**q-Value Diffusion of Myelin-Deficient Spinal Cords**

I.E. Biton, I.D. Duncan, and Y. Cohen

The apparent water diffusion anisotropy in white matter (WM) of excised spinal cords of myelin-deficient (md) rats and their age-matched controls was studied by high-b-value q-space diffusion MRS and MRI at different diffusion times. Non-monoexponential signal decay was observed at long diffusion times. The mean displacements in the md spinal cords were found to be higher than those of the controls. The apparent anisotropy (AA) of the fast-diffusing component was found to decrease more dramatically with the increase in diffusion time for the md spinal cords as compared with controls, whereas the AA of the slow-diffusing component in the controls was found to increase with the increase in diffusion time while that of the md cords decreased with the increase in diffusion time. When diffusion MRI was performed, similar diffusion anisotropy was extracted for the md and control spinal cords at diffusion times of 22 and 50 ms. Only at a diffusion time of about 200 ms was a significant difference obtained in the AA of the two groups. This originates from the much larger increase in the mean displacement perpendicular to the fiber direction in the control group vs. the md group when the diffusion time was increased. Magn Reson Med 58:993–1000, 2007. © 2007 Wiley-Liss, Inc.

**Key words:** diffusion MR; q-space diffusion; myelin-deficient; spinal cord; apparent anisotropy

Diffusion anisotropy is used to study the structure and pathology of the central nervous system (CNS) (1–4). Although anisotropic water diffusion was observed in central nervous system (CNS) tissues in the early stages of diffusion MRI (5,6), the origin and relative importance of different contributors to water diffusion anisotropy in these tissues remain to be resolved (7). It seems plausible, however, to assume that this anisotropy originates from structural elements of the tissues such as the myelin sheaths, axonal membranes, and neurofilbrils that can restrict water diffusion. Water anisotropy in white matter (WM) was previously attributed mainly to myelin (6,8). However, studies on myelinated and nonmyelinated nerves as well as on the developing brain demonstrated that myelin is not a prerequisite for observing this diffusion anisotropy (9,10). In addition, a diffusion tensor imaging (DTI) study of preterm and newborn human brains showed that although myelination is not required for observing this anisotropy, since it is present before myelination occurs, it does have a significant effect on the observed diffusion coefficient (11).

Gulani et al. (12), who studied the diffusion characteristics of water in the spinal cords of myelin-deficient (md) rats, also concluded that although myelination is not a prerequisite for diffusion anisotropy, it does influence the magnitude of the observed anisotropy. Song et al. (13) used shiverer mice to monitor the effect of dysmyelination on the observed anisotropy in WM tracts using DTI. Importantly, for the shiverer mice, in which the main observed pathology is reduced myelin, they found increased radial diffusivity (Λr), no change in the axial diffusivity (Λa) and lower diffusion anisotropy relative to their wild-type controls, again demonstrating the relative importance of myelin to the observed anisotropy in WM (13). Based on this and other studies, Song et al. (13) suggested that dysmyelination results in increased radial diffusivity whereas axonal damage is associated more with decreased axial diffusivity (14). A recent DTI study performed on formaldehyde-fixed shiverer mouse brains by Tyszka et al. (15) demonstrated increased radial and axial diffusivities in those mice, as compared with their age-matched controls, thus emphasizing the differential results that may be obtained when somewhat different preparations are studied by somewhat different protocols. Note, however, that most of these interesting studies, including some of the most recent ones (9–15), were performed with different b-value ranges; mostly with relatively low b values (bmax < 2000 s mm−2), sometimes with very few b values per direction, and often with relatively short diffusion times.

