation of neuronal pathways signaling ON and OFF responses to light (Hartline, 1938). Visual-evoked signals divide into two parallel pathways at the first synapse in the retina. Light activation of photoreceptors depolarizes ON bipolar cells and hyperpolarizes OFF bipolar cells. ON and OFF pathways remain segregated to a large extent in the retina, the lateral geniculate nucleus, and to at least the input layers of the visual cortex (Stryker and Zahs, 1983; Norton et al., 1985). Morphological specializations reflect the functional separation of these pathways. In retina, synaptic transfer of the ON and OFF signals from bipolar cells occurs in distinct layers. The axonal terminations of ON bipolar cells and the dendrites of their synaptic target neurons, ON retinal ganglion cells (RGCs), ramify as monostratified processes in sublamina *b* of the inner plexiform layer (IPL). In contrast, OFF bipolar cell and OFF RGC dendrites ramify as monostratified processes in sublamina *a* of the IPL (Famiglietti and Kolb, 1976; Nelson et al., 1978). A subset of RGCs signal both the onset and termination of light. The dendrites of these ON-OFF cells are bistratified with arborizations in both sublaminae.

In spite of well-characterized maturational refinements of function and neuronal connectivity in higher visual centers of mammals after eye opening, it is often assumed that mammalian retinae are fully mature by eye opening (Daw, 1995). Many aspects of synaptic signaling in retina reach maturity by eye opening. For example, by the time of eye opening in rodents, rabbits, cats, and ferrets, most of the morphological features and the expression of synthesizing enzymes, transporters, and receptors for neurotransmitters resemble those in adult animals (Fisher 1979; Greiner and Weidman, 1981; Redburn and Madtes, 1987; Pow and Barnett, 2000; Sassoe-Pognetto and Wässle, 1997; Johnson et al., 2003). Other functional and anatomical features underlying synaptic signaling can continue to mature for several weeks after eye opening. Amplitudes of RGC light responses in cat, ferret, and mouse increase after eye opening (Tootle, 1993; Wang et al., 2001; Tian and Copenhagen, 2001). The rate of spontaneous synaptic inputs to RGCs is low for a few days after eye opening but then increases approximately 4-fold in a period from 1.5 to 2 weeks after eye opening in mouse (Tian and Copenhagen, 2001). A further postnatal development is the ongoing restriction of RGC dendrites from bistratified processes into monostratifed, sublamina-specific arborizations after birth. This "pruning" is one of the best examples of the maturational reorganization of neuronal processes after birth (Wong and Ghosh, 2002) and has been found in cat (Maslim and Stone, 1988; Dann et al., 1988; Bodnarenko et al., 1995) and ferret (Bodnarenko et al., 1999). There are no reports that similar pruning occurs in mouse.

Consistent with the anatomical loss of bistratified RGCs, a recent study shows the percentage of RGCs having light-evoked ON-OFF responses in ferret retina declined from ~40% prior to eye opening to ~20% after eye opening. In contrast, earlier studies found no evidence for a developmental reorganization of light-evoked signaling of the ON and OFF pathways of cat or rabbit (Tootle, 1993; Bowe-Anders et al., 1975). One goal of our study was to examine whether the developmental transition of ON-OFF to ON and OFF-responsive RGCs occurs in postnatal mouse retina.

Visual deprivation dramatically alters the normal refinement of connections and function in visual centers of the cortex (Cynader and Mitchell, 1980; Mower, 1991; Fox et al., 1991; Carmignoto and Vicini, 1992; Gordon and Stryker, 1996; Kirkwood et al., 1996). These modifications have served as a paradigmatic model of activity-dependent plasticity in the nervous system. Plasticity in precortical regions of the visual system has recently been reported. Dark-reared ferrets have a higher percentage of ON-OFF cells in the dorsal lateral geniculate nucleus (dLGN), the principal target of RGC axons, than control animals raised in cyclic light (Akerman et al., 2002). Although the ON-OFF responses recorded on dLGN are generated in the retina, no studies have examined the effects of dark rearing in the maturational transformation of ON-OFF cells to ON and OFF cells in retina.

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Morphologically, it has been shown that dark rearing enlarges the diameter of RGC dendritic fields in turtle retina (Sernagor and Grzywacz, 1996). However, no studies have addressed the issue of whether dark rearing can modify the stratification patterns of RGC dendrites in the IPL of mammalian retinas. There is some evidence that synaptic activation of ON bipolar cells might play a role in the stratification of the RGC dendrites (Bodnarenko and Chalupa, 1993; Bodnarenko et al., 1995). The interpretation of these earlier studies, using intraocular injections of APB to activate the metabotropic glutamate receptors (mGluR6) of the ON bipolar cells, is somewhat enigmatic, however. Namely, a later study of mice having a genetically engineered deletion of the mGLuR6 receptor, which also blocks ON bipolar cell function, failed to find any difference in stratification patterns of RGCs (Tagawa et al., 1999). It remains an unanswered question whether other pharmacological or nonpharmacological means of altering inputs to ON bipolar cells can influence RGC dendritic stratification patterns. However, the initial formation and refinement of stratified dendrites before eye opening may depend on activation of nicotinic acetylcholine receptors expressed on third-order retinal neurons. Mice lacking $\beta 2$ subunits of nicotinic receptors exhibited delayed refinement of ganglion cell dendrites (Bansal et al., 2000).

In this present study we used a microelectrode array (Meister et al., 1994; Nirenberg and Meister, 1997) to record light responses from mouse retina. As a measure of developmental changes in light-evoked signaling in the ON and OFF pathways, we focused on how the relative population of ON-OFF RGCs varied with age before and after the time of eye opening. We also sought to ascertain whether visual deprivation affected the maturation of these pathways. To determine whether development and visual deprivation influenced the stratification patterns of RGC dendrites, we examined retinas from a mouse strain in which a fluorescent chromophore, yellow fluorescent protein (YFP), is constitutively expressed in a sample of RGCs (Feng et al., 2000).

Results

Action potentials from 2312 individual RGCs were recorded from the retinas of 19 different animals using a multielectrode array (see Experimental Procedures). The retinas were stimulated with full field steps of green light (LED, 567 nm wavelength, 1 s duration). In the first series of experiments, shown below, we determined how the relative percentage of RGCs that responded to both the onset and termination of light (ON-OFF cells) varied with postnatal age.

The Proportion of ON-OFF Cells Declines after Eye Opening

Light responses were recorded from mice in three different age groups. These groups encompassed ages before, immediately after, and 2 weeks following eye opening (postnatal days 10–12 [P10–12], P13–15, and P27–P30, respectively). In all three groups, more than 95% of the cells that spiked spontaneously also exhibited robust light-evoked responses. This provided strong evidence that, in mouse retina, similar to cat and rabbit (Tootle, 1993; Masland, 1977), a large proportion of the RGCs are light responsive by 2 to 3 days prior to eye opening. Figure 1A shows representative spike frequency histograms of RGC light responses from an ON (top), an ON-OFF (middle), and an OFF cell (bottom).

The percentage of ON-OFF cells was 3.5-fold less in the P27–30 group compared to the P10–12 group. Before eye opening 76% $\pm$ 3.5% of the light-responsive RGCs produced action potentials at both ON and OFF. Just after eye opening, the proportion of ON-OFF RGCs dropped to 40.8% $\pm$ 6.5%. Two weeks after eye opening the percentage of ON-OFF cells declined to 21.5% $\pm$ 3.5% (Figure 1B, Table 1). This age-related decline in the percentage of ON-OFF cells after eye opening in mice is similar to the developmental trend observed in ferret retina and to the developmental reduction in the percentage of ON-OFF cells recorded in LGN of cat (Wang et al., 2001; Daniels et al., 1978).

To quantify the relative predominance of the ON or OFF versus ON-OFF responsiveness, we calculated the response dominance index (RDI) for each individual RGC (cf. Akerman et al., 2002; see Experimental Procedures). In brief, cells with an RDI near zero exhibited more balanced ON and OFF components, while cells with an RDI near 1 had correspondingly more ON or OFF dominated responses. RDIs for the three cells of Figure 1A are noted in the upper right corner of each trace. The graphs in Figure 1C plot the mean RDIs for the entire sample of cells recorded at the three different ages. The RDI was 1.87-fold higher in the P27–30 group (0.84 $\pm$ 0.01) than the P10–12 group (0.45 $\pm$ 0.01), indicating a maturational transition to more ON and OFF-dominated RGCs.

