Three-Dimensional Neuronal Structure of Human Cerebral Cortex Determined by Synchrotron-Radiation Microtomography

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Neuronal circuits are responsible for brain functions including cognition, reasoning, and decision making. Neurons constitute neuronal circuits by forming three-dimensional networks in the cerebral cortex tissue. Therefore, the functional mechanism of human brain can be revealed by visualizing and analyzing the three-dimensional structure of the cerebral cortex. Here, we report on the three-dimensional structure of human cerebral-cortex tissue, as determined by synchrotron-radiation microtomography at resolutions up to 100 nm.

Post-mortem cerebral tissues were collected with informed consent from the legal next of kin using protocols approved by ethical committees of the related organizations. The cerebral tissues were subjected to Golgi impregnation, as described previously [1]. The stained tissues were sequentially immersed in ethanol, *n*-butylglycidyl ether, and epoxy resin. The samples were transferred to a borosilicate glass capillary filled with resin and kept at 90ºC for 40 h to cure the resin.

X-ray microtomography equipped with Fresnel zone plate (FZP) optics was performed at the BL37XU beamline of the SPring-8 synchrotron radiation facility. An FZP with an outermost zone width of 50 nm and diameter of 250 μm [2] was used as an objective lens, and an x-ray guide tube [3] as a beam condenser. A total of 900 transmission images were recorded with a CMOS-based detector using monochromatic radiation of 8 keV. Tomographic sections were reconstructed with the convolution back-projection method of the RecView program (available from http://www.el.u-tokai.ac.jp/ryuta/). Spatial resolution was estimated to be 100 nm by using test objects [4].

The obtained three-dimensional structure of human cerebral tissue is shown in Figure 1. Neurons and neuronal processes were clearly visualized as a three-dimensional distribution of x-ray attenuation coefficients. Dendritic spines were observed as small protruding structures from the dendrites. These spines have claviform heads with longitudinal lengths of 300-950 nm and cross-sectional diameters of 150-350 nm. The heads are connected to the dendrites through thin cords with typical widths of 150 nm and lengths of 300-1500 nm. These spines are responsible for neurotransmission in neural circuits.

Simple-projection x-ray microtomography with a wider field of view (1 mm) was performed at the BL20XU beamline of SPring-8. A total of 1800 transmission images per dataset were recorded using 12-keV radiation. The spatial resolution was estimated to be 1.2 μm by using test objects. The obtained structure (Fig. 2a) illustrated complicated neuronal networks that cannot be comprehended at a glance. As a result, the image had to be further analyzed to reveal the neuronal circuits embedded in the cerebral tissue. Such neuronal network models can be built by placing and connecting nodes in the three-dimensional map of x-ray attenuation coefficients that represent electron densities. Series of nodes corresponding to neuronal processes and somas were traced to construct skeletonized models of neurons (Fig. 2b). Since the resultant models are represented in three-dimensional Cartesian coordinates, the distances between neuronal processes or somas can be readily calculated from the coordinates. This will allow us to determine neuronal circuits in the human brain tissue by analyzing the positional relationships of the neurons [1,5].

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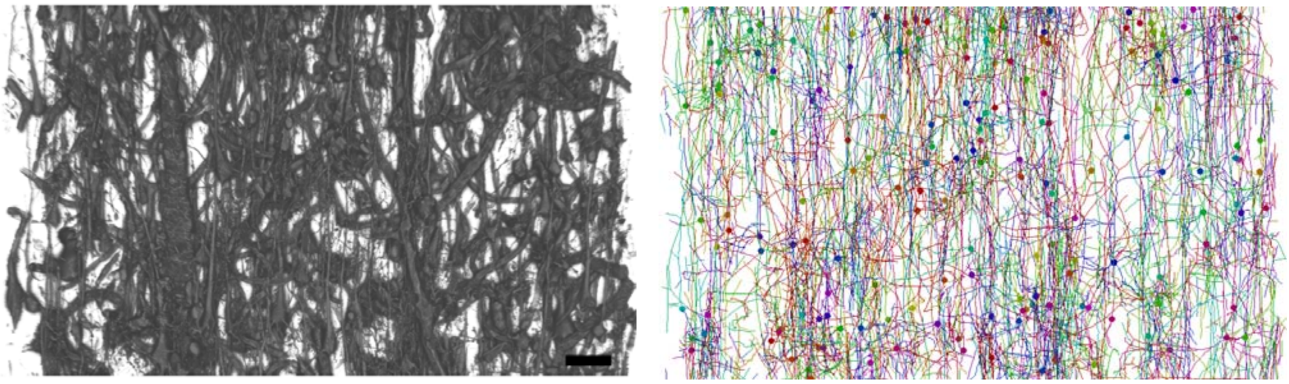
Figure 1. Stereo rendering of the three-dimensional structure of a pyramidal neuron and neuronal processes in the human cerebral cortex. Scale bar: 5.0 µm.



