Structural analysis of the higher-order visual center and the visual projection pathways in the *Drosophila* central brain

Kazunori Shinomiya
Student ID : 47-56908
Research supervisor : Prof. Kei Ito

Aim of research

The sensory systems of the brain of the fruit fly (*Drosophila melanogaster*) have been investigated intensely using molecular genetic methods. Recent studies have revealed that one neuropile called the ventrolateral protocerebrum (vlpr) in the brain (Fig. 1) is projected from all of visual, olfactory and auditory systems. The vlpr have been regarded as one of the higher-order visual centers since a number of visual projection pathways project to this region. The vlpr can be an optimal system to investigate neuroanatomical basis of integration of the sensory information, because it is only known neuropile so far that is projected from all of these three modalities. This study aims to describe the orientations of the sensory pathways that project to the vlpr, as well as to identify the internal structures of the vlpr.

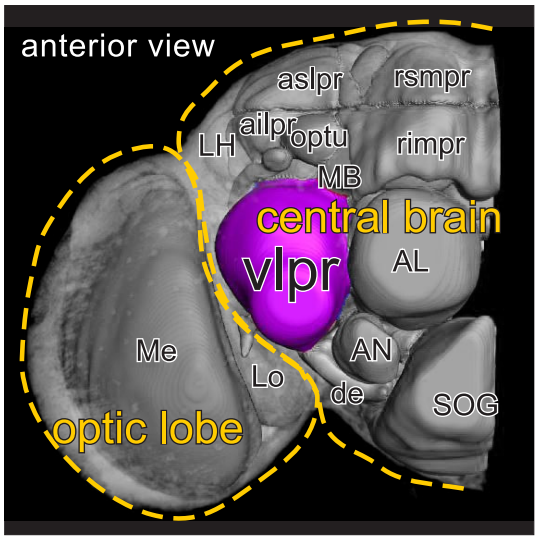

Fig. 1: *Drosophila* brain and the location of the vlpr.

Results

(1) Identification of internal structures of the vlpr

Before describing the morphology of the sensory pathways, I identified the internal structures of the vlpr, which has been hardly studied systematically so far. The internal structures can serve as landmarks in the region at determination of the projection sites of the sensory projection pathways. Glial tissue and synaptic region (neuropiles) were labeled by combining GAL4 enhancer-trap system and immunostaining (Fig. 2). As a result, eleven synapse-rich structures surrounded by glial processes and six structures lacking synapses were found. The former structures were named the glomeruli in this study by morphological analogy with the glomeruli in the antennal lobe, which is the primary center for processing the olfactory information. The latter ones were identified as tracts, or bundles of axons. The spatial orientations of the identified structures are shown in Fig. 3.

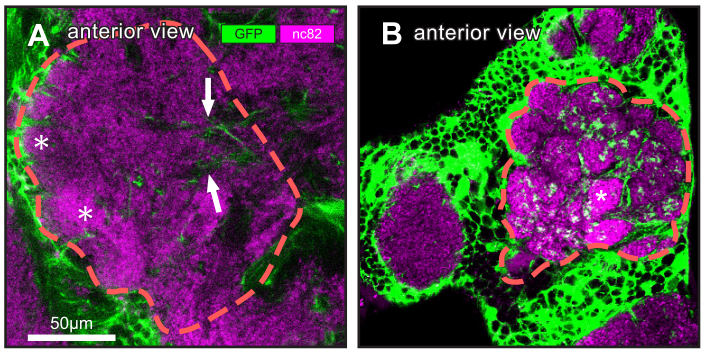

Fig. 2: Comparison of the internal structures in the vlpr (A) and the antennal lobe (B).

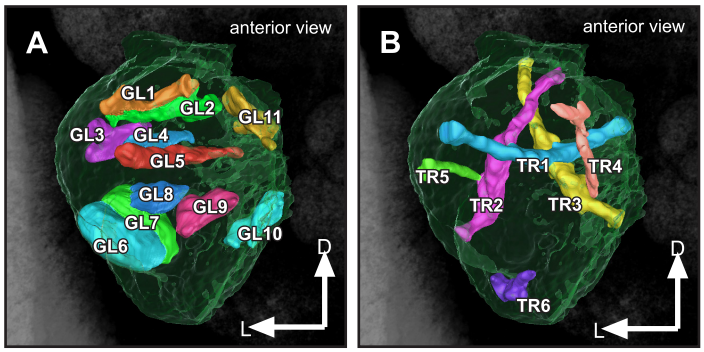

Fig. 3: Spatial orientation of the glomeruli (A) and the tracts (B).

(2) Analysis of projection sites of the sensory projection pathways

Thirteen sensory pathways are known to project to the vlpr. Nine pathways out of these are visual pathways, three are olfactory pathways and one is an auditory pathway. Next I determined the projection sites of these pathways relatively to the identified structures.

