4. Discussion

4.1 Neuroactive substance in the antennal lobe neural pathways

4.1.1 Organization of antennal lobe neural pathway

Glomeruli in the insect antennal lobe (AL) are mainly composed of local interneurons (LNs) and projection neurons (PNs) with cell bodies in the AL (Anton and Homberg, 1999; Homberg *et al.*, 1988; Matsumoto and Hildebrand, 1981; Kanzaki and Shibuya, 1983, 1986a; Kanzaki *et al.*, 2003) . In the male *B. mori*, I could classify the cell bodies in the AL into three cell clusters; i.e. the medial cell cluster, lateral cell cluster and anterior cell cluster (Fig. 1A, B). Similar cell clusters have been described in *M. sexta* (Homberg *et al.*, 1988).

In previous studies of *B. mori*, the ACT has been classified into three different pathways, i.e. IACT, MACT and OACT (Kanzaki and Shibuya, 1986a; Kanzaki *et al.*, 2003; Seki *et al.*, 2004). Among these pathways, the IACT is regarded as the most prominent ACT and the main output pathway from the AL to the protocerebrum in many species (Mobbs, 1982; Boeckh *et al.*, 1990). In *B. mori*, the IACT is thought to form a single ascending bundle to the protocerebrum. However, the present immunocytochemical and anatomical studies revealed that the IACT was classified into the IACT-a and IACT-b due to the originating positions (Fig. 1C). In *M. sexta* the IACT was classified into the dorsal root (DR) and the ventral root (VR) (Homberg *et al.*, 1988). However, this nomenclature could not be applied to *B. mori* due to the difference of the neural structural

layout. So, I named IACT-DR as IACT-a and IACT-VR as IACT-b in the *B. mori* (Fig. 1C, D).

Previous intracellular-recording studies and the present study indicate the following neural organizations in the AL of male *B. mori*. A majority of LN cell bodies are located in the lateral cell cluster (Fig. 1B; Seki and Kanzaki, 2002). Cell bodies of the PNs passing through the IACT-a are located in the medial cell cluster or anterior cell cluster (Fig. 1B, D; Kanzaki and Shibuya, 1983, 1986a; Kanzaki *et al.*, 2003; Seki *et al.*, 2004; Namiki and Kanzaki, 2004). Cell bodies of the PNs passing through the IACT-b are located in the lateral cell cluster (Fig. 1B, D; Namiki and Kanzaki, 2004). Cell bodies of the PNs passing through the MACT and OACT are located in the lateral cell cluster (Fig. 1B, D; Kanzaki and Shibuya, 1986a; Kanzaki *et al.*, 2003).

These cellular organizations in the AL neurons suggest a strong linkage between the cell body position and the projection pathway. Besides, a majority of fibers passing through the ACT were composed of ascending fibers of PNs with cell bodies in the AL (Christensen and Hildebrand, 1987; Homberg *et al.*, 1988; Kanzaki and Shibuya, 1983, 1986a; Kanzaki *et al.*, 1989; Kanzaki *et al.*, 2003).

4.1.2 Cellular organization of immunoreactive neurons in the AL

Cell bodies with GABA immunoreactivity were observed exclusively in the lateral cell cluster, but not in the medial cell cluster and anterior cell cluster (Fig. 2A). In the

projection pathways to the protocerebrum, GABA immunoreactivity was observed only in the MACT (Fig. 2D) but not in the IACT-a, IACT-b and OACT. Since the cell bodies were stained only in the lateral cell cluster, the cell bodies of PNs passing through the MACT originated in the lateral cell cluster. Thus, GABA immunoreactivity in the AL was composed of PNs passing through the MACT and LNs terminating in the AL, both of which had cell bodies in the lateral cell cluster (Table. 1). I can speculate that the inhibitory neural information is transmitted from the AL to higher olfactory center through the MACT.

Cell bodies with FMRFamide immunoreactivity were observed in the lateral cell cluster and anterior cell cluster, but not in the medial cell cluster (Fig. 3A, B, C). In the pathways to the protocerebrum, immunoreactivity was observed in the MACT (arrow in Fig. 3F) and OACT (arrow in Fig. 3E), but not in the IACT-a and IACT-b. The number of fibers in these stained ACT was smaller than the number of cell bodies stained in the lateral cell cluster and anterior cell cluster. Thus, FMRFamide immunoreactivity in the AL is probably composed of PNs passing through the MACT or OACT and LNs terminating in the AL, both of which had cell bodies in the lateral cell cluster or anterior cell cluster (Table. 1).