It has been shown for more than a decade that, at sufficiently high b values (bmax > 2500 s mm−2), in diffusion experiments of neuronal tissues water signal decay appears to be non-monoexponential and therefore cannot be analyzed by the simple Stejskal-Tanner equation (16–18). The q-space approach (19,20), first applied by King et al. (21) to study neuronal tissues (22), was suggested as a means to analyze such diffusion MR data (23). Subsequently, the q-space approach was extended to MRI (24–28). These studies indicated that high b-value q-space DWI is sensitive to the integrity of myelin sheaths. However, this information originated from spinal cord maturation (24), spinal cord trauma (25), experimental allergic encephalomyelitis (EAE) (26), multiple sclerosis patients (27–29), and patients with dementia (30,31). In the hemi-crush and the EAE models we found increased radial diffusivity (Λr) and decreased axial diffusivity (Λa) in comparison with the control group (25,26). Such changes should result in reduced anisotropy. For the EAE model, Kim et al. (32) recently observed decreased axial diffusivity and no change in radial diffusivity when the entire brain was analyzed. For regions of interest, in which Luxol-fast-blue staining was diminished, radial diffusivity (Λr) was elevated (32). However, it should be noted that

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these effects are multifactorial and myelin damage is only one of the processes that occur. To further evaluate the effect of lack of myelin on the diffusion characteristics, as obtained from high b-value q-space DWI, we used \( md \) excised rat spinal cords. These \( md \) rats, which are X-linked recessive Wistar rat mutants, show nearly a total lack of myelin in their CNS (33). In a preliminary study, we found an increase in both the axial and the radial diffusivities and surprisingly, a very small decrease in the anisotropy, as compared with controls (34). A recent study showed, however, that water anisotropy in excised spinal cords is diffusion time-dependent (35). Here, we report on the interplay between diffusion time, myelin, and the apparent diffusion anisotropy, as obtained by high b-value q-space diffusion spectroscopy and imaging, in the excised \( md \) and control rat spinal cords.

**MATERIALS AND METHODS**

Sample Preparation

This study was approved by the Animal Care Committee of the School of Veterinary Medicine of the University of Wisconsin-Madison. A total of eleven 21-day-old \( md \) and age-matched control rat spinal cords were used in this study, but only part of them were used in the different protocols performed. The spinal cords were excised and fixed with 4% paraformaldehyde and then inserted into 5-mm NMR tubes, with their long-axis parallel to the \( z \)-direction (the \( B_0 \) direction) of the magnet. For MRI measurements, the spinal cords were immersed in Fluorinert (Sigma Chemical Co., St. Louis, MO, USA). The temperature in the magnet was maintained at 25.0 \( \pm 0.1 \)\(^\circ\)C throughout the duration of the experiments.

Magnetic Resonance Experiments

MR diffusion experiments were performed on an 8.4T NMR spectrometer (Bruker, Germany) equipped with a micro5 imaging probe (Bruker, Germany) capable of producing pulse gradients of up to 190 gauss cm\(^{-1}\) in each of the three directions. The MR protocol included a series of diffusion MRS and MRI experiments and was performed in all cases on 25-mm samples of the cord.

**Diffusion MRS Experiments**

First, we measured the effect of short diffusion times (\( \Delta-\delta/3 \)) on the WM anisotropy. The experiments were performed using the \(^1\)H stimulated-echo (STE) diffusion MRS sequence with the following parameters: \( \text{TR} = 3000 \) ms, \( \text{TE} = 40 \) ms, and \( \delta = 2 \) ms. The pulse gradient strength was also incremented from 0 to 160 gauss cm\(^{-1}\) and the diffusion times (\( \Delta-\delta/3 \)) were 22, 50, 200, and 400 ms with TM values of 3.5, 31.5, 181.5, and 381.5 ms, respectively. Again, 24 \( b \)-values were acquired and diffusion was measured perpendicular and parallel to the long axis of the spinal. The \( b_{\text{max}} \) values in these diffusion experiments were 8.16 \( \times 10^5 \), 1.86 \( \times 10^6 \), 7.42 \( \times 10^6 \), and 1.48 \( \times 10^7 \) s cm\(^{-2}\), when the diffusion times were 22, 50, 200, and 400 ms, respectively, and \( q_{\text{max}} \) was 3065 cm\(^{-1}\). Here again, the \( q \)-values were incremented linearly. The signal-to-noise ratios (SNRs) of the water signal in diffusion spectra were typically about 10,000 and 100 for the minimal and maximal \( b \)-values, respectively, for the spectra acquired with the longest diffusion time. The entire MRS protocol was completed within 3 h.

