Mouse spinal cord ventrolateral white matter diffusion anisotropy indices do not change in situ up to 10 hours postmortem

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Introduction

It has recently been reported that water diffusion anisotropy indices were preserved in mouse brain after perfusion fixation, a common practice in histological analysis of tissue morphology and pathology[1]. Furthermore, the *ex vivo* diffusion anisotropy indices obtained from fixed tissues showed pathological and structural correlations[2, 3]. Of course, the standard intra-cardiac perfusion fixation of rodents is not an option for human patients. Thus, effect of variable postmortem delay before immersion fixation of autopsy specimens is of interest. In the present study, we examined mouse spinal cords using DTI *in vivo*, *in situ* postmortem at various time points prior to fixation, and *ex vivo* immersion fixed at 10 hours postmortem. Diffusion anisotropy indices obtained from spinal cords at vertebral segments T12 – L1 were examined.

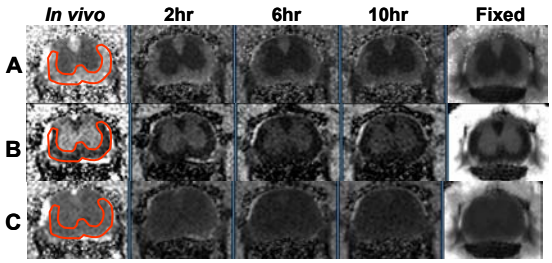

Fig. 1 Representative $\lambda_{||}$ (A), $\lambda_{\perp}$ (B), and trace (C) maps. Red line indicates ventrolateral white matter ROI. Same intensity scale is applied across the images in each row.

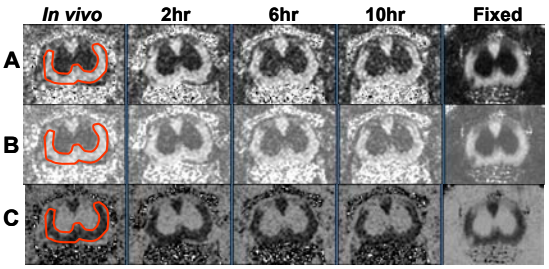

Fig. 2 Representative RA (A), $D_{||}$ (B), and $D_{\perp}$ (C) maps. Red line indicates ventrolateral white matter ROI. Same intensity scale is applied across the images in the same row.

Methods

Diffusion Tensor Imaging

A cohort of five normal C57BL/6 mice were imaged *in vivo* then sacrificed for *in situ* measurement. Following postmortem *in situ* measurements, all cords underwent immersion fixation with 10% formalin in phosphate buffered saline and were reexamined. DTI experiments employed Oxford/Magnex/Varian outfitted 4.7T MR scanners. A conventional spin-echo imaging sequence was modified by adding Stejskal-Tanner diffusion weighting gradients with respiratory gating control. The same acquisition parameters were used for all DTI measurements except diffusion-sensitizing factors (b = 0.785 ms/μm^2 - *in vivo*, b = 1.813 ms/μm^2 - *in situ* and *ex vivo*) to compensate for the decreased postmortem ADC.

Data analysis

The eigenvalues (λ_1, λ_2, and λ_3) and eigenvectors of the diffusion tensor were calculated from each diffusion-weighted image and tensor element map. On a pixel-by-pixel basis, trace of the diffusion tensor (Tr(D) = $\lambda_1 + \lambda_2 + \lambda_3$), axial diffusivity ($\lambda_{||} = \lambda_1$), radial diffusivity ($\lambda_{\perp} = 0.5 \times (\lambda_2 + \lambda_3)$), trace-normalized axial diffusivity ($D_{||} = \lambda_{||} / \mathrm{Tr}(D)$), trace-normalized radial diffusivity ($D_{\perp} = \lambda_{\perp} / \mathrm{Tr}(D)$), and relative anisotropy (RA), which is defined by equation 1 were derived.

$$RA = \frac{\sqrt{(\lambda_1 - <D>)^2 + (\lambda_2 - <D>)^2 + (\lambda_3 - <D>)^2}}{\sqrt{3}<D>} \quad [1]$$

Where $<D> = \mathrm{Tr}(D)/3$. Parameters were derived from the ventrolateral white matter of each of the spinal cord segment (T12 – L1). Differences between postmortem and *in vivo* DTI measurements were examined using the Bland and Altman analysis[4].

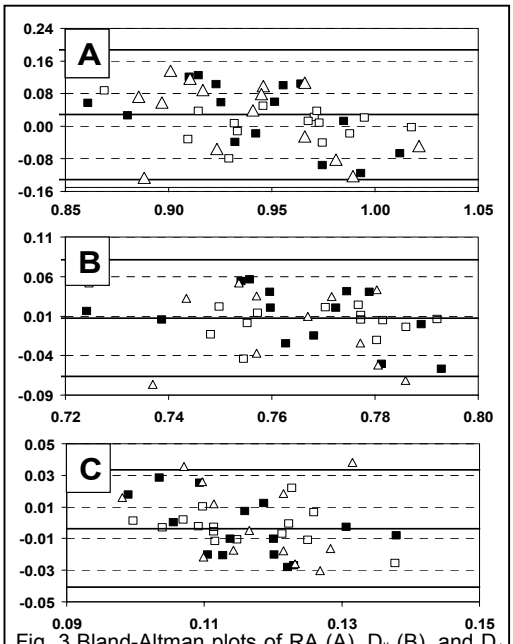

Fig. 3 Bland-Altman plots of RA (A), $D_{||}$ (B), and $D_{\perp}$ (C) demonstrate that measurements of diffusion anisotropy in mouse spinal cord *in vivo*, *in situ* 10 hours postmortem, and postmortem after immersion fixation are not different. The vertical axes represent the difference of two measurements. The horizontal axis is the average of two measurements. The center solid line is the mean difference of the measurements. The top and bottom solid lines mark the 95% confidence interval. Open squares represent comparison between *in vivo* and *in situ* 10 hrs postmortem. Filled squares represent comparison between *in vivo* and postmortem after immersion fixation. Open triangles represent comparison between *in situ* 10 hrs postmortem and postmortem after immersion fixation.

Results and Discussion

The *in vivo* $\lambda_{||}$ is about six times the value of $\lambda_{\perp}$, similar to a previous report[5]. Differences are observed in $\lambda_{||}$, $\lambda_{\perp}$, and trace maps of ventrolateral white matter between *in vivo* and postmortem *in situ* measurements (Fig. 1). This finding is consistent with the previous reports that water diffusion decreases by 50 – 70% after death and fixation[1]. Contrast between gray and white matter was preserved in $\lambda_{||}$ and $\lambda_{\perp}$ but not trace. The contrast between gray and white matter is excellent in $D_{||}$ and $D_{\perp}$ and RA and there was no apparent signal intensity difference in ventrolateral white matter (Fig. 2). There is no significant *in vivo vs.* postmortem differences in all diffusion anisotropy indices based on Bland-Altman ananlysis (Fig. 3). Trace, $\lambda_{||}$, and $\lambda_{\perp}$ all decreased significantly (~ 50%) after death *in situ* but remain unchanged at later postmortem time points. In contrast, anisotropy indices - including RA, $D_{||}$, and $D_{\perp}$ - did not change between in vivo and postmortem (*in situ* and/or fixed) states. In summary: anisotropy in cord ventrolateral white matter does not change up to 10 hours postmortem *in situ*.

References

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