Discussion

This study shows that under dark-rearing condition, the dendritic field size of comparable RGCs in the rabbit retina, such as G1, G4, G5, G7, G9, G10, and G11, does not differ from that of RGCs rearing in the normal light/dark cycle. In other words, light experience may not contribute to the development of dendritic field size of RGCs in the rabbit retina. This also implies that receptive field size of rabbit RGCs might mature without visual inputs.

Visual deprivation and retinal development

Effects of visual experience on the development of visual system have been extensively investigated over decades (Sernagor et al., 2001; Tian, 2004). In addition to their impacts on the CNS, there are also several studies on the retina in the past. With chronic exposure to different levels of darkness, two out of three chimpanzees have been reported to almost completely lose their GCLs in the retina, but all animals showed significant retardation in their visual development and their visual learning ability (Chow et al., 1957). In the non-mammalian studies, RGCs of dark-rearing turtles have significant larger dendritic fields and receptive fields than of control ones (Sernagor and Grzywacz, 1996; Mehta and Sernagor, 2006). On the contrary, alpha and beta ganglion cells of the monocular sutured eye developed normal dendritic morphologies in cats (Leventhal and Hirsch, 1983). Similarly, physiological development of X-, Y-, W –cells was also unaffected by the lid suture in cats (Sherman and Stone, 1973). Furthermore, it has been shown that visual deprivation couldn’t exert any morphological change on the dendritic pattern of type I RGCs in hamsters (Lau et al., 1990). In consistent with these studies in cats and hamsters, the present study reveals that the development of dendritic field sizes of most rabbit RGCs examined is not susceptible to light deprivation. In a separate study,

we have shown that light deprivation has no observable effect on tracer coupling patterns of DSGCs (YC Chan and CC Chiao, unpublished data). This reinforces the idea that light input may not play a significant role in the morphological maturation of mammalian RGC dendrites.

The branching pattern of dendrites is a distinguishing trait of RGCs. Under the dark-rearing condition, a group of aberrant RGCs in hamster retina increase the dendritic complexity, whereas size of the soma area and dendritic arbor remained unchanged (Wingate and Thompson, 1994). Studies on xenopus reveal that dendritic complexity may change with level of brain-derived neurotrophic factor (BDNF) in the retina or the tectum (Cohen-Cory, 2004; Lom et al., 2002). Another study shows that retinal BDNF elevated upon light input (Seki et al., 2003). These results indirectly indicate that light deprivation increases dendritic complexity of RGCs via the BDNF-dependent pathway. The present study did not quantitatively analyze the branch points of each RGC, but there is no apparent difference in their dendritic complexity of each RGC types. Hence whether visual stimulation reduces the dendritic complexity is still a debating issue.

There are a few reports about structural change of retinal neurons under visually deprived conditions. The segregation of ON and OFF layers in the IPL of mouse retina is suppressed in the absence of visual experience (Tian and Copenhagen, 2003). Similarly, light deprivation result in shrinkage of inner retina and loss of cholinergic amacrine cells in mice (Zhang et al., 2005). In this study, it is improper to evaluate the effect of light deprivation on the alteration of RGC stratification in the IPL, because we used the stratification pattern as a criterion to classify RGCs. However, according to the numbers of cells labeled in each cell type, there was no dramatic increase in the frequency of bistratified RGCs and broadly-stratified RGCs (G3, G7, and G4, Table 1). Therefore, light input may not be an important factor contributing to the maturation of ON and OFF

layers of IPL in the rabbit retina.

One way to measure the physiological response of the retina is to measure electroretinogram (ERG). Research on mice indicates that light deprivation reduces the amplitude of a and b waves and oscillatory potentials (OPs) at early developmental stages (Vistamehr and Tian, 2004), while ERG results of control and dark-reared rabbits do not differ significantly (Reuter et al., 1971; Reuter, 1976). One possible explanation of this difference is that the influence of light deprivation on physiology of retina may be species dependent. Rabbits are diurnal animals and mice are nocturnal animals, thus this behavioral difference may explain the variation in the effect of light deprivation.

Overall visual experience seems to exert no effect on dendritic morphologies of RGCs in mammalian retinas, but certain physiological responses of RGCs may be influenced by dark rearing. Light deprivation maintained the NMDA current of RGCs in the adult rat retina, which is reduced in the normal condition (Xue and Cooper, 2001; Guenther et al., 2004). The specific characteristic physiology of DSGC, such as surround inhibition, motion surround inhibition, and contextual tuning, is altered in dark-reared rabbit retinas (YC Chan and CC Chiao, unpublished data). Though it is not certain whether light deprivation would influence the general development of each RGC types, it is likely that maturation of some signal pathways within the retina may be subject to visual experience.

Unbiased sampling of gene gun?

There are two ways in which RGCs could be labeled by the diolistic gene gun technique. The direct way is based on the fact that single dye-coated tungsten particles hit the soma or primary dendrites of RGCs and then DiI fills the whole cell. The indirect way is caused by occasional events that the tungsten clumps fall on the axon bundle of RGCs

and stain they the cells via retrograde dye filling. Smaller cells were usually identified in this indirect way as they need less dye to fill completely than larger cells do. Some RGCs were labeled by tungsten particles but indistinguishable due to messily overlapping with other cells. However, the resulting label frequency of each RGC type in postnatal retinas was inconsistent with the predicted cell density of adult retinas in Rockhill et al. (2004) (Table 1). Certain cell types were even not encountered in this study. One possible reason is that the present study was conducted by one labeling technique. In comparison with Rockhill et al. (2002), which they used four different labeling methods, our result inevitably shows certain degree of bias because of the experimental design. Concerning the shortcomings mentioned above, some cell types may be more easily stained whereas some may not. This indicates that gene gun labeling technique is not free of bias as initially proposed (Gan et al., 2000). Alternatively, the predicted numbers of all cell types in the previous study were calculated based on an assumption of a uniform coverage factor of 1.8 for all cells (Devries and Baylor, 1997). This may not be true in the real condition. Furthermore, Rockhill et al. (2002) worked on the mid-periphery part of the rabbit retina, yet we analyzed cells labeled from the visual streak to all peripheral parts of the retina. The intrinsic variation of dendritic field size of RGCs at different regions may also lead to different predicted cell density. Thus the actual cell numbers of all RGC types in the rabbit retina remain further investigation.