# ショウジョウバエ中枢神経系の三次元構造解析
# Three-dimensional microstructure analysis of the *Drosophila* central nervous system

水谷　隆太[1]，竹内　晃久[2]，上杉　健太朗[2]，鈴木　芳生[2]

Ryuta Mizutani[1], Akihisa Takeuchi[2], Kentaro Uesugi[2] & Yoshio Suzuki[2]

[1]東海大学工学部生命化学科；[2]高輝度光科学研究センター

[1]Department of Applied Biochemistry, School of Engineering, Tokai University;

[2]Life and Environmental Division, JASRI/SPring-8

アブストラクト

ショウジョウバエ中枢神経系の神経回路を明らかにすることを目的としてＸ線トモグラフィー解析を行った。Ｘ線ＣＴでは、切片化などの機械的介入なしに生体組織の構造を解析できるが、神経系は軽元素から成るため、コントラスト改善の工夫が必要となる。本研究では、古典的な神経組織染色法である鍍銀法により神経線維を染色し、良好なコントラストのＣＴ像を得ることができた。組織内部には神経線維の微細構造が観察され、今後解析を進めれば、ショウジョウバエの行動を司る神経回路を明らかにできると考えている。本法はどのような神経組織にも適用可能であり、中枢神経系に埋め込まれた神経回路網を解明するものとして今後の発展が期待できる。

Abstract

An x-ray microtomographic analysis of the *Drosophila* larvae central nervous system (CNS) was performed to visualize the entire neural network. Transparency of the nerve tissue to hard x-rays enables tomographic analysis of the intact CNS without any mechanical intervention. However, the nerve tissue is composed of light elements that give little contrast in a hard x-ray transmission image. Accordingly, the contrast was enhanced by staining the neurons with metal elements. The obtained structure revealed the internal architecture of the CNS, which governs the insect's behavior. This metal-staining microtomographic analysis can be applied to any type of nerve tissue, thereby shedding light on the underlying structural basis of neural functions.

In the central nervous system (CNS), a great number of neurons comprise complicated neural networks, making it difficult to dissect the connectivity of each neuron that contributes to a particular cerebral function. Selective staining with fluorescent dye confines visualization of projection patterns to a specific neuron, so it enables optical observation of the internal structure of the CNS. Since such optical microscopic imaging requires transparency at the observation wavelength, elaborations are needed to attain high-resolution deep imaging.

A number of methods can be used for staining biological tissues with fluorescent and pigment probes. Labeling of the target tissue with an electron-dense probe facilitates acquiring contrasty x-ray images of biological objects. The neuropil features in the CNS can be microscopically observed by impregnating them with metal elements. The structure of the nerve tissue in the CNS can therefore be visualized by the use of metal staining and CT analysis. Here we report an x-ray microtomographic analysis of the metal-stained *Drosophila* CNS.

**Results**

We used the CNSs of *Drosophila melanogaster* larvae for three-dimensional structure analysis since the sample size is limited to less than about 1.0 mm with the present micro CT setup. The CNSs (composed of a ventral nerve cord and brain hemispheres, called supraesophageal ganglions) were dissected from third instar larvae. The fixed CNSs were then stained by the silver impregnation method.[1] After clearing with xylene, they were embedded in epoxy resin and subjected to micro CT analyses.

Projection geometry radiographs for micro CT analysis were recorded using a CCD-based x-ray-imaging detector. The spatial resolution of the three-dimensional structure was estimated to be 1.0 um in each direction. The reconstructed images revealed the three-dimensional structure of the CNS, as shown in **Fig. 1**. The metal-stained tissue is clearly distinguishable from the epoxy-resin surroundings, indicating that metal impregnation can be used for radiographic observation of the CNS.

The image in **Fig. 2.** shows a section of the supraesophageal ganglion and ventral nerve cord. The neurons forming the network are well resolved in each CT section, meaning that the three-dimensional structure can serve as the basis for unraveling the neural circuits.

