INTRODUCTION

Diffusion, which measures micrometer-scale motion, has become an important contrast mechanism in MRI of the central nervous system (CNS), and in the last decade diffusion MRI has been extensively used for studying CNS pathologies and white matter structure (1–6). In most diffusion studies of neuronal tissues, the data were analyzed using the well-known Stejskal–Tanner equation (7), which assumes the existence of a single isotropic and unrestricted diffusing component. Indeed, at low $b$ values ($b_{\text{max}} < 2000 \text{s/mm}^2$), only a single apparent diffusion coefficient (ADC) is generally observed for water in neuronal tissues. In most of these studies, no dependence of the ADC on the diffusion time was observed. However, when high $b$ values ($b_{\text{max}} > 2000 \text{s/mm}^2$) are used to follow water diffusion in neuronal tissues, the water signal decay is found to be non-mono-exponential, and thus the experimental data cannot be analyzed by a mono-exponential function (8–12). In such cases, a different approach is needed to analyze the data. In most cases in which the water signal decay was found to be non-mono-exponential, the signal decay was fitted to a bi-exponential function (8–12). Such an analysis implies that water diffusion represents two components which exhibit free diffusion with no exchange between them. Although Niendorf et al. (8,9) pointed out that water diffusion represents two components which exhibit free diffusion with no exchange between them, in some studies the slow and fast diffusing components were assigned to the intracellular and extracellular compartments, respectively. Several more recent studies have contested this assignment (13,14). Others have used models that take into account geometric factors, permeability, and exchange to analyze such diffusion data (15–17).

The $q$-space approach, originally developed by Callaghan et al. (18) and Cory & Garroway (19), was first used by King et al. (20,21) to study diffusion in CNS. Kuchel and co-workers (22–24) used this approach to study diffusion in red blood cells. Subsequently $q$-space diffusion MRS was used to study diffusion in white matter in the CNS (25). This approach relies on the fact that the echo attenuation $E_3(q)$ in the MR diffusion experiment

**High $b$-value $q$-space diffusion MRS of nerves: structural information and comparison with histological evidence**

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Received 10 September 2006; Revised 1 March 2007; Accepted 1 March 2007

ABSTRACT: High $b$-value $q$-space diffusion MRS was used to study the diffusion characteristics of formalin-fixed swine optic and sciatic nerves over a large range of diffusion times (3.7–99.3 ms). The very short diffusion time range was studied with a 1 ms resolution. The displacement distribution profiles obtained were fitted to a bi-Gaussian function, and structural parameters were extracted from the $q$-space diffusion MRS data. This structural information was correlated with axon sizes obtained by histological examination. It was found that high $b$-value $q$-space diffusion MRS can easily distinguish between the two nerve types. The root mean square displacements (rmsds) of both the slow and fast diffusing components of the optic nerves were found to be smaller than those of the sciatic nerves. When the rmsd was plotted against the square root of the diffusion time ($t_{1/2}^2$), it was found that all four components showed an increase in rmsd; this increase was significantly smaller than expected from the Einstein equation. However, the most restricted component is the slow diffusing component of the optic nerve. This is also the only diffusing component that shows a large change in the slope (i.e. a ‘breaking point’) of the plot of rmsd as a function of $t_{1/2}^2$. This rmsd is very similar to the mean axon size of these optic nerves determined histologically. Such a change in slope was less apparent for the slow diffusing component of sciatic nerves, which showed a wider distribution of axon size in histological images. The fast diffusing components of both nerve types showed only a small gradual change in the slope of rmsd plotted against $t_{1/2}^2$. These findings are discussed in the context of component assignment, origin of restriction, and relationships between the structural information extracted from $q$-space diffusion MRS and histological examination. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: diffusion MRS; $q$-space diffusion; central nervous system; optic nerve; sciatic nerve; micro-structure

