Direct imaging of spinal cord axons in intact lamprey by diffusion-weighted µ-MRI

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Introduction

The larval sea lamprey (*Petromyzon marinus*) is a long-standing research model for spinal cord injury (1-2), exhibiting spontaneous and robust axonal regeneration after cord transection. The animal's small size (Fig. 1) and large reticulospinal axons (20-40 µm) make it ideal for MRI studies of the mechanisms of axonal degeneration and regeneration, and of growth promotion therapies within the native environment, if individual axons could be clearly resolved. While axons have been resolved by µ-MRI in excised lamprey cord (3), with white matter-axon contrast generated by diffusion weighting (4), visualizing axons *in vivo* remains a challenge (5) and will require methods to increase SNR. Nevertheless, as a step toward feasibility, we present here the first evidence of axons clearly resolved by µ-MRI in a lamprey spinal cord still within the body of the animal.

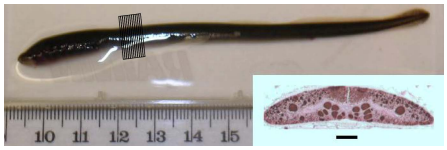

Fig. 1. Larval lampreys (~ 5 mm dia.) can fit in a small RF coil for high SNR. MRI locations are shown. Inset: spinal cord cross-section with axons stained by neurofilament immunohistology (bar = 100 µm).

Methods

For these experiments a lamprey larva (4-5 years old) was euthanized in tricaine and fixed overnight in 2% paraformaldehyde + PBS. The lamprey was ~ 10 cm long and < 5 mm diameter (Fig. 1) and fit in a 5 mm NMR tube after removing the head and gills. This allowed use of a 5 mm dia. RF birdcage coil for high SNR. The µ-MRI scans were performed on a 9.4 T vertical-bore NMR microimaging system: an Avance DMX400 (Bruker-Biospin, Inc.) with Micro2.5 tri-axial gradients (100 G/cm peak) and BAFPA40 amplifiers. Both spin echo (SE) and gradient echo (GRE) sequences were used without explicit bipolar diffusion gradient pulses, yet the imaging gradients produced intrinsic diffusion weighting in each sequence by different amounts, with effective *b*-values generated mostly by the read gradients perpendicular to the long axis of the cord. Specific parameters were for **SE:** TE = 16.9 ms, *b*-value = 1109 s/mm^2, 12 slices, 500 µm thick; and for **GRE:** TE = 7.3 ms, 60° flip, *b*-value = 20 s/mm^2, 16 slices, 250 µm thick. Remaining parameters were identical for both: TR = 2 s, FOV = 5x5 mm^2, matrix = 512x512, BW = 50 kHz, in-plane resolution = 10x10 µm^2, 64 averages, scan time ≈ 18 hrs. Image data were zero-filled to 5x5 µm^2 in-plane resolution using IDL (ITT Corp.). Additional image processing, including volume-rendering, was performed using ImageJ (NIH) and OsiriX (www.osirix-viewer.com).

Results and Discussion

Transverse high-resolution MR images of a lamprey are shown in Fig. 2, in which (a, d) show the full field of view, and (b, e) are zoomed regions of the spinal cord (S) dorsal to the notocord (N). Also clearly visible are the lateral line nerves (L) and fiber striations in the body musculature. In the spin echo µ-MRI of Fig. 2b, individual axons are resolved, including Mauthner and Müller giant axons as well as smaller axons throughout the white matter (WM). Well-differentiated gray matter (GM) also can be seen as a central "M" pattern of the neurons. There is thus a high degree of resemblance with histology (Fig. 1). Resolution, SNR (~ 16) and contrast were sufficient to volume render the largest axons (Fig. 2c). In the gradient echo µ-MRI of Fig. 2e, however, axons are not resolved, even though WM SNR is also ~ 16 and there is some GM-WM contrast producing a faint "M" pattern.

The cause of intra-axonal signal loss and the high axon-WM contrast in Fig. 2b is likely a relatively higher apparent diffusion coefficient (ADC) of water in the axons than in WM, resulting in greater signal decay in the axons. Lamprey WM is composed of fine-caliber non-myelinated ascending and descending axons within a glial cell matrix. It has been shown that for a PGSE diffusion time Δ = 11.2 ms, ADC is isotropic within large lamprey axons but highly anisotropic in the surrounding WM, and furthermore that T2-weighting at long TE (21.5 ms) is not a major contributor to axon-WM contrast (4). Since the effective read axis diffusion time in the spin echo sequence of Fig. 2 also was ~ 11 ms, we attribute the axon-WM contrast as mostly due to this difference in ADC's perpendicular to the cord.

