Freeze substitution with tannic acid/osmium (long incubation) January 2004

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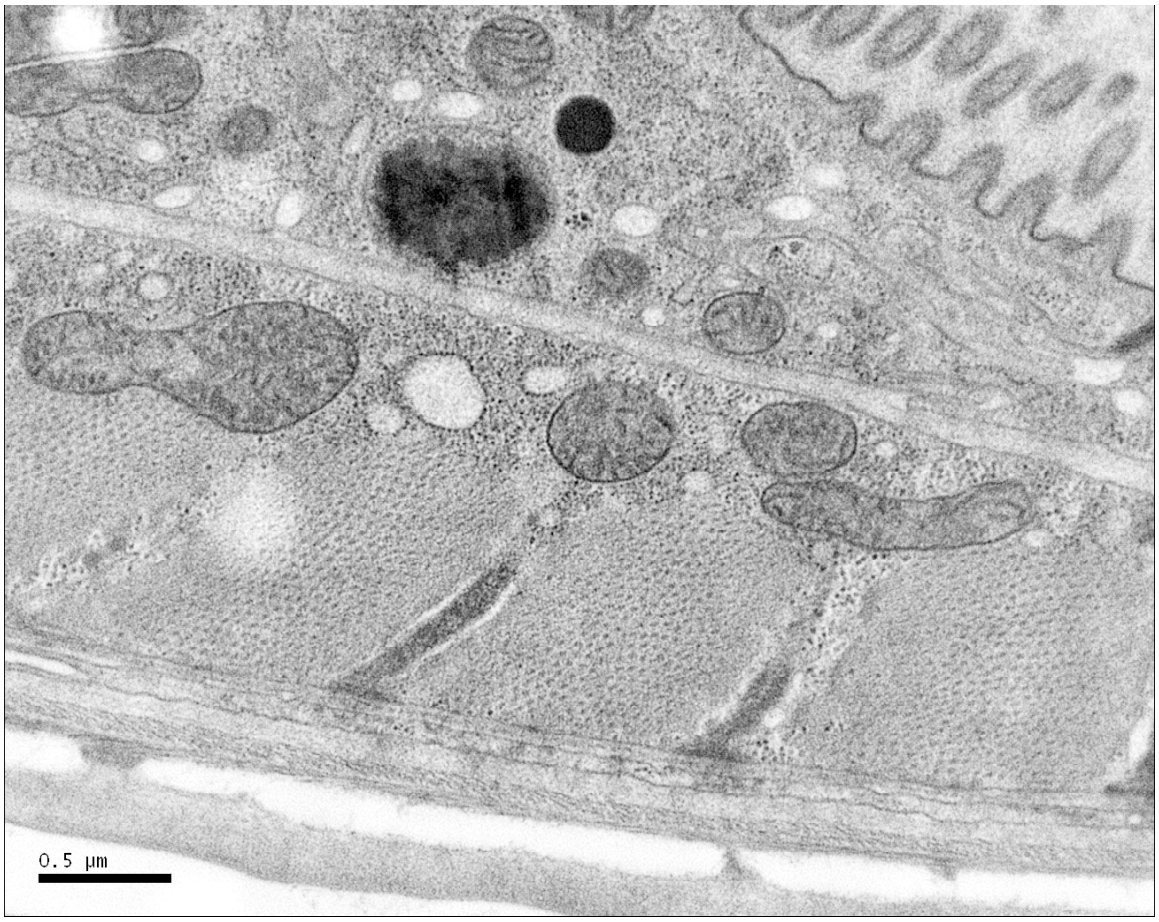
