Electron microscopic studies have revealed synaptic vesicle recycling by endocytosis following neurotransmitter release by exocytosis. Imaging methods using fluorescent dyes have enabled monitoring of synaptic vesicle endocytosis and recycling in living neurons. Measurement of plasma membrane capacitance by electrophysiological recording has further elucidated mechanisms of synaptic vesicle endocytosis. However, many aspects of synaptic vesicle mobility during exocytosis and endocytosis are still a matter of debate. For example, where endocytosis occurs in synapses is not wholly understood. To analyze ultrastructural changes that occur during synaptic activity, observation of fine structure inside intact synapses is necessary. Reconstruction of synapses by serial sectioning provides good resolution, but much information is lost because sections cannot be made thinner than ~50 nm. Focused ion beam milling and scanning electron microscopy (FIB-SEM) has enabled 3D imaging of whole synapses; however, resolution has not been satisfactory. Here I present 3D images of synaptic vesicles of the calyx of Held presynaptic terminus examined by FIB-SEM, with satisfactory resolution and contrast. Reducing the probe current and acceleration voltage permitted visualization of fine ultrastructure comparable to that of TEM. It is easy to recognize the synaptic cleft, synaptic vesicles, including clathrin-coated vesicles, mitochondrial cristae, and ribosomes on rough-endoplasmic reticulum.

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Fig. 1: Top surface view of rat brainstem slice embedded in epon (left, top). Side view of specimen during ion beam milling (left, bottom). An image obtained during ion milling shows two principal neurons of the medial nucleus of the trapezoid body surrounded by the calyx of Held presynaptic terminus.

Fig. 2: FIB-SEM ion milling of a single synaptic vesicle. Six representative serial sections out of 674 taken without drift or loss of focus. The milling thickness is estimated at ~10 nm.

Fig. 3: Resolution of FIB-SEM cut at 10 nm (right) is close to that of a TEM epon section cut at 50 nm (left).