Cumulative probability plots of response dominance indices show that the ensemble population of RGCs in the older age group were more dominated by responses at ON or OFF. These curves demonstrate that the changes in the mean RDIs (Figure 1C) did not merely reflect changes in a subset of cells. The plots in Figure 1D reveal that 78% of the RGCs in the P27–30 group had an RDI $>$ 0.8. In contrast, only 19% of the RGCs in the P10–12 group had an RDI $>$ 0.8.

These data illustrate a profound and significant age-dependent reduction in the percentage of ON-OFF responding RGCs. This maturational change occurs over the 2.5 week period starting from just before eye opening and indicates a functional transition of light-evoked signaling to ON or OFF dominated pathways.

Dark Rearing Retards the Maturational Decrease in the Percentage of ON-OFF RGCs

To test whether the maturational changes in the percentage of ON-OFF RGCs is regulated by visual activity, we recorded from 4-week-old mice raised in constant darkness from birth (P0 to P27–P30). We compared light-evoked RGC responses recorded from the dark-reared mice to age-matched control animals raised on a 12/12 hr light/dark cycle. The raster plots and the histograms in Figure 2A show light responses from ON-OFF cells recorded from a P30-aged control reared animal (left) and a P29-aged dark-reared animal (right). The most notable difference between these two groups of mice was that the percentage of ON-OFF cells in dark-reared

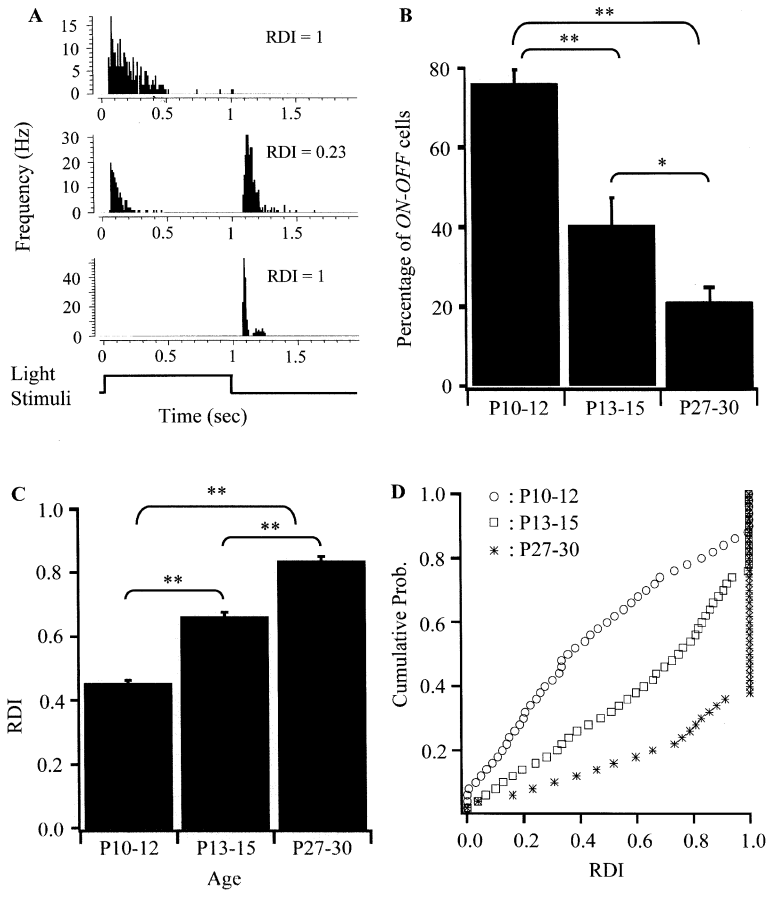

Figure 1. The Percentage of RGCs with ON-OFF Responses Declines after Eye Opening

Light-evoked RGC action potentials were recorded from retinas of mice at ages before, immediately after, and 2 weeks following eye opening (P10–12, P13–15, and P27–30). (A) Representative frequency histograms of RGC light responses of an ON cell (top), which had increased spike activity at the onset of light stimulus, an ON-OFF cell (middle), which had increased spike activity at both the onset and the offset of the stimulus, and an OFF cell (bottom), which only responded to the offset of the light, recorded from control-reared P30-aged mice. (B) Mean percentage of RGCs that responded to both the onset and offset of light stimulus from the three groups of mice. The percentage of RGCs with ON-OFF responses decreased from 76% ± 3.5% before eye opening to 40.1% ± 6.5% immediately after eye opening to 21.5% ± 3.5% at the ages of P27–30, respectively. ANOVA test shows that the differences between these three groups were highly significant ($p < 0.0001$). The data for the P10–12 group included 974 cells from seven retinas; the P13–15 group included 578 cells from five retinas; and the P27–30 group included 415 cells from four retinas. (C) Mean RDI from all RGCs, including ON, OFF, and ON-OFF cells in the three age groups. The differences between these three groups were highly significant ($p < 0.0001$). (D) Normalized cumulative distribution curves of RDI from the same three groups of RGCs as in (C). Note, for clarity, only 50 points with equal interval (every 20th, 10th, and 8th points for the P10–12, P13–15, and P27–30 groups, respectively) are plotted for each group.

P27–30 group was much higher (82.5% ± 2.5%; dark group in Figure 2B). This value was significantly higher than the percentage of ON-OFF cells in age-matched controls (P27–30; $p < 0.0001$) but not different from the percentage of ON-OFF cells in P10–12-aged animals ($p = 0.4006$). The most parsimonious explanation of the above data is that dark rearing prevented the normal maturational decrease in the percentage of RGCs that were activated at both ON and OFF.

Figure 2C plots the cumulative probability of the response dominance indices for the three groups of animals. The entire curve of dark-reared animals is very close to that for the P10–12 group. ANOVA test shows that the difference of the average RDI between the dark-reared group (0.48 ± 0.02) and the age-matched control group (0.84 ± 0.01) is highly significant ($p < 0.0001$). However, the average RDI of the dark-reared group is not different from that of control-reared P10–12 mice (0.45 ± 0.01, $p = 0.2057$). These findings add additional credence to the idea that dark rearing essentially froze the ON and OFF synaptic pathways by preserving the RGCs in a state comparable to that at P10–12.

In summary, these electrophysiological data show that the percentage of ON-OFF cells is reduced with age over the period from just before eye opening to 2 weeks after eye opening. On the assumption that the

Table 1. Effects of Age and Dark Rearing on the Percentage of ON-OFF Responsive Cells

P10-12 Control	% ON-OFF Cells	P13–15 Control	% ON-OFF Cells	P27–30 Control	% ON-OFF Cells	P27–30 Dark Rear	% ON-OFF Cells
a	61.76 (84/136)	h	33.11 (49/148)	m	13.27 (13/98)	q	85 (102/120)
b	75.63 (90/119)	i	45.83 (66/144)	n	25.19 (34/134)	r	77.65 (66/85)
c	86.29 (151/175)	j	31.34 (21/67)	o	18.52 (10/53)	s	85 (119/140)
d	73.42 (58/79)	k	29.55 (39/132)	p	28.91 (37/125)		
e	67.59 (98/145)	l	64.38 (56/87)				
f	82.72 (134/162)						
g	84.81 (134/158)						
Average ± SE	76.03% ± 3.48%		40.84% ± 6.54%		21.47% ± 3.48%		82.55% ± 2.45%
% of Total	749/974 = 76.9%		231/578 = 40%		94/415 = 22.7%		287/345 = 83.2%

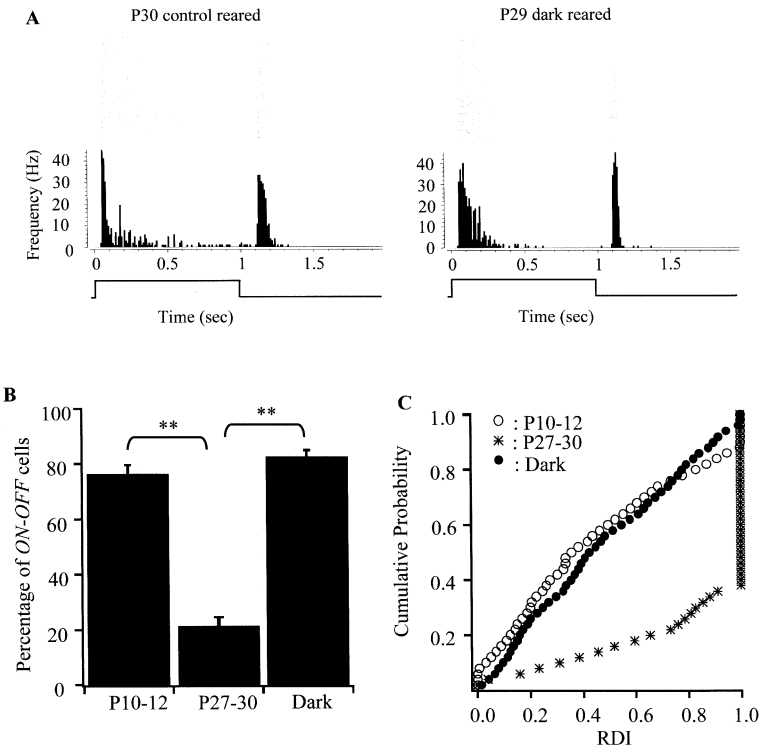

Figure 2. Visual Deprivation Suppresses the Developmental Decrease of ON-OFF RGCs

