4. Discussions

4.1 Strength of spatially offset inhibition responsible for generating direction selectivity is developmentally modulated

Compare to the magnitude of DSI measured in adult rabbits (mean = 0.56; (Taylor and Vaney, 2002), similar value at the stage group of P22-adult in the present study was observed (Fig. 2C; mean = 0.50). However, half DSGCs measured at P15-21 showed sharper directional tuning (mean = 0.64). Recent studies on the mechanism of direction selectivity in the rabbit retina reveal that spatially offset inhibition is provided by starburst amacrine cells (Fried et al., 2002, 2005), and such asymmetric signal is shaped at multiple levels of processing (Fig. 10A; Fried et al., 2005). In addition to spatially offset cholinergic inputs, two other presynaptic glutamatergic and GABAergic inputs to the DSGC (further crafted by inhibitory signals from the suppressive pathways) and postsynaptic processing along the dendrites of the DSGC underlie the circuitry of direction selectivity. The DSGC receives reducing glutamatergic input for movement in the null direction and reducing GABAergic input for movement in the preferred direction. Our finding that directional tuning was higher at P15-21 indicates that the circuitry of suppressive pathways provides stronger inhibition before the DSGC reaches maturity (Fig. 10B). Thus, glutamatergic and GABAerguc inputs to the DSGCs may be strongly inhibited

by the suppressive pathways in the developing retina. In adult rabbits, such great strength of direction tuning was less encountered.

GABA is the major inhibitory neurotransmitter in the retina. Earlier studies suggest that GABA neurotransmission gains its functional maturity around eye opening, and reaches to adult level after P20 in the rabbit retina (Crook and Pow, 1997; Hu et al., 1998, 1999). Although the DSGCs exhibited surround inhibition induced by preferred direction motion right after eye opening, the inhibition fluctuated through maturation (Fig. 5B). At P15-21, some DSGCs received higher strength of inhibition. However, static surround inhibition was equally strong throughout development (Fig. 4B). Therefore, our findings imply that the strength of certain kinds of inhibition may be modulated after eye opening.

4.2 Complex receptive field properties of the immature DSGCs is correlated with stronger asymmetric synaptic inputs

Premature responses of the DSGCs to complex stimuli were often observed in this study. For example, the responses of the DSGCs to motionless surround annulus stimulus was higher than the responses of the DSGCs to center-alone stimulus (Fig. 6B), given that the DSGCs have already displayed a strong center-surround interaction around eye opening (Fig. 4B), and contextual tuning of the DSGCs was

not present or reversed in the earlier stages (Fig. 7B). We noticed that cells with such premature responses to complex stimuli are correlated well to cells with sharper directional tuning. Almost all DSGCs showed premature responses to complex stimuli had DSI larger than 0.65. On the other hand, we also observed that the center size of the receptive field was not match the dendritic field described by previous studies in the adult rabbits (Yang and Masland, 1992, 1994). The center size for stimuli in Figures 6A and 7A was 70% larger than the cell's actual dendritic field (n = 24). Recent pharmacological experiments reveal that the cholinergic input directly to the DSGC could expand its receptive field size, but this excitatory input is usually blocked by GABAergic inhibition (Fried et al., 2005). Estimated expansion ratio (60%) based on the findings of Fried et al (2005) is close to our observation. This indicates that the DSGCs with large receptive field could receive additional cholinergic input in the developing retina (Fig. 10B). In addition, the displacement of receptive field toward the preferred side of the DSGC rarely found in adult rabbit retinas was also observed (Yang and Masland, 1994). Nondirectional zone on the preferred side of receptive field (Barlow and Levick, 1965) was thought to supply this asymmetric shift (Oesch et al., 2005). We speculate that this displacement is further enhanced by extremely strong spatially offset inhibition during development.

Based on our assumption of large displacement of receptive field, the square

wave grating presented on the center of receptive field used in Figures 6A and 7A would only excites the preferred side of the genuine receptive field. According to previous studies, stimulus moving within the receptive field would create an inhibitory zone on the preferred side of the stimulus to inhibit subsequent stimulation appeared in that zone (Wyatt and Daw, 1975; Stasheff and Masland, 2002). Therefore, motionless surround annulus falling on the null side of genuine receptive field in Figure 6A would not produce any inhibitory zone (Fig. 11B). Extra stimulation to the DSGCs could evoke greater response for the motionless windmill stimulus than for the center-alone stimulus. In contrast, reversed response of contextual tuning may be due to additional inhibition generated by the null side of the genuine receptive field which strongly inhibited the center response (Fig. 11C). Furthermore, the inhibitory zones created by surround stimuli of in-phase grating in Figure 7A would merely suppress the center response (Fig. 11D).

4.3 Light deprivation alters the maturation of suppressive pathway in the DSGCs

Results of dark-rearing condition indicate that the development of direction selectivity do not require early visual experience. However, shaper directional tuning and premature responses to complex stimuli observed in normal-reared rabbits were even more profound after two weeks of dark-rearing (Fig. 2C, 6C, and 7C). Some

earlier evidences have shown that maturation of synaptic pathway in the inner retina is visual-dependent. For example, in the mouse and rat retina, the conventional synaptic density in the inner plexiform layer is upregulated by light deprivation (Sosula and Glow, 1971; Fisher, 1979). Moreover, the oscillatory potentials (OPs) of ERG experiment is suppressed in dark-reared mouse retina, and this suppression is reversible after offering normal visual stimulation (Vistamehr and Tian, 2004). Furthermore, the development of cholinergic amacrine cells in the mouse retina after eye opening has been reported to require visual activity (Zhang et al., 2005). Although there is no effect of dark-rearing on ERG in the rabbit retinas (Reuter, 1976), our results suggest the suppressive pathways of the DSGCs in the inner retina may be altered by light deprivation.

Previous studies also suggest that remodeling of synaptic connection and dendritic complexity is visual activity dependent. For instance, developmental pruning of bistratified ganglion cells in the mouse retina is retarded by light deprivation (Tian and Copenhagen, 2003). In the dark-reared hamster retina, aberrant ganglion cells would not undergo age-dependent dendritic modification (Wingate and Thompson, 1994). However, there is no effect on the morphology of type I ganglion cells in the dark-rearing hamster retina (Lau et al., 1990). According to our results (Fig. 8 and 9), the DSGCs in dark-reared rabbits display similar morphological

patterns found in normal-reared rabbits. Thus, the DSGCs in the rabbit retina apparently do not require visual stimulation for dendritic remodeling during development.