Diffusion MRI of the spinal cord: from structural studies to pathology

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Diffusion MRI is extensively used to study brain microarchitecture and pathologies, and water diffusion appears highly anisotropic in the white matter (WM) of the spinal cord (SC). Despite these facts, the use of diffusion MRI to study the SC, which has increased in recent years, is much less common than that in the brain. In the present review, after a brief outline of early studies of diffusion MRI (DWI) and diffusion tensor MRI (DTI) of the SC, we provide a short survey on DTI and on diffusion MRI methods beyond the tensor that have been used to study SC microstructure and pathologies. After introducing the porous view of WM and describing the q-space approach and q-space diffusion MRI (QSI), we describe other methodologies that can be applied to study the SC. Selected applications of the use of DTI, QSI, and other more advanced diffusion MRI methods to study SC microstructure and pathologies are presented, with some emphasis on the use of less conventional diffusion methodologies. Because of length constraints, we concentrate on structural studies and on a few selected pathologies. Examples of the use of diffusion MRI to study dysmyelination, demyelination as in experimental autoimmune encephalomyelitis and multiple sclerosis, amyotrophic lateral sclerosis, and traumatic SC injury are presented. We conclude with a brief summary and a discussion of challenges and future directions for diffusion MRI of the SC.

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Keywords: diffusion MRI (DWI); diffusion tensor imaging (DTI); q-space diffusion MRI (QSI); spinal cord; microstructure; spinal cord pathology

INTRODUCTION

MRI is currently by far the most important imaging modality of the central nervous system (CNS), and diffusion is one of the major contrast mechanisms used to non-invasively image and study brain architecture and pathologies \((1,2)\). Diffusion MRI is an established method for studying brain microstructure and for characterizing neurological disorders and diseases both preclinically and in the clinic \((1,2)\). In contrast to the enormous number of diffusion MRI studies of the brain in both animals and human subjects, the number of diffusion MRI studies of the spinal cord (SC), which has increased in recent years, is still dramatically lower than that of the brain. The SC is an integral part of the CNS that interconnects the brain with the peripheral nervous system. Water diffusion, at least in the white matter (WM) of the SC, appears highly anisotropic. The fact that diffusion MRI has not been more widely used to study the SC is partially due to the fact that in vivo diffusion MRI of the SC is significantly more difficult than that in the brain \((3,4)\). The SC, which runs deep in the body, is a much smaller organ in the axial plane (about 15 mm in human and about 3 mm in rat, for example) than the brain. In addition, the bony structures that surround the SC contribute to significant susceptibility artifacts \((3)\), and respiratory motion, cardiac pulsation, and other physiological and physical motions that cause artifacts are significantly more pronounced in the SC than in the brain \((3,4)\). Furthermore, many of the diffusion tensor imaging (DTI) studies of the brain have been devoted to diffusion tensor tractography \((1,2,5)\), which appears, at least at first glance, less relevant to the SC than to the brain. In addition, many of the early diffusion-weighted MRI (DWI) studies in the CNS have focused on cerebral ischemia, a common pathology of the brain.

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Dedicated to the memory of Professor Sir Paul T. Callaghan – a superb scientist and a great man.