**Diffusion MRI Experiments**

Diffusion-weighted images were acquired using the stimulated-echo (STE) diffusion imaging pulse sequence with the following parameters: \( \text{TR} = 2000 \) ms, \( \text{TE} = 30 \) ms, \( \delta = 2 \) ms, \( \Delta = 50 \) ms, an FOV = 0.85 cm \( \times \) 0.85 cm, a matrix size = 256 \( \times \) 128 (transformed into a 256 \( \times \) 256 matrix), and three slices of 1.35-mm thickness with a 0.65-mm gap. The diffusion gradient strength, \( G \), was incremented from 0 to 50 gauss cm\(^{-1}\) in 16 steps, yielding a maximal \( b \)-value (\( b_{\text{max}} \)) of 3.53 \( \times 10^5 \) s cm\(^{-2}\) and a \( q_{\text{max}} \) value of 426 cm\(^{-1}\). The \( q \)-values were incremented linearly. We also measured the effect of \( \Delta-\delta/3 \) on WM anisotropy. In these experiments, \( \Delta \) was set to 22, 50, and 250 ms and diffusion was measured perpendicular and parallel to the long axis of the spinal cord. The SNRs of the water signals in the WM were typically \( \sim 24 \) and \( \sim 12 \), for the minimal and maximal \( b \)-values used, respectively, when \( \Delta = 50 \) ms and diffusion was measured perpendicular to the long axis of the cord.

**Data Analysis**

The signal decay was analyzed using the q-space approach (19,20) and image analysis of the q-space data set was performed as previously described (22,26). Note that all q-space displacement values reported herein are 0.425 times the full-width at half-height (FWHH) of the displacement distribution profile. Anisotropy maps were also calculated from the displacement maps in both orientations for each pixel according to the following Eq. [1]:

Anisotropy = \( \frac{\text{Displacement}(z) - \text{Displacement}(x)}{\text{Displacement}(x) + \text{Displacement}(z)} = \frac{D_z - D_x}{D_z + D_x} \) [1]

Note that Eq. [1] assumes a cylindrical symmetry of the fibers, i.e., \( D_y \neq D_z = D_x \). The statistical significance of the differences in the extracted values, obtained from the q-space analysis of the diffusion data, between controls and the \( md \) spinal cords, was established using Student’s t-test.
RESULTS

In a preliminary study, we found, contrary to our initial expectation, a nonsignificant difference between the anisotropies observed in the WM of the 

\[ \text{md} \]

and age-matched control spinal cords at a diffusion time \((\Delta \approx 5/3)\) of 50 ms (34). Knowing that the diffusion time should affect the apparent anisotropy (AA) (35,36), we decided to examine whether the diffusion time used in these diffusion experiments affects the diffusion characteristics of the 

\[ \text{md} \]

and control spinal cords. To this end, we first performed diffusion MRS measurements at different diffusion times as a guide for the diffusion MRI studies, which are much more time-consuming and suffer from lower SNR.

The Effect of Diffusion Time \((\Delta \approx 5/3)\)

Short diffusion time \((\Delta \approx 5/3)\) effect: First, the effect of diffusion times, in the short diffusion time regime, on the diffusion characteristics of the spinal cords of the two groups was examined. In the short diffusion time regime \((8–12\text{ ms})\), the water signal decays were found to be nearly monoexponential in the two spinal cord groups (Fig. 1a), even when diffusion was measured perpendicular to the direction of the fibers of the spinal cords. In fact, a single Gaussian function fitted well the obtained displacement distribution profile obtained by Fourier transform (FT) of the signal decay as a function of \(q\), and only one component of water was extracted for the two groups. Table 1 summarizes the mean displacement of the control and 

\[ \text{md} \]

spinal cords, measured at short diffusion times. These data indicate that there is a near-linear increase in the mean displacement, with an increase in the square root of the diffusion times, in both spinal cord groups. Although the mean displacement was generally lower in the controls, as compared with the 

\[ \text{md} \]

spinal cords, we did not find statistically significant differences between the mean displacements of the two spinal cord groups; even when diffusion was measured perpendicular to the spinal cord fiber directions.

