QSI and DTI of excised brains of the myelin-deficient rat

Amnon Bar-Shira, Ian D. Duncan, Yoram Cohen

**ABSTRACT**

High b-value q-space diffusion imaging (QSI) and conventional DTI methodologies were used to study the MRI diffusion characteristics of excised brains of 21-day-old myelin-deficient (md) rats and their age-matched controls. Three different indices were calculated from the QSI data, i.e., Displacement, Probability and Kurtosis, for the purpose of evaluating the effect of the myelin sheaths on the MR diffusion characteristics in white matter (WM) ROIs of the md versus control brains. The examined WM ROIs were the corpus callosum, the external capsule, and the internal capsule. In all examined WM ROIs, significant differences were observed between the md and control brains for all QSI indices. These differences reveal that myelin sheaths surrounding the axons in WM ROIs mostly affect the component exhibiting restricted diffusion, which is manifested by low mean displacement values and high probability and kurtosis values. Such differences were found to be more pronounced in long diffusion times, i.e., Δ = 200 ms. Conventional DTI performed with relatively low $b$-values ($b < 1500 \text{s/mm}^2$) was also used to study md versus control brains. Interestingly, the fractional anisotropy (FA) index, which was calculated from DTI data, did not reveal any significant difference between the groups in the examined WM ROIs. However, some distinctions were revealed by the three eigenvalues ($\lambda_1$, $\lambda_2$, and $\lambda_3$) obtained from the tensor analysis. These findings were supported by Voxel-based analysis using SPM. Finally, MRI-guided histology showed very good agreement between myelin-stained regions and regions with highly restricted diffusion detected by QSI.

**Introduction**

Water diffusion in neuronal tissues was found to be anisotropic almost 20 years ago (Moseley et al., 1990) and this observed anisotropy was found to be more prominent in white matter (WM) rich tissues (Assaf and Cohen, 2000; Beaulieu, 2002; Moseley et al., 1990; Stanisz and Henkelman, 1998). With the advent of the DTI technique by Basser et al. (1994), the anisotropy of water diffusion became an important and widely used tool for studying central nervous system (CNS) connectivity (Basser et al., 2000) and pathologies, (Assaf and Pasternak, 2008; Le Bihan et al., 2001; Sun et al., 2008; Zhang et al., 2003) despite the fact that the origin of this observed anisotropy in neuronal tissues is still under debate (Beaulieu, 2002). Various structural components of the WM tissue may contribute to the restricted diffusion observed when water diffusion is measured perpendicular to the long axis of the WM fibers. These components include myelin sheaths, axonal membranes, microtubules, and neurofilaments, all of which have the same directionality (Beaulieu, 2002).

Although Beaulieu and Allen (1994) reported on diffusion anisotropy in non-myelinated garfish nerves, and despite the fact that different levels of anisotropy have been observed even in poorly or unmyelinated neuronal tissues (Neil et al., 1998; Wimberger et al., 1995), myelin sheaths surrounding the axons have been suggested as a relatively important factor in determining water anisotropy within neuronal tissues (Biton et al., 2006; Moseley et al., 1990; Sakuma et al., 1991).

In recent years, several specific animal models, characterized by demyelination and dysmyelination, have been used to study the effect of myelin on the MR diffusion characteristics of WM tissues. Ono et al. examined the diffusion anisotropy in jimpys and twitcher mice, animal models for dysmyelination and demyelination, respectively (Ono et al., 1995). This study demonstrated that the diffusion anisotropy of optic nerves of jimpys does not differ significantly when compared with age-matched control mice, while in twitcher mice, diffusion anisotropy was reduced significantly in the optic and trigeminal nerves, compared with their controls (Ono et al., 1995).

