6. Discussion

Male silkmoths, *Bombyx mori* exhibit a programmed zigzagging behavior as they walk upwind toward pheromone released by conspecific females. The walking consists of a straight-line walking, zigzagging turns and a looping behavior (Kanzaki *et al*., 1992; Kanzaki, 1998). By observing the overlapping area of the DNs and a cvl-NMN, whose activity pattern is an index of this programmed behavior, past studies propose that this programmed behavior is controlled by two types of descending interneurons; that is, the straight-line walking is induced by a phasic excitation of GI-A DNs, and the zigzagging and the looping behaviors are by flipflopping activities of the GI-A DNs (Kanzaki, 1998; Mishima and Kanzaki, 1998, 1999). However, this speculation was derived from the observation of projection area of the DNs and dendritic field of the cvl-NMN in different preparations.

6.1 Double-labeling of GI, GII DNs and a cvl-NMN using a newly developed staining method

In order to understand the relationship between the DNs and the cvl-NMN, it is necessary to make a double-labeling, which reveals precise connection between the DNs
and the cv1-NMN. However, because an intracellular staining of the target DNIs in the brain was difficult, we adopted a developed staining method. Cell bodies of GI and GII were visualized using a differential interference contrast microscopy. With this technique cell clusters on the surface of the brain could be clearly visualized, allowing us to select the cells for inserting the microelectrode and stain target DNIs efficiently. Thus, I could make double-labeling of DNIs and the cv1-NMN.

Smooth dendritic arborizations are presumably of postsynaptic dendrites and the varicose processes are of presynaptic arborization, as reported in crayfish neurons (Kondoh and Hisada, 1986). In order to clarify the activity flow from GI or GII to a cv1-NMN, a confocal microscopy was used to comprehensively investigate the overlapping regions between each type of DNIs and the cv1-NMN. Similar analysis was made in starburst amacrine cells and direction-selective ganglion cells in a rabbit retina, and vertical system cells in fly optic lobe (Dong et al., 2004; Fried et al., 2002; Haag and Borst, 2004).

6.2 Two types of Flipflopping DNIs

In the previous study Mishima and Kanzaki (1999) characterized physiologically and morphologically 3 types of GI DNIs (A-C) and 3 types of GII DNIs (A-C, Fig.10). In the present I found one additional GII DN, i.e., GII-D DN which showed a typical
flipflopping activity pattern in response to pheromonal stimulation to antennae (Fig. 13). Moreover, we found that the GII-A DN was also excited with a typical flipflop pattern although a previous study reported that the GII-A DN showed only a phasic excitation to the pheromonal stimulation (Fig. 14).

Morphological and physiological classifications of the GI and GII DNs are summarized in Table 1. A typical flipflopping response was observed in GI-A, GII-A and -D DNs. These 3 types of DNs had common morphological characteristics, that is, they all had wide field dendritic arborizations in the LAL (Fig. 10; Table 2). Other types of DNs, which showed a phasic excitation (GII-C) or a phasic inhibition (GII-B), had no arborization in the LAL (Fig. 10; Table 2; Mishima and Kanzaki, 1999). The DNs showing long-lasting inhibition had dendritic arborizations in the LAL but the arborizations were confirmed within a part of the LAL (Fig. 10; Table 2; Mishima and Kanzaki, 1999). These results suggest that the flipflop activity may flow into the LAL or may be organized and generated in the LAL.

In the previous studies, two different types of flipflop activity patterns, called 'FF' and 'ff', were reported using extracellular recordings from the cervical connectives (Kanzaki et al., 1994; Kanzaki and Mishima, 1996; Mishima and Kanzaki, 1998). The 'FF' activity pattern is in high state when the ipsilateral cv1-NMN is activated. Simultaneous
recording from 'FF' and 'ff' DNs demonstrated an antiphase relationship (Kanzaki et al., 1994; Mishima and Kanzaki, 1998). So far only a single type of flipflopping DN (GI-A) has been intracellularly characterized by Mishima and Kanzaki (1999). In the present study I succeeded in identifying an additional type of flipflopping DNs, i.e., GII-A and GII-D (Figs. 13, 14). A previous study of staining neurons by backfilling with cobalt dye after extracellular recording demonstrated that 'FF' is consistently shown by some GII DNs (Kanzaki et al., 1994). Although the notion requires verification with simultaneous recording from the cv1-NMN and each type of flipflopping DNs, there is a possibility that the flipflop activity pattern recorded from the GI-A is 'ff' and the GII-A and GII-D are 'FF'.