Light-activated RGC spike activities recorded from retinas of mice raised in constant darkness since birth (P0 to P27–P30) are compared to those from mice raised in a control 12 hr dark/12 hr light cycle (P10–12 and P27–30 groups). Average light intensity illuminating the cages during subjective day was 40 lux for control mice. (A) Representative raster plots and frequency histograms of RGC light responses of two ON-OFF cells from a control-reared P30-aged mouse (left) and dark-reared P29-aged mouse (right), respectively. The raster plots show the 100 trials of spike activity evoked by a 1 s light stimulus. The frequency histograms show the frequency of the light-evoked spike activity averaged from the 100 trials. (B) Mean percentage of RGCs having ON-OFF responses in the three groups of mice. The age-matched dark-reared group had 4.8-fold more ON-OFF RGCs than the P27–30 control group. The difference was statistically significant ($p < 0.0001$). The difference between P10–12 control groups and the dark-reared group was not significant ($p = 0.4006$). (C) Normalized cumulative distribution curves of RDI from the same three groups of RGCs as in (B).

sampling of RGC types by the multielectrode array is equivalent at all ages, we interpret this finding as an indication that there is an age-dependent refinement of the ON and OFF pathways in mouse retina. Specifically, with increased age fewer of the RGCs are driven by both ON and OFF bipolar cells. These experiments also indicate that dark rearing suppresses the normal developmental decline of light-evoked signaling in the ON-OFF pathways. A complementary action of visual deprivation was observed in ferret LGN, where a larger percentage of ON-OFF cells were found in dark reared animals (Akerman et al., 2002). To the extent that similar developmental mechanisms exist in mouse and ferret, our results raise the possibility that at least part of the dark rearing effects on the ON and OFF pathways that are observed in LGN originate in the retina.

To determine whether there are morphological correlates of the refinement of ON-OFF pathways presented above, we examined the stratification patterns of RGC dendrites in the IPL as functions of age and also in response to dark rearing.

Laminar Stratification Patterns of RGC Dendrites Are Revealed in Thy1-YFP (H) Mice

Feng et al. (2000) generated 25 lines of transgenic mice in which spectral variants of green fluorescent protein (GFP) are expressed in neurons of retina and brain. These fluorescent proteins label dendrites, somata, axons, and axonal arbors of the neurons. Expression within individual cells remains stable for at least several months and has no apparent effects on synaptic structure (Feng et al., 2000). We used one of these mouse lines, Thy1-YFP (H), to visualize and quantify the stratification patterns of RGCs in the IPL of the retina.

Figure 3A is a low-power image of YFP-labeled cells in a whole-mount retina. Dendritic arbors, somata, and axons are readily evident. An enlarged view in the same retinal preparation, magnified from the marked area in Figure 3A, shows minimal overlap of YFP-expressing RGCs. This separation allowed us to examine the stratification of dendrites from individual RGCs without the confounding problem of isolating and identifying dendrites from overlapping adjacent RGCs (Figure 3B). Figure 3C shows expression of YFP and tyrosine hydroxylase, a marker for dopaminergic amacrine cells, in one area of a retina. The images were accumulated from successive confocal scans that progressed from the ganglion cell layer to a plane distal to the IPL/inner nuclear layer (INL) border. In this composite image, the red-labeled processes mark dopaminergic amacrine cells. The cell bodies (*) are localized at the border of the IPL and the INL (Tagawa et al., 1999; Haverkamp and Wässle, 2000). The pseudocolored green and blue processes are obtained from another channel of the same stack of confocal images scanned with a shorter wavelength (argon, 488 nm) laser, which shows two YFP-expressing RGCs. One of these has dendrites in sublamina *a* and the other in sublamina *b*. The green image was obtained from z sections across the outer 49% of the IPL. The blue image was obtained from z sections across the inner 51% of IPL and the ganglion cell layer. Thus, the OFF RGC shown on the left has green-colored dendrites and a blue soma and axon. The ON RGC, having dendrites, a soma, and an axon confined to the inner retina is colored entirely blue. Note

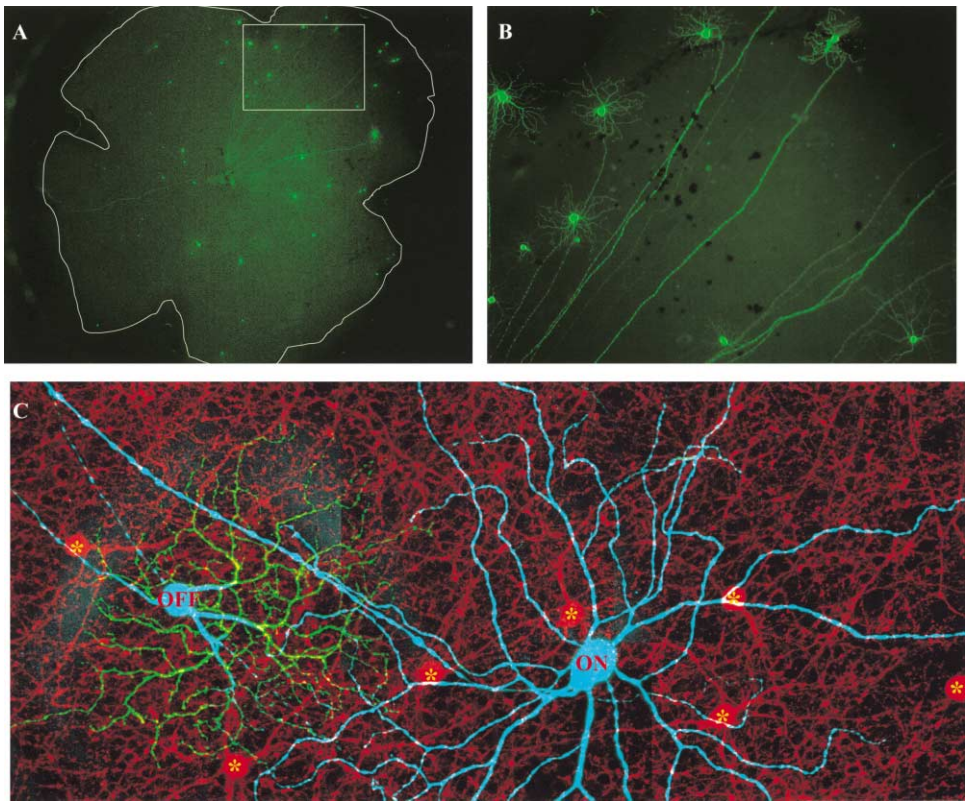

Figure 3. Dendritic Ramification Patterns of RGCs Can Be Readily Identified Using Confocal Microscopy

(A) View from vitreal side of a flat mounted retina harvested from a P30-aged, control-reared Thy-1-YFP-expressing mouse (see Experimental Procedures for details). (B) An enlarged view of the area indicated by the box in (A). Axons from individual RGCs cross the retina from each somata to the optic nerve head. (C) Stacked image of two YFP-expressing RGCs from a control-reared P30-aged mouse retina. Red label denotes the immunolabeling pattern of TH-positive cells. These dopaminergic cells have their somata positioned at the INL/IPL border and their processes localized along the INL border of the IPL. TH-positive staining was used here as a marker for the distal edge of the IPL. The green processes show stacked images of YFP-expressing processes obtained from sequential z scans between the IPL/INL border to the middle of the IPL (see inset). The blue processes and somata show stacked images of YFP-expressing RGCs obtained from z scans from the middle of the IPL to the vitreal/RGC border. The OFF RGC is shown with monostratified dendrites (green) in sublamina *a* and a somata and axon (blue) in the proximal retina. The ON RGC has dendrites (blue) restricted exclusively to sublamina *b*.

that no dendrites of the RGC labeled as ON were found in sublamina *a* and no dendrites of the RGC labeled as OFF were found in sublamina *b*. RGCs with monostratified dendrites comprised 70% of the RGCs in the P30 mice (see below). The remaining YFP-expressing RGCs were bistratified, having dendritic arbors in both sublaminae.

