Labeling Transected Axons with Intracellular Gd-DTPA for Micro-MRI of Lamprey Spinal Cord Injury

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Introduction

A well-characterized model for spinal cord injury research is the larval sea lamprey (*Petromyzon marinus*) (1). It is useful for studying repair as its axons spontaneously regenerate even after complete cord transection, accompanied by sensorimotor recovery. Although its axons are non-myelinated, the animal's small size makes it ideal for studying inhibition of acute axonal dieback and chronic Wallerian degeneration, in addition to growth promotion therapies. The large (20-40 μm) reticulospinal axons can be retrogradely labeled with fluorescent dyes and optically traced histologically and in vivo (2). Yet, limitations of these techniques prohibit detailed longitudinal study of axonal injury response within the native environment.

Recent advances in μ-MRI, however, have achieved sufficient SNR to clearly resolve individual axons in excised lamprey cord (3), with white matter (WM)-axon contrast manifesting at high in-plane resolution due in part to read gradient diffusion weighting (4). However, visualizing axons *in vivo* remains a challenge (5) and will require methods to increase SNR. In addition, a means to selectively identify injured or regenerating axons is desirable. For these reasons, we have investigated a novel method to essentially invert the WM-axon contrast by labeling severed axons at the transection site with the MRI contrast agent Gd-DTPA. A strongly T_1-weighted spin echo pulse sequence with very short TR then could be used to acquire the short-T_1 intra-axonal signal while the long-T_1 WM signal is effectively saturated. A similar concept recently was demonstrated by G. A. Johnson, et al., who obtained a 6-fold SNR gain for short-TR 3D spin echo μ-MRI of a whole mouse, perfusion fixated with formalin and Gd-DTPA (6).

Materials and Methods

In six separate animals, spinal cords were transected at the 5th gill and axons labeled by blotting the transection site (TX) for 30 minutes with Gelfoam soaked in 100 or 500 mM Gd-DTPA (gadodiamide, Omniscan, Nycomed, Inc.), or control Ringer's solution. Severed axons can remain open for 30 min before their membranes spontaneously re-seal, a process mediated by Ca^{++} ions (7). Animals were returned to fresh water tanks and allowed to recover for 5 days, providing opportunity for retrograde active transport toward the brain of any Gd-DTPA which was taken up by the severed axons. At 5 days, the spinal cords were excised with the full brain, fixed in 2% paraformaldehyde overnight, and placed in a 1 mm capillary in PBS for μ-MRI, using a 1.5 mm dia. solenoidal RF coil as described in (3). The μ-MRI experiments were carried out on a commercial 9.4 T vertical-bore (89 mm) NMR microimaging system (Bruker Avance DMX400 with Micro2.5 tri-axial gradients and BAFPA40 amplifiers).

Results

Two image sets in Fig. 1 show 0, 100, 500 mM-labeled cords from two anatomic locations: a) 0.5 mm caudal to the 4th ventricle of the brain and 7.5 mm rostral to the TX, b) 2 mm caudal to the 4th ventricle and 6 mm rostral to the TX. In the 100 mM-labeled cord, some Müller axons in the ventral column appear brightened, while others are dark. There is also brightening in the dorsal column, consisting of fine, densely-packed axons.

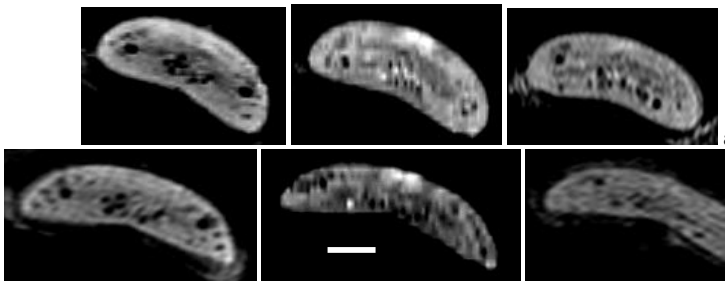

Fig. 1. 3D spin echo μ-MRI of excised lamprey cord: (a) 7.5 mm rostral to TX; (b) 6 mm rostral to TX.

Left: 0 mM Gd-DTPA (control Ringer's solution)
Middle: 100 mM Gd-DTPA labeling at the TX
Right: 500 mM Gd-DTPA labeling at the TX

TR = 50 ms, nominal voxel = 10x20x172 μm^3, zero-filled to 5x5x86 μm^3. Frequency-encode axis horizontal in all. Scale bar = 100 microns.

Additional results are shown in Fig. 2 demonstrating Müller axons labeled using 100 mM Gd-DTPA. In the left image, there is an apparent 3-4 pixel shift along the frequency-encode axis (vertical). A reformatted longitudinal section from the 3D μ-MRI data set reveals two Müller axons clearly labeled, while larger Mauthner axons above and below appear dark, indicating possibly too much labeling or the lack thereof.

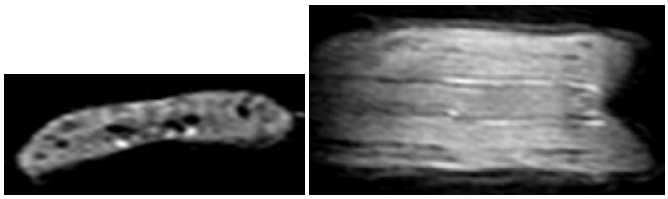

Fig. 2. 3D spin echo μ-MRI of excised lamprey cord, 6 mm rostral to TX labeled with 100 mM Gd-DTPA.

Left: cross-section, showing bright axons shifted along vertical frequency-encode axis.
Right: reformatted longitudinal section, showing Müller axon traces.

Discussion

In this preliminary study, only two or three large axons appear to have been labeled with Gd-DTPA. This might be due to premature closing of the open transected axon ends, induced by Ca^{+2} ions in the applied buffer solution. An initial experiment adding EDTA as a Ca^{+2} complexing agent gave more robust labeling (Fig. 2). Also, 100 mM Gd-DTPA, if taken up undiluted by an axon, could drastically reduce T_1 and T_2 to the point where no signal would be detected. At present, we do not know the intra-axonal concentration of Gd-DTPA. It has been demonstrated, however, that Gd-DTPA does not cross cell membranes (8), and so once axons have re-sealed after transection (~ 30 min), whatever Gd-DTPA was taken up by an axon will likely remain. We assume Gd-DTPA, a relatively small molecule (MW=574), will be distributed in the axoplasm by active transport, but this has yet to be determined. The intra-axonal Gd-DTPA concentration might be quantifiable via the induced chemical shift artifact (CSA, in pixels), expected for axons labeled with Gd-DTPA. This can be estimated for the 3D spin echo (195 Hz/pixel), given the molar magnetic susceptibility of Gd-DTPA-BMA (3140 x10^{-10} m^3/mol) (9). Assuming the molar χ for WM and water = -1.64 x10^{-10} m^3/mol, then for a cylindrical axon perpendicular to the static field B_0, the maximum field perturbation ΔB_Z inside the axon is given by $\Delta\chi\ B_0/2$. This results in a CSA = 323 pixels for 1 M Gd-DTPA, and thus 3 pixels for 10 mM Gd-DTPA.

Acknowledgements

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