

Semi-Automated Reconstruction of the Neuromuscular Junctions in the *C. elegans*

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For a nervous system to function, it must be wired properly. Specifically, neurons need to find their targets and form synapses. The neuron maintains such connections for years, accommodating growth of the organism and making allowance for other neurons that synapse to access the same target. Fulfilling these functions make topological demands on neurons and their targets. To study this process we are reconstructing the neuromuscular junctions in the nematode *C. elegans*.

In this worm, the motor neurons remain in the ventral nerve cord and their processes interdigitate along the lateral edge of the nerve bundle to make contact with the muscles. Multiple muscles in turn must send processes to these motor neurons to receive input. There are three motor neuron inputs into these muscles: the VA neurons release acetylcholine during backwards movement, the VB neurons release acetylcholine during forward movement, and the VD motor neurons release GABA to relax the muscle to allow sinusoidal movement. We are interested in answering the following questions: How do the motor neurons accommodate the needs of other motor neurons that must form contacts to the same muscles? How do the multiple muscles accommodate the needs of other muscles to receive innervation from the same motor neurons?

To determine the topology of this complex synaptic region we have reconstructed a segment of the ventral nerve cord from serial electron micrographs. The data are registered and assembled automatically [1] and then reconstruction of individual neurons is performed using a modified path finding approach [2]. The first step of the algorithm is to segment the individual neurons in the 2D images. An edge map of the membranes is created using thresholding and hand editing techniques. The regions inside the membranes are segmented using connected components. Next, the algorithm connects the neuron and muscle regions across slices by treating each region as a vertex in a graph and the correlation between regions in adjoining slices as weights on edges in the graph. A minimum spanning tree algorithm adds edges to the graph that contain a high correlation between regions, connecting neurons and muscles across slices. This results in series of strands that represent continuous segments of neurons and muscles through the dataset. Finally, by hand, a user can connect the remaining segments to form a full 3D reconstruction of the selected *C. elegans* anatomy (Figure 1).

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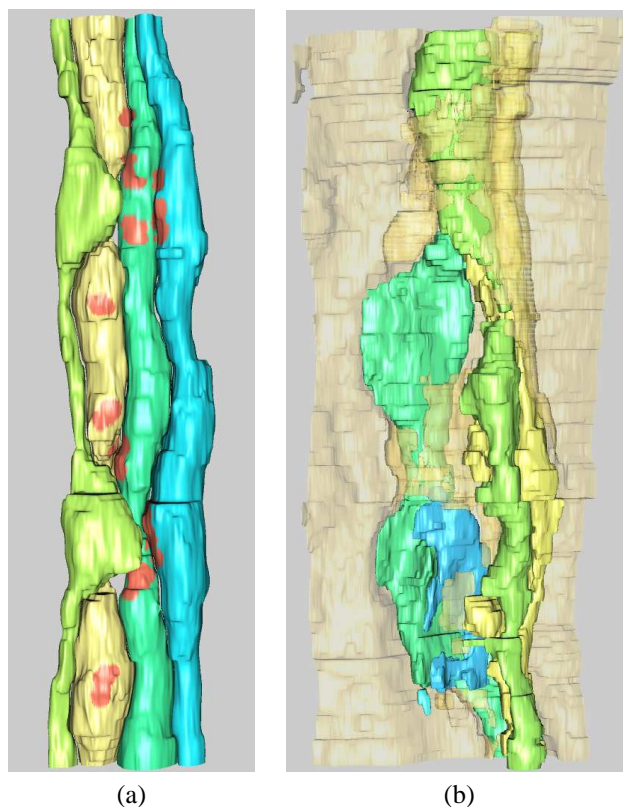


Fig. 1. (a) 3D renderings of the four neurons competing for information from the muscles. The location of the synapses, which were extracted from user specified locations, are shown in red on the neurons. (b) Similar rendering of the muscles that run alongside the motor neurons.

These data demonstrate that axons do not precisely interweave. GABA neurons run alongside a group of muscle arms and form multiple synapses to differing subsets of muscles before giving way to acetylcholine motor neurons. By contrast, the two types of acetylcholine neurons usually form contacts to the muscles simultaneously. Again, they form 2 to 3 contacts to the muscles for a segment of axon before giving way to the GABA motor neuron.

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