Song et al. studied diffusion anisotropy, using DTI, in the case of dysmyelination in shiverer mice and found increased radial diffusivity without changes in axial diffusivity (Song et al., 2002). Later, DTI studies by Nair et al. (2005) and Tyszka et al. (2006) also demonstrated that lack of myelin in shiverer mice does affect water diffusion anisotropy. Chahboun et al., using C57B/Ld mouse brains, demonstrated the increased fractional anisotropy (FA) extracted from three different WM regions during the first 7 weeks after their birth. In that study, significant changes in diffusion anisotropy were found in the corpus callosum and cingulate cortex during the myelination of axons that occurs in maturation (Chahboun et al., 2007).
The aforementioned studies and many others examined dysmyelination and demyelination but not myelin deficiency per se. However, Gulani et al. (2001), who examined myelin-deficient (md) rat spinal cords, found that myelin sheaths do affect water diffusion anisotropy. Moreover, note that in all the aforementioned studies, which dealt with the influence of myelin on the MR diffusion characteristics, the diffusion MR data were acquired using relatively low b-values ($b_{\text{max}}<1500$ s/mm$^2$) where the signal decay is mono-exponential. In those studies the signal decay was analyzed by the Stejskal–Tanner equation, assuming Gaussian diffusivity of a single component.

High b-value q-space diffusion imaging (QSI), which emphasizes the slow diffusing component, has revealed a pronounced anisotropy and a high degree of restriction in WM regions (Assaf et al., 2000; Biton et al., 2005; Nossin-Manor et al., 2002). Both the mean displacement and the probability for zero displacement, obtained from QSI, were used for this purpose and very recently kurtosis has been added to the arsenal of parameters used for describing restricted diffusion in neuronal tissues (Lu et al., 2006). The QSI approach was used to obtain micro-structural information in neuronal tissues and to characterize neurological pathologies and disorders that involve WM damage (Assaf et al., 2002a,b, 2005; Biton et al., 2005; Cohen and Assaf, 2002; Farrell et al., 2008; Mayzel-Oreg et al., 2007; Nossin-Manor et al., 2002). Very recently, very good agreement has been found between axon sizes obtained by using the q-space approach and histology performed on swine optic nerves and on mouse spinal cord (Bar-Shir and Cohen, 2008; Ong et al., 2008).

Biton et al. have demonstrated that QSI can easily distinguish between myelinated and md spinal cords, in vitro (Biton et al., 2006, 2007). In the more recent study, where different experimental parameters have been examined, it was found that the diffusion time affects the apparent displacement anisotropy and that longer diffusion times yield better differentiation between mds and their age-matched controls. Based on these observations, the authors suggested the use of apparent anisotropy (AA) and apparent FA (AFA) terms. Nair et al. (2005) have also shown that the diffusion times affect the MR diffusion indices extracted from DTI experiments in WM ROIs.

Glycolate and lactate are natural metabolites of energetic processes in the brain tissue and have been measured in the ventricles of many species including humans and mice. The increased levels of lactate and glycolate in vitro have been confirmed using single-photon emission computed tomography (SPECT) imaging, which has the potential to map the difference between the two major brain systems: energy-requiring and energy-saving. The increased levels of lactate and glycolate may indicate a change in metabolism due to increased energy demand. Also, the relationship between the two biomarkers may indicate a change in the metabolic state of the brain.

Table 1

<table>
<thead>
<tr>
<th>Δ [ms]</th>
<th>Control [μm]</th>
<th>md [μm]</th>
<th>% difference</th>
</tr>
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<tbody>
<tr>
<td>40</td>
<td>2.9 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>100</td>
<td>3.3 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>6.1</td>
</tr>
<tr>
<td>200</td>
<td>3.9 ± 0.3</td>
<td>4.3 ± 0.2</td>
<td>10.3</td>
</tr>
</tbody>
</table>

MRI experiments

MRI experiments were performed using a 7T/30 cm BioSpec System (Bruker, Germany) equipped with a BGU20 gradient system capable of producing pulse gradients of 40 Gcm$^{-1}$ in each of the three dimensions (x, y and z). Eight continuous 1-mm slices were acquired with a field of view (FOV) of 2.56×2.56 cm and 256×128 digital resolution reconstructed to a 256×256 matrix. The MRI protocol included one $b_0$ image (no diffusion gradients) and high b-value QSI and conventional DTI collected in 6 directions ((1,0,0), (1,0,1), (0,1,1), (−1,0,0), (−1,0,1), (0,1,−1)) using the pulse gradient stimulated echo (PGSTE) MRI diffusion sequence.