Serotonin immunoreactivity was observed in a single cell body, which lied on the boundary region between the AL and protocerebrum (Fig. 4B) but not in the medial cell cluster, lateral cell cluster and anterior cell cluster. The cell body belonged to the

protocerebrum (Fig. 4B). This neuron has been identified by intracellular recording and staining (Hill *et al.*, 2003; Fig. 6A, B) . Immunocytochemical double labelling for serotonin revealed that this intracellular stained neuron was in fact serotonin immunoreactive (Fig. 5). A pair of serotonin immunoreactive neurons is thought to be AL centrifugal neurons judging from the following properties; a) the cell body belongs to the protocerebrum (Figs. 4B, 6A) and b) the cell shows mechanosensory responses with much longer delay than other mechanosensory AL neurons (Hill *et al.*, 2002; Fig. 6B). I found that the axon of this neuron passed through the IACT-b (arrow Fig. 4D). There is a possibility that the IACT-b contains centrifugal neurons to the AL (Table. 1).

Tyramine immunoreactivity was observed in the lateral cell cluster but not in the medial cell cluster and anterior cell cluster (Fig. 7A). In the pathways to the protocerebrum, immunoreactivity was observed only in the IACT-b (arrow 1 Fig. 7D), but not in the IACT-a, MACT and OACT. Tyramine immunoreactive neurons passing through the IACT-b may be AL centrifugal neurons judging from the followings morphological characteristics; a) I could not find any cell bodies connected to the IACT-b fibers in the AL and b) the fibers were connected widely in the brain including the protocerebrum and the SOG (arrow 2 in Fig. 7D). Therefore, a majority of tyramine immunoreactive neurons with cell bodies in the lateral cell cluster might be LNs terminating in the AL (Table. 1). Here again, I can speculate that the IACT-b contains axons of centrifugal neurons to the AL from other area of the brain. It is possible that the IACT-b transmits feedback information with

serotonin and tyramine to the AL from other areas of the brain.

I could not find any histamine immunoreactivity in the cell cluster, glomerular structure and projection pathways in the AL (Table. 1). These results indicate that histamine has no functional role in the AL of *B. mori*.

4.1.3 Neuroactive substances in the olfactory processing in the AL

In the present study, I revealed GABA, FMRFamide, serotonin and tyramine immunoreactive neurons in glomerular structures in the AL (Table. 1). I could observe particular immunoreactive patterns in glomerular structures of the AL. For example, serotonin immunoreactive branchings were restricted to some small partitions in the MGC compartments (arrow 2 in Fig. 4C). Similar immunoreactive patterns were also observed in the GABA (arrows in Fig. 2B) and tyramine (arrows in Fig. 7C) immunoreactivity. In addition, PNs with arbors in the MGC (MGC-PNs) had axons passing through the IACT-a (Kanzaki and Shibuya, 1986a; Kanzaki *et al.*, 1989; Kanzaki *et al.*, 2003). I can speculate that restricted immunoreactive patterns in the MGC are equivalent to restricted dendritic arborization patterns of MGC-PNs in the MGC. In a previous study, a high-speed optical recording with voltage-sensitive dye suggested that the amplitudes of the excitatory postsynaptic activities in the partitions of MGC compartments are significantly different (Ai and Kanzaki, 2004). These significant difference of the neural activities in the partitions of MGC compartments and restricted immunoreactive patterns of neuroactive

substances in the partitions of MGC compartments suggest the existence of local neural units for olfactory neural processing in the MGC.

4.1.4 Neuroactive substances in the projection pathways to higher olfactory center

In the present study, I clarified the existence of GABA, amine and peptide immunoreactivity in the projection pathways to higher olfactory center. The cell bodies of these immunoreactive PNs and centrifugal neurons passing through the ACT were localized in the lateral cell cluster or outside the AL (Table. 1). The axons with GABA immunoreactivity passed through the MACT (Fig. 2D). FMRFamide immunoreactive axons passed through the MACT and OACT (Fig. 3E, F). Serotonin immunoreactive centrifugal axons passed through the IACT-b (arrow in Fig. 4D). Tyramine immunoreactive axons, which were thought to be centrifugal neurons, also passed through the IACT-b (arrow 1 Fig. 7D). In the present immunocytochemical study, I could not identify immunoreactive axons in the IACT-a, which is classified as the most prominent ACT. However, it is remarkable that GABA immunoreactive PNs always passed through only the MACT and amines immunoreactive centrifugal neurons were passing through the IACT-b. These results show that each projection pathway to a higher olfactory center contains specific combination of neuroactive substances.