Long diffusion time \((\Delta \approx 5/3)\) effect: In the next step, we evaluated the effect of diffusion time, in the long diffusion time regime, on the diffusion characteristics of water in the two spinal cord groups. Figure 1b and c show the normalized signal decays of water in the 

\[ \text{md} \]

and control spinal cords as a function of \(b\)-values, for diffusion times of 22, 50, 200, and 400 ms, when diffusion was measured perpendicular and parallel to the long axis of the spinal cord. The signal-decays curves were found to be non-monoexponential and two different diffusing components of water were extracted for each case in both groups. Figure 1b and c show that the signal decay of water in the 

\[ \text{md} \]

spinal cords is more dramatically affected than in the control group by changes in the diffusion time. The difference between the two groups is larger when diffusion was measured perpendicular \((x\text{-direction})\) to the long axis of the spinal cord. Better discrimination between the fast- and slow-diffusing components of water, and between the two groups, was observed for longer diffusion times.

Figure 2 depicts the displacement profiles extracted for diffusion measured perpendicular and parallel to the long axis of the spinal cord, obtained by Fourier transformation of the data shown in Fig. 1b and c. These data indicate that more significant differences are obtained between the two spinal cord groups when the diffusion time is increased. Interestingly, this trend is observed for diffusion measured both parallel and perpendicular to the long axis of the spinal cord. However, the differences are less pronounced for diffusion measured parallel to the long axis of the spinal cord.

Figure 3 shows the mean displacements extracted from the displacement distribution profiles, as a function of the square root of the diffusion time, for the two diffusing water components for the two groups. Diffusion was measured perpendicular and parallel to the long axis of the spinal cord. The measured values of the mean displacement are presented in Table 2. We found that the mean displacement of the fast- and the slow-diffusing compo-
nents of water, in both directions sampled, increased with an increase in the square root of the diffusion time (Fig. 3). Interestingly, we found that the mean displacements of all four diffusing components (slow and fast, at the two diffusion directions sampled) are higher in the *md* spinal cords. In addition, we found that the difference between the displacement of the fast-diffusing component of the *md* and control spinal cords is somewhat larger in the long diffusion time range (200–400 ms). This observation, however, is much more pronounced for the slow-diffusing components. Moreover, the slow-diffusing water component of the control spinal cords shows more restriction in both directions than the slow-diffusing component of the *md* spinal cords. An important result, presented in Fig. 3 and in Table 2, is that the most pronounced differences between the mean displacements of the slow-diffusing component of the control and *md* spinal cords were obtained at diffusion times of about 200 ms or higher, when diffusion was measured perpendicular to the long axis of the spinal cord (compare Fig. 3c with Fig. 3d, for example).

Moreover, the slow-diffusing water component of the control spinal cords shows more restriction in both directions than the slow-diffusing component of the *md* spinal cords. An important result, presented in Fig. 3 and in Table 2, is that the most pronounced differences between the mean displacements of the slow-diffusing component of the control and *md* spinal cords were obtained at diffusion times of about 200 ms or higher, when diffusion was measured perpendicular to the long axis of the spinal cord (compare Fig. 3c with Fig. 3d, for example).

Figure 4a and 4b show the anisotropies ([D<sub>f</sub>–D<sub>s</sub>]/(D<sub>f</sub>+D<sub>s</sub>)) of the fast- and slow-diffusing components of water in the two spinal cord groups as a function of the diffusion time, respectively. These data indicate that the slow-diffusing component is more anisotropic. Moreover, the mean anisotropies of the fast-diffusing component of water in both groups of spinal cords decreased as a function of the diffusion time. Interestingly, the decrease is more pronounced in the *md* spinal cords than in the control spinal cords. For example, it was found that the mean anisotropy of the fast-diffusing component of water in the control spinal cords decreased from 0.14 ± 0.01 arbitrary units (a.u.) to 0.10 ± 0.01 a.u., when the diffusion time was increased from 22 ms to 400 ms, respectively. In contrast, the mean anisotropy of the fast-diffusing component of water in the *md* spinal cords decreased from 0.16 ± 0.02 a.u. to 0.06 ± 0.01 a.u., when the diffusion time increased from 22 ms to 400 ms, respectively. The differences in the anisotropies of the fast-diffusing component of water in the two groups were found to be more significant for the longer diffusion times.

