4. DISCUSSION

In this study, we aimed to elucidate the patterns of synaptic connection between SACs and DSGCs. Our results demonstrate that dendrites of a single SAC contact with a DSGC equally in all directions. Furthermore, SACs symmetrically provide direct inhibitory currents to DSGCs in the rabbit retina: the SAC dendrites of any portion arrange equal inhibitory input sites to the DSGC.

4.1 Co-fasciculation may not be adequate for the estimation of synaptic transmission

The pattern of co-fasciculation between SACs and DSGCs has been debated in recent years. Fried et al. (2002) showed that a null side SAC contacts with a DSGC by half of its dendrites, and has higher degree of co-fasciculation, whereas the contacts between a preferred side SAC and DSGC are mainly crossings. On the contrary, Dong et al. (2004) provided evidence that the dendritic relationship remains constant no matter which sides the SAC locates.

Rather than examining co-fasciculation of partial dendrites of SAC, we used a single SAC to address this problem. When somata of the pair (a DSGC and a SAC) are in a close distance, the SAC dendrites of all directions usually could cover the dendritic field of a DSGC. Therefore, the uncertainty can be relatively narrow down: the SAC dendrites of any portion were belonged to one individual cell. Next, we found that the contacts under lower magnification or a single image could be falsely identified. Some seemingly contact appearances were found to be wrong using higher

magnification and fine optical sectioning. The dendrites may locate very closely but have no contact at all (Fig.16). Therefore, it is necessary to acquire high magnification images with optical stacks for the estimation of dendritic contacts. However, we neglected the appearance of co-fasciculation and count the amount of contact regions directly when quantifying.

Pairs of DSGC and SAC labeled with different dyes exhibit a similar result. All directions have equivalent dendritic contact indices (*DCI*s), especially the opposite direction (difference less than 20%). Moreover, the lowest *DCI* value was above half of the maximum value. This suggests that SACs contact with DSGCs equally in all directions.

4.2 SACs provide similar inhibitory inputs to DSGCs in all directions.

There is no doubt that inhibition asymmetry and SACs play an important role in generating direction selectivity. Surprisingly, we found that there is no apparent pattern of the inhibitory synaptic connections between SACs and DSGCs. The SAC dendrites do not connect with DSGCs selectively, seemingly the prerequisite for direction selectivity is lacking. However, the existence of synaptic connections does not equal to the synaptic current actually measured. The inhibition asymmetry can be resulted from the behavior of the pre-synaptic neurons. A possible mechanism generating this asymmetry was depicted in Fig. 20. On the basis of the even-weighted inhibitory synaptic connections between SACs and DSGCs at either side (Fig. 20, red), there might be some other upstream driving factors (Fig. 20, light gray) that suppress the GABA release from the SAC

dendrites which projected from the preferred side, but none to those from the null side. A clue can be found in an experiment of Fried et al. (2005), in which the inhibitory input of the preferred response arose in the presence of curare – an antagonist of the ACh receptor. The cholinergic drive is normally an excitatory input, how can it suppress others? One possible explanation is that the cholinergic drive acts through an inhibitory interneuron which functions as a sign inverter. Apparently, the existence of this interneuron coincides with the upstream suppressor of our argument. Furthermore, by the facilitation of the cholinergic drive, the interneuron can suppress the GABA release from SACs before the stimuli arrive without owning a wide range dendritic field. By contrast of the mechanism in the preferred side, the GABA release from SAC dendrites projected from the null side is not affected. In addition, the null side inhibition could be enhanced by an inhibitory interneuron (Fig. 20, dark gray) mediated by the cholinergic drive, because curare declined the inhibitory input on the DSGC in the null movement (Fried et al., 2005). Since the reciprocal ACh current between SACs is diminished in adult rabbit (Zheng et al., 2004), it is unlikely for the cholinergic drive to inhibit DSGC through the SAC dendrite (actually, the cholinergic drive we mentioned is SAC itself, since SACs are the only cholinergic neurons in the rabbit retina). Notably, although the interneuron in the preferred side and that in the null side share an identical source (ACh from SACs), they have different targets (SAC and DSGC, respectively). Therefore, it is not clear whether the preferred side interneuron and null side interneuron belong to one cell type or not.

The whole population of SACs has matured and co-fasciculated early in the development. Since

they comprise a tight meshwork, the SAC-SAC interaction through GABAergic pathway may play a certain role in direction selectivity (the cholinergic interaction between SACs is invalid in adulthood). The SAC dendrites under electron microscope exhibit synaptic couplet in which two processes synapse with each other before finally synapsing with the DSGC dendrites (Dacheux et al., 2003) provides an evidence. Later, dual patch experiment in which two adjacent SACs were shown to receive Ca^{2+}-dependent GABA current reciprocally (Zheng et al., 2004) firmly supported the aspect. According to our investigation and recent studies, direction selectivity in the retina is no longer simply the issue about the interaction between the DSGC and the SAC. Instead, other retinal neurons and complicate mechanisms might be involved in the coding process, and it remains to be further elucidated.

4.3 ON and OFF responses of direction selectivity may be mediated by different mechanisms

It has been shown that the responses of direction selectivity in ON and OFF system are generated in different ways. Kittila and Massey (1995) showed that when the activity of the ON system was blocked by the metabotropic glutamate agonist L-AP4, the OFF responses in the DSGC remain directional. Apparently, the computation of direction selectivity is performed independently in the OFF system. This suggests that the ON system may also has its own mechanism of direction selectivity. Patch-clamp studies (Taylor and Vaney, 2002; Fried et al., 2005) suggested that ON and OFF responses are accomplished by the use of different strategies. In normal condition, the

cholinergic drive of DSGC is regulated by GABA, silent when light ON, but turn into active at OFF response. In our physiology experiments, the ON response of direction selectivity usually had distinct strength compare with the OFF response. Morphologically, we also found that the OFF arbors of DSGCs are usually larger than the ON arbors, as well as the dendritic field size between the OFF SACs and ON SACs (data not shown). Therefore, direction selectivity may be accomplished by very different mechanisms for the ON and OFF systems.