# Characterization of the gray matter in spinal cords of Long Evans shaker rats by double-pulsed-field gradient MRI

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**Introduction:** Conventional single-pulsed-field-gradient (s-PFG) methodologies like DWI and DTI are capable of faithfully depicting diffusion anisotropy in coherently ordered structures, providing important structural information[1]. In cases where macroscopic organization is absent, microstructure is more difficult to characterize using these methods. Double-PFG (d-PFG) MR methodologies have been recently emerging as an alternative for studying microstructure in scenarios where macroscopic anisotropy is absent[2,3]. The Long Evans shaker (*les*) rats are considered a model of dysmyelination[4] and can be used to challenge new approaches that provide microstructural information. The white matter (WM) of the *les* spinal cords was recently studied by QSI[5]. Here, the spinal cords of the *les* rats and their controls were characterized by double-pulsed-field-gradient spin-echo (d-PGSE) MRI focusing on the gray matter (GM). The d-PGSE MRI results are compared with conventional DTI.

**Methods:** MRI experiments were conducted on a Bruker Avance-III 14.1T scanner capable of producing pulsed field gradients of up to 300 G/cm in each direction. Fixed ex-vivo control and *les* rat spinal cords of 33 days of age were immersed in PBS overnight and then placed in a 5 mm glass tube filled with Fluorinert, to assure fiber orientation along the z-direction. All d-PGSE MRI experiments were conducted with a slice thickness of 800 μm, a field of view (FOV) of 4.8x4.8 mm, and an in-plane spatial resolution of 50x50 μm$^2$. The angular d-PGSE MRI experiments were performed when the $G_1$ was fixed in the x-direction and the orientation of $G_2$ was varied in the x-z plane (d-PGSE$_{xz}$). The measurements were conducted for 13 different values of φ between $0^o$ and $360^o$. The images were collected using the following parameters: TR/TE = 3200/47 ms, $\delta_1 = \delta_2 = 1$ ms, 4 segments, $\Delta_1 = \Delta_2 = 15$ ms, $t_m$=5 ms, $G_1 = G_2 = 180$ G/cm and 160 averages (total acquisition time of about 7 hours). This results in b=2550 s/mm$^2$ and 2q=1500 cm$^{-1}$. DTI images were collected with the same parameters with the exceptions being that the TE was set to 22 ms and G to 165 G/cm. DTI was collected with 30 directions and 66 averages (total acquisition time of about 7 hours), resulting in b= 2143 s/mm$^2$ and q=685 cm$^{-1}$. Double-PGSE MRI data was analyzed using an in-house Matlab® code[3]. The analysis was performed either for clusters, using the k-means clustering routine or pixel by pixel. In both cases, maps of the fitted parameters were generated. DTI was analyzed using the DiVa tool. Regions of interest (ROIs) were placed according to the clusters. An interval of 100 was chosen for the colorbar, resulting in 6 clusters. Then, the ROIs were copied to the d-PGSE and DTI maps and their average values were extracted.
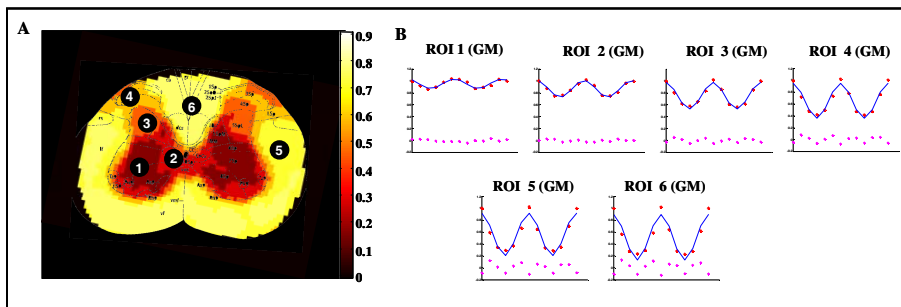