Importantly, the anisotropy of the slow-diffusing component of water in these two groups exhibited very interesting behavior (see Fig. 4b). More specifically, we found a
Data obtained from q-space diffusion MRS data.

Table 2

<table>
<thead>
<tr>
<th>$\Delta - \delta/3$ (ms)</th>
<th>Fast component</th>
<th>Slow component</th>
<th>$A_{\text{slow}}/A_{\text{fast}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{Control (}\mu\text{m)}$</td>
<td>$md$ ($\mu$m)</td>
<td>$\text{Control (}\mu\text{m)}$</td>
</tr>
<tr>
<td>X direction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>$3.4 \pm 0.1$</td>
<td>$3.5 \pm 0.1$</td>
<td>$0.8 \pm 0.1$</td>
</tr>
<tr>
<td>50</td>
<td>$4.6 \pm 0.1$</td>
<td>$5.1 \pm 0.1$</td>
<td>$0.9 \pm 0.1$</td>
</tr>
<tr>
<td>200</td>
<td>$7.7 \pm 0.2$</td>
<td>$9.3 \pm 0.1$</td>
<td>$1.1 \pm 0.1$</td>
</tr>
<tr>
<td>400</td>
<td>$9.5 \pm 0.1$</td>
<td>$11.2 \pm 0.1$</td>
<td>$a$</td>
</tr>
<tr>
<td>Z-direction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>$4.4 \pm 0.1$</td>
<td>$4.9 \pm 0.1$</td>
<td>$1.3 \pm 0.1$</td>
</tr>
<tr>
<td>50</td>
<td>$6.1 \pm 0.2$</td>
<td>$7.3 \pm 0.1$</td>
<td>$1.6 \pm 0.1$</td>
</tr>
<tr>
<td>200</td>
<td>$10.0 \pm 0.2$</td>
<td>$11.2 \pm 0.2$</td>
<td>$2.3 \pm 0.2$</td>
</tr>
<tr>
<td>400</td>
<td>$11.7 \pm 0.2$</td>
<td>$12.7 \pm 0.1$</td>
<td>$a$</td>
</tr>
</tbody>
</table>

*Data obtained from q-space diffusion MRS data.

aAt a diffusion time of 400 ms, the extraction of the displacements of the slow-diffusing component in the two directions was not robust due to noisy data and a very low population ratio.

FIG. 4. The anisotropies $(D_z - D_x)/(D_z + D_x)$ of the fast (a) and slow (b) diffusing components of water in the age-matched control ($N = 4$) and $md$ ($N = 4$) spinal cords as a function of the square root of the diffusion time. The symbols * and *** represent $P$ values smaller than 0.05 and 0.001, respectively. Data extracted from the bi-Gaussian fit of the q-space diffusion MRS experiments.

The MRS results led us to speculate that computing the diffusion anisotropy at longer diffusion times would enable us to better discriminate between the control and $md$ spinal cords. Therefore, we performed high $b$-value q-space diffusion MRI and measured the anisotropy maps of these two rat groups at three different diffusion times, i.e., when $\Delta$ was set to 22, 50, and 250 ms. Note that, in the imaging experiments, the displacement profiles were, due to lower SNR, fitted by a single Gaussian function, which emphasizes the slow-diffusing component. Figure 5a depicts the anisotropy maps at three different diffusion times, for representative control and $md$ spinal cords. As shown, the anisotropy values are all positive but are significantly higher in the WM of the control spinal cords only at a diffusion time of 250 ms (see Figs. 5 and 6). These results are also depicted in Fig. 6, which presents the anisotropy values extracted for regions of interest (ROIs) in the WM (for a definition of the WM ROI, see Fig. 5b) of the control and $md$ spinal cords for the three different diffusion times used ($N = 6$ for $\Delta = 50$ ms and $N = 4$ for $\Delta = 22$ and 250 ms). This graph shows that the anisotropy in the WM increases with increasing diffusion times in the spinal cords of both rat groups.