QSI

(i) The effect of Δ: For examining the effect of the diffusion time on the displacement, and probability maps, obtained from q-space analysis, one brain from each group was tested with the following parameters: TR/TE/δ = 1800/20/4 ms, an FOV of 1.92×1.92 cm, and a
matrix of $128 \times 64$ (zero filled to $128 \times 128$). $\Delta$ was set to 40, 100, or 200 ms. (ii) Since we found that $\Delta$ of 200 ms is the most effective diffusion time for our goal, we applied the following QSI protocol for all ten examined samples: TR/TE/$\Delta/\delta = 1800/20/200/4$ ms and four averages. The diffusion gradient was incremented from 0 to 30 Gcm$^{-1}$ in 16 equal steps for all six directions, resulting in maximal $b$- and $q$-values of 18,440 s/mm$^2$ and of 511 cm$^{-1}$, respectively. The total imaging time for each QSI protocol was 24 h.

**DTI**

The raw data of the QSI experiments were used for DTI analysis. For this goal, we used the same $b_0$ image (no diffusion gradients) as was used for the QSI protocol and one low $b$-value image per direction ($b \sim 1500$ s/mm$^2$) to fit the linear part of the signal decay versus the $b$-values, as is generally done in conventional DTI (Basser et al., 1994, 2000).

**Image processing**

After the MRI protocol was completed, the raw data were analyzed using an in-house Matlab® program tool (www.cs.tau.ac.il/~oferpas/DiVa).

**QSI image analysis**

The QSI raw data were analyzed using the QSI methodology developed by Assaf et al. (2000). After Fourier transformation of the signal decay with respect to $q$, three different QSI indices were extracted from the displacement probability profiles for each voxel, resulting in three QSI maps: the Displacement-map, the Probability-map, and the Kurtosis-map. The Kurtosis maps were calculated according to Jensen et al. (2005).

For the Displacement-maps, the smallest mean displacement value out of the six diffusion directions was selected for each voxel in the map. For the Probability-maps, the highest probability for the zero displacement value out of the six diffusion directions was selected for each voxel in the map. For the Kurtosis-maps, the highest kurtosis value, which characterizes the deviation from Gaussian diffusion, i.e., maximum restriction, out of the six diffusion directions, was selected for each voxel in the map.

**DTI image analysis**

DTI indices, i.e., FA, $\lambda_1$, $\lambda_2$ and $\lambda_3$ were calculated according to Basser and Pierpaoli (1996).

**ROI analysis**

Three WM ROIs from two regions of the rat brain, as shown in Fig. 1, were selected to challenge the differences between the examined groups. These ROIs represent the external capsule (ec, 60±20 voxels) and the corpus callosum (cc, 125±30 voxels) from the frontal part of the rat brain, and the internal capsule (ic, 135±20 voxels) from the median part of the rat brain (according to Fig. 1). The examined ROIs were determined on the corresponding FA map of each brain according to the ROIs depicted in Fig. 1. Then these ROIs were copied to the other MR diffusion maps. This procedure was performed on each examined sample and the average value of the examined index was extracted for each ROI.

**Voxel-based analysis (VBA)**

The Statistical Parameter Mapping (SPM2) program was also used to compare the md and the control groups for both the QSI and DTI results. First, a co-registration procedure with a representative $T_2$WI data set of one representative control brain as template was applied for each diffusion (QSI or DTI) data set, i.e., displacement, probability, kurtosis, FA, $\lambda_1$, $\lambda_2$, and $\lambda_3$ maps. After the co-registration procedure was completed, we used the VBA procedure to compare the md and control groups. Regions that expressed a statistical difference ($P<0.001$) after a one-way-ANOVA test between the groups were highlighted on the $T_2$WI template.

**Fig. 3.** Displacement, probability, and kurtosis maps obtained from the QSI experiments of one representative md and control brains acquired with a $\Delta$ of 200 ms. Also shown are MRI-guided histological images stained for myelin by PLP and MBP immuno-staining.

