

Synaptic Ultrastructural Reconstruction Using Serial Electron Microscopy

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Abstract:

The goal of this project was to create a three-dimensional representation of the neuromuscular junction (NMJ), taken from a five-day and seven-day-old mouse, using serial transmission electron microscopy and the image-manipulation software Reconstruct. A reconstruction of the week-old NMJ helps us to understand the neuroanatomy of the target region prior to synapse elimination, an important process that occurs in the second week of post-natal nerve development [1, 2]. Practical use of thin section electron microscopy also gives us insight into a process we hope to automate.

Introduction:

The first few weeks of post-natal nervous development are marked by a process known as synapse elimination. This phenomenon, during which two or more existing axonal branches compete for control of one post-synaptic cell, ends the polyneuronal innervation established in pre-natal life [1]. At any given target cell, one axon becomes the sole input; all other axon branches retreat by releasing axonal materials into nearby glial cells or by other forms of degeneration [3]. Though individual postsynaptic cells are losing innervating axons, it is important to note that the winning axons create enough new contacts to offset any net loss in synapses [1]. On a broader level, there is evidence that synaptic elimination plays a role in learning and memory formation.

In this study of the NMJ, we use serial transmission electron microscopy (TEM), because it offers a level of resolution and detail of cellular structures not afforded by light-microscopy methods. Though synaptic competition occurs in many areas throughout the nervous system, researchers frequently use samples of the NMJ for its easy accessibility [3]. This project uses NMJ samples extracted at post-natal five (P5) and seven (P7) days. The reconstructions will show the neuroanatomy in the vicinity of a NMJ prior to axon removal, when some or most muscle fibers are still innervated by more than one motor axon.

Methods and Procedures:

Imaging of the NMJ using serial TEM requires a series of time- and labor-intensive steps which cover sample preparation, the acquisition of images with TEM and CCD

cameras, and image reconstruction.

A sample epoxy block embedded with muscle tissue was cut into ultra-thin sections of 50 nm using a Leica UCT ultramicrotome and diamond knife. Meticulous technique was essential for collecting the thin sections and preserving continuity in their sequence. The delicacy of both the sections and grids made it difficult to bring both components through the preparation process intact.

Section-staining with uranyl acetate and lead citrate is a well-known technique used to improve the TEM imaging of skeletal muscle fibers. The dense electron clouds of the heavy metal atoms interact with the TEM electron beam to produce a detailed, high-contrast image [4]. Stain-prepared grids were viewed under the TEM, where target structures were pinpointed, captured with a CCD camera, and imported into Reconstruct.

The computer algorithms of Reconstruct allowed us to crop, montage, scale, and realign images from serial electron microscopy [4]. In addition to adjusting for spatiotemporal differences in images, Reconstruct also fixed problems associated with specimen and optical distortions [4]. Completed 2D sections, cross-sectional profiles of the specimen, were stacked on top of each other to mimic the original orientation of the sample in three dimensions.

Results and Conclusions:

While sectioning through the P7 sample, we troubleshooted problems associated with the preparation of ultra-thin sections for electron microscopy. In the first few weeks, we improved the quality and readability of our sections by introducing heavy metal dyes and semi-thin survey sections. The semi-thin sections, which were 750 nm thick and easier to prepare, afforded us a quick survey image of our tissue cross-section under the high magnification of an optical microscope. We were then able to find a promising region around which we fine-trimmed the specimen block face. Because the specimen was so information-dense, we could not properly identify a NMJ under the TEM. To solve this problem, we used the focused ion beam (FIB) to etch fiducial marks on the surface of our specimen block. This allowed us to orient ourselves in the section geography around a reference point, which was especially useful under the high magnification of the TEM and in Reconstruct. Trimming the block face into an unsymmetrical trapezoid