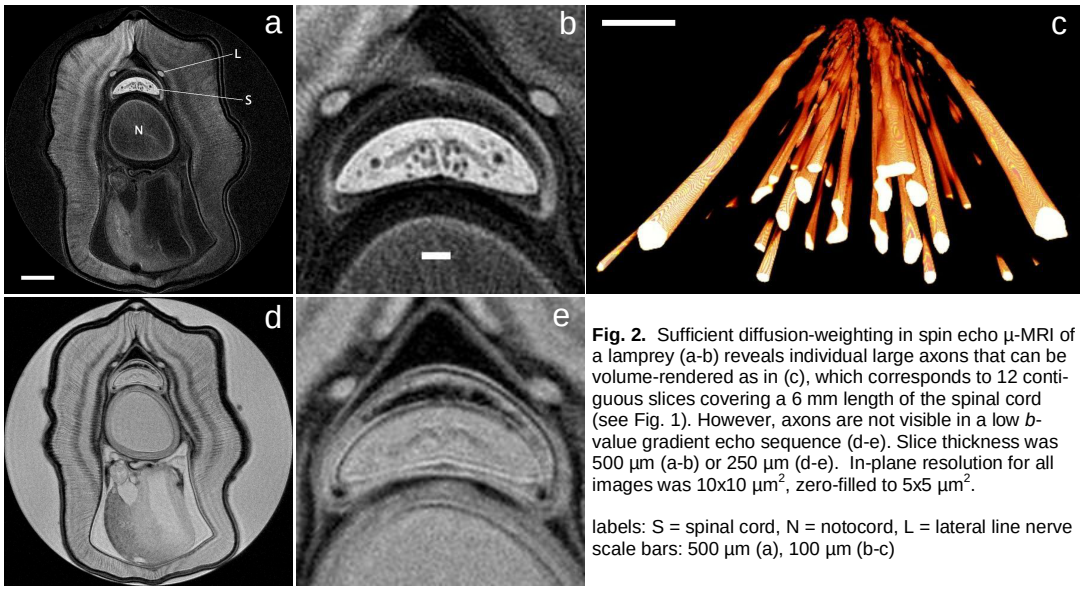

Fig. 2. Sufficient diffusion-weighting in spin echo µ-MRI of a lamprey (a-b) reveals individual large axons that can be volume-rendered as in (c), which corresponds to 12 contiguous slices covering a 6 mm length of the spinal cord (see Fig. 1). However, axons are not visible in a low *b*-value gradient echo sequence (d-e). Slice thickness was 500 µm (a-b) or 250 µm (d-e). In-plane resolution for all images was 10x10 µm^2, zero-filled to 5x5 µm^2.

labels: S = spinal cord, N = notocord, L = lateral line nerve
scale bars: 500 µm (a), 100 µm (b-c)

In conclusion, results presented here suggest the feasibility of *in vivo* µ-MRI of spinal cord axons in lamprey. High-resolution spin echo cross-sectional images (voxel size = 10x10x500 µm^3), acquired in 18 hrs, demonstrated clearly resolved large axons (10-40 µm diameter) and excellent detail of anatomical structures. These data represent the first direct visualization of axonal architecture in a non-excised lamprey spinal cord by µ-MRI. For *in vivo* imaging, a scan time no longer than 2 hrs would be required to avoid motion artifacts and limit the dose of anesthesia. This would require an SNR gain of a factor of $\sqrt{(18/2)}$ = 3x. While this will be difficult to achieve, it may be possible using a 5 mm solenoidal RF coil (no need to remove the head and gills) together with optimization of the pulse sequence. Higher field strength also would be beneficial. The longer T1 and T2 of fresh vs. fixed tissue may have compensatory effects at the relatively long TR/TE used here. Furthermore, thinner slices may be possible with a 3D sequence. The prospects therefore are promising for serial measurements evaluating recovery from injury in the same animal.

Acknowledgements

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References

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