Structure / function analysis of *C. elegans* synapses

J. Hegermann, S. Eimer

European Neuoscience Institute Göttingen; Center for Molecular Physiology of the Brain
Grisebachstr. 5, 37077 Göttingen

jhegerm1@gwdg.de
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Using HPF and three-dimensional reconstruction we are investigating the overall ultrastructure of the neuromuscular junction (NMJ) in *C. elegans* as model system. At the NMJ, synaptic vesicles gather around an electron-dense specialization, called "presynaptic density" (PD). It is located in the center of the "active zone", where vesicles bind tightly to the membrane. The electron dense material forming the PD is not homogeneous. It consists of filamentous material extending into the cytoplasm, tethering the nearest vesicles. From these the filamentous material extends further, binding and also interconnecting the next vesicles. Thereby an irregular meshwork is formed, inside which vesicles are trapped and thereby concentrated around the PD. We are aiming to understand the mechanism how vesicles are brought from the cytoplasm to the membrane, where they are finally exocytosed. Therefore we investigate the fine-structure of the PD by thin sectioning and threedimensional reconstructions from tomography and serial sectioning. Comparing the vesicle distribution and the structure of the PD in different mutants we are seeking for proteins possibly involved in maintaining the structure and function of synapses.

Additionally we are adressing the question where neuropeptide-containing dense core vesicles (DCV) derive from and where they go. Some of these appear also near to synapses, however, we find them to be distributed all along the axons.

C. elegans is a very well and extensively described model organism concerning its biochemistry, genetics and ultrastructure. Its organs and different tissues have been subject to numerous electron microscopic investigations [1]. However, convenstional electron microscopic methods based on chemical fixation often do not provide satisfying results, as nematodes have a nearly impermeable cuticule and infiltration with chemical fixative takes up to several hours. Nerve tissue like the nerve cord and the nerve ring (corresponding to the spinal cord and the brain in higher animals) is suffering particularly from chemical fixation, dehydration and plastic embedding, resulting in collapse of axons and loss of synaptic vesicles. High Pressure Freezing (HPF) has overcome these drawbacks of the conventional preparation of biological material for electron microscopy. During HPF the tissue is not chemically fixed but frozen rapidly within milliseconds. Afterwards it can be investigated either in frozen-hydrated state [2] or can be embedded in plastic without further alteration. The process of freezing is fast enough to capture highly dynamic cellular processes like endo- and exocytosis, the real-life state of golgi stacks and the distribution of synaptic vesicles at the synapse.

1. http://www.wormatlas.org/index.htm
2. Tokuyasu, K. T. (1973). A technique for ultracryotomy of cell suspensions and tissues. J Cell Biol **57**, 551-65.

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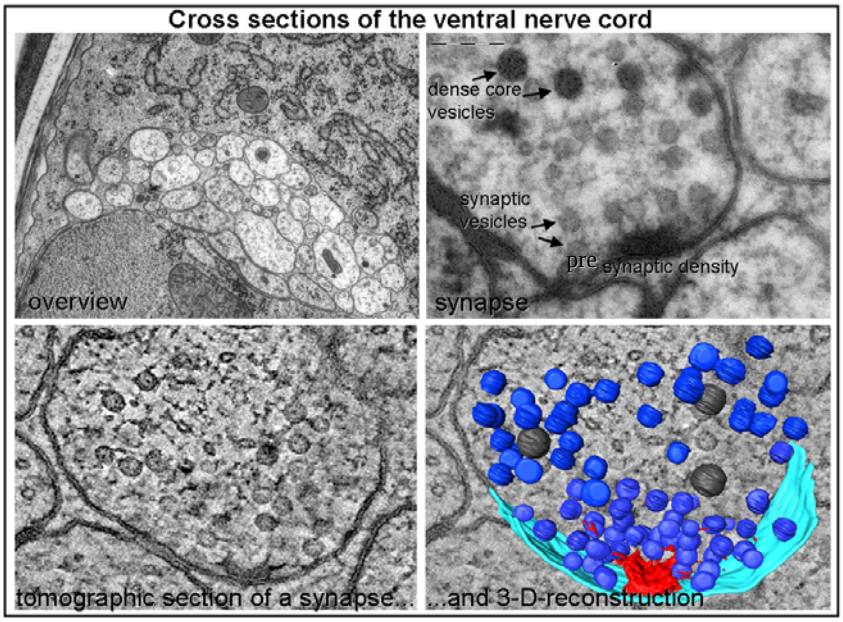

Figure 1. Cross sections of the ventral nerve cord in *C. elegans*. Top left: Overview of the nerve cord. Top right: A synapse in the nerve cord, showing the presynaptic density as well as synaptic and dense-core-vesicles. Bottom left: tomographic z-slice of a synapse in the ventral nerve cord, bottom right: three-dimensional reconstruction from the tomogram derived from a 250 nm section of an osmium-stained sample.

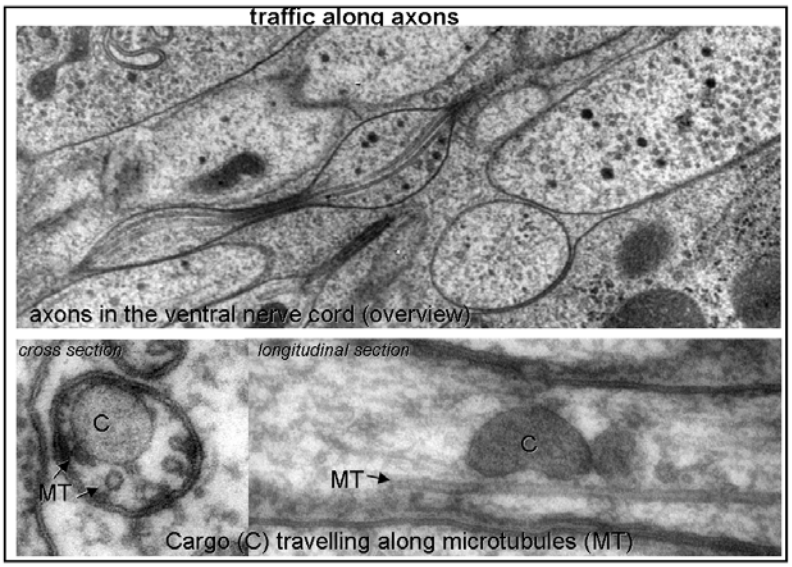

Figure 2. Traffic along axons in the ventral nerve cord of *C. elegans*. Top: Overview of axons along the nerve cord, note the necklace-like appearance of the axons with narrow sites where only the microtubules can pass. Bottom: Vesicular structures travelling along microtubules.

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