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Abbreviations used: ADC, apparent diffusion coefficient; CNS, central nervous system; rmsd, root mean square displacement; PGSTE, pulsed gradient stimulated echo; WM, white matter.
relates to the ‘reciprocal spatial vector’, \( \mathbf{q} \), according to eqn (1) (18,19)

\[
E_\Delta(q) = \int \overline{P}_r(R,\Delta) \exp(i2\pi qr)dR
\]

where \( E_\Delta(q) \) represents the echo decay as a function of \( \mathbf{q} \), defined as \( (2\pi)^{-1} \gamma \delta g, \overline{P}_r(R,\Delta) \) is the displacement probability, and \( R \) is the net displacement \( (R = r - r_0) \) during the diffusion time (\( \Delta \)). Therefore, the displacement probability profile can be obtained by performing a Fourier transformation on the signal decay with respect to the reciprocal wave vector, \( \mathbf{q} \). From this profile, one can extract the root mean square displacement (rmsd) of the diffusing spins from the full-width at half height (18,19).

Kuchel and co-workers (22–24) observed diffraction patterns in \( q \)-space diffusion NMR experiments performed on red blood cells. Diffraction patterns of the signal decay with respect to \( \mathbf{q} \) have not yet been reported in neuronal tissues, but King et al. (20,21), Assaf et al. (25,26) and others (27) have used this approach to obtain some structural information on neuronal tissues.

We have extended the \( q \)-space approach to diffusion MRI and found that the slow diffusing component is larger in areas rich in white matter (28–30). This diffusing component showed pronounced anisotropy and a high degree of restriction (26–28). Therefore it was suggested that the slow diffusing component observed in high \( b \)-value \( q \)-space diffusion MR of water in neuronal tissues represents mainly axonal water and could provide a more specific means of obtaining structural information on neuronal tissues (26,28). It has been shown that high \( b \)-value \( q \)-space diffusion MRI is useful in detecting white matter-associated disorders and studying CNS maturation (28–30).

The \( q \)-space approach was derived using the short gradient pulse approximation (i.e. \( \delta \rightarrow 0, \delta < < \Delta \)) and the long diffusion time condition (i.e. \( t_d > l^2/2D \)) where \( l \) is the size of the compartment in which the diffusion takes place, and \( D \) is the diffusion coefficient (18,19,31,32). Under these conditions, one should be able to extract real structural information. In a recent study, micro-cylinders, with inner diameter of 10–20 \( \mu \)m, were used as a model system to test the accuracy of the structural information obtained from such a \( q \)-space analysis (33). When the diffusion was measured perpendicular to the long axis of the cylinders and the diffusion times were long enough, diffusion patterns were observed, and the structural information extracted from the diffusion patterns and the displacement distribution profiles were found to be in good agreement with the expected radii of the microtubes. In addition, it was found that deviations from physical size were similar for the two methods of analysis (33). Therefore, the main goals of this study were to: (a) evaluate the ability of high \( b \)-value \( q \)-space diffusion MRS to provide structural characteristics of different nerve types; (b) examine the structural information extracted from the \( q \)-space analysis of high \( b \)-value diffusion data and compare it with structural information obtained from histological examination; (c) evaluate the effect of diffusion time, especially in the very short diffusion time range, on the extracted rmsds and verify whether this rmsd dependence enables determination of the real sizes of the axons of these nerves. For very short diffusion times \( t_d < l^2/2D \), the diffusing water molecules should, in principle, exhibit unrestricted diffusion because, in this time, a water molecule has only a small probability of reaching the boundaries of the compartment in which diffusion is taking place. Therefore, we evaluated the effect of diffusion time on the extracted rmsd for a large range of diffusion times, starting from very short times. In the short diffusion time regimen, the diffusion time increment was 1 ms.