YFP is expressed in several different classes of mouse RGCs (Figure 4A). Monostratified RGCs with arborizations in sublamina *b* or *a* are illustrated as Mono (*b*) and Mono (*a*). Two bistratified RGCs are shown in the right column. The processes of all six of these cells are color coded for depth. Blue processes arborize in the inner region of the IPL and green ones in the outer IPL. Although extensive classification of RGC types was beyond the scope of this present study, comparisons of YFP-expressing cells to RGC classes identified by Sun et al. (2002), using diolistic staining with the fluorescent dye DiI, or by Doi et al. (1995), using HRP uptake as a marker, reveal that YFP is expressed in a moderately extensive subset of previously identified cell classes. For example, the top cell in Mono (*b*) could easily be a member of Sun et al.'s RG_A or RG_C classes, while the lower cell is very similar to Sun et al.'s. RG_B type. Likewise, the lower cell in Mono (*a*) could be a member of the RG_A or RG_C classes.

To determine whether RGC dendrites were mono- or bistratified, we examined whole-mount retinas and recorded the location of the arbors along the radial dimension as we focused through the IPL from the ganglion cell layer to the IPL/INL border. In mouse retina, the radial depths of RGC dendrites across the IPL are reasonably confined, which makes identification of the stratification patterns straightforward. Sun et al. (2002), who examined the radial depth of dendrites carefully, found the average radial spread, or "thickness," was $\pm$13.6% of the IPL for all RGC cells. Different cell types ranged between $\pm$9% to $\pm$18%. Sun et al. (2002) also showed a clear gap between the dendrites of bistratified dendrites having one arborization in sublamina *a* and a second in sublamina *b*. The radial extent of RGC dendrites in mouse appears qualitatively similar to that reported for rabbit (Rockhill et al., 2002). RGC dendrites of the YFP-expressing mice we used in this study were

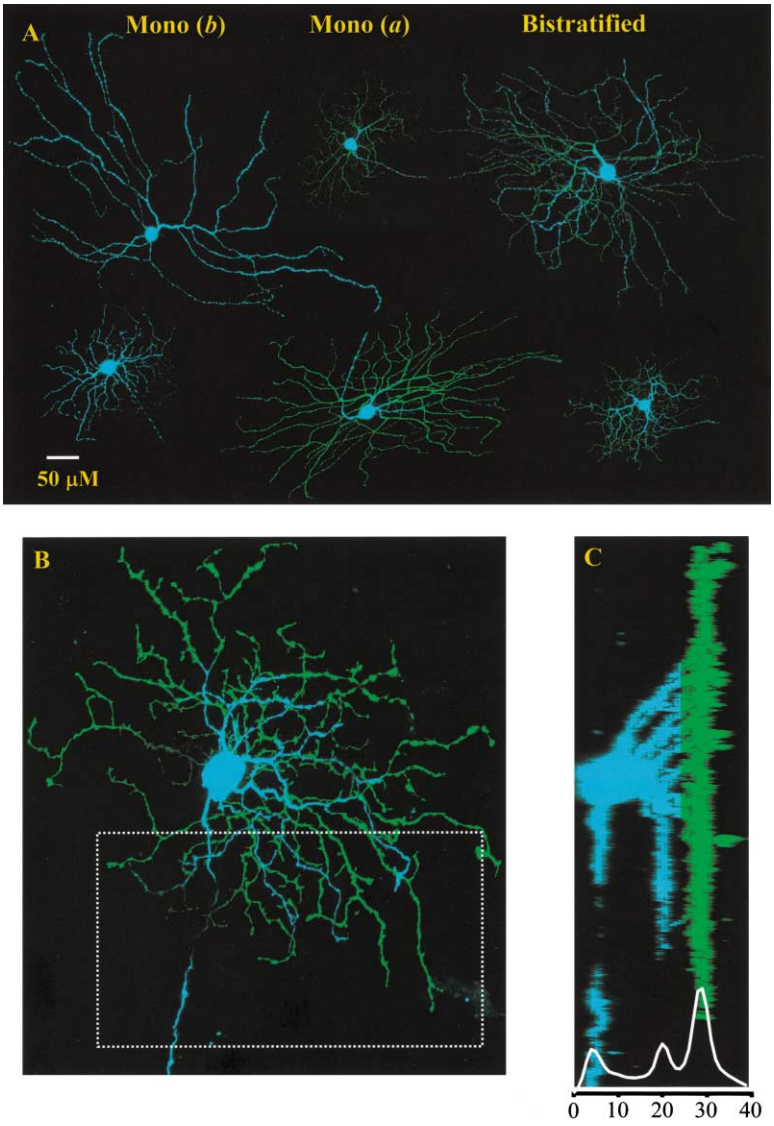

Figure 4. Thy1-YFP-Positive RGCs Include Large Samples of Cell Types Having either Monostratified or Bistratified Dendrites

Thy1-YFP is expressed in most classes of morphologically identified ganglion cell types in mouse retina. (A) Stacked z section images of RGCs that ramified only in sublamina *b* (Mono b), only in sublamina *a* (Mono a), and both sublamina *a* and *b* (Bistratified) of IPL with different size and morphology of dendritic fields. The dendrites of the RGCs are color coded based on the ramification depth in IPL. Dendrites in the outer 50% of IPL (sublamina *a*) are color coded green. The dendrites in the inner 50% of IPL (sublamina *b*), soma, and axons are color coded blue. All cells are obtained from control-reared P30-aged mice. (B) Image of a YFP-expressing bistratified RGC derived from the maximum projection of a stack of images with color-coded dendrites, soma, and axon. (C) The 90° rotation view of (B). The plot at the bottom quantifies the normalized pixel intensity as a function of IPL depth from the area indicated by the box in (B). The number indicates the number of frames in z axis.

similarly confined to a narrow depth of the IPL. Thus, RGCs with bistratified dendrites could be easily identified. Figures 4B and 4C illustrate a whole-mount and xz view of a bistratified RGC. The separation between the dendrites in each sublamina is evident in the xz view and in the intensity profile drawn at the bottom of Figure 4C.

The Percentage of Bistratified RGCs in Mice Declines after Eye Opening

We compared the stratification patterns in P10 YFP-expressing mice to those in P30 mice. In P10 mice, 53% of the RGCs were bistratified (53/100 cells, four retinas). In P30 mice, only 29% of the RGCs were bistratified (58/198 cells, five retinas, Table 2). Figure 5 plots the percentage of bistratified and monostratifed RGCs in mice at the two different ages. A similar age-dependent loss of bistratified RGCs has been observed in ferret and cat retinas (Bodnarenko et al., 1995, 1999). Given that this pruning occurs in mouse, it is plausible to assume that comparable changes take place in retinas of other mammals as well.

Dark Rearing Blocks the Age-Related Loss of Bistratified RGCs in Postnatal Mouse Retina

Mice were dark reared from birth until P30. Stratification patterns from dark-reared mice were compared to those of age-matched control animals raised in 12:12 hr light:dark cycles. In the dark reared P30-aged mice, 53% of the RGCs exhibited bistratified dendrites (207/396 cells, six retinas, Table 2). This percentage was significantly higher than the age-matched control animals (53.2% $\pm$ 2.5% versus 28.9% $\pm$ 3%; $p < 0.0001$) but not significantly different from the proportion of bistratified RGCs in the P10 control mice (53.2% $\pm$ 2.5% versus 52.8% $\pm$ 2.7%; $p = 0.9277$). Thus, dark rearing not only eliminated the developmental loss in the percentage of ON-OFF cells responding to light (Figure 2), it also retarded the "pruning" process that normally reduces

Table 2. Effects of Age and Dark Rearing on the Percentage of Bistratified RGCs

P10 Control	% Bistratified Cells	P30 Control	% Bistratified Cells	P30 Dark Rear	% Bistratified Cells
A	52.38 (11/21)	E[a]	18.75 (6/32)	J	60.26 (47/78)
B	52.27 (23/44)	F[a]	32.35 (11/34)	K[a]	60.53 (23/38)
C	46.67 (7/15)	G	32.65 (16/49)	L[a]	48.57 (17/35)
D	60 (12/20)	H	35 (14/40)	M	48.48 (48/99)
		I	25.58 (11/43)	N	54.35 (25/46)
				O	47 (47/100)
Average ± SE	52.83% ± 2.74%		28.87% ± 2.98%		53.2% ± 2.5%
Total Cells	53/100 = 53%		58/198 = 29.3%		207/396 = 52.3%

[a] Ramification patterns of cells from these retinas were confirmed with confocal images.

the number of RGCs with bistratified dendrites. These results support the hypothesis that dark rearing retards the normal functional and morphological loss of ON-OFF cells in the retina that occurs after eye opening.

