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Note

Efficient three-dimensional reconstruction of synapse with high-voltage electron microscopy

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Abstract Three-dimensional (3-D) information on nervous tissue is essential for the understanding of brain function. Especially, 3-D synaptic analyses on serial ultrathin sections with transmission electron microscopy (TEM) have contributed to the knowledge on neural plasticity associated with various pathophysiological conditions. The 3-D reconstruction procedures, however, not only require a great amount of expertise but also include time-consuming processes. Here, we carried out computer-assisted 3-D reconstruction of parallel fibre–Purkinje cell synapses based on 250 nm serial sections using high-voltage electron microscopy (HVEM). The 3-D synapse models were constructed more efficiently and rapidly compared with conventional serial TEM reconstruction. This result suggests that 3-D reconstruction with thicker sections and HVEM is a useful method to study synaptic connectivity.

Keywords 3-D reconstruction, HVEM, synapse, Purkinje cell, cerebellum

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Synapses are specialized intercellular junctions between neurons where signals are propagated from one cell to another. Most excitatory synapses consist of presynaptic axon terminals and postsynaptic dendritic spines in the mammalian central nervous system. The structure and number of synapses are changed drastically under various physiological or pathological conditions, such as development [1], environmental enrichment [2], learning [3], hormonal states [4], ataxia [5] and mental retardation [6].

Since the structural modification of synapses could modulate their functional properties, the morphological investigation of synapses is crucial to the understanding of synaptic transmission, synaptogenesis and synaptic plasticity.

Although transmission electron microscopy (TEM) of ultrathin sections has produced high-resolution images of synaptic profiles, access to three-dimensional (3-D) information is limited. The 3-D reconstruction of synapses using serial ultrathin sections has provided more-comprehensive information on the structural dimensions and composition of synapses with high-resolution images of synaptic profiles. Recently, several studies reported ultrastructural characteristics of synapses in the hippocampus CA1 area and in the cerebellum using the reconstruction of serial TEM sections [7–9]. Unfortunately, the 3-D reconstruction of synapses using conventional TEM includes prohibitively time-consuming processes requiring tools and expertise. High-voltage electron microscopy (HVEM) provides an increase in resolution, specimen-penetrating capability and depth of information [10]. Consequently, HVEM has been applied effectively to the study of neuronal structures by using thick sections and taking advantage of its high resolution and penetrating power [11,12]. In this context, we have considered the application of serial thick sections and HVEM for the 3-D reconstruction of synapses in cerebellar cortex. If thick sections are used for reconstruction, it would be possible to reduce time-consuming processes, such as the construction of numerous serial sections and the operation of stacks of images using computer programs. However, we have to consider low contrast in HVEM and overlapping of neural profiles due to the use of thick sections.

Male Sprague–Dawley rats (4 months old) were anaesthetized with sodium pentobarbital (100 mg kg^{-1}) and intracardially perfused with 2% paraformaldehyde/2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4).

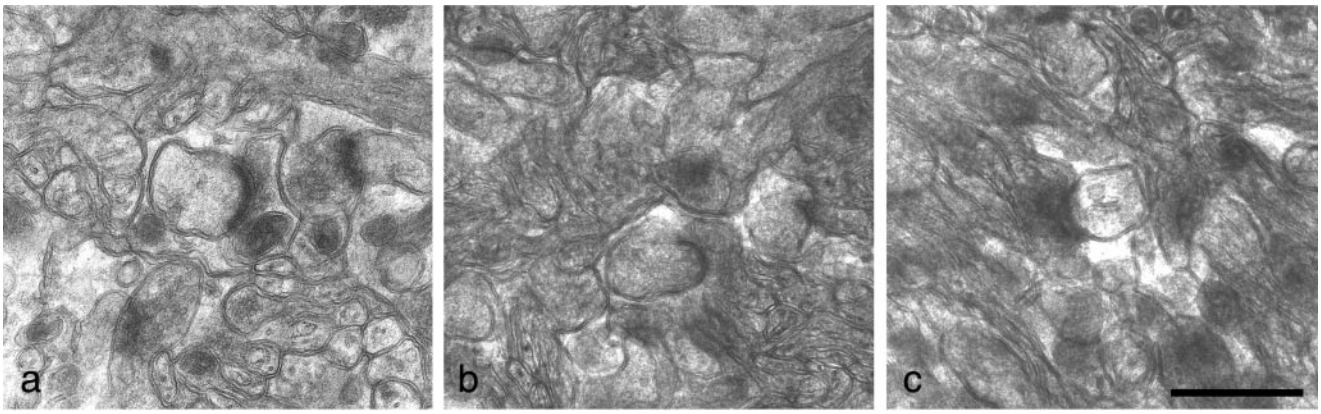

Fig. 1 The decision of an optimal section thickness for 3-D reconstruction of parallel fibre–Purkinje cell synapses using HVEM. The overlapping of synaptic profiles is observed in proportion to the increase in section thickness: (a) 250 nm, (b) 500 nm and (c) 1000 nm. Bar = 2 μm.

