3. RESULTS

Dye injection was the first attempt for characterizing bipolar cell morphologies in the initial phase of this study. Since bipolar cells of earlier postnatal stages (prior to P6) were difficult to fill completely, the gene gun labeling technique later replaced the dye injection method to become the main tool in this investigation. We grouped both results from dye injection and gene gun labeling together. Regardless the methods, cells were used for the analysis have to meet two criteria. First, the cell was clearly filled or labeled, and can be distinguished from neighboring cells as possible. Second, the cell has "bipolar" processes, i.e., one process extended to the outer plexiform layer (OPL), and the other process went the opposite direction and reached to the inner plexiform layer (IPL). The second criterion thus cannot separate bipolar cells and Muller cells apart, especially in the earlier postnatal stages, when both cell types are developing. Total 84 injected cells and 335 gene gun labeled cells from 58 rabbits were included in this study.

Cells identified in different retinas were grouped together according to the postnatal days (P0-1, P2-3, P4-5, P6-7, P8-9, and P10-P14) and rearing conditions (normal and dark). In consistent with previous morphological studies on bipolar cell development in chicks (Quesada et al., 1981), ferrets (Miller et al., 1999), and mouse (Morgan et al., 2006), our results showed a similar morphological differentiation

pattern (Fig. 1a). There was no distinguishable bipolar cell at birth in the rabbit retina. The cell soma was elliptical or elongated. Their processes are branching and extend significantly into both the neuroblastal layers (NBL) and the ganglion cell layer (GCL). In contrast to an early immunohistochemical study on bipolar cell development in the rabbit retina that the rod bipolar cell was not identified until P6 (Casini et al., 1996), the first recognizable bipolar cells could be detected as early as P1 in the present study, and most likely are cone bipolar cells. Development of bipolar cells in the rabbit retina typically takes 4-6 days to mature in the normal reared condition, and by P8 most bipolar cells have reached their adult-like morphology (see more DiI labeled cells in Appendix 1).

In contrast to morphological differentiation of bipolar cells in the normal reared rabbits, maturation of bipolar cells shows a different temporal pattern in dark (Fig. 1b). Development of bipolar cells is delayed rather than arrested in the light deprivation condition. Initially, cell morphologies in the normal and dark reared rabbits are similar. From P2 to P5, cells in the dark reared rabbits retard their morphological differentiation. However, after P5 most cells resume their normal developmental process, and reach similar mature level at P10 as their normal reared counterparts (see more DiI labeled cells in Appendix 1).

To quantify the dark rear effect on bipolar cell development described above, we

analyzed several morphological parameters of identified bipolar cells. Table 1 gives a summary result of this analysis. Since we could not confidently separate bipolar cells from Muller cells in earlier developmental stages, all cells with "bipolar" appearance were put into the "B/M" category. We therefore used the percentage of B/M cells as an indication of bipolar cell maturation, i.e., the higher the B/M percentage, the less recognizable bipolar cells could be counted at that developmental stage, thus less mature the retina is. All cells with recognizable bipolar cell characteristics were separately placed into either ON or OFF type category, depending on their axonal stratifications in the IPL. However, sometimes cells with distinct bipolar cell characteristics have processes ramifying in both ON and OFF layers, or their axonal terminals diffusely stratifying across the border of ON and OFF layers of the IPL, these cells were put into another category, called "undefined BP" cells.

As expected, the percentage of B/M cells is high in P1-3 of the normal reared rabbits, falls significantly in P4-7, and all cells can be easily identified as either bipolar cells or Muller cells after P8 (Fig. 2a). On the contrary, while the percentage of B/M cells falls gradually throughout the developmental stages in the dark reared rabbits, the proportion of B/M cells remains a significant degree even at P8-9 (Fig. 2b). Nevertheless, all cells can be confidently recognized after P10. This indicates that light deprivation delays morphological differentiation in bipolar cells. Another way of

looking at this result is to compare the percentage of all bipolar cells in the total labeled cells for both normal and dark reared conditions. Figure 3 shows this comparison. At birth, both normal and dark rearing conditions have low percentages of identified bipolar cells. However, from P2-3 to P4-5, the percentage increases significantly in the normal reared rabbits, and level off after P4-5. By P8-9, all bipolar cells are adult-like. In contrast, the percentage increases steadily in the dark reared rabbits, but shows a significant delay in P4-9. Nevertheless, all bipolar cells are eventually adult-like by eye opening around P10-11.

In addition to this light deprivation effect on morphological differentiation of bipolar cells, both rearing conditions show a similar pattern of ON/OFF developing asymmetry. Recognizable OFF bipolar cells appear to have higher proportion than ON bipolar cells in earlier developmental stages (Fig. 2). This difference diminishes after P6-7 in the normal rear rabbits and P8-9 in the dark reared rabbits. By eye opening, ON and OFF bipolar cells reach roughly equal amounts. This observation is in consistent with other studies that OFF bipolar cells develop earlier than ON bipolar cells (Sherry et al., 2003).

Earlier studies of bipolar cell development in other animals have shown that somata of immature bipolar cells are more elliptical than of mature bipolar cells (Quesada et al., 1981; Miller et al., 1999; Morgan et al., 2006), thus we sought to

quantify the soma aspect ratio as an index of bipolar cell maturation. The length and width of an individual cell soma was measured and used to calculate the soma aspect ratio (soma length divided by soma width). The less the soma aspect ratio, the more mature the cell is. Our results show that both normal and dark reared rabbits display decreasing soma aspect ratio from birth to eye opening (Fig. 4). However, between P2 and P5, the dark reared animals have more elongated somata than the normal reared counterparts, but the difference diminishes after P6-7.

The soma location of bipolar cells in the adult retina is typically in the upper two-third of the INL. However, the somata of developing bipolar cells could range in all levels of the INL (Quesada et al., 1981; Miller et al., 1999; Morgan et al., 2006). We divided the INL into three layers (N1, N2, and N3), and recorded the cell locations of all injected or labeled cells. It appears that around 20% of labeled cells have their somata in the lower one-third of the INL (N3) between P0 and P3 in the normal reared rabbits (Fig. 5a). This percentage drops in P4-5 and P6-7, and disappears after P8. By contrast, significant portion of somata in the lower one-third of the INL (N3) are present from birth to P8-9 in the dark reared rabbits (Fig. 5b). This result indicates that immature bipolar cells are more abundant in the dark reared condition than in the normal reared condition, especially between P4 and P9.

In summary, light deprivation has a significant effect on the morphological

maturation of bipolar cells in the rabbit retina. Our results suggest that visual stimulation is a facilitating factor for the normal development of bipolar cells, but visual deprivation does not permanent arrest the maturation of bipolar cells.