**EXPERIMENTAL**

**MRS experiments**

Experiments were performed on 4% formaldehyde-fixed swine optic and sciatic nerves (four in each group). Each optic nerve (diameter \( \sim 4 \) mm) was placed in a 5-mm NMR tube so that its fibers were parallel to the \( B_0 \) direction (\( z \) direction). The 2-cm-long sciatic nerves were first attached to a glass tube and then inserted into 10-mm NMR tube to ensure correct alignment. NMR diffusion experiments were performed using an 8.4-T NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a micro5 gradient probe capable of producing pulse gradients of up to 190 G/cm in each of the three axes. NMR diffusion experiments were conducted when the diffusion gradients were perpendicular to the fiber orientation of the nerve (\( x \) direction) using the stimulated echo sequence with the following parameters: \( TR/TE/\delta = 3000\text{ ms}/50\text{ ms}/2\text{ ms} \). Pulsed gradient strength (G) was increased from 0.5 to 160 G/cm in 24 steps. Diffusion time (\( t_d \)) was increased from 3.7 to 8.7 ms in 1 ms steps and then to 14.3, 29.3, 49.3 and 99.3 ms. A series of such experiments with \( \delta = 4 \) ms were also performed but the minimum \( t_d \) was 5.0 ms. The displacement probability profiles obtained by performing a Fourier transformation on the signal decay with respect to \( \mathbf{q} \) were fitted to a bi-Gaussian function. The signal-to-noise ratio was higher than 10 000 and 2800 for the maximal and minimal \( b \) values used (\( b_{\text{min}} \) and \( b_{\text{max}} \), respectively), allowing us to determine the two components with relatively high accuracy using a bi-Gaussian fit. From the full-width at half height of these profiles, we calculated the rmsd of the fast and slow diffusing components (19). The rmsds for each component were plotted against the square root of the diffusion time (\( t_d^{1/2} \)). For each component, the slopes of this graph for the 0–\( t_d \) and \( t_d = 100 \) ms ranges were calculated and termed \( p_1 \) and \( p_2 \), respectively. Then the diffusion time for which \( p_1/p_2 \) is maximal was determined. If this value was higher than
10 ± 1, this point was termed a ‘breaking point’, i.e. a dramatic change in the slope of the graph of rmsd versus $t_d^{1/2}$.

**Histological examination**

After completion of the diffusion MRS experiments, the nerves were placed in 2.5% glutaraldehyde solution (in phosphate-buffered saline), and then postfixed with 1% osmium tetraoxide. The samples were then rinsed with buffer, dehydrated in graded ethanol solutions, and embedded in Epon at 60°C for 60 h. For light microscope images, 1 μm sections were cut and stained with toluidine blue. The specificity of toluidine blue staining to myelin enabled us to measure mean axon size using the Image J software developed at the National Institute of Health (NIH). Eight different sections were randomly chosen from each group and used to analyze axon size. Areas of 60 × 45 μm² were sampled in each case. The mean inner diameter of the axons was estimated by measuring the mean area of the axon and calculating its diameter by approximating the shape of the axon to a circle. Over 2000 axons were analyzed for each nerve type.

**RESULTS**

Figure 1 shows the water signal decay in diffusion experiments of one representative optic and sciatic nerve. The difference between the signal decays of the two nerve types is evident, showing that signal decay is indeed much slower for the optic nerve (Fig. 1A) than for the sciatic nerve (Fig. 1B).

Figure 2A shows the experimental water signal decay with respect to $q$ at a diffusion time of 99.3 ms for both groups of nerves. Figure 2B shows the displacement distribution profiles obtained by a Fourier transformation of the data shown in Fig. 2A. The displacement profiles were fitted to a bi-Gaussian function, and the rmsd of the two diffusing components in each nerve type was determined. The mean displacement of the slow and fast diffusing components in the optic nerve were 1.39 and 6.82 μm, respectively, when the diffusion time was 99.3 ms. For the sciatic nerve, higher values were obtained: 2.17 μm and 9.35 μm, respectively.