Discussion

ON-OFF Pathways Are Refined after Eye Opening

A fundamental physiological finding that emerges from this study of mouse retina is that there is an age-dependent reduction in the percentage of ON-OFF RGCs. A 70% reduction occurs during the maturational period spanning the time from just before eye opening to 2 weeks after eye opening. The essential anatomical result made evident here is that the percentage of RGCs having bistratified dendrites in both sublamina *a* and *b* decreases after eye opening. Similar physiological and anatomical refinements have been observed in other mammalian retinae. In ferret retina and in kitten LGN, the percentage of ON-OFF responding cells decreases postnatally (Wang et al., 2001; Daniels et al., 1978). A reduction in the proportion of bistratified RGCs has been observed in ferret and kitten (Bodnarenko et al., 1995, 1999). In kitten, the refinement continues after eye opening (Bodnarenko et al., 1995). It is a strong inference that the ON-OFF responding RGCs must receive synaptic inputs from presynaptic neurons arborizing in both sublamina *a* and *b* (Famiglietti and Kolb, 1976; Nelson et al., 1978). It is to be expected therefore that the loss of light-evoked ON-OFF responsive RGCs would be commensurate with the reduction in the number of bistratified RGCs.

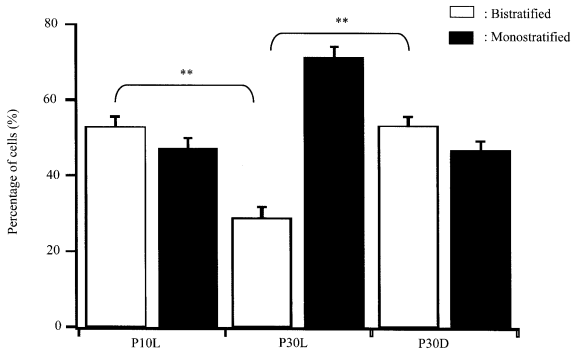

Figure 5. The Percentage of Bistratified RGCs Decreases after Eye Opening; Dark Rearing Blocks This Maturational Decrease

Mean percentage of RGCs that monostratified in sublamina *a* or *b* (filled bars) or which were bistratified (open bars). Three sets of data are shown: one from P10-aged control-reared mice (P10L), one from P30-aged control-reared mice (P30L), and one from P30-aged mice that were reared in constant darkness from birth (P30D). The percentage of bistratified RGCs was 53% ± 2.7% (mean ± SE, n [number of retinas] = 4; total of 100 cells), 29% ± 3% (n = 5; 198 cells), and 53% ± 2.5% (n = 6; 396 cells) for the P10L, P30L, and P30D groups, respectively. The differences between control-reared mice at P10 versus P30 and control-reared mice at the age of P30 versus dark-reared mice at the age of P30 were statistically significant ($p < 0.0001$). The difference between control-reared mice at the age of P10 and dark-reared mice at the age of P30 was not significant ($p = 0.9277$).

Mechanistically, the maturational alterations of ON and OFF pathways that occur after eye opening likely reflect refinements of the postsynaptic elements of bipolar/RGC synapses. In mouse retina, the numbers of ribbon and conventional synapses in the IPL assume adult levels by eye opening (Fisher, 1979). Therefore, the changes in synaptic signaling observed after eye opening is probably not due to a loss of bipolar cell axon terminals. Moreover, experimentally induced destruction of RGCs via optic nerve lesions has no effect on the stratification of bipolar cell axonal terminals (Gunhan-Agar et al., 2000). Although the RGC population as a whole declines due to developmentally regulated cell death after birth, the changes we observe after eye opening cannot be attributed to the loss of cells. Cell death is complete in the mouse retina by P10 (Young, 1984). Further work will be required to ascertain whether a maturational loss in the efficacy of particular bipolar cell/RGC synapses leads to the retraction of dendritic processes from that synapse or whether extrasynaptic extrinsic factors might control the retraction of the dendrites. Nonetheless, this report does establish that the refinement of ON-OFF pathway is controlled by visual inputs. It will be interesting to learn whether the maturational changes in RGC receptive field sizes (Bowe-Anders et al., 1975; Masland, 1977; Rusoff and Dubin, 1977) are also controlled by visual inputs.

One logically consistent, but unlikely, explanation for the electrophysiological data is that the sampling properties of the microelectrode array were different at P10–12 compared to P27–30. In this model, if one assumed that there was no decrease in the percentage of ON-OFF cells with age, the sampling characteristics of the array would have to be biased to record at least

3-fold more ON or OFF cells in P27–30 retinas versus P10–12 retinas. Several observations make this scenario unlikely. Examination of histological sections of the retina reveals no distinct age-dependent difference in the displacement of RGC somata from the vitreal/retinal border along the ganglion cell layer (our unpublished data). The number of light-responsive cells recorded per retina in P10–12 versus P27–30 was comparable. Finally, the decrease in the percentage of anatomically defined bistratified cells provides strong correlative evidence that a large component of the decline in the percentage of ON-OFF cells could be attributed to a loss of synaptic inputs from either sublamina *a* or *b*.

Although the anatomical and electrophysiological results reveal an age-dependent loss of ON-OFF cells, the magnitudes of the declines seem disparate between the two different types of experiment. One major source of this discrepancy is the undersampling of OFF cells by the multielectrode array (see below). While we believe that we observe a reasonable sampling of RGCs in the YFP-expressing mice, based on comparisons to other anatomical studies, we judge that we do not sample equally all the RGC types with the microelectrode array. Under these circumstances our estimate of the percentage of light-responsive ON-OFF cells is inflated.

Visual Deprivation and the Development of Retinal Circuits

These results establish convincingly that visual excitation of the eye is required for the normal development of ON and OFF pathways in mouse retina. In an earlier study, we showed that dark rearing regulated the frequency of spontaneous synaptic inputs to mouse RGCs. This present study investigates the much more compelling question of whether dark rearing affects light-evoked signaling in the retinal pathways and the structure of RGC dendritic arbors. To our knowledge, no previous study has shown an effect of dark rearing on light-evoked responses in mammalian retina after eye opening. Recent work in ferret shows a greater preponderance of ON-OFF units in dLGN of dark-reared animals (Akerman et al., 2002). Our findings in retina would predict this result. Many questions remain about which parameters of the visual deprivation are most significant. We chose an extensive period of dark rearing that covered the period from birth to the peak of the critical period for the effects of monocular deprivation on cortical reorganization in mouse (Gordon and Stryker, 1996). Will shorter periods of dark rearing affect the refinement of the ON-OFF pathways? Is there a critical period over which a short period of darkness will inhibit development in the retina? Is the effect of 4 weeks of dark rearing reversible? We have not tested explicitly for recovery of the ON-OFF responses in mouse, but we did find that the effects of dark rearing on spontaneous synaptic activity reversed after 6 days of control 12:12 hr light/dark rearing (Tian and Copenhagen, 2001). Therefore, it would not be surprising that the refinement of ON-OFF pathways might be reversible as well.

From the multielectrode array recordings it is not possible to ascertain whether equal numbers of ON-OFF RGCs become ON and OFF cells. Analysis of the YFP-expressing RGCs provides some support for the idea that ON-OFF RGCs are preferentially converted to OFF cells. In the P10 group of mice, 4% (4/100) of the RGCs monostratified in sublamina *a*. In the P30 group, 30% (61/202) of the RGCs were similarly stratified. The percentage of ON RGCs remained about the same, with 43% and 41% stratifying in sublamina *b*, respectively, at P10 and P30.