**Fig. 4.** Displacement, probability, and kurtosis maps obtained from the QSI experiments of one representative md and control brains acquired with a $\Delta$ of 200 ms. Also shown are MRI-guided histological images stained for myelin by PLP and MBP immuno-staining.
the sections were incubated with biotin-conjugated goat anti-rabbit secondary antibody (Jackson Immunoresearch, 1:1000), treated with avidin–biotin complex (ABC) reagent (Vector Laboratories, 1:1000), and then developed with 3,3′-diaminobenzidine (DAB). For negative-control staining, the primary antibodies were omitted, and no staining was observed.

Results

Selecting the optimal diffusion time

First, we examined the effect of the diffusion time on the displacement maps obtained from QSI data, to choose the best Δ for distinguishing between the md and their age-matched control rat brains. For this purpose, we tested one brain from each group with Δs of 40, 100, and 200 ms, since it had been shown previously that the diffusion time may have a significant effect on the apparent displacement anisotropy of water in the spinal cord (Biton et al., 2007; Nossin-Manor et al., 2005).

Fig. 2 shows the differences in the mean displacement values observed from the ic ROI for the two examined brains, i.e., the md and control brains. More pronounced differences were found in WM ROI when the QSI data were acquired with a relatively long diffusion time (Δ = 200 ms), as previously observed (Biton et al., 2007). These findings are summarized in Table 1 and show that the longer the diffusion time, the larger is the difference between the md and the control brains in the ic ROI. At the shortest diffusion time used (Δ = 40 ms), the mean displacement of the water in the ic ROI was found to be the same for both investigated brains, namely, 2.9 ± 0.1 μm. The largest difference was found when Δ was 200 ms (i.e., more than a 10% difference).

QSI characteristics and histology of md and control brains

Fig. 3 depicts displacement, probability, and kurtosis maps obtained from the QSI experiments of one representative md and one control brain sample, at Δ of 200 ms. MRI-guided histological images, which were stained for myelin by PLP as well as by MBP immuno-staining, are also shown. This figure clearly shows that in WM ROIs, the obtained displacement values were smaller in the control brains than in the non-myelinated md brains. Interestingly, the opposite trend was observed for the probability and kurtosis maps, where higher values were found for the myelinated control brains than for the md brains. These findings imply that water diffusion is more restricted in WM ROIs of the myelinated control brains than in the md brains. Regions that exhibited high degrees of restricted diffusion in QSI data also showed high content of myelin by MRI-guided histological images.

Histology

Different group of md and control brains were used for histology. Briefly, at 21 days of age, the md affected and control rats were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were frozen and the striatal and hippocampal levels were cut coronally at 20 μm on a cryostat. The free-floating sections were immunolabeled with the following primary antibodies: rabbit anti-myelin basic protein (MBP) polyclonal antibody (Chemicon, 1:30,000) and rabbit anti-proteolipid protein (PLP) polyclonal antibody (gift from Dr. Ian Griffiths, 1:100,000). After overnight incubation with the primary antibodies diluted in 0.1 M PBS containing 0.3% Triton X-100 (PBS-T, pH 7.4),

Table 2

QSI indices, i.e., kurtosis, displacement, and probability, observed for the md and their age-matched control brains (N = 5 for each group) and the extracted P-values.

<table>
<thead>
<tr>
<th>QSI index</th>
<th>ROI</th>
<th>Control</th>
<th>md</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kurtosis [a.u.]{lc</td>
<td>cc</td>
<td>11.4±0.2</td>
<td>10.1±0.3</td>
<td>0.004**</td>
</tr>
<tr>
<td></td>
<td>ic</td>
<td>11.6±0.7</td>
<td>9.4±0.1</td>
<td>0.001**</td>
</tr>
<tr>
<td></td>
<td>ic</td>
<td>12.2±0.2</td>
<td>9.7±0.7</td>
<td>0.0006**</td>
</tr>
<tr>
<td>Displacement [μm]</td>
<td>cc</td>
<td>3.9±0.1</td>
<td>4.2±0.2</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td>ic</td>
<td>3.8±0.1</td>
<td>4.4±0.1</td>
<td>0.0002***</td>
</tr>
<tr>
<td></td>
<td>ic</td>
<td>3.7±0.1</td>
<td>4.3±0.3</td>
<td>0.0003***</td>
</tr>
<tr>
<td>Probability [a.u.]{lc</td>
<td>cc</td>
<td>1.7±0.2</td>
<td>1.6±0.1</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td>ec</td>
<td>1.7±0.2</td>
<td>1.6±0.1</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td>ic</td>
<td>1.8±0.1</td>
<td>1.6±0.1</td>
<td>0.0001***</td>
</tr>
</tbody>
</table>