It is well known that PNs have different projection patterns in the calyces of mushroom bodies (Ca) and LPC depending on their ascending pathways (i.e. IACT, MACT,

OACT etc.) to the protocerebrum (Christensen and Hildebrand, 1987; Homberg *et al.*, 1988; Kanzaki and Shibuya, 1983, 1986a; Kanzaki *et al.*, 2003). In *B. mori*, Kanzaki *et al.*, (2003) characterized MGC-PNs, which have an axon passing through the IACT-a, MACT and OACT. MGC-PNs passing through IACT-a project to the Ca and the LPC. In contrast, MGC-PNs passing through MACT and OACT project to the LPC bypassing the Ca. My immunocytochemical results indicate that, besides its projection patterns in the protocerebrum, each projection pathway can be defined by the presence of specific neuroactive substances. I speculate that each pathway ought to have different functional properties in the olfactory processing.

In the present study, I reported basic distribution patterns of major neuroactive substances in the AL. These basic results provide valuable information for understanding functional roles of neuroactive substances in the olfactory processing. I am planning to identify other putative neuroactive substances in the AL. This study underlines the importance of investigating functional roles of neuroactive substances in dynamic olfactory processing in the AL.

4.2 Odor-evoked locomotion control mechanism in the protocerebrum

4.2.1 Summary of LAL-VPC neural network

The morphological properties and cellular organization of the LAL-VPC intrinsic neurons allowed me to consider that two LAL-VPC neural structures are symmetrical and

linked to each other by the neural commissure. Therefore in the present study, I defined one side of the LAL-VPC neural structure as a LAL-VPC neural unit. The whole LAL-VPC neural structure, which linked both sides of LAL-VPC neural unit, defined as LAL-VPC neural system.

4.2.2 Long-lasting activity generation hypothesis

Pervious studies showed that the descending interneurons with an input region on one side of the LAL-VPC exhibit a long-lasting activity pattern to the pulsed pheromone stimulation (Kanzaki *et al.*, 1991b; Mishima and Kanzaki, 1999). Especially these descending interneurons show a characteristic "flip-flop activity pattern" (Olberg, 1983; Kanzaki *et al.*, 1994; Kanzaki and Mishima, 1996; Mishima and Kanzaki, 1999). Moreover, the activity states of both sides of flip-flopping descending interneurons show an antiphasic relationship (Kanzaki *et al.*, 1994; Kanzaki and Mishima, 1996).

These results suggest that the specific long-lasting activity pattern will be generated and maintained in one side of the LAL-VPC. Morphological properties of the LAL-VPC intrinsic neurons suggest that this long-lasting activity should be induced by the neurons, terminated only in one side of LAL-VPC. Among the LAL-VPC intrinsic neurons, LAL-VPC LNs showed this unilateral morphological properties and some LAL-VPC LNs elicited a long-lasting activity (Fig. 22). Therefore, there is a possibility that the long-lasting activity patterns may be induced by the LAL-VPC LNs.

In the present study, I roughly classified LAL-VPC LNs into two types according to their morphological properties, i.e., Type-A (input from LAL and output to VPC) (Fig. 12) and Type-B (input from VPC and output to LAL) (Fig. 13). Moreover these roughly classified LAL-VPC LNs were subdivided into three types according to their physiological properties, i.e., Type-A-α, Type-A-β, Type-B-α and Type-B-β. Type-A LAL-VPC-LNs usually showed a recurrent brief excitation, which repeated a brief excitation at regular intervals in response to pheromone stimulation (Fig. 22). Type-A-β LAL-VPC LNs showed a long-lasting excitation for pheromone stimulation. Type-B-α LAL-VPC LNs usually showed a delayed brief excitation in comparison with Type-A-α LAL-VPC LNs in response to pheromone stimulation (Fig. 22). Type-B-β showed a long-lasting excitation to pheromone stimulation (Fig. 22).

In these characteristic physiological properties of Type-A-α and Type-B-α LAL-VPC-LNs, the activating timing usually showed anti-phasic relationship (Fig. 22). Morphological and physiological properties of these two types of neurons suggest an existence of reciprocal neural transmissions between the LAL and VPC in each side of the LAL-VPC.

