

3. Results

3.1 The DSGCs can be found in both normal-reared and dark-reared rabbits around eye opening, but the strength of directional tuning is significantly higher in dark-reared rabbits after P22

Consistent with previous studies (Masland, 1977), direction selectivity was robust around eye opening (P10) in normal-reared rabbits. It is difficult to be sure if the DSGCs show direction selectivity before eye opening, because the light response was weak and rapidly adapted (Masland, 1977). We also found ganglion cells with direction selectivity in dark-reared rabbits at the same stage. To quantify the strength of directional tuning of the DSGCs throughout developmental stages, we calculated the direction selective index (DSI) used by (Taylor and Vaney, 2002) in adult rabbit retinas. We found that the magnitude of DSIs in normal-reared rabbits around eye opening was similar to adult, but slightly fluctuated through maturation (Fig. 2C; $P = 0.0425$ for three stage comparison). DSIs were higher at P15-21 ($P = 0.0228$ for P10-14 vs. P15-21; $P = 0.0294$ for P15-21 vs. P22-adult) since half DSGCs had DSIs greater than 0.65. In dark-reared rabbits, similar DSIs were observed at early stage. However, after P22, we found that more than half DSGCs had DSIs larger than 0.65. Thus, the DSGCs showed sharper directional tuning in dark-reared rabbits after P22 ($P = 0.0067$ for NR vs. DR; normal-rearing vs. dark-rearing).

3.2 Velocity tuning of the DSGCs is adult-like around eye opening, and is not affected by light deprivation

It is well known that DSGCs are tuned for broad range of image motion velocities and respond strongly to higher speed in adult rabbit retinas (Wyatt and Daw, 1975). Although directional selectivity of the DSGCs was already presented around eye opening, it is uncertain that all DSGCs attain their adult velocity tuning right after eye opening. In the present study, we found that velocity tuning was adult-like in normal-reared rabbits around eye opening (Fig. 3B), and slightly shifted toward higher velocity through maturation ($P = 0.0085$ for three stage comparison; $P = 0.0209$ at 0.12 mm/sec for P10-14 vs. P15-21; $P = 0.0261$ at 0.18 mm/sec for P10-14 vs. P22-adult; $P = 0.0122$ and $P = 0.0008$ at 0.27 mm/sec for P10-14 vs. P15-21 and P10-14 vs. P22-adult, respectively; $P = 0.0092$ at 0.41 mm/sec for P10-14 vs. P22-adult; $P = 0.0248$ at 0.62 mm/sec for P10-14 vs. P22-adult). We also noticed that the variation was greater in higher velocity ranges at early stage ($P = 0.0049$ and $P = 0.0052$ at 3.18 mm/sec for P10-14 vs. P15-21 and P10-14 vs. P22-adult, respectively). In dark-reared rabbits, the DSGCs had similar velocity tuning as in normal-reared rabbits (Fig. 3C). Developmental modifications ($P = 0.0209$ for three stage comparison) such as shifting toward higher speed ($P = 0.0098$ at 1.40 mm/sec for

P15-21 vs. P22-adult; $P = 0.0115$ at 2.11 mm/sec for P15-21 vs. P22-adult), and larger variation at higher velocity ($P = 0.0282$ and $P = 0.0312$ at 4.80 mm/sec for P10-14 vs. P15-21 and P15-21 vs. P22-adult, respectively) were observed as well.

3.3 Classical center-surround organization of the DSGCs attains its adult form around eye opening, and is independent of rearing conditions

Although the direction selectivity is the main trigger feature of the DSGCs, the DSGCs in the adult rabbit retina also have a classical center-surround organization of the receptive field. It has been reported that half ganglion cells show silent surround or surround absent shortly after eye opening (Masland, 1977). We therefore are interested in determining if the DSGCs equip this center-surround receptive field property early in the development. We found that the response of the DSGCs was inhibited from steady surround inhibition while the size of stimulus expanded beyond the center region (Fig. 4B and 4C). Thus, the DSGCs already exhibited strong silent surround inhibition around eye opening in both normal-reared and dark-reared rabbits. In addition, we found that the center size of receptive field was larger in normal-reared rabbits at early stage ($P = 0.0123$ for three stage comparison; $P = 0.0040$ at 126 μm for P10-14 vs. P15-21; $P = 0.0023$ at 254 μm for P10-14 vs. P15-21). However, many DSGCs still remained larger center size in dark-reared

rabbits after P22 ($P = 0.0203$ for three stage comparison).

3.4 Light deprivation enhances surround inhibition induced by the preferred direction motion of the DSGCs

Like classical center-surround interaction, the DSGCs were inhibited as long as the moving stimulus was extended to the surround area in adult rabbit retinas (Wyatt and Daw, 1975; Chiao and Masland, 2003). However, such moving surround inhibition induced by the preferred direction motion was weaker than static surround inhibition in the postnatal retinas, and gradually declined to adult form in normal-reared rabbits (Fig. 5B). The pattern of developmental fluctuation was similar to the tuning of direction selectivity seen in Fig. 2C ($P < 0.0001$ for three stage comparison; $P = 0.0065$ and $P = 0.0147$ at $180\ \mu\text{m}$ for P10-14 vs. P22-adult and P15-21 vs. P22-adult, respectively; $P = 0.0299$ at $540\ \mu\text{m}$ for P15-21 vs. P22-adult; $P = 0.0235$ at $720\ \mu\text{m}$ for P15-21 vs. P22-adult; $P = 0.0179$ at $900\ \mu\text{m}$ for P15-21 vs. P22-adult). In contrast, such inhibition remained strong with little fluctuation through maturation in dark-reared rabbits (Fig. 5C; $P < 0.0001$ for three stage comparison; $P = 0.0134$ and $P = 0.0236$ at $180\ \mu\text{m}$ for P10-14 vs. P22-adult and P15-21 vs. P22-adult, respectively; $P = 0.0236$ at $360\ \mu\text{m}$ for P10-14 vs. P22-adult). Thus, in dark-reared rabbits, the DSGCs received stronger inhibition induced by the preferred direction