The influence of diffusion time ($t_d$) on mean displacement was tested as shown in Fig. 3 for a representative optic nerve. Figure 3A presents the experimental signal decay with respect to $q$ at different diffusion times, and Fig. 3B shows the displacement probability profiles, obtained by Fourier transformation, for the data shown in Fig. 3A from which the two diffusing components from each profile were extracted. Figure 3C shows the bi-Gaussian shape of the displacement distribution profile for Δ of 9.35 and 100 ms. The rmsds extracted from these profiles were plotted as a function of $t_d^{1/2}$ as shown in Fig. 3D. This figure, which depicts one of the most interesting findings of this study, shows that, at very short diffusion times, i.e. 3.7–5.7 ms, the rmsd of the slow diffusing component increases dramatically with the increase in diffusion time. In fact, this increase is as expected from the Einstein equation. For diffusion times >5.7 ms, the rmsd of the slow component increases only slightly, as expected for restricted diffusion. Indeed, the slow diffusing component of the optic nerve shows a clear change in the slope (i.e., a breaking point) of the plot of rmsd as a function of $t_d^{1/2}$. This breaking point, which represents the point after which much greater restriction is found, reflects the mean size of the axons of the optic nerves (1.12 ± 0.08 μm). However, larger values for the mean displacement were found for the fast component, which increased more linearly with the increase in $t_d^{1/2}$. In these cases no clear breaking point is observed.

Figure 4 shows the same information for a representative sciatic nerve. Higher rmsd values for both components were obtained, and the fast diffusing component showed a more linear increase in rmsd with the increase in $t_d^{1/2}$ than the slow diffusing component. In addition, no clear leveling-off, or breaking point, is observed in the case of the slow diffusing component when rmsd is plotted as a function of $t_d^{1/2}$. This may suggest larger variability in axon size (vide infra).

Figure 5 depicts the changes in rmsd as a function of $t_d^{1/2}$ for the slow and fast diffusing components for both nerve types (n = 4), along with theoretical rmsd curves calculated on the basis of the Einstein equation in which the ADC at the shortest diffusion time was taken as the intrinsic ADC of the tissue. The values of the...
experimental, theoretical, and percentage difference in rmsd of the four components for different diffusion times are given in Tables 1 and 2. These results show that the rmsds of both components, at all diffusion times, are significantly higher in the sciatic nerves than the optic nerves. Figure 5C also shows that the slow diffusing component in the optic nerves is the most restricted for diffusion times >5.7 ms, but it is the least restricted in the range 3.7–5.7 ms. For this diffusing component, there is a linear increase in rmsd as a function of $t_1^{1/2}$ for the short diffusion time range followed by a leveling-off and a large deviation from values expected from the Einstein equation in the long diffusion time range (Fig. 5C and Table 1). This results in a clear change in the slope (breaking point) of the plot of rmsd as a function of $t_1^{1/2}$. For this component, the breaking point, i.e. the maximal.
The $p_1/p_2$ value, was $>16$. All the other components exhibit less dramatic changes in the $p_1/p_2$ values, and their mean rmsds increase significantly, although not to the same extent, with the increase in $t_{d}^{1/2}$. Figure 5 and Tables 1 and 2 show that the second most restricted component is the slow diffusing component of the sciatic nerve, for which the maximal $p_1/p_2$ value was found to be 6.4. The fast diffusing component of the sciatic nerve is the least restricted one. Indeed, the maximal $p_1/p_2$ values for the fast diffusing component of the sciatic and optic nerves were found to be about 2 and 3, respectively, indicating that most water molecules in these components are not restricted. The tables also show that the proportion of fast diffusing component in sciatic nerves is larger than in optic nerves. In addition, it was found, for both nerve types, that the proportion of slow diffusing component remains relatively constant up to a diffusion time of 8.7 ms, but then decreases with the increase in diffusion time.

We also examined the effect of the duration of the gradient on the rmsd values extracted from the $q$-space analysis of the diffusion data to evaluate the effect of the violation of the short gradient pulse approximation on the structural information obtained. We found that, when gradient pulses of 4 ms were used, smaller rmsd values were obtained for both the slow and fast diffusing components of the two nerve types. These findings are shown in Fig. 6, which depicts the extracted rmsds of the slow components in the two nerve types for $\Delta$ of 2 and 4 ms. This figure shows that, as expected from theory, (31–35) significantly smaller rmsd values are obtained when the duration of the pulse gradient is increased from 2 to 4 ms. These predictions have also been found experimentally in microtubes and neuronal tissues. (29,33,35) More importantly, Fig. 6A also shows that, when gradient pulses of 4 ms are used, and consequently longer diffusion times, the clear change in the slope (breaking point) of the rmsd of the slow diffusing component of the optic nerves, when plotted against $t_{d}^{1/2}$, is not apparent.