Previous Studies Showing No Effect of Visual Deprivation on Retina Did Not Focus Specifically on the Properties of ON and OFF Pathways

Many earlier studies have concluded that short-term visual deprivation has no discernible effect on either retinal morphology or light-evoked responsiveness (Hendrickson and Boothe, 1976; Lau et al., 1990; Baro et al., 1990; Sherman and Stone, 1973; Leventhal and Hirsch, 1983). On the basis of these and other investigations, it is commonly assumed that the retina is immune to visual deprivation. Do the inferences drawn in these earlier studies conflict with findings reported here? On the whole, none of these prior investigators considered the proportion of ON-OFF responding cells nor did they examine whether the stratification patterns of RGC dendrites in the IPL were changed. Hendrickson and Boothe (1976) reported that the retinas of dark-reared monkeys were no different from control-reared monkeys in terms of cell number, size, or staining characteristics, as identified with the soma-selective Toluidine blue staining. Lau et al. (1990) found no difference in the field diameters, branching patterns, or total lengths of type I RGC dendrites of hamsters raised in normal, visually deprived, or light-deprived environments. Baro et al. (1990) used the electroretinogram to investigate whether lid suture changed the amplitude of the b wave, an indicator of photoreceptor to ON bipolar cell synaptic transmission. They reported no changes. We similarly found that dark rearing did not reduce but slightly increased b wave amplitudes in mice (Tian and Copenhagen, 2001). Sherman and Stone (1973) found that conduction velocities, relative numbers, and receptive field properties of X and Y cells and soma sizes of RGCs were unaffected by visual deprivation induced with lid suture. Leventhal and Hirsch (1983), who injected the marker horseradish peroxidase into the LGN to retrogradely label RGCs, found no difference in the dendrites or axonal terminals of α or β RGCs in eyes visually deprived by monocular suture. Thus, although numerous studies infer that there are no retinal changes induced by visual deprivation, the properties of ON-OFF pathways were not examined in detail. Therefore, there is no apparent conflict between our present results and the findings in earlier studies. However, the conclusions derived from our study seriously challenge the commonly held belief that retinal structure and function is immune to visual deprivation.

The Effects of Dark Rearing Result from the Lack of Visual Inputs and Are Not Likely Attributable to Indirect Actions on the Mice

Are the actions of dark rearing on the ON-OFF RGCs due to indirect effects altering retinal function? Previously, we addressed this question pointing out that animals' weights, cellular morphology, and outer retinal responses were not affected by dark rearing (Tian and

Copenhagen, 2001). Analysis of our present results shows that dark rearing did not discernibly modify the sampled population of RGCs. In the P27–30 group of control-reared animals, we recorded light responses from 104 RGCs on average from each retina. In the dark-reared mice, we recorded light response from 115 RGCs on average. Thus, we have no experimental findings to indicate that dark rearing effects on the signaling and dendritic stratifications were due to anything but the loss of visual inputs to the bipolar cells and RGCs.

How Accurately Have the Absolute Percentages of RGC Types Been Measured in Mouse Retina?

Without further study, it is problematic to assess whether we or other investigators accurately sampled all RGC types. Our multielectrode array sampled a much different proportion of ON, OFF, and ON-OFF RGCs than Stone and Pinto (1992), who utilized single extracellular recording electrodes in mouse retina. They report, in a sample size of 87 cells from adult mice, that 44% of the cells were OFF RGCs. In our control P27–30 group, we find in a sample size of 426 cells that 2% of the RGCs were OFF RGCs. In the P30-aged YFP-positive mice, 31% of the RGC dendrites were monostratified in sublamina *a*, while Sun et al. report that 60% of the cells of DiI-labeled RGCs were monostratified in sublamina *a*. The disparity between the microarray and the anatomical results makes it apparent that the multielectrode array undersampled OFF RGCs. This problem makes it difficult to discern electrophysiologically any maturational or visual deprivation-dependent changes on the OFF RGC population. We theorize that the OFF cells were undersampled because their somata were displaced away from the electrode surfaces by about 1/3 of a soma diameter compared to the ON and ON-OFF somata identified in the YFP-positive mouse retinas (our unpublished data). There is better agreement in the various estimates of ON-OFF RGCs. In P27–30 mice, we find 21.5% light-responsive ON-OFF RGCs compared to 28% bistratified YFP-expressing RGCs and 20% bistratified DiI-labeled RGCs (Sun et al., 2002). The lower percentage of ON-OFF RGCs reported by Stone and Pinto, 9% (1993), may reflect the smaller soma sizes of the bistratified cells (Sun et al., 2002) that caused these cell types to be undersampled with their single electrode approach. Further work will be required to determine which of the RGC staining methods yields the most accurate measure of actual RGC cell types. Nonuniform expression of YFP in different RGC types or preferential staining certain classes of RGCs with the diolistic technique could give a distorted picture of the actual populations of RGC types. Nonetheless, both methods do label enough RGCs to allow sampling from moderately large numbers of cells with bistratified or monostratified dendrites. Furthermore, the reasonable agreement between the two anatomical techniques and the microarray recordings supports the basic premise of this study—that we have obtained a fair estimate of the number of ON-OFF RGCs and how their numbers change with development and visual deprivation.

Could the effects of age and dark rearing on the percentages of light-responsive ON-OFF cells be reflecting the properties of displaced amacrine cells rather than RGCs? Although up to 60% of the somata in the ganglion cell layer of mouse retinas may be displaced amacrine cells (Jeon et al., 1998), it is not likely that this cell type can account for a major portion of the effects we find here. Amacrine cells have smaller voltage-activated sodium currents than RGCs when compared in culture (Taschenberger and Grantyn, 1995) and in mouse retinal slice preparation (Tian et al., 1998). In concordance with the smaller sodium currents, displaced amacrine cells exhibit a very limited capacity for repetitive firing (Taschenberger and Grantyn, 1995). We can't rule out the possibility that at least some light-responsive cells were displaced amacrine cells; however, given the electrophysiological characteristics mentioned above, it is a reasonable assumption that a majority of the cells we recorded were RGCs. The anatomical findings in the YFP-expressing mice also argue strongly for age and dark rearing actions on RGCs. All of the 694 YFP cells we examined had a distinct and easily identifiable axon. For these morphological experiments, there is no chance that RGCs could have been confused with displaced amacrine cells.

If Thy-1 expression itself were regulated by light, could this explain the $>$3-fold greater percentage of bistratified RGCs in dark-reared animals? To account for the results, it would have to be the case that the relative level of Thy-1 expression in the bistratified but not the monostratified RGCs was suppressed during cyclic light rearing. Although direct experimental verification or refutation of a light-dependent regulation of Thy-1 expression was not attempted in this study, several lines of evidence suggest this is improbable. In P27–30 aged mice, we found that 29% of the YFP-labeled RGCs were bistratified. An independent study using DiI labeling reported that 20% of the RGCs were bistratified (Sun et al., 2002). These two sets of data do not support the idea of a selective downregulation of Thy-1 expressing bistratified RGCs. In addition, developmental studies of Thy-1 expression using an antibody to Thy-1 demonstrated that the density of Thy-1-positive RGCs reached its peak by P15, the time of eye opening, and remained at this elevated level through adulthood (Liu et al., 1996). On the basis of these above arguments, we assumed for the data analysis that Thy-1 expression was not regulated by light.

Summary

These experiments demonstrate that significant maturational changes in the ON and OFF pathways occur after eye opening in mouse retina. The number of ON-OFF RGCs declines more than 3-fold, and the number of RGCs having bistratified dendrites also drops significantly. In dark-reared animals, the functional and morphological developmental changes are absent. We conclude that visual stimulation is therefore essential for the normal maturation of the ON-OFF pathways in retina.

Experimental Procedures

Animals

Retinas for electrophysiological recordings were obtained from C57BL/6 wild-type mice (The Jackson Laboratory, Bar Harbor, ME). Transgenic mice expressing yellow fluorescent protein (YFP) in a subset of RGCs (YFP [H] line; The Jackson Laboratory) were used

for studying the stratification patterns of RGC dendrites (Feng et al., 2000). All animals were dark adapted for 30 min before euthanization. Adult mice (P27–30) were killed by cervical dislocation, and young mice (P10–15) were killed by decapitation. The handling and maintenance of animals and tissue preparation met the NIH guidelines and were approved by the Yale University and the University of California, San Francisco, Committees on Animal Research.

Preparation of Retina for Multielectrode Array Recordings

For electrophysiological recordings, eyes were enucleated and hemisected at the ora serata under very dim red light illumination immediately after the animals were euthanized. The cornea, iris, lens, and vitreous were removed from eyes immersed in a petri dish filled with oxygenated extracellular solution, which contained (in mM) NaCl, 124; KCl, 2.5; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4, 1.25; $NaHCO_3$, 26; and glucose, 22 (pH 7.35 with 95% O_2 and 5% CO_2). Within 2 min, the retina was detached from the eyecup using forceps and placed photoreceptor side down on a piece of nitrocellulose filter paper (8 μm pore size, Millipore Corp., Bedford, MA). The mounted retina was placed in the MEA-60 multielectrode array recording chamber (Multi Channel System MCS GmbH, Reutlingen, Germany), with the ganglion cell layer facing the recording electrodes. The filter paper was held in a fixed position with a miniature manipulator, and the retina was continuously perfused in oxygenated extracellular solution at 34°C during all experiments. Procedures after enucleation were performed under infrared illumination. With this preparation, we have been able to record stable ganglion cell light responses for at least 4 hr.

