Diffusion tensor tractography identifies demyelination and remyelination in the spinal cord of a mouse model of multiple sclerosis

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Target audience: Those using diffusion tensor tractography (DTT) for assessing demyelination/remyelination, particularly in the spinal cord.

Purpose: To show that DTT is useful for assessing lesions in mouse models, and to determine if DTT can be used to differentiate a demyelinating lesion from a lesion that is undergoing remyelination.

Methods: We induced focal demyelination in C57BL/6 mice by injecting lysolecithin in the dorsal column of the spinal cord between T3 and T4.[1] Mice were sacrificed at d7 and d14 post-injection (n=7 for each time point). There is maximal demyelination at d7, while at d14 there is ongoing remyelination. Cords were fixed in 4% PFA then transferred to a 30% sucrose solution for at least 48 hours before imaging. The day of imaging, spinal cords were rinsed to remove sucrose then were incubated in a 1:200 solution of gadolinium:PBS for 45 minutes to enhance grey/white matter contrast. Spinal cords were embedded in agarose gelatin using a method described previously[2] in a syringe which fit 4 spinal cords. A DTI EPI sequence was used on a 9.4T Bruker Avance console with a 35mm volume coil (matrix=128x128, FOV=1.5cmx1.5cm, TE/TR=35.66/5000ms, flip angle=90°, 15 directions, 4 segments, NEX=12, slice thickness=0.5mm). MedINRIA software (version 1.9.4, France) was used for analysis. The fractional anisotropy (FA) threshold was set at 300 (corresponding to an actual FA value of 0.300), minimum length at 15, smoothness at 20 and sampling at 1. ROIs were drawn on the lesion area and on the area superior to the lesion area (internal control) from which we obtained the following scalar measures: FA, apparent diffusion coefficient (ADC), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD). Independent t-tests were used to compare the control area and the lesion area for each of the five scalar measures.

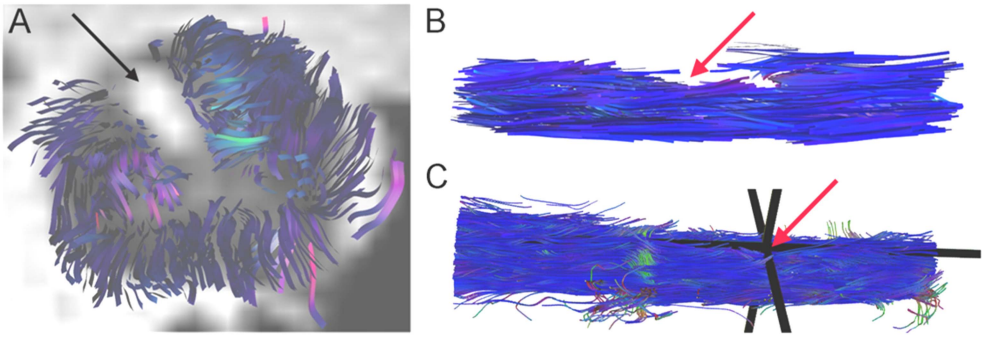

Figure 1. DTT differentiates a demyelinated lesion in d7 lysolecithin spinal cord from a lesion that is undergoing remyelination in d14 lysolecithin spinal cord. A provides the axial view of a lesion at d7 where the loss of myelin is visible with a gap in tractography in the lesion area (black arrow). **B** shows a d7 spinal cord lengthwise, where the lesion area is devoid of white matter tracts (red arrow). **C** shows a d14 spinal cord, where the lesion can be seen (red arrow) only when the 3 axes are present. By d14, the lesion area contains more white matter tracts than were seen in the d7 spinal cord.

Table 1. Scalar measures for d7 lysolecithin spinal cords comparing control area to lesion area.

Scalar measure	Control area (mean ± SEM)	Lesion area (mean ± SEM)	p value
FA	0.50 ± 0.06	0.21 ± 0.03	0.001
ADC (mm^2/s)	1.11 ± 0.20	2.93 ± 0.42	0.002
AD (mm^2/s)	0.56 ± 0.08	1.14 ± 0.16	0.006
RD (mm^2/s)	0.27 ± 0.06	0.89 ± 0.13	0.001
MD (mm^2/s)	0.37 ± 0.07	0.98 ± 0.14	0.002

Results: DTT of d7 lysolecithin spinal cord (**Fig. 1**) showed that no white matter tracts were present in the lesion area as seen on an axial slice (**Fig. 1A**). In the reconstructed tracts from d7, the lesion also appears as a large gap devoid of white matter tracts, indicative of demyelination (**Fig. 1B**). By d14, the lesion was less obvious with white matter tracts filling in part of the lesion area (**Fig. 1C**). For all five scalar measures, the lesion area was significantly different from the control area superior to the lesion area at d7 (**Table 1**). By d14 there was no significant difference between the control area and the lesion area for any of the scalar measures (data not shown).

Discussion: This is the first time DTT has been used to differentiate a demyelinated lesion from one undergoing remyelination in the lysolecithin model. The significant differences between the lesion area and the area superior to the lesion for FA, ADC, AD, RD, and MD observed in d7 spinal cords are reflective of demyelination and an overall loss of myelin integrity, as has been reported by others previously.[3] That these scalar measures are no longer significantly different when comparing the lesion area to the control area by d14 is likely due to remyelination.

Conclusion: DTT can be used to assess demyelination/remyelination in mouse spinal cord. It could be implemented in clinical trials for remyelinating therapies in MS to determine how effective such therapies are by visually observing their effects at the level of white matter tracts.

References:

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