motion throughout development ($P < 0.0001$ for NR vs. DR at P10-14 with $P = 0.0058$ at $360\ \mu\text{m}$; $P = 0.0190$ for NR vs. DR at P15-21; $P < 0.0001$ for NR vs. DR at P22-adult with $P = 0.0340$ at $720\ \mu\text{m}$, $P = 0.0435$ at $900\ \mu\text{m}$, and $P = 0.0436$ at $1080\ \mu\text{m}$).

3.5 Motion surround inhibition of the DSGCs is apparent around eye opening, but the strength of inhibition is weaker in dark-reared rabbits after P22

In addition, we also used windmill vanes to isolate motion surround inhibition (Werblin, 1972; Chiao and Masland, 2003). Similarly, when windmill vanes start rotating, the response of the DSGCs in both normal-reared and dark-reared rabbits was suppressed by motion surround inhibition (Fig. 6B and 6C). Reducing strength of inhibition was also observed while the windmill rotated rapidly. However, we found that the response of certain DSGCs stimulated with both center grating and static windmill surround annulus was even greater than center alone in normal-reared and dark-reared rabbits. Motion surround inhibition also gradually matured to adult form, though the immature response of steady windmill surround annulus still could be seen after P22 (Fig. 6B; $n = 1$). However, this phenomenon was even more pronounced in dark-reared rabbits after P22 (Fig. 6C; $P < 0.0001$ for three stage comparison; $P = 0.0087$ and $P = 0.0038$ at 0 rotation/min for P10-14 vs. P22-adult and P15-21 vs.

P22-adult, respectively; $P = 0.0318$ at 15 rotations/min for P10-14 vs. P22-adult; $P = 0.0284$ at 20 rotations/min for P10-14 vs. P22-adult; $P = 0.0305$ at 25 rotations/min for P10-14 vs. P22-adult; $P = 0.0209$ at 30 rotations/min for P10-14 vs. P22-adult; $P = 0.0238$ and $P = 0.0235$ at 35 rotations/min for P10-14 vs. P22-adult and P15-21 vs. P22-adult, respectively; $P = 0.0248$ and $P = 0.0196$ at 40 rotations/min for P10-14 vs. P22-adult and P15-21 vs. P22-adult, respectively). Thus, weak steady windmill surround inhibition remained in dark-reared rabbits after P22 ($P = 0.0366$ for NR vs. DR at P15-21 with $P = 0.0281$ at 15 rotations/min; $P = 0.0005$ for NR vs. DR at P22-adult).



3.6 Contextual tuning of the DSGCs is not mature until P22, and dark rearing has a profound effect on this property

On the other hand, it has been shown that motion contrast inhibition is stronger as the surround grating is in phase with the center grating in adult rabbit retinas (Chiao and Masland, 2003). Although this contextual tuning effect can be seen in normal-reared rabbits at early stage, we also noticed that certain DSGCs showed no such contextual tuning (Fig. 7B). Contextual tuning gradually matured to adult form after P22. However, after two weeks of dark-rearing, the response was strongly suppressed when the surround grating was shifted 180° from the center grating, yet

became weakly suppressed toward the in-phase grating (Fig. 7C). After P22, this phenomenon was extremely profound ($P = 0.0008$ for three stage comparison; $P = 0.0244$ at 0° for P15-21 vs. P22-adult; $P = 0.0095$ and $P = 0.0030$ at 45° for P10-14 vs. P22-adult and P15-21 vs. P22-adult, respectively; $P = 0.0322$ at 315° for P10-14 vs. P22-adult). Therefore, the DSGCs appeared to show reversed contextual tuning in dark-reared rabbits after P22 ($P = 0.0088$ for NR vs. DR at P22-adult with $P = 0.0325$ at 0° , $P = 0.0026$ at 45° , and $P = 0.0411$ at 315°).

3.7 Light deprivation has no observable effect on dendritic features and tracer coupling patterns of the DSGCs

Characteristic morphological features of the DSGCs in adult rabbit retinas (Amthor et al., 1989; Wong, 1990) were observed in developing rabbits throughout developmental stages (Wong, 1990). We found that the DSGCs had bistratified and lattice-like dendritic trees in both normal-reared and dark-reared rabbits at different stages (Fig. 8). Both ON and OFF dendritic arbors of the DSGCs bore many high order branching dendrites and some dendrites were recursive toward the somata. Further systematical analysis of morphological maturation in both rearing conditions is currently investigated by another lab member (HJ Yang, unpublished results). In agreement with previous studies (Amthor et al., 1989; Yang and Masland, 1992, 1994),

the DSGCs did not show dendritic polarization to the preferred direction in the developing rabbit retinas. Moreover, tracer coupling patterns of the DSGCs in both normal-reared and dark-reared rabbits were alike at P8 (Fig. 9). In consistent with a recent study in the developing rabbit retina (DeBoer and Vaney, 2005), the coupled somata were both inside and outside dendritic fields of the injected DSGC's.