I obtained detailed three-dimensional images of the LAL-VPC intrinsic neurons by a confocal laser-scanning microscope (Figs. 12, 13, 14). Therefore, I could recognize detailed three-dimensional structures in the moth brain, and could compare morphological arborization patterns of LAL-VPC intrinsic neurons with surrounding neural structure

layout. As a result of this analysis, I confirmed that several Type-A and Type-B-α LAL-VPC-LNs have secondary output arborization in the particular border region between the LAL and the VPC (Fig. 18A, B). On this boundary region, output neurites of some Type-A-α and Type-B-α LAL-VPC LN probably overlapped (Fig. 18A). I called this specific area the mVPC anterior region (mVPCa).

I paid special attention to this particular border region between the LAL and the VPC. I investigated the detailed structure of the dendritic patterns of the LAL-VPC LNs. I recognized that the two Type-B-β LAL-VPC LNs (Figs. 14, 22) had varicose terminals (input) only in the mVPCa (Fig. 18A, B). Then, these LAL-VPC LNs showed long-lasting excitation (Fig. 22). These two neurons out of four LAL-VPC-LNs showed the long-lasting excitation (Fig. 22). Moreover, three neurons out of four LAL-VPC-LNs, which showed the long-lasting excitation, had input in the mVPC including the mVPCa (Fig. 22). In the present study, I have no direct evidence of monosynaptic connection between these LAL-VPC-LNs. However, it is possible to suggest that many neurons, which have input terminals on the mVPCa, showed long-lasting excitation. Therefore, I conclude that the specific mVPCa area may be the generation point of a long-lasting excitation.

Then, I suggest a neural circuit model that accounts for the generation of the long-lasting activity in the LAL from these results. The following process may generate the long-lasting activity observed in each side of the LAL-VPC. 1) Reciprocal neural transmissions occurres between the LAL and VPC region through Type-A LAL-VPC LNs

(transmit an activity from LAL to VPC) and Type-B-α LAL-VPC-LNs (transmitted an activity from VPC to LAL). 2) The two distinct sequential activities may be transmitted to the mVPCa region by Type-A and Type-B-α LAL-VPC-LNs, which have secondary output arborization in the mVPCa. 3) Combined continuous or alternating activities may be transmitted to Type-B-β LAL-VPC-LNs, which have varicose terminals (input) in the mVPC (especially the mVPCa), and finally may generate the long-lasting activity (Figs. 18C, 20).

4.2.3 Hypothesis for generating the alternated activity pattern

Anti-phasic relationship activities of the DNs have been recorded from ventral nerve cord (VNC) (Kanzaki *et al.*, 1994; Kanzaki and Mishima, 1996). I can consider that this anti-phasic relationship reflects the reversed long-lasting activity between both sides of the LAL-VPC neural units. For generating the alternative long-lasting activities, each LAL-VPC neural unit should receive the long-term reciprocal controls, which regulate switching back a force the state of the activity pattern. From morphological properties of the LAL-VPC intrinsic neurons, this reciprocal control should be induced by the neurons which link each side of the LAL-VPC neural units bilaterally. In the LAL-VPC intrinsic neurons, LAL-BLs showed this bilateral morphological properties and some LAL-BLs elicited long-lasting activity patterns (Fig. 21). There is a possibility that the long-term reciprocal control may be induced by the few LAL-BLs, which elicite the long-lasting

activity.

In the present study, I classified the LAL-BLs into two groups according to the morphological and physiological properties (Figs. 17, 18C, 21). One is the Type-A LAL-BLs group which have inputs from the vmLAL and outputs to vmLAL. They showed characteristic long-lasting excitation induced by pheromone stimulation (Figs. 12, 17, 18C, 21). The other one is the TypeB LAL-BLs group which has input from dlLAL and lVPC and output to vmLAL and dlLAL. They showed inhibitiory responses including the long-lasting inhibition (Figs. 13, 17, 18C, 21). Then, what kind of long-term reciprocal control is necessary to generate the alternating long-lasting activities between the LAL-VPC neural units?

