In Vivo MRI of Lamprey Spinal Cord

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Introduction

Larval sea lampreys (*Petromyzon marinus*) have been used for some time as a model for axonal degeneration and regeneration following spinal cord trauma (1, 2), since, unlike those in mammals, axons in the spinal cords of these animals have the ability to regenerate spontaneously following injury. During such studies, micro-architectural changes accompanying axonal injury and regeneration are typically traced histologically. Recent applications of MR microscopy, however, have demonstrated direct visualization of the axon tracts in excised lamprey spinal cord (3), and similarly diffusion measurements in excised lamprey cord have shown regional differences in diffusion anisotropy (4). This suggests the possibility of imaging the progression of the largest axons (10-40 μm diameter – Müller and Mauthner fibers), either directly or by changes in diffusion anisotropy, during recovery from spinal cord transection in a single live lamprey over time, provided sufficient SNR and contrast can be achieved. Here we report the application of MR microscopy techniques toward imaging the spinal cord in a live lamprey and present initial results.

Materials and Methods

Animal Preparation

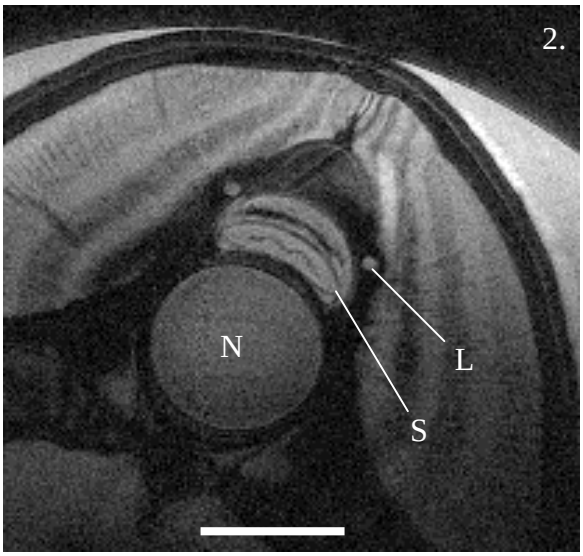
Lamprey larvae (4-5 years old), prior to transformation to adult stage, are about 10 cm long and about 1 cm in diameter and thus could be supported in a vertical 1 cm diameter sample tube (Fig. 1). Anesthesia was accomplished by immersing an animal in a 0.1% tricaine solution for approximately 10 min., followed by transfer to a lamprey Ringer's solution also used in the imaging experiment. This anesthesia protocol resulted in a sedation lasting about 1 hr.

MR Microscopy

Imaging was performed on a 9.4 T vertical bore spectrometer/microimaging system (Avance DMX400, Bruker-Biospin, Inc.) equipped with 100 G/cm peak tri-axial gradients. To achieve high SNR, a small transmit/receive surface coil, consisting of a 400 MHz single copper loop (8x9 mm^2), was constructed and interfaced with the vendor's RF insert (Fig. 1). Optimal coil size was determined from a calculation of SNR vs. coil radius using a quasi-static approximation for RF losses. A fixed tuning capacitor and two variable matching capacitors are located below the coil. The coil was mounted on a plastic tube concentric to the sample tube holding the animal. This allowed free positioning of the animal so that its dorsal surface (the most shallow location of the spinal cord) was within the coil's sensitive region and held near to the coil with a plastic spacer in the sample tube. The anesthetized animal was nearly motionless, nevertheless a 2D gradient echo sequence was used to rapidly acquire 16 high-resolution images in 12.8 minutes to avoid potential motion artifacts and minimize anesthesia requirements.

Results and Discussion

An axial MR image of a live lamprey, in which the spinal cord (S) is clearly visible just above (dorsal to) the notocord (N), is shown in Fig. 2. Exquisite detail can be seen, including the lateral line nerves (L) and fiber striations in the body musculature. In the spinal cord, while individual axons were not resolved, good contrast was achieved between gray and white matter, clearly revealing the central "M" pattern of the gray matter neurons and the surrounding "white matter", composed of non-myelinated ascending and descending axons within a glia cell matrix. There is very little evidence of motion artifacts, however at this resolution (9.7x9.7x250 μm^3) significant chemical shift artifact was seen for the epidural fat pad just dorsal to the spinal cord. While the fat signal in Fig. 2 had mostly decayed at the long TE used here, fat signal overlap onto the spinal cord region can be avoided by judicial choice of the frequency-encode direction. An axial histological section through a lamprey spinal cord (Fig. 3) demonstrates anatomical correspondence with the MRI of Fig 2, in particular the gray matter "M" pattern.



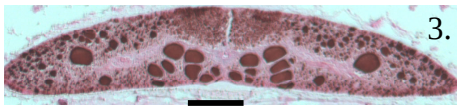


Fig. 1 Photograph of lamprey sample tube and 400 MHz RF surface coil, attached to the RF probe insert. Scale bar = 10 mm.

Fig. 2 Axial gradient echo image (cropped) of a live lamprey, showing the spinal cord (S) with a characteristic "M" pattern of the central gray matter, the notocord (N), and lateral line nerves (L). Scan parameters: 512 μs sinc RF pulse, 1x1 cm^2 FOV, 512x512 matrix, TE = 11.2 ms, TR = 1500 ms, 16 slices, 1 NEX, scan time = 12 min. 48 sec. Voxel = 9.7 x 9.7 x 250 μm^3. Scale bar = 1 mm.

Fig. 3 Immunohistochemical stain for neurofilaments in a lamprey spinal cord cross section, showing the "M" shape of the gray matter, surrounded by "white matter" consisting of axons perforating a glia cell scaffold. Scale bar = 0.1 mm.

In conclusion, results presented here demonstrate the feasibility for in vivo MR microscopy of the spinal cord in sea lamprey eels. A custom-designed 400 MHz coil is described and MR micro-images are shown of an anesthetized lamprey. High-resolution gradient echo cross-sectional images (voxel size = 9.7x9.7x250 μm^3), acquired in reasonable scan times, demonstrated high SNR and excellent detail for anatomical structures. Such techniques will facilitate longitudinal studies of spinal cord injury and regeneration in this well-characterized animal model.

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References

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