Abbreviations used: AD, axial diffusivity; ADC, apparent diffusion coefficient; ALS, amyotrophic lateral sclerosis; ASIA, American Spinal Injury Association; AxD, axon diameter; BBBS, Basso–Beattie–Bresnahan score; BDNF, brain-derived neurotrophic factor; BMS, Basso mouse scale; CNS, central nervous system; CSA, cross-sectional area; CST, cortico-spinal tract; DAPI, 4\textsuperscript{v},6-diamidino-2-phenylindol; DBS, diffusion basis spectrum imaging; DC, dorsal column; DFER, DTI-based fiber estimate ratio; DKI, diffusion kurtosis imaging; d-PFG, double-pulsed field gradient; DTI, diffusion tensor imaging; DTT, diffusion tensor tractography; DWI, diffusion-weighted imaging; EAE, experimental autoimmune encephalomyelitis; EDSS, Expanded Disability Status Scale; EPI, echo planar imaging; FA, fractional anisotropy; FWHM, full width at half maximum; GFA, generalized FA; GM, gray matter; HARDI, high-angular-resolution diffusion imaging; H&E, hematoxylin and eosin; IADC, longitudinal diffusivity; LDD, Lernaldekar; LFB, Luxol fast blue; MAD, mean axon diameter; MBP, myelin basic protein; MD, mean diffusivity; Md, myelin deficient; MRM, magnetic resonance microscopy; MS, multiple sclerosis; MT, magnetization transfer; MTR, MT ratio; NAA, N-acetyl aspartate; NAWM, normal-appearing white matter; NF, neurofilament; NODDI, neurite orientation dispersion and density imaging; NOGSE, non-uniform oscillating-gradient spin echo; OXWM, primary progressive MS; QBI, q-ball imaging; QSI, q-space diffusion MRI; RA, relative anisotropy; RD, radial diffusivity; Rmsd, root-mean-square displacement; ROI, region of interest; SC, spinal cord; SCI, SC injury; SGP, short gradient pulse; SNR, signal-to-noise ratio; STE, stimulated echo; TADC, transverse diffusivity; VLWM, ventrolateral white matter; WM, white matter.
In fact, some of the problems one encounters when studying SC with diffusion MRI are similar to those faced when studying other organs outside the brain with diffusion MRI, as discussed in several papers of this volume. However, as stated, water diffusion in the WM of the SC appears highly anisotropic, and this makes diffusion MRI an attractive method to study SC architecture and pathologies (3,4). In addition, the SC is suitable for in vitro and ex vivo diffusion MRI microscopy (MRM). Since the SC has a more organized WM than does the brain and well-defined areas of gray matter (GM), it is not surprising that in vitro and ex vivo SC samples were also used to test new diffusion MRI methodologies.

In this short review, we will survey the applications of diffusion MRI to study SC microstructure and pathologies. We will describe both DTI applications and also the applications of diffusion methodologies beyond the tensor. As there are numerous comprehensive reviews on DTI (1–10), including several devoted to DTI of the SC (3,4,10), here, despite the fact that DTI is the diffusion MRI method by far the most used to study the SC, we will also present applications of less conventional diffusion MRI approaches used to study the SC, both in animals and in human subjects. Because of space limitations the application section covers the literature only on some SC pathologies. We also hope that the selected examples demonstrate what has been achieved and what can be anticipated when imaging the SC with the plethora of diffusion MRI methodologies currently available.

## DIFFUSION ANISOTROPY IN STUDY OF THE SC: THE EARLY DAYS

Wesbey et al. were the first to measure diffusion by MRI (11), Le Bihan and Breton were the first to present diffusion MR images of the human brain (12), and Thomsen and co-workers were the first to evoke restricted diffusion and the notion of diffusion anisotropy in the WM area of the human brain (13). However, the first firm demonstration of the anisotropy of water diffusion in the SC, as well as in other WM areas of the brain, was reported by Moseley et al. in 1990 (14), along with the first demonstration of the ability of DWI to detect early cerebral ischemic events (15). After this, most of the early DWI studies of SC involved measurement of the apparent diffusion coefficients (ADCs) parallel and perpendicular to the main direction of the fibers of the SC (16). Many early diffusion MRI studies in the SC were performed on in vitro and ex vivo samples (17–22), and in vivo diffusion MRI studies were performed using implanted coils (23–27). Use of implanted coils increased the signal-to-noise ratio (SNR) and mitigated, to some extent, motion artifacts.

These early studies, despite the slightly different ADC values obtained, demonstrated that water diffusion is indeed highly anisotropic in the WM of the SC. These analyses also showed that the longitudinal diffusivity (LADC), often called axial diffusivity (AD or $D_A$ or $λ_∥$), is much larger than the transverse diffusivity (TADC), often called radial diffusivity (RD or $D_R$ or $λ_⊥$). All these early in vitro, ex vivo, and in vivo studies (17–27), collected with different experimental parameters, demonstrated that diffusion is an informative contrast mechanism that can reveal WM structure and WM damage due to pathologies in the SC. Many of these early diffusion MRI studies of the SC were reviewed by Schwartz and Hackney (16).