Reciprocal control function of Type-A LAL-BLs

Previous studies suggested neural models for generating the rhythmic activity using a reciprocal inhibition (Perkel and Mulloney, 1974; Brown, 1914; Arbas and Calabrese, 1987; Peorson and Ramirez, 1990). Detailed analysis in the present study by confocal laser scanning microscope showed that the majority of Type-A LAL-BLs passed by the frontal region of the LALC (Fig. 12B), where I observed several GABA immunoreactive axons (Fig. 15A). GABA immunoreactivity is usually regarded as the existence of inhibitory neurotransmission in invertebrate (Kerkut *et al.*, 1969; Waldrop *et al.*, 1987). In addition, a detailed analysis about three-dimensional data obtained by

confocal laser scanning microscope revealed that the position of cell bodies was similar between Type-A LAL-BLs and GABA immunoreactivity cell clusters which innervated the LAL region (Fig. 10A, arrow1 in Fig. 15B). I expect that they probably belong to the same cell cluster. In these circumstantial evidences, I suggest that some neurons in the Type-A may be GABAergic.

For disproof, I presume the majority Type-A LAL-BLs, which showed the long-lasting excitation, have excitatory neurotransmitter. In this case, they would perform to induce excitatory state to the opposite side of LAL-VPC neural unit when the input side of the LAL-VPC neural unit is activated. This condition has great contradiction to generate the alternating long-lasting activity state, because in both sides of LAL-VPC neural units and anti-phasic relationship activity state was recorded in VNC (Kanzaki *et al.*, 1994; Kanzaki and Mishima, 1996).

From these results, I can consider that the majority of Type-A LAL-BLs that showed long-lasting excitation probably have inhibitory neurotransmitter. Probably, when one side of the LAL-VPC neural unit is activated, Type-A LAL-BLs would perform the function of the long-term suppression against to the opposite side of the LAL-VPC neural unit. The Type-A LAL-BLs may perform an reciprocal inhibition and would be the bare bones of a rhythm generation system (Fig. 20).

Reciprocal control function of Type-B LAL-BLs

Then what is the role of the other bilateral neurons Type-B LAL-BLs? The physiological property of Type-B LAL-BLs was a long-lasting or brief inhibitory response (Fig.17). It is reported that a neural circuit that works as a reciprocal inhibition is composed of a combination of neurons having a low resting potential and high resting potential. This reciprocal inhibition was classified into the asymmetric reciprocal inhibition (Manor *et al.*, 1999). Do the Type-B LAL-BLs perform a part of asymmetric reciprocal inhibition system composed of Type-A LAL-BLs in the LAL-VPC neural system?

However, I can exhibit many differences between the LAL-VPC neural system and asymmetric reciprocal inhibition system. First, I recognize there is no connection between Type-A and Type-B LAL-BLs. Their simplified morphologies of input and output region would not support a direct connection in each subregion (Figs. 19C, 21). Especially, I cannot expect connections between output sides of Type-A (vmLAL) and input sides of Type-B LAL-BLs (dlLAL and lVPC). In this part, they showed completely different regional properties. Second, the appearance ratio between the long-lasting excitation and inhibition are not balanced (Figs. 17, 21). If Type-A and Type-B LAL-BLs composed a pair of neural circuit, the appearance ratio should be well balanced.

These Type-B LAL-BLs might perform a part of the asymmetric reciprocal inhibition system with an inhibitory neurotransmitter. Type-B LAL-BLs may perform an inhibitory influence to the opposite side of the LAL-VPC neural unit. When the input side of LAL-VPC neural unit may be activated, Type-B LAL-BLs, which showed a long-lasting

inhibition, will not release an inhibitory neurotransmitter to the opposite side of LAL-VPC neural unit. On this condition, Type-B LAL-BLs may not perform any effective reciprocal control function in the LAL-VPC neural system. When the input side of LAL-VPC neural unit is inactivated, Type-B LAL-BLs may resume their spontaneous activity. However this reactivation would have an inhibitory function to the opposite side of LAL-VPC neural unit. In this situation, both sides of LAL-VPC neural units are inactivated for a long period. This situation is contradiction to the sequential zigzag behavior in *B. mori* and the long-lasting activity of LAL-DNs elicited by single stimulation (Kanzaki *et al.*, 1991b; Kanzaki *et al.*, 1994; Mishima and Kanzaki, 1999).

In the other case, these Type-B LAL-BLs would perform excitatory transmission to the opposite side of LAL-VPC neural unit. Type-B LAL-BLs would not have any inferences to the output side of LAL-VPC neural unit when they receive the excitatory activities from the input side of LAL-VPC neural unit. However, when the long-lasting activity of the input side of LAL-VPC neural unit is inactivated, Type-B LAL-BLs would be able to start an excitatory transmission and transfer the excitatory state from one side to the opposite side of the LAL-VPC. On these perspectives, Type-B LAL-BLs, which elicited the long-lasting inhibition, perform important function to transfer the excitatory state from one side to the opposite side of LAL-VPC neural unit. Therefore, Type-B LAL-BLs probably perform reciprocal excitation rather than reciprocal inhibition.