Light-evoked action potential recordings were started 60 min after the retina was positioned in the recording chamber. Action potentials were simultaneously recorded from 60 channels with a multielectrode array having 10 μm diameter electrodes spaced 100–200 μm apart. A green LED (567 nm) was used to stimulate the retina with a 1 s diffuse full-field light. Responses were averaged from 20 or 100 2 s recordings started at the onset of a 1 s step of light. The interval between onsets of each light stimulus was 10 s. Data were collected using a PC-based interface card and software (Multi Channel System MCS GmbH, Reutlingen, Germany). The signals were filtered between 100 Hz (low cut off) and 3 kHz (high cut off). ON cells generated visually identifiable transient or sustained increases in spike frequency during the time the light was on. ON-OFF cells exhibited increased spike activity following both the onset and the termination of the stimulus. OFF cells produced an increase in spiking only following the termination of the stimulus. Basically, if we saw additional spikes within 300 ms of light onset and within 500 ms of light termination on at least 30% of the trials, we considered the unit an ON-OFF cell. In mouse retina, even though the RGCs have center-surround receptive fields, the response to a diffuse light is dominated by the central region (Stone and Pinto, 1993). On this basis, our classification reflects the response properties of the central region of the RGC receptive fields.

The response dominance index (RDI) was calculated following the method used by Akerman et al. (2002). Responses of each identified RGC to 20 or 100 repeats of 1 s light steps were averaged as described above. The peak spike frequency of the ON and OFF responses were calculated from the averages. The RDI was defined as

$$\mathrm{RDI} = \frac{|(\mathrm{ON}_{\text{peak frequency}} - \mathrm{OFF}_{\text{peak frequency}})|}{|(\mathrm{ON}_{\text{peak frequency}} + \mathrm{OFF}_{\text{peak frequency}})|}$$

MEA Data Analysis

Offline data analysis to isolate the responses from individual neurons was carried out on a PC computer using Off-line Sorter (Plexon Inc, Dallas, TX) and NeuroExplorer (Nex Technologies, Lexington, MA). The action potentials recorded by each electrode were first sorted from the raw data on a PC computer using Off-line Sorter. During a first pass through the data, a threshold set to 68μV detects the action potentials. The first and second principal components were calculated from the wavelets (0.5 ms before the onset to 2 ms after the onset) detected in the first pass. Action potentials having similar principal components form clusters in 2D plots of the principal components. We defined templates based on the cluster distributions for the second sorting. Using these templates, we sorted the action potentials from individual neurons.

The results were further analyzed with NeuroExplorer. The timestamps of the action potentials from neuron were crosscorrelated with each other and plotted as response histograms with a 10 ms bin width.

Preparation of Retinas for YFP Imaging

Eyes were enucleated and hemisected at the ora serata immediately after the animals were euthanized. The cornea, iris, lens, and vitreous were removed from eyes immersed in a petri dish filled with 0.1 M phosphate buffer solution (PBS). Then the excised retinas were immediately immersed in 4% paraformaldehyde (PFA) in 0.1 M PBS and fixed for 30 min at room temperature. The retinas were then stored overnight in 30% sucrose in 0.1 M PBS at 4°C. A rabbit polyclonal antibody for GFP conjugated with Alexa Fluor 488 (Molecular Probes, Inc., Eugene, OR) was used to enhance the YFP signal in fixed retinas. A sheep polyclonal antibody for tyrosine hydroxylase (anti-TH, Chemicon International, Inc., Temecula, CA) was used to label dopaminergic amacrine cells.

Fixed retinas were washed in 0.1 M PBS, incubated 6 days in primary antibodies (1:500 for anti-GFP antibody and 1:200 for anti-TH antibody) with 0.2% Triton X-100 and 10% normal donkey serum (NDS) at 4°C, and then washed in 0.1 M PBS. Retinas were then incubated at a 1:50 dilution in secondary antibody (donkey polyclonal anti-sheep antibody conjugated with Texas red, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) with 0.2% Triton X-100 and 10% NDS overnight at 4°C. Retinas were flattened, mounted on Super-Frost Plus slides (Fisher Scientific, Pittsburgh, PA) with Vectashield (Vector Laboratories, Burlingame, CA) to retard fading.

Detection and Analysis of YFP-Expressing RGCs

YFP-expressing RGCs were identified visually using an Olympus BX51WI microscope (Optical Analysis Corporation, Nashua, NH) with a PlanApo 60× oil objective (N.A. 1.4). The anti-TH-labeled somas were used to establish the border between the IPL and the INL. RGCs were classified into monostratified and bistratified cells based on their dendritic ramification within the IPL, measured by taking the z axis reading on the microscope. To define the midpoint of the IPL, we took half the distance between the best focus positions of the TH-positive amacrine cells and the RGC axons. We found that the RGC axons generally emanated from a position near the midpoint of the somata of ON and ON-OFF RGCs. Thus, the vitreal surface of the cell bodies were further from the inner retina than the axons (see Figure 4C for example). Preliminary measurements in which we compared the midpoint derived from using the INL/IPL and IPL/ganglion cell border to that derived from looking at the best focus planes as described above yielded very similar estimates. Since the focus of the TH-positive cell bodies and the axons was easier to see, we routinely used these landmarks as the anchor points to calculate the midpoint of the IPL. Midpoint measurements were performed on each RGC.

The classification of visually identified YFP-expressing RGCs of four retinas (E, F, K, and L in Table 2, total of 139 cells) were confirmed by reexamining the retinas taken with a confocal laser-scanning microscope. Confocal laser-scanning immunofluorecent images of RGCs were collected using the same 60× oil objective on an Olympus FV5-PSU microscope (Optical Analysis Corporation, Nashua, NH). For cells with large laterally expansive dendritic fields, several z section images were taken at the same focus plane so the entire cell was covered. Software IPLabs (Scanalystics, Inc., Fairfax, VA) was used to align multistacks of images together, examine dendritic branch distribution, adjust levels and curves of confocal images, and make pseudocolor overlay images. From those 139 cells, three of them (2%) could not be reliably identified by visual examination.

Statistical Analysis

Analysis of variance (ANOVA) was used to determine the significance of difference between more than two means, and Student's t test was used to examine the difference between two means using software StatView (Abacus Concepts, Berkeley, CA).

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References

Akerman, C.J., Smyth, D., and Thompson, I.D. (2002). Visual experience before eye opening and the development of the retinogeniculate pathway. Neuron *36*, 869–879.

Bansal, A., Singer, J.H., Hwang, B.J., Xu, W., Beaudet, A., and Feller, M.B. (2000). Mice lacking specific nicotinic acetylcholine receptor subunits exhibit dramatically altered spontaneous activity patterns and reveal a limited role for retinal waves in forming ON and OFF circuits in the inner retina. J. Neurosci. *20*, 7672–7681.

Baro, J.A., Lehmkuhle, S., and Kratz, K.E. (1990). Electroretinograms and visual evoked potentials in long-term monocularly deprived cats. Invest. Ophthalmol. Vis. Sci. *31*, 1405–1409.

Bodnarenko, S.R., and Chalupa, L.M. (1993). Stratification of ON and OFF ganglion cell dendrites depends on glutamate-mediated afferent activity in the developing retina. Nature *364*, 144–146.

Bodnarenko, S.R., Jeyarasasingam, G., and Chalupa, L.M. (1995). Development and regulation of dendritic stratification in retinal ganglion cells by glutamate-mediated afferent activity. J. Neurosci. *15*, 7037–7045.

Bodnarenko, S.R., Yeung, G., Thomas, L., and McCarthy, M. (1999). The development of retinal ganglion cell dendritic stratification in ferrets. Neuroreport *10*, 2955–2959.

Bowe-Anders, C., Miller, R.F., and Dacheux, R. (1975). Developmental characteristics of receptive organization in the isolated retina-eyecup of the rabbit. Brain Res. *87*, 61–65.

Carmignoto, G., and Vicini, S. (1992). Activity-dependent decrease in NMDA receptor responses during development of the visual cortex. Science *258*, 1007–1011.

