Electron Microscopy of the neuromuscular junction:

NMJs can also be visualized by transmission electron microscopy for a more detailed look at pre- and postsynaptic anatomies.

1. Muscles should be prepared for electron microscopy using standard techniques, including rapid fixation (preferably by perfusion) with glutaraldehyde-based fixatives.
2. The muscle should be dissected free and trimmed for cross sections at the point where the nerve enters the muscle. The endplate band is only a narrow region near the middle of the muscle (*see* **Note below**).
3. Samples should be post-fixed, osmicated, and embedded in plastic, cross-sectioned, and mounted on EM grids using standard procedures (for example, (1)).

The challenge to viewing sections is finding NMJs. They can be spotted by scanning the grids at 10-12K magnification and concentrating on areas where axons, fat, or blood vessels are also present. NMJs are rarely found in areas where the muscle fibers tightly stacked. Detailed images can be obtained at 30-60K magnification.

Normal NMJs have a generally polarized nerve terminal with accumulations of 40-50 nm small clear vesicles near the presynaptic membrane and mitochondria located farther away (Fig. 1). In mice, the terminal Schwann cell capping the nerve terminal can be difficult to resolve. The postsynaptic membrane has a series of junctional folds invaginating into the muscle fiber. At the mouth (crest) of each fold, the membrane appears electron dense because of the accumulation of AChRs. The synaptic cleft is pronounced and contains a visible basal lamina. Pathological deviations include an absence of junctional folds, partial innervation (folds without an overlying nerve terminal), and vacuolated mitochondria. Assessing more subtle defects, such as changes in vesicle number, requires a statistical analysis on many junctions.

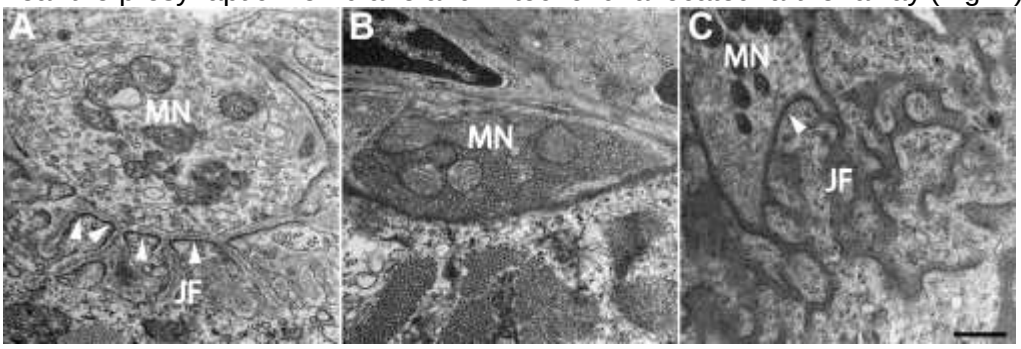

Fig. 1 - Neuromuscular junctions analyzed by transmission electron microscopy . (a) In wild-type mice, the motor nerve terminal (MN) is depressed into the muscle fiber surface. The terminal is polarized, with small clear vesicles near the presynaptic membrane and mitochondria in the more proximal portion of the terminal. The postsynaptic membrane has deep convolutions (junctional folds, JF) and the membrane near the tops of these folds is very electron dense because of the high density of acetylcholine receptors (arrowheads). (b) In some myasthenias where the nerve sprouts but remains in contact with the muscle, terminals with mitochondria and vesicles are observed in the absence of any postsynaptic specialization. Presumably these are sprouting terminals that have not established a functional connection. (c) Partial innervation of postsynaptic sites is evident as elaborate junctional folds in the muscle membrane with no overlying nerve terminal. In these examples, the interpretations were aided by light microscopy examination of other samples as described in Fig. 8 in parallel with electron microscopy . The mutation shown in (b), (c) is an unpublished ENU-induced allele of agrin

Note: *It is better to lose some NMJs than to waste a lot of effort sectioning regions of the muscle where there are no synapses.*

References

1. Patton BL, Cunningham JM, Thyboll J, Kortesmaa J, Westerblad H, Edstrom L et al (2001) Properly formed but improperly localized synaptic specializations in the absence of laminin alpha4. Nat Neurosci 4:597–604