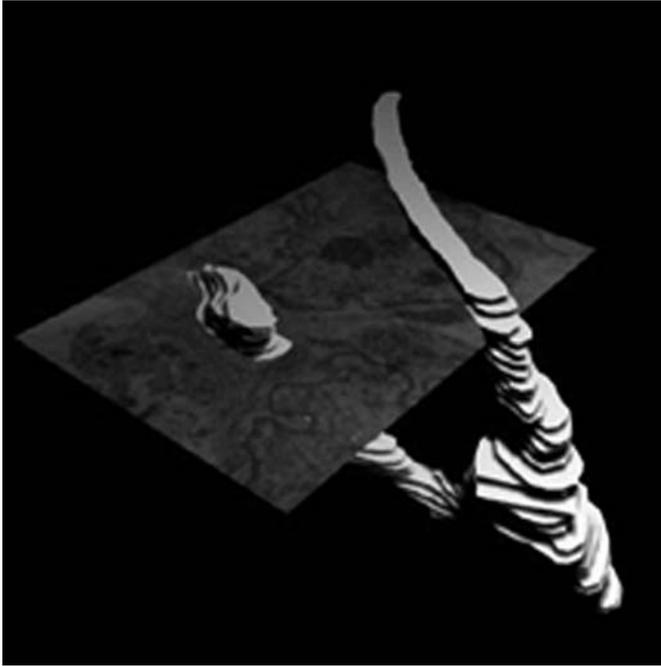


Figure 1: 3D reconstruction of axonal branch, superimposed onto corresponding electron micrograph.

also aided in the identification of section orientation.

An axon from 45 sections—previously prepared, imaged, and montaged—was reconstructed for proof-of-concept. Figure 1 shows the 3D axon reconstruction superimposed onto one of the 2D electron micrographs from which it originated. Additional reconstruction is possible and intended with the other structures found within this stack of data.

We also began work on the P5 sample. After prominent axon bundles were found on survey sections, we prepared ultra-thin sections from the P5 block and viewed them under the TEM. At present, at least one NMJ and many axon profiles have been found in both the optical and TEM images. The myelin sheath, which encapsulates the axon cross-section, makes a distinct boundary around the axon cross-section, which allows for easy identification. The presence of the sheath around most of the observed axons also indicates that we are imaging parts of the branch away from the NMJ. As we cut and image deeper into the sample, we should be able to witness some axons making synaptic contacts on muscular fibers. Figure 2 locates these notable regions on the P5 TEM image. Using these leads to specify our search, collection, and eventual 3D reconstruction will be the next endeavor.

Future Work:

In the immediate future we plan to fully reconstruct NMJs found in the P5 sample. Ultimately, the full 3D reconstruction of the NMJ ultrastructure will yield a great

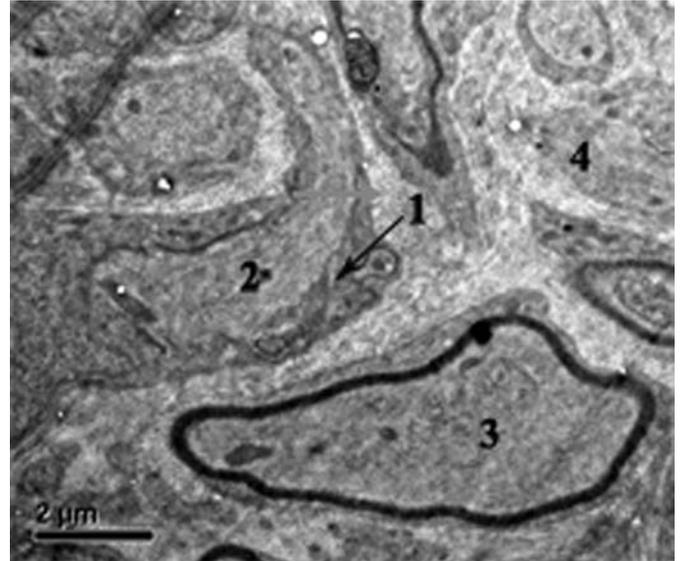


Figure 2: 1. NMJ site at interface of light-gray axonal profile and distinctive pattern of muscular tissue; 2. cross-section of axon contacting muscle fiber; 3. profile of proximal axon, which is not yet contacting muscle fiber; 4. surrounding cellular and sub-cellular material, including the organelles of Schwann cells and capillaries.

deal of anatomical information about this region prior to synaptic elimination. While we expect to see polyneuronal innervation at so early a post-natal stage, the detail of 3D reconstructions using electron micrographs will give us confirmation. In addition to improving the quality of collected data, the procedural solutions and experience with ultra-thin sections for serial TEM will eventually allow us to automate this useful, but labor-intensive process.

Acknowledgments:

Thank you to Prof. Jeff Lichtman and Dr. Richard Schalek for their expert guidance and enthusiasm throughout this summer. Thank you also to the Lichtman lab—particularly Kate Mahoney, Bobby Kasthuri, and Ju Lu—for their time, information, and help. This program would not have been possible without the Harvard CNS, NNIN, and NSF.

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