1. INTRODUCTION

1.1 Vision

Light signals are one of the innumerable information that the world comprises. Most animals possess a visual system which not only can sense the streams of light but also can reconstruct the original scenes. Generally speaking, mammalian vision is initially formed in the retina; subsequently, the visual information travels through the optic chiasm and the lateral geniculate nucleus in the thalamus, and finally reaches primary visual cortex of the brain. Retina, a sheet of neural tissue lies on the back of the vertebrate eye, has been thoroughly investigated since Ramón y Cajal described it over a century ago.

Similar to other central nervous system tissues, the retina is a layered structure and contains diverse cell types (Masland, 2001). All vertebrate retinae are mainly composed of three layers of nerve cell bodies and two layers of synaptic networks. The outer nuclear layer (ONL) contains somata of photoreceptors, the inner nuclear layer (INL) contains somata of the bipolar, horizontal and amacrine cells, and the ganglion cell layer (GCL) contains somata of ganglion cells and displaced amacrine cells. Dividing these nerve cell layers are two neuropils where the neurons ramify their processes and the synaptic connections occur. The first area of neuropil is the outer plexiform layer (OPL) where connections between photoreceptors, bipolar cells and horizontal cells occur. The second neuropil is the inner plexiform layer (IPL) where the bipolar cells relay the

carried information to the ganglion cells. In addition, amacrine cells influence the signals by lateral interacting with those vertically running neurons. Before projecting to higher centers, a great deal of visual encodings was accomplished by the highly organized circuitry in the IPL.

1.2 Direction selectivity in the visual system

From retina to brain, a variety of visual features such as color, form, depth, and motion are processed in parallel (Boycott and Wassle, 1999; Werblin et al., 2001; Wassle, 2004). Direction selectivity is inherently a division of motion sensitive property, which can be defined as an ability to discriminate the direction of a moving object. In other word, a direction selective neuron will fire strongly when an object moves in one direction (the preferred direction), but weakly or not at all when the object moves in the opposite way (the null direction).

Cells that respond directionally to the image motion can be found in many parts of the visual system. Hubel and Wiesel (1962) presented evidences that certain cortical neurons in cats respond only to movement in a particular direction. Later, Barlow and colleagues (1963 and 1964) showed that retinal ganglion cells in the rabbit exhibited directionality as well. In addition to mammals, this type of response was encountered in many vertebrate retinae, including birds (Maturana and Frenc, 1963; Holden, 1977), reptiles (Jensen and deVoe, 1983; Kittila and Granda, 1994; Ammermuller et al., 1995), fish (Cronly-Dillon, 1964; Jacobson and Gaze, 1964) and amphibians (Barlow, 1953; Lettvin et al., 1960; Werblin, 1970).

1.3 Direction selective ganglion cells

The ON-OFF direction selective ganglion cells (DSGCs) are probably the most well studied ganglion cells in the rabbit retina (Vaney et al., 2001). They are capable of discriminating the direction of a moving object, and respond well to both positive (ON) and negative (OFF) contrast stimuli.

Like other ganglion cells, DSGCs lie on the ganglion cell layer, and ramify their dendrite in the inner plexiform layer, which was further divided into five sub-laminae. Functionally, the two strata below the amacrine cell bodies can be grouped into sub-lamina a (the OFF sub-lamina), whereas the other 3 strata stretched to the ganglion cell bodies are sub-lamina b (the ON sub-lamina) (Famigletti and Kolb, 1976). In order to keep the ON and OFF signals separated, interactions are only allowed between invagination-contacting cone bipolar cells (the ON bipolar cells) and ON ganglion cells in the ON sub-lamina, while basal-contacting cone bipolar cells (the OFF bipolar cells) can only interact with OFF ganglion cells branching in the OFF sub-lamina. The DSGCs, however, arborize their dendrites in both the ON and OFF sub-laminae of the inner plexiform layer (Amthor et al., 1984; Oyster et al., 1993; Famiglietti, 1992).

The ON-OFF DSGCs comprise four subtypes. Each population responds preferentially to image motion in one of the four cardinal ocular directions (Oyster and Barlow, 1967). The retina is covered by four different subtypes of DSGCs all over the place, where DSGCs of the same subtype

tile the retina in a territorial manner, with little overlap of their dendrites (Vaney, 1994; Amthor and Oyster, 1995).

1.4 Starburst amacrine cells

Starburst amacrine cells (SACs) are ubiquitous among vertebrate retinae (Perry and Walker, 1980). Like most amacrine cells, SACs are free of axon, and therefore synaptic outputs and inputs are intermingled on the dendrites (Famiglietti, 1991). Their dendrites project outward in all directions from the centrally located somata, therefore the dendritic arbors possess a radially symmetric appearance.

The SACs can be divided into two populations, one lies in the ganglion cell layer, another is in the inner nuclear layer, with their dendrites stratified mirror symmetrically in the ON and the OFF sub-laminae of the inner plexiform layer, respectively (Masland and Mills, 1979; Vaney et al., 1981). The ON-SACs and OFF-SACs stratify at precisely the same levels as the bi-stratified DSGCs (Famiglietti, 1992). The processes of numerous overlapping SACs and those of DSGCs are reported to co-fasciculate together, (Tauchi and Masland, 1985; Vaney and Pow, 2000). Moreover, the SACs are known to release acetylcholine (ACh), the excitatory neurotransmitter (Tauchi and Masland, 1984), as well as γ-amino butyric acid (GABA), the inhibitory neurotransmitter (Brecha et al., 1988; O'Malley et al., 1992).

