Axon analysis

The femoral nerve provides an excellent system for evaluating peripheral neuropathy when there is hind limb involvement. In combination with counts of ventral roots, femoral axon counts can be used to distinguish peripheral neuropathy from motor neuron death. The nerve has a primarily motor branch that innervates the quadriceps, and a primarily sensory branch that becomes the saphenous nerve more distally **(Fig. 1**).
Each branch can be easily dissected free (the animals can be transcardially perfused before, or the nerves can be fixed by immersion after dissection).

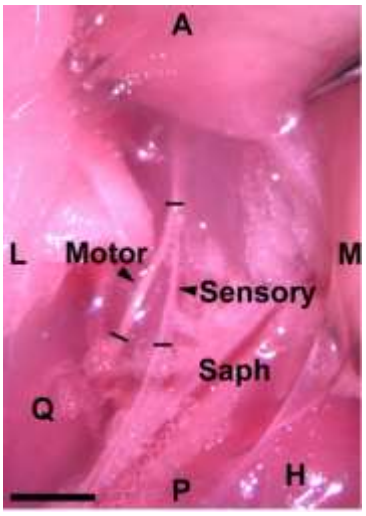

Fig. 1 Femoral nerve dissection. The motor and sensory branches of the femoral nerve are exposed. The mouse is supine and the right hip is shown (forceps are retracting the abdominal wall, A, P, M, L are anterior, posterior, medial, and lateral respectively, H is hamstring muscles). Some adipose tissue has been removed for clarity. The motor branch of the femoral nerve innervates the quadriceps (Q). The sensory branch becomes the saphenous nerve, which runs adjacent to the saphenous vein (Saph) on the medial side of the thigh. Dissecting the nerve where the tick marks provides a reasonable length of nerve to work with. Note the sensory branch sometimes runs as two fascicles and both should be taken to get

1. Nerves should be plastic embedded and cross-sectioned.
2. Axons can be counted from Toluidine Blue stained sections.
3. The distribution of axon diameters, myelin thickness, and G-ratios (inner/outer diameters, the inner being the axoplasm, the outer including the myelin) can also be determined. This may be done most accurately by low magnification (4000-6000X) transmission electron microscopy.
4. The assessment of axon diameters may reveal general axonal atrophy, or a missing class, such as large diameter, fast motor axons.
5. The assessment of myelin layering and myelin thickness may reveal a demyelinating or hypomyelinating neuropathy.
6. The G-ratio may indicate abnormal reciprocal signaling between the axon and the myelinating Schwann cell, or may highlight thin myelin or conversely, thin axons, since there is normally a rough correlation between axon diameter and myelin thickness.
7. Other peripheral nerve pathologies such as onion bulbs (indicating rounds of demyelination/remyelination), Schmidt-Lanterman Incisures (indicating abnormal myelin packing), myelinating Schwann cells wrapping multiple axons, and bundles of regenerating axons can also be seen in these sections. See examples **Fig. 2** and references such as (1,2).

Internodal Distance

In addition to axon loss/atrophy and defects in myelination, the intermodal distance can also affect nerve conduction velocities.

1. To determine intermodal distance, dissect a 1-2 cm segment of peripheral nerve such as the femoral or sciatic, and fix as above.
2. Tease the nerve longitudinally to individual fibers using No. 5 forceps or a 30-gauge needle. Keeping the nerve immersed in a drop of PBS, tease the nerve directly on a microscope slide.
3. Coverslip the teased nerves and view using Nomarski-DIC optics.
4. Measure internodal distances and correlate them with axon diameters.
5. Again, there should be a rough correlation, with larger axons having longer internodal distances. This analysis requires software calibrated for digital image analysis to determine the distances (**Fig. 2**).

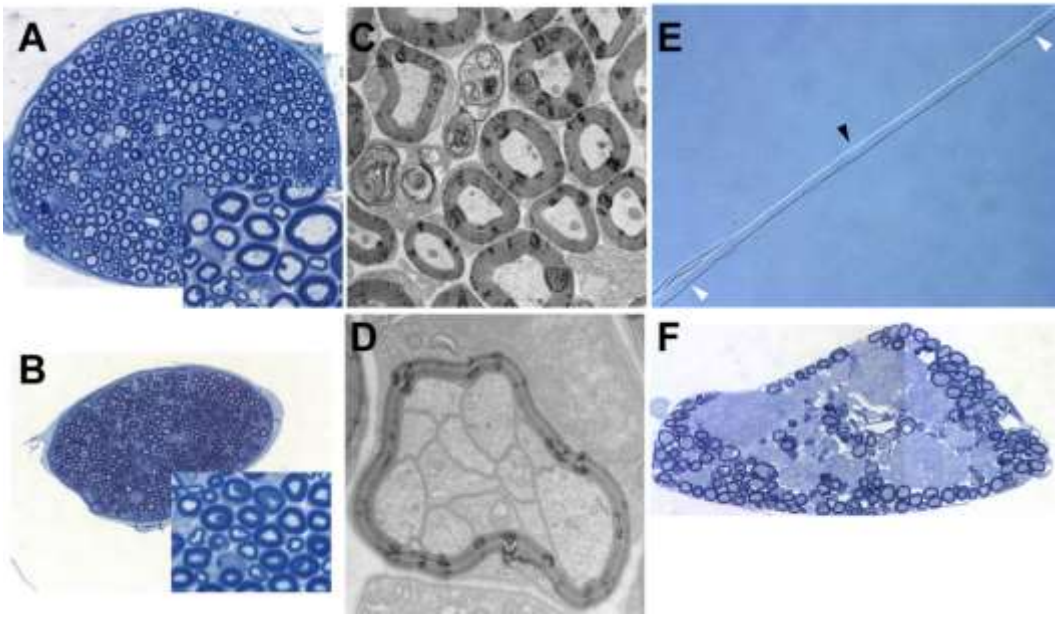

Fig. 2 Peripheral neuropathy phenotypes . A cross section of the motor branch of the femoral nerve in a control mouse (a) and a Gars Nmf249/+ model of Charcot-Marie-Tooth 2D (b) are shown. The insets highlight the varied axon diameters in a control nerve and the almost complete absence of large diameter axons in the mutant nerve. (c) The same mutation examined by transmission electron microscopy demonstrates degenerating axon profiles. Note, the rregularities in the myelin are fi xation artifact and not pathology , highlighting the need to always process control samples in parallel. (d) A myelinating Schwann cell that has ensheathed multiple axons, probably representing a failure in radial sorting during early postnatal development. Note, this is different from a Remak bundle of small sensory axons, in which a non-myelinating Schwann cell enwraps a number of axons in a single basal lamina (see the bottom right corner of c). (e) Nodes of Ranvier can be examined by light microscopy in teased nerve preparations. The nucleus of the Schwann cell (black arrow) is typically midway between the nodes (white arrows). (f) An example of hypomyelination in the ventral root of a Lama2 dy/dy mouse. Normally, 100 % of the ventral root axons are myelinated. In this mutation , bundles of large but unmyelinated axons are evident.

References

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