GAL4 enhancer-trap system was applied to label these pathways (Fig. 4). Various reporter genes lying downstream of UAS sequence are expressed in specific group of cells depending on expression of GAL4 so that the cell can be visualized. In this study, the entire neurons and the presynaptic sites were specifically labeled with DsRed and n-synaptobrevin::GFP (n-syb::GFP), respectively (Fig. 5). As a result of analysis of the pathways, five visual pathways were turned out to project to the glomeruli independently. The projection pattern of the other visual and the olfactory pathways were sparse. The terminal of the auditory pathway was condensed but not overlapped with the glomeruli.

(3) Structural analysis of the projection pattern of the visual projection pathways

All of the nine visual projection pathways project to the vlpr from the lobula, a neuropile in the optic lobe. Five of these pathways are called lobula-specific columnar type pathways (LC pathways) and one LC pathway consists of approximately 30-330 neurons. The two-dimensional topology is known to be conserved in the lobula, while it is still unclear whether the topological information is also conserved in the central brain. LC pathways possibly transfer such information because the information of the visual field can be divided into small potions by large numbers of the neurons.

To confirm this possibility of conservation, I analyzed the orientation of the axons of the LC pathways by single-neuron labeling using flip-out system. Flip-out system is a method to label single cells out of a group of cells dependding on a heat shock (Fig. 6). The results of the analysis of one LC pathway revealed that the orientation of the axons in the projecting pathway and the glomerulus was regular to a certain extent. This means that the two-dimensional visual topology may be conserved even in the central brain. Standardized view of the single neurons of the LC4 pathway is indicated as Fig. 7.

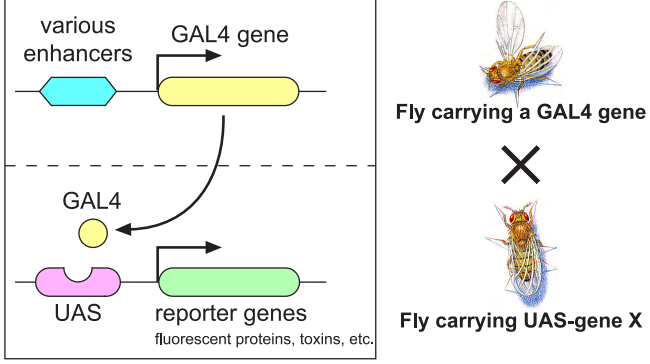

Fig. 4: GAL4 enhancer-trap system.

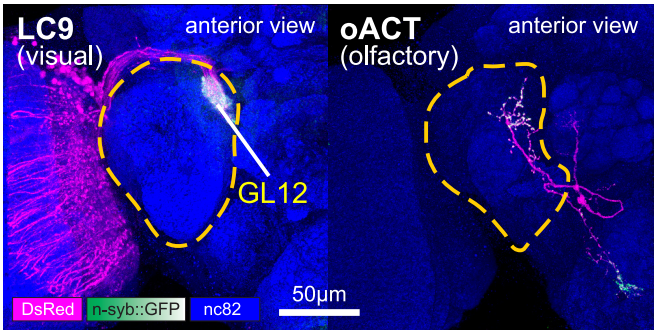

Fig. 5: Identification of the projection sites of the sensory pathways.

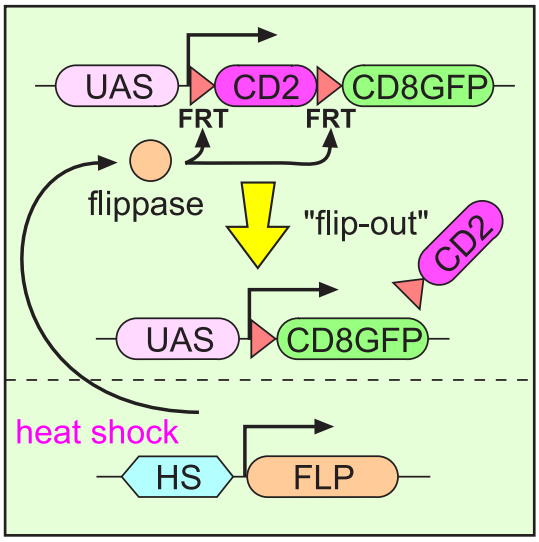

Fig. 6: Scheme of flip-out system.

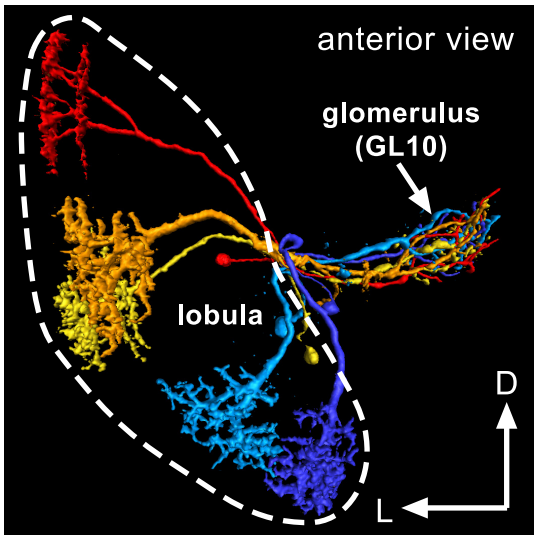

Fig. 7: Structural analysis of a visual projection pathway by standardization.