In the present study, I have no information about the neurotransmitter of Type-B

LAL-BLs. However as described above, I observed several GABA immunoreactivity axons in the "frontal area" of the LALC (Fig. 15A). In the whole area of the LALC, especially in the posterior area, I could not observe any GABA immunoreactivity axons. Probably many other LAL-BLs ought to have different neurotransmitters. Especially, Type-B LAL-BLs always pass the posterior area of the LALC (Fig.13B). Therefore, Type-B LAL-BLs do not have any possibility that they include inhibitory neurotransmitter and perform a reciprocal inhibition or play a part of reciprocal inhibition system. Although it is necessary to identify their neurotransmitter, there is a strong possibility that they include excitatory neurotransmitter and perform a reciprocal excitation system in LAL-VPC neural system (Fig. 20).

These hypotheses about dual reciprocal systems LAL-BLs (Fig. 20) should still be confirmed by immunocytochemical and a single-cell double-labeling technique, which allow the identification of the neurotransmitter of individual cells. I have just established this double labeling technique supported by the Neuron Database. One example was demonstrated in the AL serotonin immunoreactive centrifugal neurons (Figs. 5, 6) (Hill *et al.*, 2002). In the near future, I will be able to integrate neurotransmitter, morphology and physiology data at once in many single neurons using the Neuron Database. However, the dual reciprocal control hypotheses that I expected on this work have been supported by various physiological previous knowledges strongly and positively.

4.2.4 Odor-orientation zigzaging behavior in the insect

In male silkworm moths, I can consider that the activities of LAL-VPC neural system are evoked exclusively by sex-pheromone and generate zigzag behavior. However, it is very hard to regard that the LAL-VPC neural system exists in moth, is activated by only pheromone information, and elicits only one particular behavior. A direct instance, LAL-VPC intrinsic neurons were invariably observed in the female silkworm moth which releases the sex-pheromone (Iwano, unpublished). In various kinds of insect species, the existence of the LAL-VPC neural system is confirmed in their nervous system (Kanzaki *et al.*, 1991a; 1991b; Homberg, 1994, Lei *et al.*, 2001). For example in the coleoptera, I identified LAL-DNs in the beetle *Allomyrina dichotoma* and LAL-BLs in the stag beetle *Prosopocoilus dissimilis* (Iwano personal observation). Besides, G1-LAL-DNs like descending interneurons already identified in the locust *Schistocerca gregaria* (Homberg, 1994). By these observations, the LAL-VPC neural system has strongly been suggested to be general and common in holometabolic and heterometabolic insects.

The function of the LAL-VPC neural system, which I analyzed in the male *B. mori*, can be represented in other words as the operation system for preserving or memorizing pheromone information as a long-lasting state in their neural network for a certain period, and evoking behavior. Then in which environmental conditions did develop the LAL-VPC neural system? Animals intermittently receive the odor, which is distributed discontinuously by the chaotic turbulence in the atmosphere. Therefore, the olfactory signal

received by an animal is definite pulses. On this condition, LAL-VPC neural system should preserve the first olfactory signal in their neural system and exploit this preserved signal to acquire the next signal. Probably the property of olfactory signals, which is received as a pulse, would develop the LAL-VPC neural system.

It is not clear in which environmental conditions, the insect or invertebrate acquired this LAL-VPC neural system. But, it is naturally considered that the searching behavior including zigzag behavior is essential for primitive animals to get their food or reproduction partner. From this background, I can consider that the LAL-VPC neural system is to have very old origins. On one side, the LAL-VPC neural system is essential for the motor steering control system. On the other side, I can consider this neural network as a kind of memory system. In many viewpoints, the LAL-VPC neural system is one of the greatly interesting neural networks in animals.

I will enforce my hypothesis about generation mechanisms of zigzag behavior by immunocytochemical neurotransmitter identification of the LAL-VPC neural system accompanied with various physiological and behavioral experiments. Furthermore, I will compare the LAL-VPC neural system of many insect species and invertebrate for clarify the origin and evolution of LAL-VPC neural system.