DTI was first used to study diffusion in the brain (1,2,5–9), and DTI slowly became the method of choice for studying diffusion in the SC of animals and human subjects (3,4,10,16). DTI results are often presented as mean diffusivity (MD) and by the fractional and relative anisotropies (FA and RA, respectively). Although these collective indices are convenient, they convey only part of the embedded spatial information and may fail to report on specific directional changes that may be specific to the underlying WM pathology. Because of the cylindrical symmetry of the axons in the WM of the SC, it was suggested that DTI can be used to compute RD and AD (i.e. diffusion perpendicular or parallel to the main direction of the fibers bundles of the SC, respectively). Clearly, one may measure diffusion in only two directions when analyzing short excised SC specimens which can be properly aligned; however, for longer samples, and more importantly for in vivo applications, a rotationally invariant method such as DTI is essential. This is even more critical if one is interested in performing fiber mapping through the use of DTI to follow SC pathology such as SC injury (SCI) for example (see later).

## DIFFUSION MRI METHODOLOGIES FOR ANALYSIS OF THE SC

### From DWI to DTI

Since DTI has been comprehensively reviewed (1,2,6–9), in the present review we will comment on the DTI method only briefly. DTI consists of performing a DWI experiment in different directions. DTI, as does DWI, uses the Stejskal–Tanner equation (Equations [1A] and [1B]) to describe the signal (echo) decay due to the application of a pair of pulsed-field gradients. In such diffusion MR experiments, which can also be classified as single-pulsed field gradient or single-diffusion encoding MR experiments, and which in fact measure the Brownian motion of the investigated molecular species, the signal (echo) decay, neglecting relaxation effects, is given by Equations [1A] and [1B]:

$$E_G = E_0 e^{-\gamma^2Dq^2G^2(\lambda-\lambda_0)^2} = E_0 e^{-bD} \quad (1A)$$

$$E_{q(\lambda)} = E_0 e^{-4\pi^2q^2/p^2(\lambda-\lambda_0)^2} \quad (1B)$$

where $E_G$ and $E_q$ are the echo intensities in the presence of the gradient pulses with magnitude and duration $G$ and $\delta$, respectively; $E_0$ is the echo intensity in the absence of $G$ and $q = (2\pi)^{-1}g \delta$. The time $t$ is the time separation between the edges of the two pulse gradients, $\gamma$ is the gyromagnetic ratio, $p$ is the coherence order (generally equal to unity), and $D$ is the diffusion coefficient. The term $(1 - \delta/3)$ represents the diffusion time, whereas $b$ represents the overall diffusion weighting. Note that Equations [1A] and [1B] are valid only for a single component exhibiting free Gaussian diffusion and monitored with rectangular diffusion gradients. For more general formulas of the signal (echo) decay, taking into account the ramp-up time of trapezoidal gradient, for example, or any other more generalized acquisition schemes, see References (10,28).

Diffusion MR experiments measure the root-mean-square displacement ($\text{rmsd}$) that a molecular species explores in a given diffusion time, which, in the absence of restriction, relates to diffusion coefficient $D$ according to the Einstein equation (Equation [2]):

$$\sqrt{\text{rmsd}} = \sqrt{nDt_d} \quad (2)$$

where $t_d$ is the diffusion time, and $n$ is 2, 4, or 6 for the one-, two-, and three-dimensional scenarios, respectively. It should be noted
that in tissues one measures only the ADC, which is related to the diffusion coefficient by $ADC = D/\eta^2$, where $\eta^2$ is the sample tortuosity. The tortuosity relates the actual displacement performed by a molecule relative to the shortest trajectory connecting two points in the sample, and in fact reflects the effect of the medium on the $D$ of the investigated species. As shown in Figure 1, diffusion is isotropic if it is free or if it is restricted in all directions (Fig. 1A, B) or is anisotropic if there is a specific direction in which the diffusion is less restricted and more preferred (Fig. 1C). However, it should be noted that diffusion of a molecular species will appear restricted only if the diffusion time is long enough to allow at least a significant fraction of the diffusing species to encounter the restricting barriers (i.e. when $t_d$ is longer than or of the order of $l^2/2D$, where $l$ is the size of the compartment in which the diffusion occurs). In such cases the microstructure of a sample impacts the apparent diffusion characteristics of the systems in a diffusion-time-dependent manner. Importantly, diffusion time is a parameter readily controllable in MR diffusion experiments. As already mentioned, when the main axes of the samples are known, it is sufficient to measure the diffusion in three directions, and in cylindrical samples or compartments measuring diffusion in two directions will suffice to characterize such anisotropy. When the alignment of the sample is not known, however, a tensor analysis is required to obtain rotationally invariant diffusion characteristics of the sample. The diffusion tensor consists of nine elements, but since it is symmetric (i.e., $D_x = D_y$, $D_z = D_x$, $D_y = D_z$) a minimum of six non-collinear directions are required to characterize the tensor. Adding the $D_y$ image implies that the minimal number of MR images required to perform the most basic tensor analysis is therefore seven (29). Acquiring the data with more directions should result in a better determination of the tensor and should give rise to more accurate fiber tracking, especially in areas with crossing and kissing fibers (1,30,31). After characterizing the tensor, the tensor is diagonalized, affording the eigenvalues ($\lambda_1$, $\lambda_2$, and $\lambda_3$) and eigenvectors ($e_1$, $e_2$, and $e_3$) of the systems, from which one can compute the rotationally invariant characteristics of the tissue such as the FA and MD according to Equations [3] and [4], respectively. Note also that FA values are between zero and unity, where a value of zero denotes isotropic diffusion. From the eigenvalues one can then determine the axial ($D_{1x}$, $\lambda_1$) and radial ($D_{xx}$, $\lambda_2 + \lambda_3/2$) diffusivities.

