## Introduction

The mammalian retina displays a diversity of cell types and functionally distinct synaptic pathways. Much of this anatomical diversity has been summarized (Fig. 1; see review in Dacey, 2000; Masland, 2001a; Wassle, 2004). In brief, at least 10 types of bipolar cell populations transmit visual signals in parallel from 2-3 types of photoreceptors in the outer retina to ganglion cells in the inner retina (Boycott and Wassle, 1991; McGillem and Dacheux, 2001; MacNeil et al., 2004; Pignatelli and Strettoi, 2004). The ganglion cells can be further subdivided into estimated 10-15 anatomically distinct populations that project to the lateral geniculate nucleus and other central visual area (Citron et al., 1988; Rockhill et al., 2002). Still more complex, the signal between photoreceptors and bipolar cells are shaped by 2-3 types of horizontal cells (Boycott et al., 1987; Famiglietti, 1990; Dacey et al., 1996; Hack and Peichl, 1999), and the link between bipolar cells and ganglion cells is further modulated by at least 30 types of the amacrine cells (MacNeil and Masland, 1998; MacNeil et al., 1999). These lateral interactions of horizontal and amacrine cells are responsible for generating receptive field of various ganglion cells. Thus, the “retinal circuit”' is not a single circuit but many “microcircuits”, comprising on the order of 60-70 neural cell types (see review in Dacey, 2000; Masland, 2001a; Wassle, 2004).

### Cell types and synaptic pathways in the rabbit retina

A typical rabbit retina contains only one type of rod and two types of cone photoreceptor cells, but there are as many as 13 different types of bipolar cells including 12 types of cone bipolar cells and 1 type of rod bipolar cell (McGillem and Dacheux, 2001; MacNeil, 2004). This diversity of bipolar cells in the retina has been suggested for the initiation of parallel processing early in the visual system (Dacey, 2000).

Cone bipolar cells are the major elements responsible for transmitting signals from cone photoreceptors to ganglion cells (Fig. 2). They are divided into two categories: ON types and OFF types by physiological responses and axonal stratification in the inner plexiform layer (IPL) (Euler and Wassle, 1995; Euler et al., 1996). The dendrite of an ON-bipolar cell is central invaginating element of a cone terminal's triad synapse that is shared with two laterally flanking horizontal cell processes. The dendrites of an OFF-bipolar cell establish flat contacts or basal junctions at the base of the cone pedicle (Fig. 3). The axon terminals of ON and OFF bipolar cells display distinct morphologies based on the positions of their stratifications within the IPL. The axons of ON bipolar cells project to sublamina b of the IPL (the lower tier near the ganglion cell layer); whereas the axons of OFF bipolar cells project to sublamina a of the IPL (the upper tier near the inner nuclear layer). Rod bipolar cells unlike cone bipolar cells have a well characterized singular morphology with snowflake-like dendrites in the outer plexiform layer (OPL) and large bulbous axon terminals located in the inner most of the IPL (the bottom of sublamina b) (Dacheux and Raviola,1986; Young and Vaney, 1991).

The most diverse cells in the rabbit retina are amacrine cells containing at least 30 types (MacNeil and Masland, 1998; MacNeil et al., 1999; Masland, 2001b). Amacrine cells are the major players in the retinal processing of visual information. They make up approximately 40% of all neurons in the inner nuclear layer (INL) of mammalian retinas, and modulate the signal transmission form bipolar cells to ganglion cells (Strettoi and Masland, 1995). Ganglion cells receive input from bipolar cells and amacrine cells and transmit them via action potential to the various central targets. They are divided into ON and OFF types as well, and are easily distinguished by their morphological features (e.g., dendritic patterns and stratifications) and physiological response (e.g., concentric receptive fields, local edge detector, direction selectivity, etc.). It is now known that there are at least 11 types of retinal ganglion cells in the rabbit retina (Rockhill et al., 2002).



**The color processing pathway in the primate retina**

Much of understanding about the color processing circuitry in the mammalian retina comes from the extensive studies of primate retina (see review in Dacey, 1999; 2000; Dacey and Packer, 2003). Since the color processing pathway in the rabbit retina has not been examined in detail before, thus the color processing circuitry known in the primate retina is an important guidance for understanding its counter part in the rabbit retina.

In macaque, as other old world monkeys, there are three types of cone photoreceptors in the

retina, namely the S cones, M cones and L cones. From many psychological and physiological evidences, it has been known that macaques have true trichromatic color vision (see review in Dacey, 1999; Dacey and Packer, 2003). In contrast, the new world money had individual variation in color vision (see review in Jacobs, 1998).

To generate a neural code of color information, the outputs of the three cone types must be compared at a second stage of neural processing. Spectral opponency begins when cone signaling pathways converge antagonistically in the receptive fields of a subset of anatomically distinct ganglion cell populations in the primate retina (Dacey, 2000). Spectrally opponent ganglion cells project subsequently their chromatic signals to the lateral geniculate nucleus in the thalamus where more complex interactions occur (Johnson et al., 2001; Conway, 2001).