(a) (b)

Figure 2. (a) Three-dimensional structure of human cerebral tissue. Scale bar: 50 µm. (b) Skeletonized model of the tissue structure. Neurons are color-coded. Soma positions are indicated with closed circles.

X-ray Tomographic Microscopy of Drosophila Brain Network and Skeletonized Model Building in the Three-Dimensional Image

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The brain consists of a large number of neurons that make up a three-dimensional network. The first step to understanding brain functions is to analyze the structure of this network. Although three-dimensional structures of brain tissues have been reported, their structures are difficult to comprehend. This is because of a lack of quantitative descriptions of the three-dimensional network, which should be represented with three-dimensional Cartesian coordinates, rather than a three-dimensional distribution of intensities. Here, we report on x-ray tomographic microscopy of the brain network of the fruit fly *Drosophila melanogaster* and its analysis by skeletonized-model building [1].

Cephalic ganglion dissected from a wild-type Canton-S adult fly was stained with aurate by reduced-silver impregnation, as described previously [2,3]. The brain was then sequentially immersed in ethanol, *n*-butylglycidyl ether, and epoxy resin (Burnham Petrographics). The obtained sample was mounted using a nylon loop (Hampton Research) and incubated at 90ºC for 16 h to cure the resin.

X-ray microtomography equipped with Fresnel zone plate (FZP) optics was performed at the BL37XU beamline of the SPring-8 synchrotron radiation facility. An FZP with an outermost zone width of 100 nm and diameter of 310 µm was used as an x-ray objective lens. Transmission images produced by 8-keV x-rays were recorded using a CMOS-based imaging detector (Hamamatsu Photonics). The tomographic slices were reconstructed from the x-ray images to determine the three-dimensional distribution of the linear attenuation coefficient. The spatial resolution was estimated to be 160 nm by using three-dimensional square-wave patterns. An example of the obtained structure is shown in Fig. 1. Neuronal processes were clearly visualized as a network structure.

In order to analyze the structure, its three-dimensional network should be further delineated in terms of Cartesian coordinates by building a skeletonized model. The model can be built by using a method like those used in crystallographic studies of macromolecular structures [1]. Since such an analysis should start by constructing an overall model that can facilitate structural analysis at a higher resolution, an initial model was built from a three-dimensional image obtained with other equipment of microtomography having a wider viewing field at BL47XU of SPring-8 [1]. Automatic tracing was applied to sparsely distributed structures such as those of peripheral nerves, while manual tracing was performed to build the neuropil model. The resulting model (Fig. 2) consists of neuronal processes with a total length of 378 mm in a volume of $0.220 \times 0.328 \times 0.314$ mm^3.

The neuronal process model was classified into groups on the basis of the three-dimensional structures. Anatomical segments can be extracted from the model by specifying neuronal processes in a group-by-group manner. Figure 2 shows some of the structures of the optic lobe, which is responsible for visual information processing. Neuronal processes of the medulla and second optic chiasma, which are proximal to the compound eye, exhibit periodical structures corresponding to repeated units of photoreceptors. On the opposite side of the optic lobe, neuronal processes are assembled into several tracts pointed toward central brain regions. These structures represent information paths from the inputs on the eye to the integrative process in the central brain. Refinements of these models by using the high resolution data should reveal finer three-dimensional aspects of the *Drosophila* brain.

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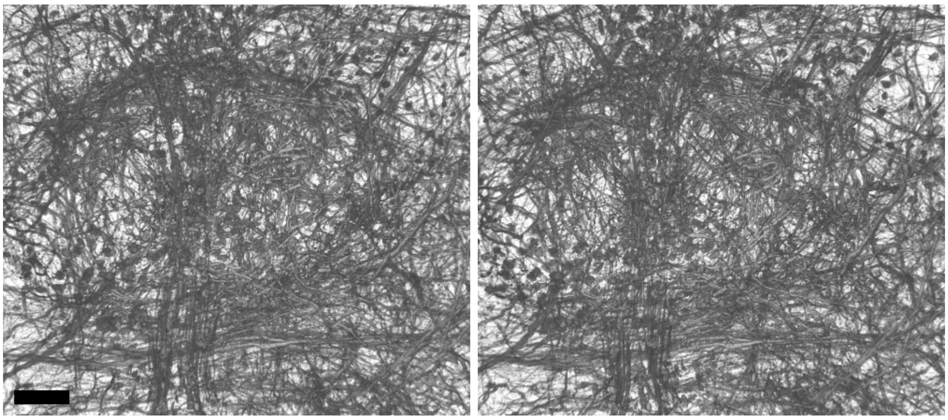

