Biological Application of the High Voltage Electron Microscope

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I) Introduction:

The following advantages are expected using the high voltage electron microscope (HVEM) in the observation of biological materials. 1) Higher resolution can be expected because of the shorter wave length and smaller aberrations. 2) Thicker specimens can be observed because of the higher penetration power of electrons. 3) Smaller beam damage to the specimen may enables observation of living cells. Actually the initial impetus toward high voltage operation came from biologists desiring to examine living cells. However,the challenge was not successful. Advanced HVEMs were built in the late 50's and early 1960's. They were mainly used in the field of material sciences where the advantage of observing material in bulk was enormous, while results of the biological application were miserable. Successful examples of the biological application appeared at the First US-Japan High Voltage Electron Microscope Conference held in 1967. Since then various types of biological applications have been tried utilizing advantageous properties of the HVEM 1, 2,3,4,5.

As for the thick specimen observation,it became clear that very thick biological specimens up to 5 μm thick could be observed at 1000 kV without severely impairing the spatial resolution. This property of the HVEM especially suits for the morphological study of the brain, because nerve cells and glia cells have complicated very fine branches which intermingle to each other to achieve brain functions 5,6. These branches are too fine for the light microscope examination and too complicated and widely spread for thin section electron microscope analysis.

II) Dendritic spines:

Dendrites of the spiny neurons in the brain are studded with numerous spines even up to 200 thousands in number. These spines are the major sits of excitatory

inputs to the cell, and thought to be the site of synaptic plasticity underlying learning and memory. Consequently, precise morphometrical data are indispensable for the better understanding of the brain function. HVEM examination revealed that the diameter of stalks of most spines of cortical neurons are less than 0.2 μm, close to the limit of the resolution of the light microscope (LM). Therefore, the LM may not be adequate for precise morphometry of dendritic spines.

We developed a computer aided 3-D morphometrical analysis system using tilted stereo paired pictures 7,8. Some of the results are as follows.

1) Number of spines are about 1.3-2.0 times larger than those reported in previous light microscope examination , which suggested that the resolution of the light microscope was insufficient to identify closely positioned spines.

2) Although the spine density per unit length of the dendrite decrease from proximal to distal ,the spine density per unite surface area of the dendrite are about equal throughout the course of the dendrite.

3) The average length of the spines measured in 3-D is about 1.4 times longer than that measured in 2-D.

4) The total surface area of the dendrite is about doubled by the addition of the spine surfaces.

III) Glia cell morphology:

Glia means glue in the brain and they always associate with nerve cells to help nerve cell functions. Here, the structure of the astrocyte will be concerned 5,9,10.

Light microscope image of the astrocyte in the cerebral cortex differ considerably according to the methods of staining. In GFAP immune-stained materials they appear as spiders with many long legs, whereas in Lucifer Yellow stained preparation and Golgi preparation, they have bushy appearance with many fine processes between large branches. The structural details of these fine processes can not be clarified by the LM.

HVEM observation of 5 μm thick Golgi preparation revealed that many thin ribbon-like or leaflet-like processes arise directly from the cell body and large processes. They frequently branch and anastomose to form complicated 3-D meshwork in which neuronal processes and blood vessels are embedded. The sizes and shapes of the mesh vary according to their target structures.

On the surface of the cell body with few synaptic inputs, the astrocytic process attenuate into very thin sheet of less than 50 nm in thickness and encapsulate neuronal cell bodies. Whereas, on the surface of the cell body with many synaptic inputs, very thin astrocytic processes separate each presynaptic bouton forming a layer of compartments around the nerve terminals,which probably correspond to the peri-neural net of Held.

IV) 3-D morphometry of astrocytic processes:

The ratio of the perimeter and surface area of the digitized image of the same specimen observed by the LM and HVEM were measured and used as a parameter of the complexity. The perimeter / area ratio of the HVEM image was 2 times larger compare to that of the LM image,suggesting that the structural details of astrocytic processes can not be analyzed precisely with the light microscope. Consequently, we tried computer tomography analysis to estimate the volume and the surface area of the astrocytic processes. 61 tilted images starting from -60 degrees to +60 degrees with 2 degrees increment were used for analysis 11. Preliminary results show that the surface volume ratio of the astrocyte are very large, 17- 33 μm-1 , which is attributed to unusual thinness of flat terminal processes of the astrocyte. The large surface area of the astrocyte is important for their functions, because various receptors, ion channels, and transporters are located on the surface plasma membranes constituting the site of neuron-glia interactions.

V) Faithfulness of Golgi method in astrocyte morphology:

Finally, thin serial sections of gold toned Golgi preparations were examined with the electron microscope at 100 kV to check whether or not the Golgi method faithfully represented the 3-D morphology of astrocytic processes. Both gold particles and bundles of glia filaments were found in the cell bodies indicating that the astrocytic nature of the Golgi stained cells. In addition, gold particles were confined within astrocytic processes as thin as less than 50 nm in thickness covered by an intact plasma membrane. These thin profiles contain no glia filaments but they could be traced to the large processesa which contained both glia filaments and gold particles in serial sections. The result strongly supported the view that the HVEM image of thick Golgi preparation faithfully represented the morphology of the astrocyte 8.

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