$$
\text{FA} = \sqrt[3]{\frac{\lambda_1}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}
$$

$$
\text{MD} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}
$$

The probability density function can be represented mathematically by the tensor. In the case of isotropic diffusion, the tensor is represented by a sphere, whereas in the case of anisotropic diffusion the tensor shape approaches an ellipsoid or a cigar-like shape. The simplest way to obtain directional information from the DTI indices on a two-dimensional image is to use red–blue–green $e_1$ maps (32). A much more suitable approach is to use DTT (1,2,33,34). DTI can be obtained from a three-dimensional data set or a continuous multi-slice two-dimensional DTI data set. DTT is based on the assumption that the principal direction of the diffusion tensor associated with the largest eigenvalue ($\lambda_1$) within a voxel provides a local estimate of the fiber orientation in that voxel. This assumption is more reasonable for relatively homogeneous WM, as found in many areas of the SC, but may fail in areas of fiber crossings and dispersion. This can be partially alleviated by collecting the DTI data using a large number of directions as in high-angular-resolution diffusion imaging (HARDI).

Both deterministic and probabilistic algorithms have been proposed to assess fiber direction. The deterministic approach,
which was developed first, requires starting and target locations in the WM. The deterministic method includes, for example, the streamline approach proposed by Basser (35), where the tract propagates from a “seed” location along the tract as long as the adjacent tensor is strongly aligned and possesses FA values that are higher than a predetermined threshold. Termination occurs when the FA value falls below this threshold value (generally FA < 0.2) or when the bending exceeds the prescribed angle for a single step. This approach is based on the assumption that WM trajectories are generally relatively smooth. Clearly, the streamline approach is more suitable for areas with high anisotropy. Several approaches have been proposed to integrate the streamline pathway. The simplest are fiber assignment by continuous tracking and the Euler approaches (36,37). Probabilistic approaches (38) have also been proposed; however, they have not been used extensively to study tractography in the SC, and so we will not elaborate on these approaches here. Important issues in DTT are the determination of the thresholds of FA and bending angle that result in termination of a tract. Tracking is generally terminated when a voxel is reached where the FA is lower than a threshold of 0.25–0.35 (in WM of the SC this threshold may even be 0.4) and when the bending angle between adjacent two principal eigenvectors is greater than 35 or 40 degrees (1,2). The fact that the SC is an elongated organ with a very small cross-section makes DTT, where cubic voxels are preferred, even more challenging.

Despite the tremendous utility of DTI in studying WM architecture and pathologies in the brain, it should be noted that DTI does have some intrinsic limitations (1,2,39,40). Perhaps the most important is that DTI assumes that diffusion is Gaussian in all directions and that the signal decay is mono-exponential and can be fitted by the Stejskal–Tanner equation (7). However, almost two decades ago it was demonstrated that, at sufficiently high diffusion weighting, the signal decay of water (and metabolites) in diffusion MR experiments performed in neuronal tissues is not mono-exponential (39–41). In these early studies, as an obvious extension, the signal decay was fitted to a bi- or a tri-exponential function (39–41). Such an analysis implies imposing a specific diffusion model on the system, where the assumptions of the model are not necessarily valid given the underlying physiology. For example, the use of a bi-exponential diffusion model implies that the diffusing components are described by two non-exchanging diffusing populations exhibiting Gaussian diffusion. Although it was proposed that extra- and intracellular water are the rapidly and slowly diffusing components, this suggestion was challenged and refuted (39).

