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Wiring Specificity and Plasticity of Developing Rod and Cone Pathways

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Vertebrate retinas possess rod and cone photoreceptors that are important for night and daytime vision, respectively. In the outer retina, rods and cones wire synaptically with distinct types or cell compartments of neurons to transmit visual signals. Rods primarily synapse with rod bipolar cells (RBCs) and cones preferentially synapse with cone bipolar cells (CBCs) [1,2]. Horizontal cells (HCs), which provide inhibitory modulation of photoreceptor transmission, contact rods with their axons and cones with their dendrites. It remains largely unknown how rod and cone wiring specificity is achieved during development. To determine the cellular mechanisms underlying photoreceptor type-specific wiring, I investigated how the outer retinal connectivity is affected when rods are transformed into cones during the development of the mouse retina.

In this study, I compared the morphology and connectivity of rod and cone photoreceptors and their postsynaptic neurons in the wildtype (WT) and a knockout of a transcription factor, neural retina leucine zipper (*Nrl*$^{-/-}$). The absence of *Nrl*$^{-/-}$ results in all rod precursors differentiating into short wavelength-sensitive cones [3]. I first investigated the connectivity between photoreceptors and bipolar cells using confocal microscopy, fluorescence reporter mouse transgenic lines, and immunohistochemistry. In *Nrl-EGFP*/*Grm6-tdTomato* double transgenics, all rods or rod precursor-derived cones (i.e. transformed cones, cone^{Trans}) express GFP and RBCs and some types of ON CBCs express tdTomato [4,5]. To visualize the synapses, I immunostained the metabotropic glutamate receptor 6 (mGluR6), which is present at photoreceptor synapses onto RBC and ON CBC dendrites. Connectivity was determined by quantifying the number of mGluR6 receptor clusters (puncta) in tdTomato-labeled bipolar cells that are apposed to GFP-positive rod/cone^{Trans} or GFP-negative normal cones. I compared the connectivity of RBCs and a type of ON CBC, the type 6 (T6) CBC. Photoreceptor connectivity at ultrastructural resolution in control and knockout animals was also obtained using serial block-face scanning electron microscopy (SBFSEM).

Immunolabeling showed that in the *Nrl*$^{-/-}$ retina, both RBCs and T6 CBCs contact cone^{Trans} and normal cones when normal rods are absent. The average total synapse number of RBCs was lower in the *Nrl*$^{-/-}$ retina compared to WT, whereas that of T6 CBCs was unchanged. Interestingly, although cone^{Trans} largely express cone genes and have cone-like physiological properties [6,7], the SBFSEM reconstructions revealed two morphologically distinct groups of photoreceptors. One group structurally resembled WT cones, whereas the other group had a mixture of rod and cone morphologies. Specifically, many *Nrl*$^{-/-}$ photoreceptors exhibited some rod-like features, such as oval shape axon terminals, high electron density and high vesicle density. These photoreceptors, however, also contained multiple active zones like WT cones, instead of WT rods which usually possess a single active zone. Therefore, it is likely that the photoreceptors with rod-cone hybrid morphology are cone^{Trans} and the others are normal cones. Consistent with observations from immunohistochemistry, the EM analysis also suggest that RBCs and T6 CBCs are contacted by both cone^{Trans} and normal cones. Moreover, HCs in the *Nrl*$^{-/-}$ retina lost their cell compartment-specific connectivity with photoreceptors; their axon and dendrites contact both cone^{Trans} and normal cones.

In conclusion, the absence of normal rods during development trigger RBCs to contact cone-like and normal cone photoreceptors to establish synaptic connections but with fewer numbers than WT. Although the rod to cone transformation is incomplete, cone genes expressed in cone^{Trans} appear sufficient to establish their connectivity with CBCs. These findings provide fundamental knowledge of the mechanisms regulating the specificity of rod and cone pathway connectivity. This knowledge also provides insight into designing cell replacement and rewiring strategies for restoring vision in retinas with photoreceptor degeneration [8].

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