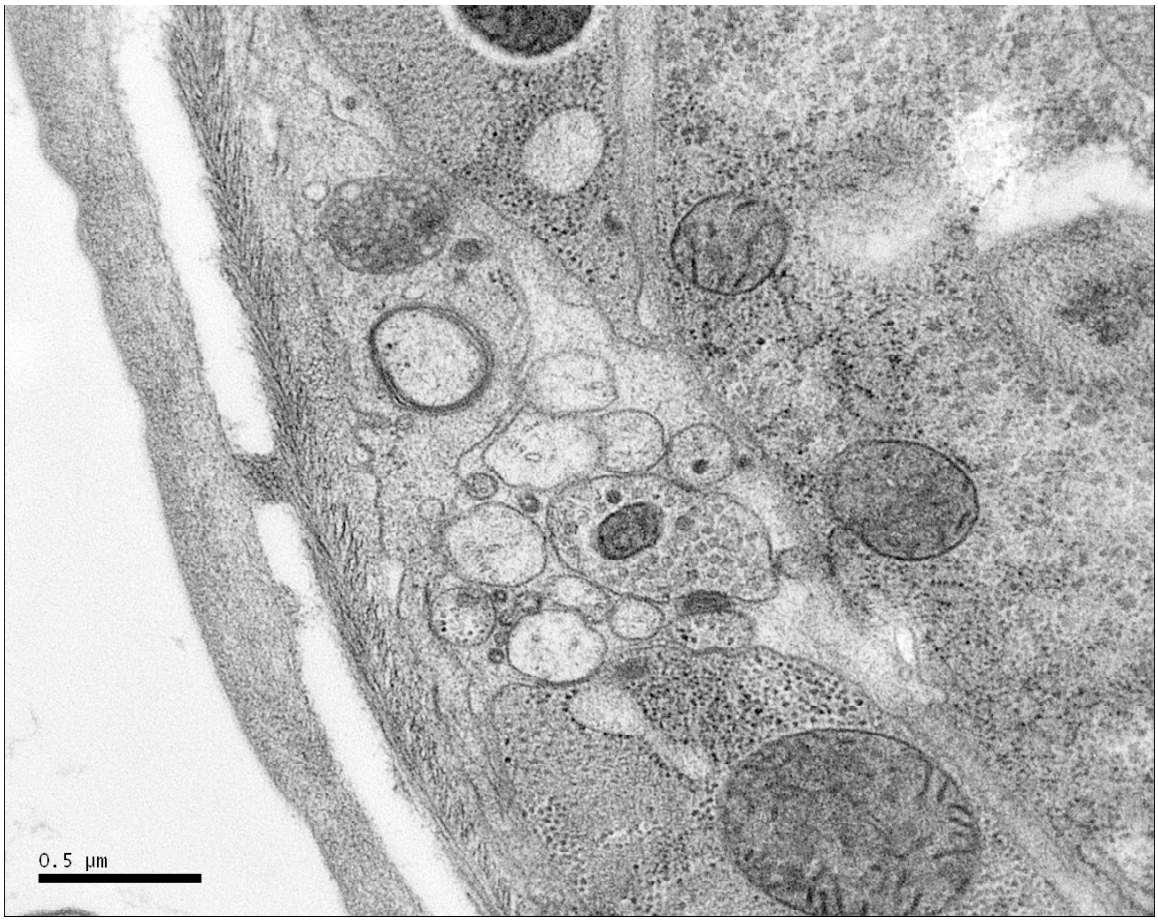
Freezing:
Several animals (~10) were loaded into a 100um deep chamber surrounded by *E. coli* and frozen in a BalTech HPM 010.