Cynader, M., and Mitchell, D.E. (1980). Prolonged sensitivity to monocular deprivation in dark-reared cats. J. Neurophysiol. *43*, 1026–1040.

Daniels, J.D., Pettigrew, J.D., and Norman, J.L. (1978). Development of single-neuron responses in kitten's lateral geniculate nucleus. J. Neurophysiol. *41*, 1373–1393.

Dann, J.F., Buhl, E.H., and Peichl, L. (1988). Postnatal dendritic maturation of alpha and beta ganglion cells in cat retina. J. Neurosci. *8*, 1485–1499.

Daw, N.W. (1995). Visual Development (New York: Plenum Press).

Doi, M., Uji, Y., and Yamamura, H. (1995). Morphological classification of retinal ganglion cells in mice. J. Comp. Neurol. *356*, 368–386.

Famiglietti, E.V., Jr., and Kolb, H. (1976). Structural basis for ON-and OFF-center responses in retinal ganglion cells. Science *194*, 193–195.

Feng, G., Mellor, R.H., Bernstein, M., Keller-Peck, C., Nguyen, Q.T., Wallace, M., Nerbonne, J.M., Lichtman, J.W., and Sanes, J.R. (2000). Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron *28*, 41–51.

Fisher, L.J. (1979). Development of synaptic arrays in the inner plexiform layer of neonatal mouse retina. J. Comp. Neurol. *187*, 359–372.

Fox, K., Daw, N., Sato, H., and Czepita, D. (1991). Dark-rearing delays the loss of NMDA-receptor function in kitten visual cortex. Nature *350*, 342–344.

Gordon, J.A., and Stryker, M.P. (1996). Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J. Neurosci. *16*, 3274–3286.

Greiner, J.V., and Weidman, T.A. (1981). Histogenesis of the ferret retina. Exp. Eye Res. *33*, 315–332.

Gunhan-Agar, E., Kahn, D., and Chalupa, L.M. (2000). Segregation of on and off bipolar cell axonal arbors in the absence of retinal ganglion cells. J. Neurosci. *20*, 306–314.

Hartline, H.K. (1938). The response of single optic nerve fibers of the vertebrate eye to illumination of the retina. Am. J. Physiol. *121*, 400–415.

Haverkamp, S., and Wässle, H. (2000). Immunocytochemical analysis of the mouse retina. J. Comp. Neurol. *424*, 1–23.

Hendrickson, A., and Boothe, R. (1976). Morphology of the retina and dorsal lateral geniculate nucleus in dark-reared monkeys (*Macaca nemestrina*). Vision Res. *16*, 517–521.

Jeon, C.-J., Strettoi, E., and Masland R.H. (1998). The major cell populations of the mouse retina. J. Neurosci. *18*, 8936–8946.

Johnson, J., Tian, N., Caywood, M.S., Reimer, R.J., Edwards, R.H., and Copenhagen, D.R. (2003). Vesicular neurotransmitter transporter expression in developing postnatal rodent retina: GABA and glycine precede glutamate. J. Neurosci. *23*, 518–529.

Kirkwood, A., Rioult, M.C., and Bear, M.F. (1996). Experience-dependent modification of synaptic plasticity in visual cortex. Nature *381*, 526–528.

Lau, K.C., So, K.F., and Tay, D. (1990). Effects of visual or light deprivation on the morphology, and the elimination of the transient features during development, of type I retinal ganglion cells in hamsters. J. Comp. Neurol. *300*, 583–592.

Leventhal, A.G., and Hirsch, H.V. (1983). Effects of visual deprivation upon the morphology of retinal ganglion cells projecting to the dorsal lateral geniculate nucleus of the cat. J. Neurosci. *3*, 332–344.

Liu, C.J., Chaturvedi, N., Barnstable, C.J., and Dreyer, E.B. (1996). Retinal Thy-1 expression during development. Invest. Ophthalmol. Vis. Sci. *37*, 1469–1473.

Masland, R.H. (1977). Maturation of function in the developing rabbit retina. J. Comp. Neurol. *175*, 275–286.

Maslim, J., and Stone, J. (1988). Time course of stratification of the dendritic fields of ganglion cells in the retina of the cat. Brain Res. Dev. Brain Res. *44*, 87–93.

Meister, M., Pine, J., and Baylor, D.A. (1994). Multi-neuronal signals from the retina: acquisition and analysis. J. Neurosci. Methods *51*, 95–106.

Mower, G.D. (1991). The effect of dark rearing on the time course of the critical period in cat visual cortex. Brain Res. Dev. Brain Res. *58*, 151–158.

Nelson, R., Famiglietti, E.V., Jr., and Kolb, H. (1978). Intracellular staining reveals different levels of stratification for on- and off-center ganglion cells in cat retina. J. Neurophysiol. *41*, 472–483.

Nirenberg, S., and Meister, M. (1997). The light response of retinal ganglion cells is truncated by a displaced amacrine circuit. Neuron *18*, 637–650.

Norton, T.T., Rager, G., and Kretz, R. (1985). ON and OFF regions in layer IV of striate cortex. Brain Res. *327*, 319–323.

Pow, D.V., and Barnett, N.L. (2000). Developmental expression of excitatory amino acid transporter 5: a photoreceptor and bipolar cell glutamate transporter in rat retina. Neurosci. Lett. *280*, 21–24.

Redburn, D.A., and Madtes, P. (1987). GABA—Its roles and development in retina. Prog. Retinal Eye Res. *6*, 69–84.

Rockhill, R.L., Daly, F.J., MacNeil, M.A., Brown, S.P., and Masland, R.H. (2002). The diversity of ganglion cells in a mammalian retina. J. Neurosci. *22*, 3831–3843.

Rusoff, A.C., and Dubin, M.W. (1977). Development of receptive-field properties of retinal ganglion cells in kittens. J. Neurophysiol. *40*, 1188–1198.

Sassoe-Pognetto, M., and Wässle, H. (1997). Synaptogenesis in the

rat retina: subcellular localization of glycine receptors, GABA(A) receptors, and the anchoring protein gephyrin. J. Comp. Neurol. *381*, 158–174.

Sernagor, E., and Grzywacz, N.M. (1996). Influence of spontaneous activity and visual experience on developing retinal receptive fields. Curr. Biol. *6*, 1503–1508.

Sherman, S.M., and Stone, J. (1973). Physiological normality of the retinal in visually deprived cats. Brain Res. *60*, 224–230.

Stone, C., and Pinto, L.H. (1992). Receptive field organization of retinal ganglion cells in the spastic mutant mouse. J. Physiol. *456*, 125–142.

Stone, C., and Pinto, L.H. (1993). Response properties of ganglion cells in the isolated mouse retina. Vis. Neurosci. *10*, 31–39.

Stryker, M.P., and Zahs, K.R. (1983). On and off sublaminae in the lateral geniculate nucleus of the ferret. J. Neurosci. *3*, 1943–1951.

Sun, W., Li, N., and He, S. (2002). Large-scale morphological survey of mouse retinal ganglion cells. J. Comp. Neurol. *451*, 115–126.

Tagawa, Y., Sawai, H., Ueda, Y., Tauchi, M., and Nakanishi, S. (1999). Immunohistological studies of metabotropic glutamate receptor subtype 6-deficient mice show no abnormality of retinal cell organization and ganglion cell maturation. J. Neurosci. *19*, 2568–2579.

Taschenberger, H., and Grantyn, R. (1995). Several types of Ca2+ channels mediate glutamatergic synaptic responses to activation of single Thy-1-immunolabeled rat retinal ganglion neurons. J. Neurosci. *15*, 2240–2254.

Tian, N., and Copenhagen, D.R. (2001). Visual deprivation alters development of synaptic function in inner retina after eye opening. Neuron *32*, 439–449.

Tian, N., Hwang, T., and Copenhagen, D.R. (1998). Characterization of spontaneous synaptic activity in mouse retinal ganglion cells. J. Neurophysiol. *80*, 1327–1340.

Tootle, J.S. (1993). Early postnatal development of visual function in ganglion cells of the cat retina. J. Neurophysiol. *69*, 1645–1660.

Wang, G.Y., Liets, L.C., and Chalupa, L.M. (2001). Unique functional properties of on and off pathways in the developing mammalian retina. J. Neurosci. *21*, 4310–4317.

Wong, R.O., and Ghosh, A. (2002). Activity-dependent regulation of dendritic growth and patterning. Nat. Rev. Neurosci. *3*, 803–812.

Young, R.W. (1984). Cell death during differentiation of the retina in the mouse. J. Comp. Neurol. *229*, 362–373.