To gain a better insight into the origin of these observations, one should inspect the effect of diffusion time on the extracted displacement in a specific direction, as shown in Fig. 7. The data in Fig. 7 show the effect of the diffusion time on the q-space displacement map, when diffusion...
was measured perpendicular to the long axis of the spinal cord for representative control and \textit{md} spinal cords. Indeed, we found higher displacement values in the WM of the \textit{md} spinal cords than in the WM of the controls for all diffusion times measured. These data clearly demonstrate that the difference between the two groups is maximal for longer diffusion times.

**DISCUSSION**

Despite the fact that for more than a decade it has been known that water diffusion in WM is macroscopically anisotropic, the origin to this observation and the relative importance of the different factors that govern it, are still not completely resolved. Conflicting results have been obtained from different models, and even recent studies utilizing \textit{shiverer} mice have led to different conclusions (13,15). To date, even the role of myelin in determining this anisotropy remains controversial (7,9,11–13,15).

The present study shows that the lack of myelin in the \textit{md} spinal cord significantly affects the diffusion characteristics and the diffusion anisotropy of water in WM in a diffusion time-dependent manner. Importantly, we can demonstrate that the myelin effect is strongly dependent on the diffusion time used in the MR diffusion experiments. For example, in the system studied here, at short diffusion times (of about 8–12 ms), only one diffusing component of water is observed in both groups, even when diffusion is measured perpendicular to the long axis of the fibers of the spinal cord. In addition, the mean displacements were slightly higher in the \textit{md} spinal cords than in the age-matched control spinal cords. However, these diff-

**Figure 8a and b** depict the mean displacements extracted for ROIs, presented in Fig. 5b, in the WM in the control and \textit{md} spinal cords, in the perpendicular and parallel directions, respectively, for three different diffusion times. As shown, the mean displacement is higher for the \textit{md} spinal cords at all diffusion times, in the two sampled directions. This data indicates that the origin of the increased anisotropy difference between the two groups at a diffusion time of 250 ms originates mainly from the much lower displacement of water molecules that diffuse perpendicular to the long axis of the spinal cord in the control spinal cords, as compared to the \textit{md} group.

**FIG. 5.** The \textit{q}-space displacement anisotropy MRI maps of representative age-matched control and \textit{md} spinal cords at \(\Delta\) values of 22, 50, and 250 ms (a). The diffusion MRI data were analyzed using a single component model. The data acquired with \(\Delta\) values of 22 and 250 ms are from the same animal whereas the data acquired with \(\Delta\) 50 ms are from another rat. b: The definition of the ROI in the WM used for the analyses is presented in Figs. 6 and 8.

**FIG. 6.** The anisotropy of the mean displacement, for different diffusion times, \(\Delta\), of age-matched control and \textit{md} spinal cords (\(N = 6\) for \(\Delta = 50\) ms and \(N = 4\) for \(\Delta = 22\) and 250 ms). Data extracted from high \textit{b}-value \textit{q}-space diffusion MRI experiments for an ROI in the WM. The \(P\) values represent the statistical significance of the differences between the anisotropies of the two groups at a given \(\Delta\).