* P < 0.05.
** P < 0.005.
*** P < 0.0005.
† Probability values are given in ×10⁻² arbitrary units [a.u.].
Fig. 4 shows the same data set as shown in Fig. 3 but in a more frontal region of the rat brain, according to Fig. 1. This figure demonstrates again that more restricted water diffusion was found by QSI indices (displacement, probability, and kurtosis) in the WM ROIs of the control brain than in the same regions in the md. In these slices, as in the case presented in Fig. 3, MRI-guided histological images show very good agreement between myelin content and restricted water diffusion.

The findings from Figs. 3 and 4 were quantitatively summarized for the two examined groups in Figs. 5A–C and in Table 2. The data show that significant differences between the groups are indeed observed for the three examined ROIs, and for all three indices extracted from QSI. These results corroborate the claims that myelin sheaths do affect the MR diffusion characteristics of water in WM tissues and restrict water diffusion perpendicular to the main axis of the fibers.

**DTI characteristics of md and control brains**

For each sample, DTI data were used to compute the FA, $\lambda_1$, $\lambda_2$, and $\lambda_3$ maps, as presented in Fig. 6. These maps show the same slices presented in Fig. 3 and were used (together with more frontal slices, which correspond to the data in Fig. 4, data not shown) to extract quantitative values from the DTI indices, for the two examined groups. These quantitative data are summarized in Fig. 7 and in Table 3.

![Fig. 6. FA, $\lambda_1$, $\lambda_2$, and $\lambda_3$ maps of one slice obtained from the conventional DTI experiments of one representative md and control brains at $\Delta$ of 200 ms.](image)

![Fig. 7. Quantitative values obtained from DTI data for controls (black columns) and md's (white columns) in all three examined WM ROIs, i.e., cc, ec, and ic, according to Fig. 1. (A) FA, (B) $\lambda_1$, (C) $\lambda_2$, and (D) $\lambda_3$ values are shown. *$P<0.05$, **$P<0.005$, ***$P<0.0005$.](image)
Surprisingly, as presented in Fig. 7A, the FA maps display no difference between the md and control brains in all three examined WM ROIs. However, differences between the md and control brains were observed in the λ₁, λ₂, and λ₃ indices (Figs. 7B–D) but in different ROIs and with varying statistical significance. Note that, as expected, lower λ₁–λ₃ values were obtained for the WM ROIs in the control brains as compared to the md brains. As expected, the differences in λ₁–λ₃ values are also expressed in the ADC values (Table 3), which represent the mean diffusivity in each ROI.

**Voxel-based analysis of QSI and DTI indices of md and control brains**

After a co-registration procedure of each individual data set (i.e., displacement, probability, kurtosis, FA, λ₁, λ₂, and λ₃ maps) the VBA procedure was performed using the SPM2 program. Such an analysis highlighted those voxels with values that are statistically different when the one-way ANOVA test is performed on the two examined groups. Fig. 8 shows representative group analysis obtained by the voxel-based comparison for QSI probability, FA, and λ₃ maps.

The highlighted regions represent ROIs in which the index is significantly different (P < 0.001) in the two groups. Fig. 8 shows that highly significant differences were, indeed, found in the ic ROI for probability values extracted from QSI and for λ₃ values that had been extracted from conventional DTI. However, no highlighted voxels were obtained in the comparison between the FA maps of the two groups. This observation implies that under the experimental parameters used in this study, the FA index, obtained from DTI, cannot distinguish between the md and control brains, even in WM ROIs.