Although selectively laser ablation of either the preferred side or the null side SACs did not

eliminate direction selectivity in rabbits (He and Masland, 1997), using toxin-mediated cell targeting techniques to specifically ablate SACs did eliminate direction selectivity in mice and rabbits (Yoshida et al., 2001; Amthor et al., 2002). Morphologically, an electron microscopic study suggests that DSGC receives the majority of synaptic inputs from a cluster of several amacring cells, and these dendrites are probably belonged to several SACs (Dacheux et al., 2003). Moreover, dual patch recording of SACs and DSGCs provide strong evidence that SACs play a key role in the computation of direction selectivity (Fried et al., 2002).

1.5 Neuronal circuitry and cellular mechanisms of direction selectivity

Since Barlow and Levick (1965) first characterized, direction selectivity of retinal ganglion cells has fascinated scientists for over four decade. In order to generate direction selective response, asymmetry of either excitation or inhibition is required. Direction selectivity has been proposed to be mediated mainly by the suppression for movement in the null direction, but not for the preferred direction (Barlow and Levick, 1965; Wyatt and Daw, 1975; Amthor and Grzywacz, 1993; Stasheff and Masland, 2002). In fact, the excitatory input to DSGCs is larger during the preferred movement (Borg-Graham, 2001; Fried et al., 2002; Taylor and Vaney, 2002), and the inhibitory input is larger during the null movement (Fried et al., 2002; Taylor and Vaney, 2002). Therefore, both the excitatory and inhibitory input currents to the DSGCs are themselves directionally selective. This suggests that the computation is initially performed presynaptically to the DSGCs.

Null direction inhibition has been considered to play a major role in generating direction selectivity for a long time (Taylor and Vaney, 2003). Pharmacological experiments, which showed that antagonists of the inhibitory transmitter GABA receptors abolish direction selectivity, provided strong supports for the importance of inhibition (Wyatt and Daw, 1976; Caldwell et al., 1978; Ariel and Daw, 1982; Kittila and Massey, 1997; Chiao and Masland, 2002). A series of dual patch recordings identified the SACs as a source of inhibitory input to DSGCs, and showed that a DSGC receives inhibitory input only from the subset of starburst processes that point in the DSGC's null direction (Fried et al., 2002).

Several evidences also revealed that facilitation of excitatory inputs occurs for the preferred-direction movement (Barlow and Levick, 1965; Grzywacz and Amthor, 1993; Taylor and Vaney, 2002). In other words, direction selectivity can not be eradicated completely in the presence of antagonists of the excitatory transmitter ACh receptors under most conditions, except half of response of DSGCs was reduced (Ariel and Daw, 1982; Kittila and Massey, 1997). Extracellular recording studies indicated that DSGCs receive about half of their excitatory drives are from bipolar cells, primarily through the activation of glutamate receptors, and receive the other half from SACs, through the cholinergic pathway (Ariel and Daw, 1982; Grzywacz et al., 1997). A recent study further indicated that the glutamatergic and cholinergic inputs are themselves directional (Fried et al., 2005).

Although the SACs receive inputs of bipolar and other amacrine cells (include other SACs) all over the dendritic arbors, they only outputs on the varicose distal tips (Famiglietti, 1991). This

nature creates an asymmetry of neurotransmitter release - more transmitter should be released when a stimulus moving outward along a branch than one moving inward. This implies that individual branches can serve as distinct computational units. Euler et al. (2002) injected SACs with a Ca^{2+} indicator dye and then used two-photon microscopy to measure Ca^{2+} transients in the SAC dendrites in response to visual stimulation. The Ca^{2+} concentration in the distal processes tend to be greater for centrifugal image motion, indicate that SACs release more transmitters for centrifugal movements rather than centripetal, and individual branches can function independently. Furthermore, the directionality of the SAC dendrites has also been suggested to be mediated by the offset distribution of K-Cl co-transporter (the Cl^- extruder) and Na-K-Cl co-transporter (the Cl^- accumulator) (Gavrikov et al., 2003).

Both the dye-injection studies (Vaney and Pow, 2000; Dong et al. 2004) and the Golgi-staining study (Famiglietti, 2002) concluded that the SACs co-fasciculate with the DSGCs on all sides of the receptive field, together with the GABA receptors are distributed equally over the dendrites of DSGCs (Jeon at el., 2002). Although Fried et al. (2005) showed that both GABAergic and cholinergic pathways shape the directionality of excitatory and inhibitory input signals, respectively, the synaptic sites of interaction is not known.

Since the inhibition asymmetry is necessary for direction selectivity, I assumed that the degree of dendritic contacts or synaptic connections might be different between the preferred side SAC-DSGC interactions (in which the SAC dendrites originated from the preferred side) and the null side ones (in which the SAC dendrites originated from the null side). First, DSGCs were

physiologically identified. Then, the whole dendritic arbors of both DSGCs and SACs were dye filled to reveal the relationship between their processes. Furthermore, observation of the actual inhibitory synaptic sites was carried out by immunolabling of $GABA_A$ receptors. In this study, I demonstrated that the SAC dendrites contacts with the ON dendritic arbors of DSGC with a symmetrical manner. Contrary to our expectations, no asymmetry of the inhibitory inputs was observed either. Therefore, neither the geometric proximity nor the inhibitory synaptic inputs between SACs and DSGCs is likely to mediate the directionality.