**FIG. 7.** \textit{q}-Space displacement MRI maps of representative control (a–c) and \textit{md} (d–f) spinal cords at three different diffusion times, measured perpendicular to the long axis of the spinal cord. Insets show the mean displacement in the WM. Note the differences in the color scale in the case of (c) and (f). The data acquired with \(\Delta\)s of 22 and 250 ms are from the same animal whereas the data acquired with \(\Delta\) 50 ms are from another rat. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
MRS experiments acquired with high SNR, we found that a larger difference is observed for the slow-diffusing component when diffusion is measured perpendicular to the long axis of the spinal cord. Indeed, in the control group, this is the component that exhibits the maximal restriction. Even for this diffusing component, larger differences between the two groups are observed at longer diffusion times. In addition, we found that the relative fraction of the slow-diffusing component is smaller in the md spinal cords than in controls. These results led us to conclude that if one wishes to observe significant differences between the two groups in terms of diffusion anisotropy, one should use relatively long diffusion times. q-Space displacement anisotropy maps shown in Fig. 5 obtained by fitting the diffusion MRI data with a single component model, emphasizing the slow-diffusion component, clearly demonstrates this important feature. Generally, it is difficult to obtain heavily diffusion-weighted MR images with sufficiently high SNR. Consequently, computing reliable separate images of the two observed diffusing components is more difficult.

The anisotropy deduced from the q-space analysis of the diffusion MRI data, which overemphasizes the contribution of the most restricted slow-diffusing component, indeed shows better discrimination between the two groups at long diffusion times. Figure 8 also shows the origin of this observation. The lack of myelin results in increased mean displacement of the water molecules. However, this increase is observed for both diffusion directions and consequently, it is less apparent in the computed diffusion anisotropy. Only at sufficiently long diffusion times, where water molecules diffusing perpendicular to the long axis of the fibers of the two spinal cord groups may translate very different distances, is anisotropy significantly different between the two groups. This conclusion is also in line with the results of a recent publication by the Duong group (Nair et al. (36)) that DTI sensitivity is increased mean displacement of the water molecules. However, this increase is observed for both diffusion directions and consequently, it is less apparent in the computed diffusion anisotropy. Only at sufficiently long diffusion times, where water molecules diffusing perpendicular to the long axis of the fibers of the two spinal cord groups may translate very different distances, is anisotropy significantly different between the two groups. This conclusion is also in line with the results of a recent publication by the Duong group (Nair et al. (36)) that DTI sensitivity is increased.

This study demonstrates that the answer to the question whether myelin affects the diffusion characteristics or, more importantly, the diffusion anisotropy in the WM, is diffusion time–dependent and even system-dependent. At very short diffusion times (i.e., less than 10 ms), one would conclude that myelin has only a marginal effect in the present system. However, at longer diffusion times, it is clear that myelin does affect the diffusion characteristics of the water molecules; but only at relatively long diffusion times are the differences in the mean displacements sufficiently large in only one of the sampled directions (generally the more restricted direction), thus resulting in a significant difference in the computed anisotropy. Thus, we concluded that myelin does affect diffusion anisotropy in a diffusion time–dependent manner, and only in the long diffusion time regime, in the model used in the present study, is this effect large enough to produce statistically different diffusion anisotropies between the two groups.

Note that we used 21-day-old rat spinal cords that do not have a fully developed myelin system (24). Here, the diffusion anisotropy, even in the control group, was relatively low. Consequently, and in order for the reduced anisotropy of the md spinal cords to be significantly different from that of the control group, relatively long diffusion times, about 200 ms (or more) were required. We

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**FIG. 8.** The mean displacement at different Δ values for age-matched control and md spinal cords measured perpendicular (x-direction) (a) and parallel (z-direction) (b) to the long axis of the spinal cord (N = 6 for Δ = 50 ms and N = 4 for Δ = 22 and 250 ms), extracted from the high b-value q-space diffusion MRI experiments for an ROI in the WM. The P-values represent the statistical significance of the differences between the observed displacements of the two groups for a given Δ value.
anticipate that for mature rats, statistically significant differences in the anisotropy between the two groups would be reached at shorter diffusion times. This may also explain why both Song et al. (13) and Tyska et al. (15) were able to show differences between shiverer mice and their age-matched controls while using shorter diffusion times and smaller diffusion weighting factors. In these cases the studied groups were seven and eight weeks of age (13,15), whereas in the present study the studied rats were only three weeks old. Diffusion is in fact a filter; therefore, differential diffusion protocols may result in different sensitivities to different pathologies. Since the observed anisotropy is diffusion time-dependent as well as model system-dependent, it is clear that in diffusion MRI, one should refer only to AA in cases of diffusion anisotropy in WM.

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