

## Abstract

For the comprehensive study of the olfactory neural pathways in the insect brain, histochemical techniques were applied to the brain of silkworm moth *Bombyx mori* with the support of Neuron Database in our laboratory. Detailed morphological analyses were executed around two neuropil structures, the antennal lobe (AL) and the lateral accessory lobe (LAL). In the AL, olfactory neurons and the antenno-cerebral tracts were studied in the context of neuroactive substances. In the LAL, new neural circuit models, which control an odor-evoked locomotion, were suggested on the base of the detailed analysis of LAL olfactory neurons.

As a first step towards understanding the functional role of neuroactive substances in the first olfactory center of the male silkworm moth *Bombyx mori*, an immunocytochemical identification of the AL neurons was carried out. Antibodies against  $\gamma$ -aminobutyric acid (GABA), FMRFamide, serotonin, tyramine and histamine were applied to detect the existence of neuroactive substances in the AL. In the present immunocytochemical study, four antenno-cerebral tracts from the AL to the protocerebrum were clarified, and the following immunoreactive cellular organizations in the AL were revealed. 1) Local interneurons with cell bodies in the lateral cell cluster showed GABA, FMRFamide and tyramine immunoreactivity. 2) Projection neurons passing through the medial antenno-cerebral tract with cell bodies in the lateral cell cluster showed GABA and FMRFamide immunoreactivity. Projection neurons passing through the outer antenno-cerebral tract with cell bodies in the lateral cell cluster showed FMRFamide immunoreactivity. 3) Centrifugal neurons passing through the inner

antenna-cerebral tract-b with cell bodies located outside the antennal lobe showed serotonin and tyramine immunoreactivity. These results revealed basic distribution patterns of neuroactive substances in the AL and indicated that each projection pathway from the AL to the protocerebrum contains specific combination of neuroactive substances.

The lateral accessory lobe (LAL) is a bilaterally symmetrical spheroidal neuropil structure in many insect brains. This neuropil structure is one of the major targets of olfactory interneurons. In *Bombyx mori*, each LAL was tightly connected to the ventrolateral protocerebrum (VPC) by unilateral LAL-VPC interneurons and formed a LAL-VPC neural unit. Both sides of the LAL-VPC neural unit were linked to each other by LAL bilateral neurons and formed a LAL-VPC neural system. A recent report suggested that pheromone-induced zigzag behavior of *B. mori* is controlled by a characteristic “flip-flop activity pattern”, which transfers from the brain to the thoracic motor center by descending interneurons. Since these descending interneurons had their major dendritic arborization only in the one side of LAL-VPC, they surely reflected an activity of one side of the LAL-VPC. Further, the activity of descending interneurons recorded from both sides of the ventral nerve cord showed an anti-phasic relationship. These results suggest the existence of “alternating activity pattern” between both sides of the LAL-VPC. However, little was currently known about how long-lasting and alternating activity patterns were generated in the LAL-VPC. In the present study, I examined the morphological, physiological and neurochemical properties of the LAL-VPC intrinsic neurons, which were extracted from the Neuron Database in our laboratory. By the analysis of

the LAL-VPC intrinsic neurons, I suggested the following possibilities. 1) In one side of LAL-VPC, unilateral LAL-VPC interneurons generate long-lasting activities with reciprocal neural transmissions and integration. 2) In both sides of the LAL-VPC, the activity states were controlled by the LAL bilateral neurons with reciprocal inhibition and/or excitation. These neural systems generate “alternating activity pattern” in both sides of the LAL-VPC and transmit the “flip-flop activity pattern” to descending interneurons.