To compare the structural information extracted from the $q$-space analysis of the diffusion MRS data with histological data, we used light microscopy to determine the size distribution of the axons in the two nerve types studied. Figures 7 and 8 show the light microscope images of four representative regions in the optic and sciatic nerves, respectively, along with the axon diameter distribution in these images. It is clear that, whereas
the optic nerve samples show a very similar axon diameter distribution with a mean value of \( \sim 1 \mu m \), the sciatic nerve samples show a much wider distribution of axon diameters. In the sciatic nerve area, an axon diameter distribution of \( \sim 1 \mu m \) and up to 8 \( \mu m \) can be found. In addition, these light microscope images show that the extracellular space, i.e. the extra-axonal space, is made up of different interconnected spaces. It should be noted that axons with a size of \( \sim \frac{1}{2} \mu m \) are less important in terms of size distribution because our \( q \)-space diffusion MR experiment cannot differentiate between them and pile them all up in one group. On the other hand, the large axons observed in the sciatic nerves have a greater effect on size distribution, which one could conclude from their low incidence as they have a larger volume and contain more water molecules. Figure 9 shows the cumulative axon size distribution in all eight samples of optic and sciatic nerves. Clearly, the mean and standard deviation of

| Table 1. Theoretical rmsd (rms\(_a\)), experimental rmsd (rms\(_e\)) and percentage difference in rmsd (%\(\Delta\)rms) for the two diffusing components of the optic nerves for different diffusion times (\(t_d\)) |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| \(t_d\) (ms) | rms\(_a\) (\(\mu m\)) | rms\(_e\) (\(\mu m\)) | \%\(\Delta\)rms | Relative population (%) | rms\(_a\) (\(\mu m\)) | rms\(_e\) (\(\mu m\)) | \%\(\Delta\)rms | Relative population (%) |
| 3.7 | 0.87 | 0.87 ± 0.06 | 0 | 69.8 ± 7.1 | 2.55 | 2.55 ± 0.20 | 0 | 30.2 ± 7.1 |
| 4.7 | 0.98 | 0.99 ± 0.09 | 0 | 69.2 ± 9.2 | 2.87 | 2.80 ± 0.29 | 2.5 | 30.8 ± 9.2 |
| 5.7 | 1.08 | 1.12 ± 0.08 | 0 | 71.0 ± 5.0 | 3.17 | 3.05 ± 0.14 | 3.9 | 29.0 ± 5.0 |
| 6.7 | 1.17 | 1.09 ± 0.07 | 7.3 | 67.5 ± 6.0 | 3.44 | 3.11 ± 0.18 | 10.6 | 32.5 ± 6.0 |
| 7.7 | 1.26 | 1.09 ± 0.11 | 15.6 | 64.2 ± 5.3 | 3.68 | 3.2 ± 0.15 | 15.0 | 35.8 ± 5.3 |
| 8.7 | 1.34 | 1.11 ± 0.09 | 20.7 | 63.5 ± 7.3 | 3.91 | 3.35 ± 0.21 | 16.7 | 36.5 ± 7.3 |
| 14.3 | 1.72 | 1.17 ± 0.08 | 47.0 | 56.0 ± 6.1 | 5.03 | 3.77 ± 0.12 | 33.4 | 44.0 ± 6.1 |
| 29.3 | 2.45 | 1.24 ± 0.09 | 97.6 | 46.7 ± 6.2 | 7.19 | 4.6 ± 0.10 | 56.3 | 53.3 ± 6.2 |
| 49.3 | 3.18 | 1.28 ± 0.14 | 248.4 | 40.7 ± 6.8 | 9.33 | 5.36 ± 0.18 | 74.1 | 59.3 ± 6.8 |
| 99.3 | 4.52 | 1.39 ± 0.20 | 325.2 | 35.7 ± 7.4 | 13.24 | 6.82 ± 0.37 | 194.1 | 64.3 ± 7.4 |