*Figure 1*



*Figure 2*

**Results and discussion:** Fig. 1A depicts a clustered aE map obtained from d-PGSE MRI, in which the ROIs are highlighted. Fig. 1B shows the E(ψ)$_{norm}$ (red asterisks), their fit (blue line) and the errors (pink asterisks) for each ROI shown in Fig. 1A. ROIs 1-4 are placed in GM areas and ROIs 5 and 6 are placed in the WM of the spinal cord. Note that the GM ROIs do show an oscillation, although relatively smaller than the oscillations of the WM ROIs. This results in a relatively small aE value for the GM areas, suggesting that they are not highly anisotropic but some organization is present. Fig. 2A shows pixel-by-pixel analyzed d-PGSE$_{xz}$ MR images, while Fig. 2B depicts FA



*Figure 3*

maps of a control and *les* spinal cord. We observed that the GM area with respect to the total area of the spinal cord is larger in the *les*. This is shown in both d-PGSE and FA maps. In addition, the WM shows lower apparent eccentricities and lower FA values in the *les* when compared to the control, although the GM seems similar when looking qualitatively. Fig. 3 shows the quantification of the aE extracted from the d-PGSE MRI and the FA value extracted from DTI for the six ROIs depicted in Fig. 1A for a control (Fig. 3A) and *les* (Fig. 3B) rat. Note that values were all normalized to the value of ROI 1. In the control rat, the aE values are slightly higher from the FA values in ROIs 1-3, which are pure GM areas. When looking at ROI 4 and the pure WM ROIs (5 and 6), the FA values are larger than the aE values. However, in the *les* rat, the aE values are much larger in all of the ROIs. Also, it seems like ROIs 1-3 show similar FA values although the aE values differ significantly. This seems to suggest that the aE derived from d-PGSE MRI provides a more detailed characterization of the *les* GM than FA derived from conventional DTI.

**Conclusions:** The FA values, derived from conventional DTI acquisitions, differentiate very clearly between WM and GM in the spinal cord of both control and *les* rats. The aE values derived from d-PGSE MRI show a bit more accentuated results for the control but seem to provide new information regarding the *les* model. Here, for the first time, we are able to clearly differentiate between three pure GM areas of the *les* spinal cord.

**References:** 1. D.K. Jones (Ed), Oxford University Press, Inc. (2011) 2. N. Shemesh et al, Magn. Reson. Med. 65(5):1216-27 (2011) 3. N. Shemesh et al, Magn. Reson. Med. 68: 794-806 (2012) 4. J.M. Kwiecien et al, J. Neurocytol. 27: 581-591 (1998) 5.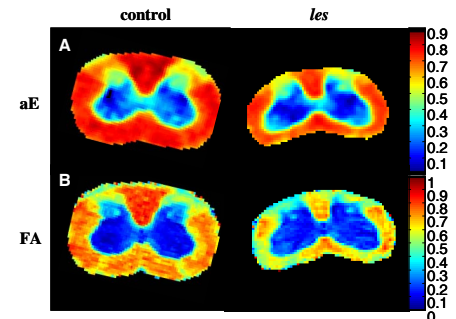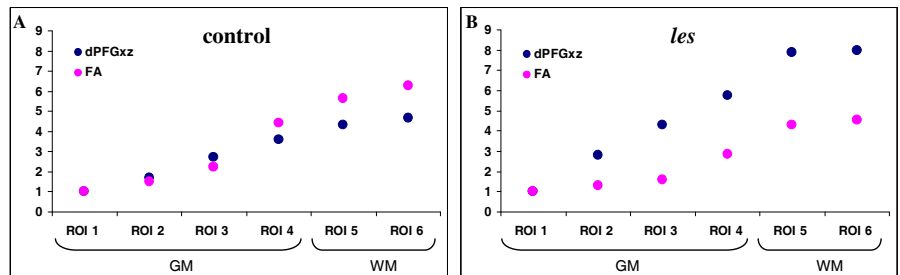 D. Anaby et al, NMR Biomed. DOI: 10.1002/nbm.3043 (2013).