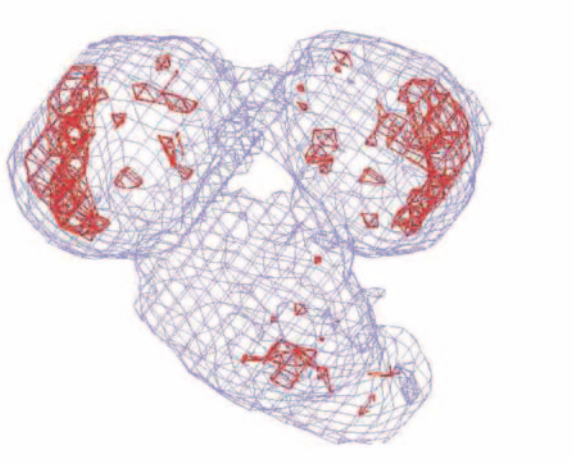

**Fig. 1.** Overall CNS structure of *Drosophila*. Absorption coefficients are contoured at 1 σ (purple) and 4 σ (red), using the program *RefineView* (http://pubweb.cc.u-tokai.ac.jp/ryuta/refview.htm).

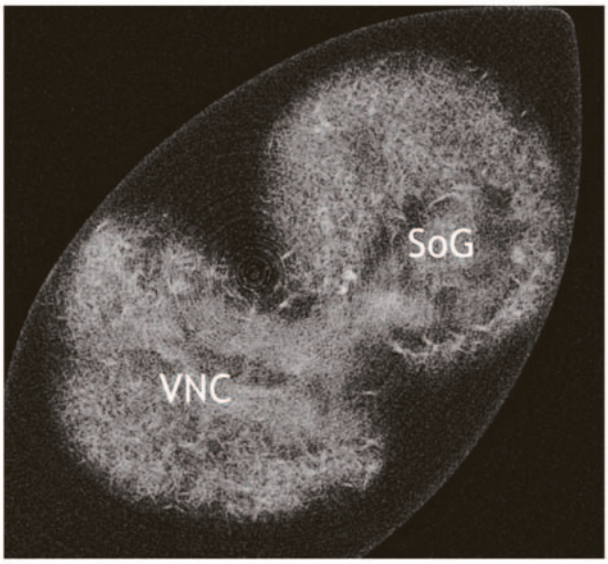


**Fig. 2.** Image showing supraesophageal ganglion (SoG) and ventral nerve cord (VNC). Linear absorption coefficients are shown in gray scale, from 0 $cm^{-1}$ (black) to 35 $cm^{-1}$ (white).

**Discussion**

The electron-density distribution reveals the overall features of the neural network in the nerve tissue, enabling visualization of the CNS internal structure. Kenyon cells and calyx of the mushroom body, a memory body in insects, were found in the dorsal cortex of the supraesophageal ganglion. The peduncle of the mushroom body was found to be exserted from the calyx. Axons in the peduncle projected medially and branched into two major lobes, which were assigned to the electron-dense regions of the dorsal and medial lobes. The optic lobe (red contour seen in **Fig. 1.**) showed a funnel-like structure in the lateral half of the ganglion. A repeated array of electron-dense tubercles was observed in the thoracic and abdominal cortex of the ventral nerve cord. These metal-stained CT images gave a complete view of the *Drosophila* CNS.

In fluorescence confocal microscopy,[2] absorbance at emission or excitation wavelengths interferes with the fluorescence detection. In contrast, transparency of the nerve tissue to hard x-rays enables tomographic analysis of the intact CNS without any mechanical intervention, such as paraffin sectioning or flattened preparation. Yet the nerve tissue is composed of light elements that give little contrast in a hard x-ray transmission image. The contrast was enhanced by staining the neurons with metal elements. This contrast enhancement visualized the entire CNS structure. However, the higher resolution (up to 100 nm) with 500 um diameter field of view is required for the complete analysis of the neural network of *Drosophila* brain.

Metal staining in conjunction with micro CT analysis can be applied to any biological system with a three-dimensional structure that controls its functions. Since uniform staining of all the tissue in the structure is essential for visualizing it, a variety of staining methods and dyes should be tested. Along with selective staining, CT analysis can shed light on the underlying microstructural basis of biological functions.

**References**


1. Heinz, T. *Biotech. Histochem.* **80**, 211-222 (2005).
2. Conchello, J.–A. & Lichtman, J.W. *Nature Meth.* **2**, 920-931 (2005).


**Publication**


Visualization of the three-dimensional microstructure of the nerve tissue, by Ryuta Mizutani, Akihisa Takeuchi, Tomohiro Hara, Kentaro Uesugi, and Yoshio Suzuki, submitted.


**Keywords**


Central nervous system (CNS), nervous tissue including brain and spinal cord; *Drosophila*, fruit fly commonly used in genetics experiments; impregnation, histological staining method using metal elements.