\(^a\)Values calculated using the Einstein equation in which rmsd observed for a diffusion time of 3.7 ms was taken as representing the intrinsic diffusion characteristics of the tissue.
Table 2. Theoretical rmsd (rms\textsubscript{t}), experimental rmsd (rms\textsubscript{e}), and percentage difference in rmsd (%Δrms) for the two diffusing components of the sciatic nerves for different diffusion times (t\textsubscript{d})

<table>
<thead>
<tr>
<th>t\textsubscript{d} (ms)</th>
<th>rms\textsubscript{t} (\textmu m)</th>
<th>rms\textsubscript{e} (\textmu m)</th>
<th>%Δrms</th>
<th>Relative population (%)</th>
<th>rms\textsubscript{t} (\textmu m)</th>
<th>rms\textsubscript{e} (\textmu m)</th>
<th>%Δrms</th>
<th>Relative population (%)</th>
</tr>
</thead>
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<tr>
<td>3.7</td>
<td>1.14</td>
<td>1.14 ± 0.08</td>
<td>0</td>
<td>46.0 ± 12.3</td>
<td>2.86</td>
<td>2.86 ± 0.13</td>
<td>0</td>
<td>54.0 ± 12.3</td>
</tr>
<tr>
<td>4.7</td>
<td>1.28</td>
<td>1.27 ± 0.04</td>
<td>0</td>
<td>46.5 ± 14.0</td>
<td>3.23</td>
<td>3.24 ± 0.06</td>
<td>0</td>
<td>53.5 ± 14.0</td>
</tr>
<tr>
<td>5.7</td>
<td>1.42</td>
<td>1.31 ± 0.06</td>
<td>8.4</td>
<td>44.2 ± 9.0</td>
<td>3.55</td>
<td>3.43 ± 0.21</td>
<td>3.8</td>
<td>55.8 ± 9.0</td>
</tr>
<tr>
<td>6.7</td>
<td>1.54</td>
<td>1.35 ± 0.05</td>
<td>14.1</td>
<td>42.5 ± 9.5</td>
<td>3.85</td>
<td>3.63 ± 0.23</td>
<td>6.1</td>
<td>57.5 ± 9.5</td>
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<tr>
<td>7.7</td>
<td>1.65</td>
<td>1.4 ± 0.05</td>
<td>17.6</td>
<td>42.0 ± 9.8</td>
<td>4.13</td>
<td>3.84 ± 0.26</td>
<td>7.6</td>
<td>58.0 ± 9.8</td>
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<tr>
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<td>1.44 ± 0.07</td>
<td>21.5</td>
<td>41.7 ± 9.3</td>
<td>4.39</td>
<td>4.04 ± 0.31</td>
<td>8.7</td>
<td>58.3 ± 9.3</td>
</tr>
<tr>
<td>9.7</td>
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<td>1.58 ± 0.08</td>
<td>42.4</td>
<td>38.5 ± 8.6</td>
<td>5.64</td>
<td>4.86 ± 0.46</td>
<td>16.0</td>
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<tr>
<td>10.9</td>
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<td>1.60 ± 0.11</td>
<td>48.9</td>
<td>34.2 ± 6.8</td>
<td>8.07</td>
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</tr>
<tr>
<td>12.3</td>
<td>4.17</td>
<td>1.97 ± 0.14</td>
<td>211.7</td>
<td>31.2 ± 5.7</td>
<td>10.46</td>
<td>7.46 ± 1.00</td>
<td>40.2</td>
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</tr>
<tr>
<td>14.3</td>
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<td>2.17 ± 0.12</td>
<td>272.8</td>
<td>27.5 ± 5.1</td>
<td>14.85</td>
<td>9.35 ± 1.34</td>
<td>58.8</td>
<td>72.5 ± 5.1</td>
</tr>
</tbody>
</table>

Values calculated using the Einstein equation in which rmsd observed for a diffusion time of 3.7 ms was taken as representing the intrinsic diffusion characteristics of the tissue.