6.3 Possible flow of physiological activity from GI and GII DN to cv1 NMN

In all the types of DNs except for the GI-C DNs we succeeded in making double-labeling with the cv1-NMN which activity is an index of the motor pattern of the pheromone-triggered programmed behavior (Table 2). The cv1-NMN had smooth and flat dendritic arborizations in the posterior-lateral part of the SOG (Fig. 15). Three types of DNs, GI-A, GII-A and GII-D showed a typical flipflopping activity pattern in response to pheromonal stimulation to the antennae, but there were some morphological differences in relation to their contact with the cv1-NMN. GII-A and GII-D DNs had remarkable
overlapping regions with the cv1-NMN (Figs. 17, 20), but no overlapping regions were observed in a GI-A DN (Fig. 16). In a previous study, it was reported that the GI-A DNs are the major DNs which will transmit the flipflop activity pattern to the cv1-NMN judging from comparison of the branching area of the GI-A DNs and the cv1-NMN in the posterior lateral par of the SOG (Mishima and Kanzaki, 1999). Double-Labeling of the GI-A DN and a cv1-NMN clarified that the cv1-NMN had no remarkable contact with the GI-A DN, but had contact with the GII-A and GII-D DNs in the posterior lateral part of the SOG. These results suggest that the cv1-NMN receives the flipflopping neural activity patterns from the GII-A and GII-D DNs but not from the GI-A. The present study also implied that the flipflopping information is transmitted to the cv1-NMN mainly along central thick neurites of the dendritic field of the cv1-NMN judging from the overlapping regions of blebby terminals of the GII-A and GII-D DNs and the dendritic field of the cv1-NMN (Figs. 17, 20; Table 2). This was commonly observed in all the double-labeled preparations (GII-A n=4; GII-D n=5; Table 2).

Furthermore, GII-C DNs, which showed a phasic excitation, also had overlapping regions with the cv1-NMN (Fig. 19), suggesting the cv1-NMN receives a phasic excitation from the GII-C DNs. The overlapping regions with the cv1-NMN were restricted within the middle and frontal parts of the dendritic area of the cv1-NMN (Fig. 19B; Table 2). Any
of the GI, and GII DNs did not show positive GABA-like immunoreactivity (Iwano M personal communications). Therefore, there is a possibility that flipflopping GII-A, -D and phasic excitatory GII-C DNs transmit excitatory information to the cv1-NMN.

On the contrary, GII-B showed a phasic inhibition and GI-B showed a long-lasting inhibition which lasted after the end of the stimulation (Table 1; Mishima and Kanzaki, 1999). Present study revealed that the GI-B DNs had remarkable varicose profiles on the dendrites of the cv1-NMNs (Fig. 16; Table 2), but the GII-B DNs did not (Fig. 18; Table 2), suggesting that the long-lasting inhibition of GI-B may flow to the cv1 NMN.

In summarizing all the results, possible connections between the GI, GII-DNs and the cv1-NMN are schematically illustrated in Fig. 26. Previous studies speculated that this pheromone-triggered programmed behavior is instructed by two types of activity pattern descending from the brain and the thoracic ganglia; one is a phasic excitation and the other is a flipflopping activity (Kanzaki et al., 1994; Mishima and Kanzaki, 1998, 1999). Present study extends the previous work and clarifies the possible control mechanisms of the pheromone-triggered programmed behavior using double-labeling techniques: the straight-line walking is controlled by the phasic excitation by the GII-C DNs and the following zigzag turns and looping are controlled by the flipflop activity of GIIB, -IID DNs. Additionally long-lasting inhibition of GI-B may also contribute to control the
programmed zigzagging behavior.

It is our ongoing study to understand the function of two types of flipflopping activity carried out by GII-A and GII-D DNs and the effect of long-lasting inhibition on controlling the programmed zigzagging behavior.

6.4 Relationship between visual interneurons and GII-A DN

The neural pathway of the visual information on the pheromone-triggered behavior in the silkmoth brain is not well known. Therefore we aimed to find the visual pathway from the optic lobe to the GI, II using intracellular recording and staining. Several visual interneurons were stained using intracellular staining. These visual interneurons had their dendritic arborization around the posterior area of the PC, which is considered to be the POF projected by many visual interneurons in the fly brain (Staufferfeld 1976). Morphological observation demonstrated that the varicose processes, considered as presynaptic area of certain groups of visual interneurons, corresponded to the smooth dendritic arborizations, considered as postsynaptic area of GI, II DNs. Although double labeling between GI, II DNs and visual interneurons is still required for the verification, it is possible that these visual interneurons may transmit their visual information to the GI, II DNs and modulate the programmed behavior controlled by GI, II DNs.
On the other hand, GII-A responded to light ON/OFF stimuli and the firing frequency depended on the light ON/OFF conditions. Similar responses were elicited in several visual interneurons (Figs. 23-25). These results suggest that the GII-A which controls zigzag turns and looping in the programmed zigzagging behavior receives the light information. Although detailed analysis of behavioral experiment is required, zigzag turns and looping in the zigzagging behavior may be modulated by the light ON/OFF stimuli. Possible connections between the visual interneurons and GII-A are schematically illustrated in Fig. 26.

In the present study, some other types of GI, II DNs in addition to the GII-A also had their dendritic arborization in the POF. This evidence suggests that other GI, II DNs which control the programmed zigzagging behavior may receive the light information in the POF. Double labeling between each type of GI, II DNs and visual interneurons will reveal more clear modulation mechanisms by visual information on the programmed zigzagging behavior.