1. Introduction

1.1 Functional structure of the vertebrate retina

Five major functional neurons form a three-layer framework in the vertebrate retina, which includes both the vertical pathway (photoreceptors to bipolar cells to ganglion cells) and the lateral pathway (horizontal cells in the outer retina, and amacrine cells in the inner retina). As the final output neurons, retinal ganglion cells (RGCs) have three discharge patterns (Hartline, 1938). The ON RGCs respond to onset of light, the OFF RGCs are excited by light cessation, and the ON-OFF RGCs respond to both onset and offset of light. The dendrites of the ON RGCs make synapses with axon terminals of the ON bipolar cells stratified in the inner part of the inner plexiform layer (IPL), while the OFF RGCs make synapses with the OFF bipolar cells in the outer part of IPL (Nelson et al., 1978). The ON-OFF RGCs stratify in both the inner and the outer IPL, thus a bistratified morphology (Amthor et al., 1989). The region of the retina where a neuron could be evoked by a stimulation is defined as the receptive field by Hartline (1938). Antagonistic surround provided by lateral inhibition from horizontal cells creates concentric center-surround receptive field organization of RGCs (Kuffler, 1953). However, other RGCs exhibit more complex receptive fields, such as direction selectivity described below.

1.2 ON-OFF direction selective ganglion cells in adult rabbit retina

The ON-OFF direction selective ganglion cells (DSGCs) in the rabbit retina have been characterized for more than 40 years (Barlow and Levick, 1965). The DSGC exhibits a vigorous spiking activity when a light or dark object moves in the preferred direction across its receptive field, and shows little or no response when the same object sweeps across in the opposite (null) direction (Barlow and Hill, 1963). There are four subtypes of ON-OFF DSGCs, each selectively responds to one of the 4 orthogonal directions (anterior, posterior, superior, inferior; Oyster and Barlow, 1967). Prevailing evidences indicate that the spatially offset inhibition in the null direction is responsible for this robust direction selectivity (Barlow and Levick, 1965; Wyatt and Daw, 1975; Fried et al., 2002). Moreover, the DSGCs can recognize object against background with the aids of both static and motion surround inhibition (Chiao and Masland, 2003). Corresponding to its characteristic physiology, the DSGCs display high order branching bistratified dendrites with loops forming by retroflexed dendrites (Amthor et al., 1989). However, the polarization of dendritic trees is not predictable for four DSGC subtypes (Amthor et al., 1989; Yang and Masland, 1992, 1994). Only one subtype of DSGCs shows tracer coupling to the same subtype (Vaney, 1994; Amthor and Oyster, 1995). Comparison of homosubtypic tracer coupling and receptive field size suggests that each subtype tiles the retina with slight overlap

(Vaney, 1994; Yang and Masland, 1994; Amthor and Oyster, 1995). In spite of extensive studies on the cellular circuitry of the DSGC, little is known about the maturation of this intricate circuit throughout development.

1.3 Development of retinal ganglion cells in the rabbit retina

Earlier studies in the receptive field properties of RGCs in the rabbit retina show that concentric center-surround receptive field and the direction selectivity can be detected around eye opening at postnatal day 10 (P10) and reach the adult level at P21 (Bowe-Anders et al., 1975; Masland, 1977). Recent whole-cell patch experiments in the developing rabbit retina also confirm that the presence of direction selectivity and input currents to the DSGC are adult-like immediately after eye opening (Lee and Zhou, 2005). However, evidences from electroretinogram (ERG) studies indicate that mature retinal function is not obtained until five weeks of age (Reuter, 1976; Gorfinkel et al., 1988). In addition, it has been known that the DSGCs undergo significant dendritic remodeling in the first three weeks after birth (Wong, 1990), and the tracer coupling pattern among DSGCs changes drastically before eye opening (Wong, 1990; DeBoer and Vaney, 2005). Taken together, these physiological and morphological evidences indicate that the elegant direction selectivity circuitry seen in the adult rabbit retina is functional after eye opening, but the entire receptive field

property may take several weeks to fully develop.

1.4 Effects of visual experience on the development of visual pathway

Numerous studies have shown that visual experience is essential for normal development of visual cortex (Cynader and Mitchell, 1980; Fox et al., 1991; Gordon and Stryker, 1996; Kirkwood et al., 1996). Recent studies further indicate that visual deprivation dramatically alters the formation of direction selectivity in ferret visual cortex (Li et al., 2006). Impacts of visual experience on the development of pre-cortical regions have also been reported. Dark-rearing prior to natural eye-opening has striking effects on the ON-OFF segregation in the ferret dorsal lateral geniculate nucleus (dLGN; Akerman et al., 2002). Furthermore, naturalistic visual stimuli presented through unopened eyelids can significantly activate the dLGN neurons (Akerman et al., 2002). These results imply that visual experience before eye-opening has developmental significance. Although it is well accepted that there is a significant plasticity on synaptic connections and circuit refinements in higher visual centers of mammals before eye opening, it is less certain if mammalian retinas are also susceptible to visual deprivation during development (Daw, 1995). Early evidences have indicated that visual experience affects the functional and morphological refinement of the retina (Chow et al., 1957; Sosula and Glow, 1971;

Fisher, 1979; Wingate and Thompson, 1994; Fujikado et al., 1996; Sernagor and Grzywacz, 1996). Recent studies further show that the development of synaptic function and RGC dendritic stratification undergo a drastic activity-dependent remodeling (Wong & Ghosh, 2002; Tian, 2004). In mouse retina, dark rearing reduces the light-evoked responsiveness of inner retinal neurons (Tian and Copenhagen, 2001). Light deprivation also reduces the maturational loss of ON-OFF responsive RGCs and the pruning of dendrites (Tian and Copenhagen, 2003). Furthermore, recent ERG study shows that the light response of inner retina in the dark-reared mice is significantly suppressed (Vistamehr and Tian, 2004). In addition to these visual experience mediated functional and morphological refinement in the retina, light deprivation has also been reported to alter the expression patterns of glutamate receptor subunits in the rat retina (Xue and Cooper, 2001; Xue et al., 2001; Guenther et al., 2004). All these emerging evidences have suggested that maturation of RGCs may be highly susceptible to visual deprivation.

1.5 Goals and summary

The primary goals of this study are to characterize the maturation DSGC receptive field throughout the developmental stages, and to examine the effect of visual deprivation on the development of the DSGC circuitry in the rabbit retina. In

consistent with previous studies, we found that direction selectivity is fully mature around eye opening, but some receptive field properties, such as motion-induced surround inhibition, is not adult-like until P21. Although the dark-reared rabbits also exhibit similar direction selectivity as the normal-reared rabbits immediately after eye opening, the maturation of surround inhibition mediated by amacrine cells in the inner retina was significantly altered. Furthermore, the injected DSGCs apparently show similar dendritic features and tracer coupling patterns regardless the rearing conditions. Overall, our results indicate that visual deprivation does not affect the maturation of the DSGC trigger features and dendritic morphologies in the developing rabbit retina, but the development of certain receptive field properties may be delayed or altered without light stimulation after birth.