Red-green opponency

The L and M cones together make up the great majority of cones (roughly 90%) randomly arranged in the cone mosaic (Roorda and Williams, 1999; Roorda et al., 2001). In about the central 10 degrees (i.e., fovea), a single midget bipolar cell gets all of its photoreceptor input from a single L or M cone and connects in turn exclusively to a single midget ganglion cell, establishing a "private-line" from a single cone to the brain (Calkins et al., 1994). Given random cone connectivity (i.e., both L and M cone inputs) to an antagonistic receptive field surround of a midget ganglion cell, strong red-green opponency would still be ensued (because the high gain and single

cone center could cancel the same cone inputs to the surround), leaving a pure L versus M cone signal at the output leave. However, in the retinal periphery from 20–50 degrees eccentricity, the private line pathway breaks down because numerous midget bipolar cells converge on single midget ganglion cell with enlarged dendritic tree diameter (Dacey, 1993). L versus M cone opponency should therefore drastically decline in the retinal periphery.

Blue-yellow opponency

The S cones make up only 5–10% of the cones and, not surprisingly, the retinal circuitry associated with this sparsely distributed mosaic has been difficult to access experimentally. The major advance of the blue-yellow opponency came from the identification of several key components in S-cone circuitry. First, a distinctive bipolar cell population (namely the blue cone bipolar cell) labeled with antisera that recognize glycine-extended cholecystokinin precursors (CCK) has dendrites connected exclusively to S cones (Fig.4; Mariani, 1984; Kouyama and Marchak, 1992). Second, a distinctive ganglion cell population (the small bistratified ganglion cells) was found to be the morphological base for the well established “blue-ON-yellow-OFF” opponent pathway (Dacey and Lee, 1994). These ganglion cells receive a direct ON input from the blue cone bipolar cell that makes selective connections with S cones (Calkins et al., 1998; Herr et al., 2003). Third, a distinct horizontal cell type (namely the H2 cell) was reported to receive strong S-cone inputs and weak L and M cone inputs (Dacey et al., 1996). These H2 cells could provide a basis for L and M cone signal feedback to the S cones, creating L + M cone surrounds in the receptive field of the S cones (Dacey, 2000),

thus the S cones can transmit color opponency signal (blue center yellow surround) to the blue cone bipolar cells. Finally, the small bistratified ganglion cell also receives weak OFF inputs from the diffused cone bipolar cells connected to L and M cones, which could contribute to the overall S versus L＋ M opponent response. But, evidences from a recent study by Dacey et al. (2003) showed that S-cone opponent signals in fact can be recorded from several other rare ganglion cell populations (e.g., large sparse monostratified ganglion cell and large sparse bistratified ganglion cell). These results imply that the blue-yellow opponency seems to be more complicated than we thought before.

**Information of color processing pathway in the rabbit retina**

In rabbits, unlike primate, there are only two types of cone photoreceptors in the retina, namely the S cones and the M cones. From behavioral evidences (Nuboer, 1971; Nuboer and Moed, 1983) and ERG experiments (Nuboer et al., 1983), it has been known that rabbits have dichromatic color vision. Despite the retinal cell types in rabbit have been carefully characterized, there are only a few reports about the neural circuitry of color processing in the past. First, the potential color encoding ganglion cells in the rabbit retina have been reported by Caldwell and Daw (1978) and DeMonasterio (1978), however the morphological identities are not clear. In addition, the presence of green center ganglion cells has been positive in DeMonasterio (1978) study but negative in Caldwell and Daw study (1978). Second, Famiglietti (1981) reported two types of wide-field

bipolar cells as the color encoding bipolar cells (both ON and OFF types) by morphological features. The results in MacNeil et al. (2004) also indirectly support this hypothesis because each bipolar cell types identified by Golgi staining have no selective cone connection. However, the physiological evidences are lacking and the specific cone input connection of bipolar cells has not been examined thoroughly. In addition, the morphological identities of two types of assumed blue cone bipolar cells are inconsistent with the primate's results. This uncertainty warrants a key investigation to re-examine the color encoding bipolar cells in the rabbit retina. Finally, Famiglietti (1990) and Famiglietti and Sharpe (1995) proposed a third type of horizontal cell which may selectively connect only S cones, therefore is responsible for providing a blue surround in color processing. However, the most recent study by Hack and Peichl (1999) showed that both A type and B type horizontal cells are non-selectively connected to the cones and the third type of horizontal cell has not been found in their study. From these studies, Hack and Peichl (1999) concluded that horizontal cells unlikely play a role in color processing of the rabbit retina, except for the third type of horizontal cell.

**Experimental goals**

In Hack and Peichl experiments (1999; described above), they used horizontal cell microinjection and immunocytochemical labeling of S and M cones to examine the connection between horizontal cells and cone photoreceptors in the rabbit retina. Using a similar technique, by

swapping the target cell of microinjection from horizontal cells to bipolar cells, we can investigate the synaptic connection between bipolar cells and cone photoreceptors. However, the approach is much harder in our study, because the more types and the smaller somata as well as processing size of bipolar cells. Using this method, I hope to morphologically identify the S cone bipolar cell types which are important for color processing research in the rabbit retina.