Results

Classification of ganglion cell types is based on morphological criteria and dendritic stratification characterized in Rockhill et al. (2002). Not all cell types were encountered or identified in equal frequency for both normal- and dark-reared rabbits. Cell numbers less than six in each ganglion cell type were not compared in this study. Summary of cell numbers in all ganglion cell types is listed in Table 1. For all comparable cell types (i.e., G1, G4, G5, G7, G9, G10, and G11), the general linear model (GLM) was applied to test whether light deprivation has any influence on the dendritic field size of ganglion cells. The results are summarized in Table 2.

The G7 ganglion cell

G7 cells, also called directionally selective ganglion cells (ON-OFF DSGCs), are the most well characterized cell type in the rabbit retina. The G7 cells labeled in this study all exhibited typical morphological features of the DSGC (Fig. 1A, 1B, 1D, and 1E), such as bi-stratification of dendrites at two different sublaminae (ON and OFF) of the inner plexiform layer (IPL), medium-sized dendritic arbors, and recurving branches making up a web-like dendritic pattern (Amthor et al., 1984; Yang and Masland, 1992, 1994; Rockhill et al., 2002). Cells in the dark-reared group showed no apparent morphological difference with those of the control group. Dendritic field areas of the ON and the OFF layers in the two groups both have inversed correlations with their nucleus densities. Data of these two groups were linearly aligned in the scatter plot and overlapped with each other (Fig. 1C and 1F). Furthermore, dendritic field sizes of the ON and the OFF layers in the dark-reared group were not significantly different from those in the control group (Table 2). Taken together, visual deprivation has no effect on dendritic field size and gross dendritic pattern of the G7 ganglion cells in the rabbit retina.

The G1 ganglion cell

G1 cells are the smallest ganglion cell type whose dendrites arborizd narrowly at stratum S3 of the IPL in the rabbit retina. Most identified G1 cells showed extensive short branches along their dendrites as shown in previous studies (Amthor et al., 1989; Rockhill et al., 2002). Overall dendritic patterns of G1 cells in the dark-reared group were similar to that in the control group (Fig 2A and 2B). Dendritic field sizes of G1 cells in two groups were both inversely correlated with their nucleus densities (Fig. 2C), and were not significantly different (Table 2).

The G4 ganglion cell

G4 cells have a distinct pattern of branching and stratification (Rockhill et al, 2002). They have lots of short branches protruding from primary dendrites and varicosities along the dendrites, especially at the terminal parts. G4 cells stratify broadly within the IPL with two subclasses, the G4 ON and the G4 OFF. In this study, G4 cells recognized in the control and the dark-reared group both possessed those morphological characteristics and thus had dense appearances in a single optic plane (Fig. 3A, 3B, 3D, and 3E). The distribution of the dark-reared cells intermingled with the distribution of the control ones in the scatter plot (Fig. 3C and 3D). Although there was only one cell identified in the control group of the G4 ON, it showed no tendency to differ from data in the dark-reared group. As for the GLM result, the effect of visual experience was not significant on the determination of dendritic field sizes of the G4 OFF cells (Table 2).

The G5 ganglion cell

The dendritic field diameters of G5 cells were around 300 μm at mid-peripheral region in the rabbit retina (Rockhill et al, 2002). Their primary dendrites hold a uniform

size from soma to periphery. A remarkable feature of G5 cells is that most of the dendrites display certain degrees of curvature. Since short branches emit from the main dendrites frequently, it appears that G5 cells arborize evenly and narrowly at the OFF sublamina of the IPL. G5 cells identified in the control and the dark-reared groups shared common morphological features described above (Fig. 5A and 5B). However, the data points of two groups in the scatter plot distributed rather sparsely and did not seem to overlap completely, yet there were still inversed correlations between dendritic field area and nucleus density in both groups (Fig. 5C). As for GLM result, not only the effect of visual experience could be omitted, but the control parameter, nucleus density part, was of no statistical significance (Table 1). This may result from small cell numbers in both the control and the dark-reared group, and some "outlier" cells in the analysis. Therefore, it was difficult to conclude that whether visual deprivation has any influence on the development of dendritic field sizes in G5 cells.

The G9 ganglion cell

The G9 cells are known to have similar dendritic patterns as the G10 cells but flatly arborize at the OFF sublamina of the IPL. G9 cells also resemble the OFF delta ganglion cells in cats (Wassle et al., 1987). The dendrites of G9 cells usually covered the dendritic field evenly. Cells identified as the G9 in the dark-reared group exhibited the same characters with those in the control group (Fig. 5A and 5B). Unlike the G7 and the G1 cells, the dendritic field sizes of G9 cells in the control and the dark-reared groups decreased slightly as their nucleus densities increase. However, the data points of two groups overlapped with each other in the scatter plot (Fig. 5C). The difference of the dendritic field size between the control and dark-reared group was not statistically significant (Table 2).

The G10 ganglion cell

The G10 cells, the ON directional selective ganglion cells (ON DSGCs), are large-size ganglion cells that monostratify in the ON sublamina of the IPL. The distinguished feature of the G10 dendritic arbor is that a large number of branches extend from the wavy proximal dendrites (He and Masland, 1998; Rockhill et al., 2002; Ackert et al., 2006). Labeled G10 cells in the dark-reared group were morphologically identical with those in the control group (Fig. 6A, 6B). Though the distribution of data point in both groups in the scatter plot was dispersive, it appears that the dendritic field size was negatively correlated to the nucleus density (Fig. 6C). The GLM result also indicates that dark rearing exerted no effect on the development of dendritic field area of the G10 cells (Table 2).

The G11 ganglion cell

The G11 cells are α cells, a conserved ganglion cell type in most mammalian retinas (Peichl et al., 1987). They are largest ganglion cells in the rabbit retina, and have ON and OFF subclasses. The dendritic pattern of G11 cells is well characterized by long and relatively straight secondary branches radiating outward the cell body. Labeled G11 cells in the control and the dark-reared groups both have similar appearances in those morphological aspects (Fig. 7A, 7B, 7D, and 7E). The scatter plots reveal that dendritic field areas were negatively associated with the nucleus density in both the control and the dark-reared groups, and data points of both groups were intermingled with each other (Fig. 7C and 7F). Furthermore, dendritic field sizes of G11 OFF cells in the dark-reared group were not significantly different from those in the control group (Table 2). However, the GLM result of the G11 ON is inconsistent between p values calculated from type **I**

error and type **III** error (Table 2). This may result from some cells analyzed in the control and the dark-reared group distribute at regions of different nucleus densities, though the linear relation can be seen. Nevertheless, it suggests that visual deprivation has no effect on the growth of dendritic area of G11 cells.

The unclassified cells

A number of ganglion cells were hard to assign into specific cell types. For example, one group of cells might represent the transition between two cell types. These "OR-type" cells lack enough information of dendritic morphology or stratification to specify their cell types. Other unclassified cells seemed to be developmental accidents. These cells did not belong to any particular ganglion cell type according to their dendritic morphologies. Interestingly, we found a distinct cell type, "Bi-GC", which stratified in both ON and OFF sublamina but not resembled the G7 cells (Fig. 8C and 8D). Due to their rare occurrences, it might be a developmental accident as well. In this study, we encountered these abnormal cells in both control and dark-reared groups (Table 1), thus it is unlikely that these developmental accidents result from the visual deprivation.