A PROTOCOL FOR THE USE OF ELECTRON MICROSCOPY IN STUDIES OF AXONAL PATHFINDING

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We have developed a protocol using electron microscopy for the analysis of axonal pathfinding in the ventral and dorsal nerve cords of *C. elegans*. We standardized our preparation technique, as described below, to compare animals of different genotypes to the wild type. For each genotype we embedded 30-40 animals, of which 10 animals were sectioned. We photographed the ribbons of sections and the individual worms at low magnification both to establish the orientation of the nerve cords (right, left, dorsal, ventral) and to determine the location of the section within the worm (e.g., at the gonadal reflex). We cut and examined two different points along the anterior-posterior axis of each worm at a distance separated by about 30 microns within a region between the gonadal reflex and the vulva, to compare defects within an animal and to check that our data were consistent within a single worm. From each worm and at each point, both dorsal and ventral cords were photographed at high resolution. Defects in axonal elongation were determined by counting the total number of axons, and defects in fasciculation were quantified by counting the number of axons separated from the main fascicles. Using this method we discovered synthetic nerve cord defects in *ced-10*; *mig-2* double mutants (see abstract by Reddien et al., for further information about these genes). We will present details of our techniques and representative pictures to demonstrate our methodology and results.