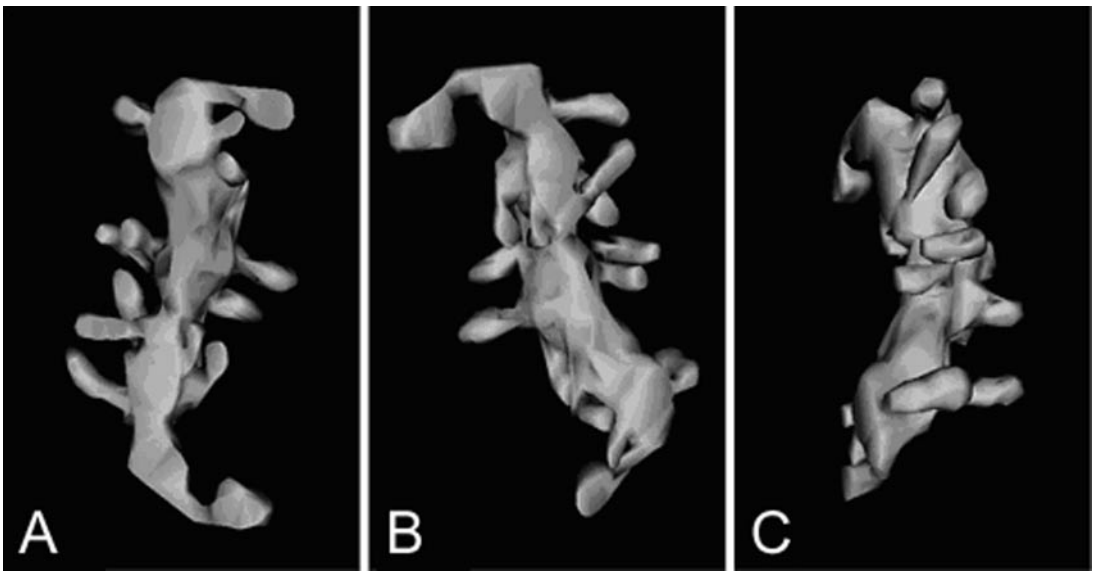

Fig. 2 Practical 3-D reconstruction of the partial dendrite and spines of a Purkinje cell.

The cerebellum was removed and stored in the same fresh fixative overnight at 4°C. Sagittal sections of each cerebellum were washed and postfixed in 2% osmium tetroxide for 3 h. *En bloc* staining was carried out with 2% uranyl acetate overnight to increase contrast during HVEM observation. Semi-thin sections were obtained to identify the paramedian lobule (PML) under the light microscope and a series of thick sections (250, 500 and 1000 nm) was taken from the molecular layer of the PML using Ultracut-E (Reichert-Jung) to evaluate the optimal section thickness preserving the ultrastructures of synapses. Although it was possible to observe synaptic ultrastructures in all section thicknesses, the overlapping of cellular membranes, organelles or synaptic vesicles was observed more frequently in proportion to the increase in section thickness (Fig. 1). The 250 nm thick sections showed well-preserved synaptic membranes in detail and this thickness was selected to avoid a complication in contouring synaptic membranes, which is caused by a pile of ultrastructures in a section. Some 25–30 consecutive serial sections were mounted on formvar-coated slot grids (2 × 1 mm; Synaptek Co.) and stained with 6% uranyl acetate for 1 h and then Reynold's lead citrate for 15 min. The sections were observed at the accelerating voltage of 1250 kV using the JEM-ARM 1300S (JEOL, Tokyo, Japan) at the Korean Basic Science Institute (Dae-Jeon, Korea). From each section, the same spot was taken serially at a magnification of ×10 000–20 000. The negatives on HVEM film were scanned at 1000 dpi using an Epson Perfection 3200 photo scanner and saved as TIFFs for further computer-assisted reconstruction processes.

'Reconstruct' software, which is a Windows® application for aligning, tracing and reconstructing objects from serial section images, was used in this investigation (http://synapses.bu.edu/tools). The scanned image files were imported into Reconstruct software using a multifile import operation and converted to sequence files. The alignment of serial images was carried out by putting a few points into correspondence between adjacent sections. This can be done by entering point traces alternately on the same organelle (for instance, mitochondria or microtubule) of adjacent

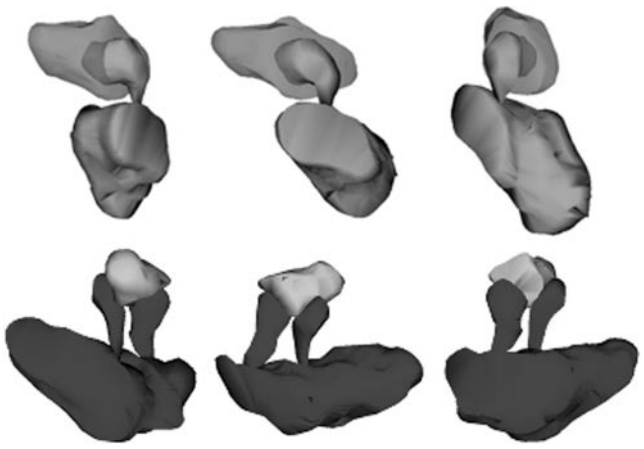

Fig. 3 The 3-D reconstruction of single and multiple contact synapses consisting of parallel fibres and Purkinje cell dendritic spines. The multiple-contact synapse shows that two dendritic spines originate from same dendrite.

sections. The membranes of presynaptic parallel fibres and postsynaptic dendritic spines of Purkinje cells were then outlined manually on each aligned section. The serial contours of each synapse were previewed in an OpenGL-based 3-D scene window. The 3-D images of synapses were finally saved as VRML files (Figs 2 and 3). The Purkinje cell dendritic fragment has been reconstructed and can be observed in any direction with the VRML browser. In addition to a dendritic tree with spines, 3-D models of the synapses between parallel fibre varicosity and Purkinje cell dendritic spines clearly demonstrate how they are connected.

The 3-D reconstruction using 50–60 nm sections imaged by TEM has been used effectively for studies on the structural dimensions and composition of synapses [7–9]. To reconstruct 4 μm of Purkinje cell dendrite shown in this result, 66–80 serial ultrathin sections would be required if conventional TEM are used, whereas only 16 serial sections were used in this study.

In conclusion, we could construct 3-D models of synapse more efficiently and rapidly compared with conventional serial TEM reconstruction. This 3-D modelling method with HVEM can be applied to not only neuroscience but also other biological researches.

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