The other options for analysis of the non-mono-exponential signal decay in diffusion MR experiments are to use either the model-free q-space approach (42–44) or to model the signal by including microstructural and geometrical features of the system under investigation. Despite the intrinsic limitations of DTT, conventional DTI, where the b values are lower than 1500 s mm⁻², is the diffusion MRI method by far the most used to study the SC. In the next sections, we will elaborate further on diffusion methods beyond the tensor.

q-space diffusion MR

In 1984, Callaghan pointed out the formal analogy between pulsed-gradient spin-echo NMR and the incoherent fraction of inelastic space neutron scattering, where q is used as the reciprocal space dimension conjugated to the dynamic displacement (45). This eventually led to the development of q-space diffusion MR (42,43). The q-space approach, developed initially for studying pseudo-periodic porous materials, makes use of the average propagator concept (46) and is based on the Fourier relationship between the signal decay and the displacement probability function. According to this approach the echo attenuation \(E_q(q)\) in NMR diffusion experiments is related to the displacement probability \(\bar{P}_s(R,\Delta)\) via the reciprocal spatial vector \(q\), defined as \((2\pi)^{-1}\gamma q\), according to

\[
E_q(q) = \int \bar{P}_s(R,\Delta) \exp(i2\pi q R) dR
\]

In Equation 5 \(E_q(q)\) represents the echo decay as a function of \(q, R\) is the net displacement vector \((R = r - r_0)\), and \(\bar{P}_s(R,\Delta)\) is the averaged displacement probability. In cases of restricted diffusion, diffusion-diffraction patterns are to be expected. In such cases and under certain experimental conditions (i.e. long diffusion times \((t > \Delta/2D)\) and the short gradient pulse (SGP) approximation \((\delta < < \Delta\) and \(\delta \sim 0)\), these diffusion-diffraction patterns can report on the size of the compartment in which the diffusion occurs. Such diffusion-diffractions patterns are indeed found in porous materials (43). Using model systems it has been shown that the q-space approach allows one to extract the size of the compartments with high accuracy (47,48). It was also demonstrated that diffusion-diffraction patterns are only apparent when diffusion is measured perpendicular or nearly perpendicular to the restricting barriers (48). Callaghan also devised the relation between \(q_{\text{min}}\) (i.e. the first diffusion-diffraction minimum) and the size of the compartment for simple geometries such as spheres, cylinders, and long parallel plates (49). More recently, the effect of violating the SGP approximation was described, and it was shown that, by fitting such data and taking into account the finite values of \(\delta\), it is possible to extract the correct size of the compartment even when diffusion-diffraction minima are pushed to higher q values (50). However, as expected, when there are multiple sizes in the sample the diffusion-diffraction minima disappear. Thus, this approximation is not generally applicable to heterogeneous systems such as neuronal tissues, in which the sizes of the compartments are polydisperse (51).

From q-space diffusion MRS to q-space MRI (QSI): the porous view of WM

As it was noted that, at sufficiently high diffusion weighting, one can identify in neuronal tissues a slowly diffusing component that appears restricted (39–41), and in view of the appearance of the histological cross-section of WM, it was suggested that the WM in the CNS should be treated, to a first approximation, as a porous material (52). Based on the early papers on q-space diffusion MR by Callaghan (41–45), it was expected that q-space diffusion MR would be a useful approach for gleaning microstructural information on the WM. Since diffusion-diffraction patterns, which are found in red blood cells, for example (53,54), are not apparent in neuronal tissues, it was decided to follow the Cory and Garway approach (44) used previously by King et al. to follow cerebral ischemia (55,56). This view was further supported by the
q-space diffusion MRS data obtained from a mature optic nerve, which are presented in Figure 2 (52). This figure shows the displacement distribution profiles of water in a mature optic nerve at different diffusion times and in two major orientations (52). As shown in Figure 2, two diffusion components with distinct displacement profiles are observed: a broad one, which becomes broader as diffusion time is increased, and a much narrower one, which is nearly constant even when the diffusion time is increased by a factor of about 10.