Figure 6. rmsd of the slow diffusing components of the optic (A) and sciatic (B) nerves as a function of \( t_{d}^{1/2} \) extracted when the pulse gradient duration (\( \delta \)) was 2 or 4 ms.

Figure 7. Light microscope images of swine optic nerves after myelin staining with toluidine blue for four representative slices of 60 \( \times \) 45 \( \mu \)m and histogram of the axon sizes obtained from the Image J software assuming a circular shape for the axons (bar: 10 \( \mu \)m). This figure is available in colour online at www.interscience.wiley.com/journal/nbm
the axon diameter of the sciatic nerves are larger than that of the optic nerves.

DISCUSSION

In this study, we demonstrate, as expected, that high \(b\)-value \(q\)-space diffusion MRS can easily distinguish between optic and sciatic nerves. The very high signal-to-noise ratio of our spectra enabled us to characterize, robustly, two major diffusion components in each case. The use of the \(q\)-space enabled us to compute the mean displacements of the two components for each of the nerve types at different diffusion times. Interestingly, we found the slow diffusing components of both nerve types to be more restricted at long diffusion times. Of the two slow diffusing components, that of the optic nerve exhibits much more pronounced restriction at diffusion times >5.7 ms but almost no restriction in the very short diffusion time range (3.7–5.7 ms, vide infra). This resulted in a clear breaking point in the slope of the graph of rmsd versus \(t_1^{1/2}\), where the maximal \(p_1/p_2\) value was found to be >16. This value was found for a diffusion time of 5.7 ms.

One of the main advantages of \(q\)-space diffusion MRS is its ability to provide micro-structural information. The size of the compartments that can be probed depends on the strength and length of the gradient pulses used. A number of studies have shown good correlation between the structural information obtained and histological evidence (24,27,28). Therefore we decided, in this study, to explore in detail the effect of diffusion time on the mean displacement extracted from \(q\)-space diffusion MRS in different nerve tissues, concentrating on the very short diffusion time range.

At very short diffusion times (\(t_d \rightarrow 0\)), diffusing water molecules should, in principle, exhibit free diffusion, and only at longer diffusion times should restriction be observed, provided that the restricting barriers can be reached by the diffusing molecular species. Here we explored the effect of diffusion time with the aim of verifying whether a definite meaningful breaking point or dramatic change in the slope can be observed when the
rmisd is plotted against $t_d^{1/2}$. Because of the relatively small diameters of axons, we tried characterizing the rmisd in a very short diffusion time range. We did find a clear breaking point, but only for the slow diffusing component of the optic nerve. Up to a diffusion time of 5.7 ms, the rmisd increases linearly with $t_d^{1/2}$ and thereafter it remains nearly constant even if the diffusion time increases dramatically. This means that, up to this point, most of the water molecules experience little restriction to diffusion, but from then on real barriers are encountered, which contribute to the slow diffusing component of these tissues. In addition, we found that the rmisd at 5.7 ms is very similar to the mean diameter of the axons in the optic nerve as deduced from histological examination.

These findings have several implications. First, they corroborate the assignment of the slow diffusing component, particularly at diffusion times >5.7 ms, to the intra-axonal milieu, and also suggest that the axon membrane and myelin represent a real barrier to diffusing water molecules under the ex vivo conditions used in this study. In addition, these findings show that very detailed relevant structural information can be obtained from q-space diffusion MRS. Indeed, the size obtained by q-space diffusion MRS, 1.12 ± 0.08 μm (Table 1), is in good agreement with the mean value obtained histologically (1.09 ± 0.5 μm). For the slow diffusing component of the sciatic nerve, however, we observe a less clear discontinuity in the rmisd versus $t_d^{1/2}$ plot. This is again in accordance with light microscope images of the sciatic nerves: we found a wider distribution of axon diameters and hence it was difficult to identify a single time point that reflected the mean axon diameter of the sample. Even in the less uniform sciatic nerves, a change in the slope of the rmisd versus $t_d^{1/2}$ plot appears for diffusion times of 5.7–6.7 ms, when the calculated mean displacement was found to be in the range 1.31 ± 0.06 to 1.34 ± 0.05 μm.