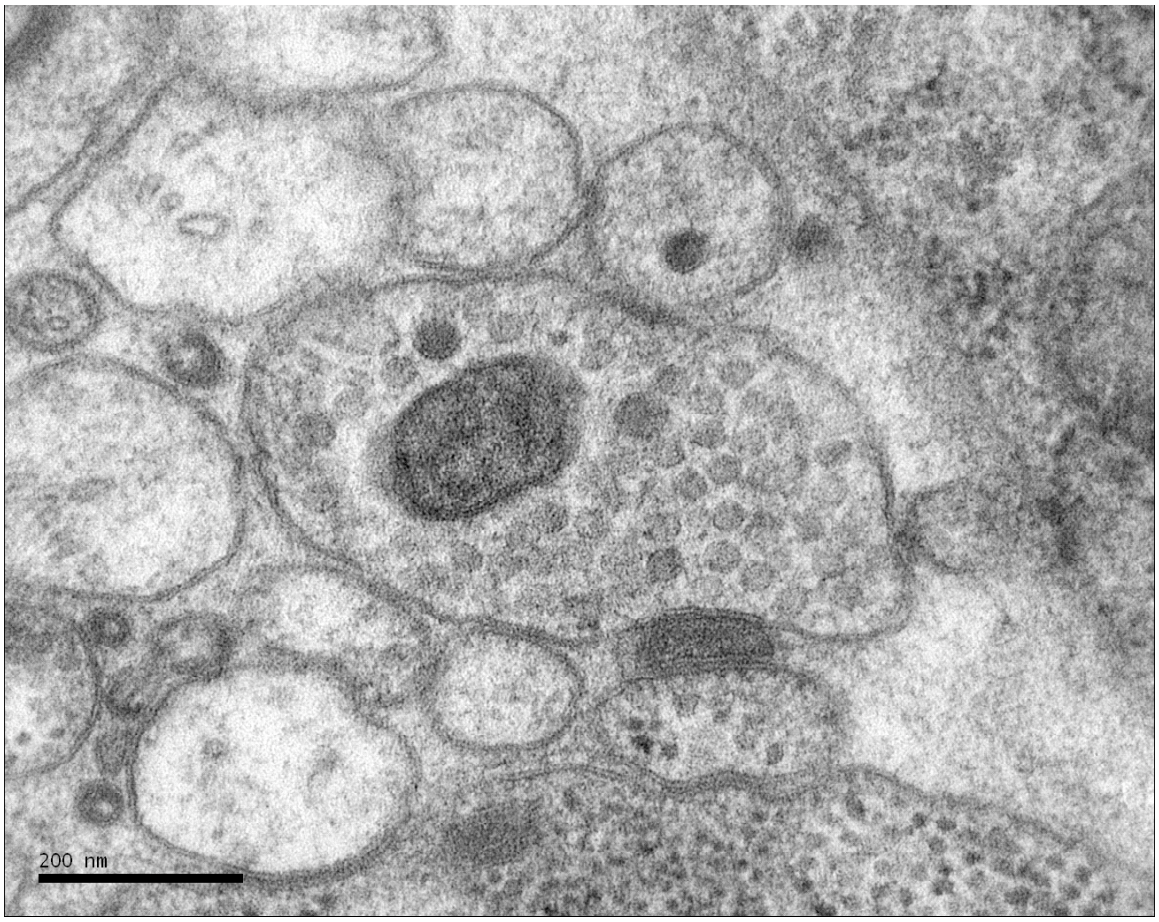
Freeze substitution and embedding:
The freeze substitution was carried out in an Reichart AFS.
-90C for 4 days in 0.1% tannic acid, 0.5% gluteraldehyde in anhydrous acetone
-90C for 4 hours in anhydrous acetone, changing the acetone several times
-90C for 4 hours in 2% osmium in anhydrous acetone
-90C to –20C at 5C/hr, total of 14 hours
-20C for 16 hours
-20C to 4C at 6C/hr, total of 4 hours
4C for 4 hours
4C for 2 hours in anhydrous acetone, changing the acetone several times
4C to room temperature over 1 hour
embedded in Araldite 502.

Sectioning:
40nm sections were collected on formvar covered grids and counterstained (4 minutes in 2.5% uranyl acetate in 70% methanol, 2 minutes in Reynolds lead). Micrographs were collected using a Gatan digital camera while the specimen was being viewed in a Techni 12 electron microscope at 80kV. The animal shown was sectioned near the anterior gonad reflex.

Observation:
Most tissues were well preserved, all membranes (specifically, synaptic vesicles and mitochondrial membranes) are well contrasted (see following micrographs), however, dense tissues are often too dark for fine observation.


0.5 µm


0.5 µm


200 nm

a different synapse