**Discussion**

Although diffusion anisotropy in neuronal tissues was demonstrated for the first time almost two decades ago and despite the widespread use of diffusion imaging techniques, especially DTI-based MR approaches, the relative importance of myelin in determining the observed water anisotropy under different experimental conditions remains elusive (Beaulieu, 2002).

In the present study we have used high b-value QSI (Assaf et al., 2000; Biton et al., 2005; Cohen and Assaf, 2002; Nossin-Manor et al., 2002) and conventional DTI (b < 1500 s/mm²) (Basser et al., 1994, 2000) to examine the MR diffusion characteristics of different WM ROIs in md rat brains and their age-matched controls for the first time. The aim was to examine and evaluate the effect of myelin sheaths on the diffusion indices extracted from both diffusion techniques.

Indeed, as expected from previous studies of control and md spinal cords (Biton et al., 2007), we found that the larger difference between MR diffusion characteristics of md and control brains is obtained when the diffusion data are acquired with relatively long diffusion times. Sufficiently long enough diffusion times are required for detecting restricted diffusion since Δ should be larger than I/2D (where I is the size of the compartment in which water molecules with diffusion coefficient D are diffusing). Taking into account the average size of axons in rat CNS (1–2 μm), it seems that a diffusion time of ~7 ms should suffice to reach the boundaries. Indeed, in a recent study, we noted a very dramatic restriction at a diffusion time of ~7 ms. In that study, however, the data were acquired in a fully myelinated optic nerve (Bar-Shir and Cohen, 2008). In the present work, however, the brains, even of the controls, are only partially myelinated and, therefore, one would expect that longer diffusion times will be required to fully observe restriction. Indeed, to amplify as much as possible the differences between the two groups, we used a long diffusion time. When Δ of 200 ms was used, the displacement values in the ic ROI (Fig. 2) showed the most significant difference between the two examined brains. However, such a difference was less prominent when Δ of 100 ms was used, and it practically disappeared when a diffusion time of 40 ms was used. These findings clearly indicate that myelin sheaths have a dramatic effect on the restriction observed perpendicular to the main axis of the axons, as we found previously with QSI (Assaf et al., 2000; Biton et al., 2005, 2007; Cohen and Assaf, 2002; Nossin-Manor et al., 2005). In the md brains, the diffusion perpendicular to the axons is less restricted because of the lack of myelin. Therefore, at sufficiently long diffusion times, the mean displacement of the water molecules in this direction is significantly larger in the md brains than in their age-matched controls. Note that the brain samples used in this study were excised from 21-day-old rats that did not reach maturity and therefore were not fully myelinated (Gulani et al., 2001). This lack of myelin in the control group is probably why relatively long diffusion times were required for a better differentiating between the two investigated groups, as explained above.

![Fig. 8](image-url) Results from the VBA procedure performed by the SPM2 program. Highlighted regions represent regions with a significant difference (P < 0.001) between the md and control groups in three representative indices: probability values extracted from QSI data, FA, and λ₃ values calculated from the DTI data.
In all examined WM ROIs, i.e., cc, ec, and ic, QSI indices can significantly distinguish between the md and control brains, however, with somewhat different sensitivities. All extracted QSI indices, i.e., mean displacement, probability, and kurtosis, displayed higher degrees of restricted diffusion in the examined WM ROIs of the control group compared with md brains. Higher kurtosis values and a higher probability for zero displacement values were found in WM ROIs of the control brains compared to the md brains.

An opposite behavior, however, was found for the displacement index. For this index, smaller values were found in the WM ROIs of the control brains compared with their age-matched md brains. These changes indicate a higher degree of restricted diffusion in the control group compared with the md group. For the more myelinated axons, as in the WM ROIs of the controls, restricted diffusion should result in higher probability and kurtosis values and in lower displacement values.