Interestingly, these values are in good agreement with the mean axon diameters obtained histologically (1.34 ± 1.05 μm). But in this case, the $p_1/p_2$ value was only ~6.5. The larger standard deviation in axon size of the sciatic nerves may be one of the reasons for the lack of a clear breaking point in the plot of rmisd against $t_d^{1/2}$ (Fig. 9). MRS averages out all regions of the observed tissue, therefore it can be expected that, in nerves with variable axon diameter, it will be more difficult to identify a clear discontinuity in the rmisd versus $t_d^{1/2}$ plot. In fact, variability in axon diameter has been suggested as a possible reason for the lack of diffraction patterns in neuronal and biological tissues (21,29,33,35–36). All these results clearly demonstrate that high $b$-value q-space diffusion MRS, with the experimental parameters used in this study, provides accurate structural information, enabling the different diffusing components detected to be assigned with greater confidence.

With regard to the fast diffusing components of both types of nerve, only a gradual change in the slope of the rmisd versus $t_d^{1/2}$ plot is observed. The low maximal $p_1/p_2$ found for these components suggests that most of their water molecules experience hindered diffusion, as could be expected for the extra-axonal space which is made up of interconnected spaces.

In this study, we used a two-component model, which implies no exchange between the two populations. Indeed we found that, for the short diffusion time range ($t_d < 10$ ms), there were no statistically significant changes in the relative populations of the different components. However, it should be noted that the relative populations of the two diffusing components depend on the diffusion time at the longer diffusion time (i.e. $t_d > 10$ ms). This is partly because at longer diffusion times exchange plays a more important role, but more importantly because discrimination between a restricted and hindered population is more apparent at longer diffusion times. Slow diffusing water molecules can, in principle, be present in both compartments (intra-axonal and extra-axonal spaces), therefore the diffusion time needs to be increased to discriminate effectively between these two water populations. As $t_d$ is increased, the rmisd of the restricted component remains almost constant, whereas the rmisd of the molecules that exhibit hindered diffusion, such as molecules in the extra-axonal space, increases with $t_d$, thus enabling differentiation between the two water pools. This is why good agreement is observed between the MRS and histological evidence in the present preparation at a diffusion time of several tens of milliseconds.

**CONCLUSION**

This study, which demonstrates the ability of high $b$-value q-space diffusion MRS to distinguish between two different nerves, shows that this approach provides accurate and detailed micro-structural information in the more regular optic nerve. It was found that the rmisd for both diffusing components are higher in sciatic nerves than optic nerves at all diffusion times, as expected from histological evidence. Only the slow diffusing component of the optic nerve shows a clear change in the slope (breaking point) in the rmisd versus $t_d^{1/2}$ plot. Interestingly, the rmisd at this point is in very good agreement with the mean axon diameter obtained histologically. This is less apparent in sciatic nerves which show a wider distribution of axon size on histological examination. These findings clearly indicate that the slow diffusing component, especially at diffusion times >5–6 ms, originates mainly from intra-axonal water and that axonal membranes and myelin act as barriers to water diffusion. For the optic nerves, the sizes obtained from the change in the slope of the rmisd versus $t_d^{1/2}$ plot are in good agreement with structural information obtained from light microscope images. For the sciatic nerves, we could not observe a clear breaking point, which is consistent with histological findings of a wider distribution of axon size.
ACKNOWLEDGEMENTS

This work was supported by grant (522/03) from the Israel Science Foundation (ISF) and by grant (353/03) from US-Israel Binational Science Foundation (BSF).

REFERENCES