Figure 1. Stereo rendering of a frontal view of the *Drosophila* brain. Networks including the midline structure called the central complex are illustrated. Scale bar: 10 µm.

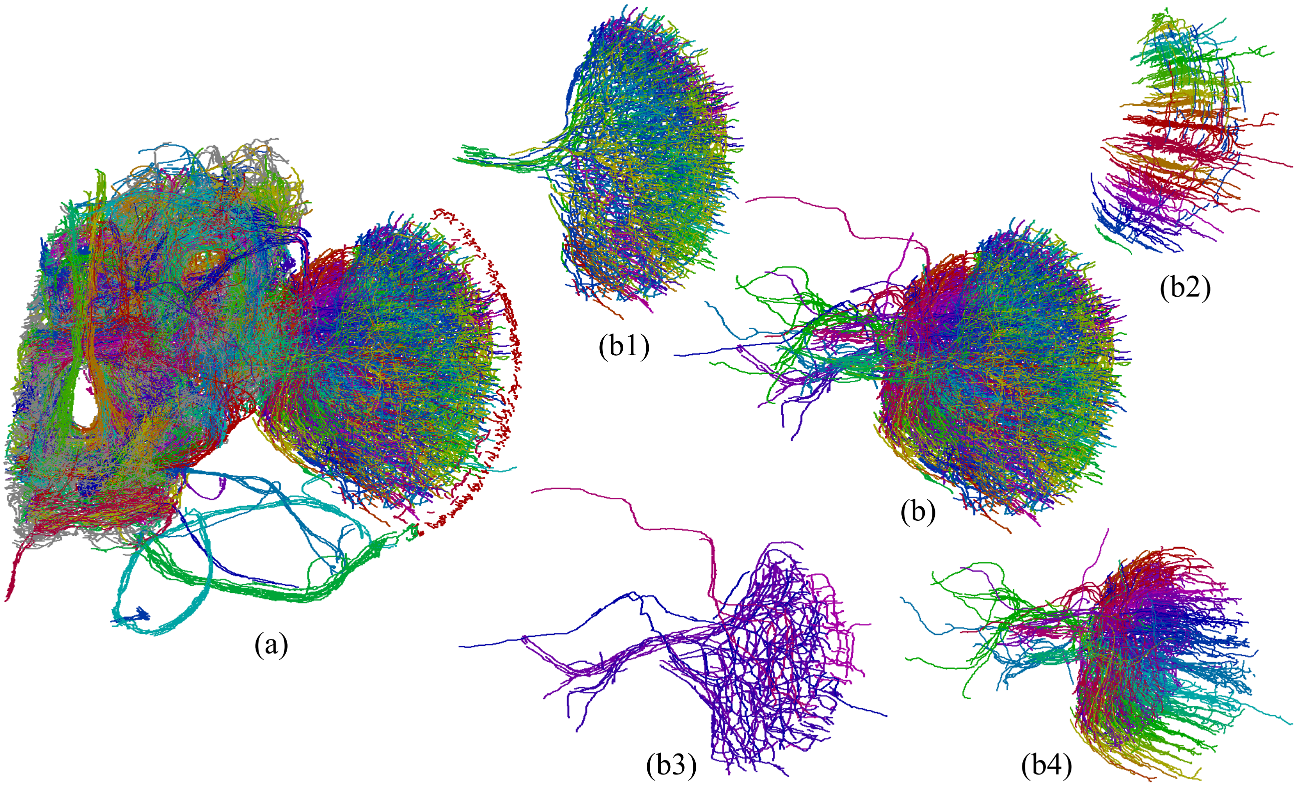

Figure 2. Skeletonized model of the left hemisphere of the *Drosophila* brain. The entire model is shown in (a). The optic lobe (b) is composed of the medulla (b1), second optic chiasma (b2), lobula plate (b3), and lobula (b4). Neuronal process groups are color-coded.