Conventional DTI experiments also revealed some differences between the md and control brains. Such differences were found to be significantly less pronounced than those found in the QSI experiments. Surprisingly, we found differences between the $\lambda_1$ values extracted for both groups. The $\lambda_1$ index represents axial diffusivity and is expected to be similar between the examined groups, since no restriction is expected parallel to the main axis of the fibers. However, in the present study we have used a relatively long diffusion time ($\Delta = 200$ ms), generally not used in conventional DTI studies. Under these experimental conditions, the mean displacement of the water molecules is about 15 $\mu$m in this direction. The data seem to indicate that there is more restriction in the control group also in this direction. Differences between the examined groups were also found for the $\lambda_2$ and $\lambda_3$ indices in the ec and ic ROIs. Both indices represent the radial diffusivity and, hence, are expected to be higher for the less myelinated WM ROIs of the md compared with controls. Interestingly, for the cc ROI no differences were observed between the examined groups even from the eigenvalues that represent radial diffusivity, i.e., $\lambda_2$ and $\lambda_3$. This observation can be explained by the fact that WM ROIs of the control rats are not fully myelinated. Recently it was demonstrated by Jito et al. (2008) that equal number of unmeyelinated and myelinated axons were found in the cc of 21 days old rats. However, the number of myelinated axons was much higher in the cc of 4 weeks rats.

An interesting and important finding of the present study is that no differences in the FA index, deduced from DTI, were observed between the md and controls in the examined WM ROIs. This surprising result can be explained by the differences observed for the different eigenvalues ($\lambda_1, \lambda_2,$ and $\lambda_3$) between the two groups. The FA index averages the changes in the three eigenvalues, and since we have found an increase in $\lambda_1-\lambda_3$ values in the md compared with controls, these differences are canceled out when the FA is computed. These results imply that under the experimental parameters used in the present study, FA cannot distinguish between the md and the control brains. The similarity in the FA maps of the two examined groups was confirmed by the VBA maps obtained from SPM methodology, as shown in Fig. 8. This figure indeed shows that FA maps are undistinguishable for both groups, whereas significant differences are observed in the $\lambda_1$ maps. Importantly, note that the VBA of the probability maps, obtained from QSI, is even more similar to the difference between the two groups observed on histology.

These findings demonstrate that FA, which is the most frequently used index of DTI in general and in clinical DTI in particular, may not always be the best index for following changes in myelination. If changes occur in all eigenvalues, i.e., $\lambda_1-\lambda_3$, FA may still not change. These results may have implication on the use of clinical MRI, however, it should be noted that the present study was performed on excised formalin-fixed brains, where diffusion characteristics may be somewhat different from those of in vivo brains (Sun et al., 2003, 2005). In addition, it should be noted that the examined brains in the present study, were excised from 21 days old rats that have not reached maturity.

Clearly QSI, which emphasizes the most restricted component, i.e., the axonal-extracellular, water, is more sensitive to myelin changes than indices extracted from conventional DTI, since the latter averaged out diffusion characteristics in all compartments. These findings clearly show the increased sensitivity of QSI compared with DTI when following myelin deficiency.

Conclusion

Understanding the contribution of myelin to the diffusion MR characteristics of neuronal tissues may increase our ability to detect WM pathologies that are found in many neurological disorders. This study clearly demonstrates that, in the present model, the lack of myelin significantly affects all the diffusion indices obtained from both high b-value QSI and only some of the indices obtained from conventional DTI. The eigenvalues calculated from the DTI differed between the two groups but not in all examined ROIs. These differences were less significant compared to the differences in the indices computed from QSI. Surprisingly, the FA was found not to be dependent on the myelin content in the present study. This means that FA is not always the best index for detecting WM changes and pathologies. This conclusion is even more significant since the data in the present study were collected with a relatively long diffusion time ($\Delta = 200$ ms) where the differences in the diffusion indices between the two groups are expected to be maximal. Therefore, we can conclude that myelin affects the diffusion characteristics of the tissue in a diffusion time-dependent manner and, therefore, the term Apparent Diffusion Anisotropy (ADA) and Apparent FA (AFA) should be used. Clearly, QSI, which is more specific toward the restricted water components, is more sensitive than DTI in detecting myelin deficiency.

Acknowledgments

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References