In addition, it was found that in the optic nerve the narrow and restricted component is much larger when diffusion is measured perpendicular to the direction of the fibers of the optic nerve than when it is measured parallel to the fiber long axis. It was also found that this narrow component has an average size of about 2 μm (52). All these results imply that, in the optic nerve, which has a structure reminiscent to the WM of the SC, there is a large population of water the diffusion of which is highly restricted and which is in slow exchange with other water populations. It was therefore hypothesized that this component/population represents mainly intra-axonal water, although some contributions from restricted populations in the extra-axonal space cannot be ruled out. Note that the exchange rate was evaluated to be of the order of 3–4 s⁻¹; a recent study on exchange of water across the myelin membranes in the WM reports even slower exchange (57). In that analysis of the optic nerve, it became apparent that one has a means to measure intra-axonal water and therefore it was decided to extend this approach to MRI and perform such an analysis on a pixel-by-pixel basis using the procedure outlined in Figure 3A (58,59). This procedure, outlined in Figure 3A, resulted in q-space diffusion MRI or QSI (59,60). In this approach the displacement distribution is characterized by two images, a mean displacement image and the image of the probability for zero displacement. Figures 3B and 3C show such QSI images of SCs excised from rats at different ages. The displacement image shows that the average displacement in the WM of the mature SC is indeed only about 2 μm, which is in good agreement with the average axon diameter (AxD) in the WM (58,59). Under these experimental conditions (high q values, short δ) and sufficiently long Δ, QSI provides a means to characterize the average size of the axonal milieu. Note, however, that Δ should be long enough that the majority of the water molecules diffusing within the axons will reach the boundaries and will report on the compartment size, and that water molecules that are not restricted will travel significantly longer distances. To obtain accurate microstructural information from QSI, however, in addition to using sufficiently high q values, it is required that during the diffusion time the exchange between the compartments will be negligible. Fortunately, most axons, at least in the CNS, are less than 3.0 μm in size, and, therefore, restriction effects are apparent even at relatively short diffusion times of 15–20 ms. However, to obtain greater contrast between the restricted and non-restricted diffusing molecules longer diffusion times that allow the non-restricted component to diffuse further and the restricted molecules to encounter multiple barriers may be preferable. Therefore, for these types of application, stimulated-echo (STE) diffusion MR schemes are more suitable (58,59). Such schemes allow for longer diffusion time while keeping reasonable level of SNR. This is possible since STE-based sequences allow increasing the diffusion time.

**Figure 2.** q-Space displacement distribution profiles. (A) Displacement distribution profiles for freely diffusing tert-butanol at different diffusion times. (B) Displacement distribution profiles of excised rat brain. (C, D) Displacement distribution profiles of excised bovine optic nerve measured (C) parallel and (D) perpendicular to the long axis of the fiber. Reproduced with permission from Reference (52).
by increasing the mixing time ($t_m$), where $T_1$, which is generally much longer than $T_2$ in biological tissues, is the active relaxation mechanism.

Additional diffusion MRI methods to study the SC

DWI, DTI, and to some extent QSI have been the major approaches used to study and image SC microstructure and pathologies. In addition, both diffusion spectrum imaging (DSI) and q-ball imaging (QBI) were presented (61,62), but only QBI was used once to study the SC (63). In the last decade, however, several additional approaches to collect and analyze diffusion MRI data have been reported. These methods include diffusion kurtosis MRI (DKI) (64,65), pioneered by Jensen and Helpern, which enables evaluation of the deviation from Gaussian diffusion. Despite the increasing use of DKI to study the brain, DKI was only very recently applied to study of the SC (66,67). Recently, both oscillating gradient diffusion MRI (68,69) and double-pulsed field gradient (d-PFG) MRI (70,71) were suggested as additional means to obtain microstructural information in the CNS (72–74). In addition, some parametrical models, that were developed mainly to study brain microstructure, have been tested to some extent in the SC. The ones that should be mentioned are the AxCaliber method developed by the Assaf group (75,76), the neurite orientation dispersion and density imaging (NODDI) method developed by Alexander and co-workers (77), and diffusion basis spectrum imaging (DBSI) developed by the Song group (78,79).

Figure 3. QSI of rat SC maturation. (A) Schematic diagram depicting the steps involved in obtaining the displacement and probability maps from a $q$-space DWI data set. (B, C) Probabilities for zero displacement (B) and displacement (C) QSI images of excised rat SC as a function of time after birth. (D) Displacement distribution profiles of representative pixels from GM and WM of SC of 3 day (a, b) and 77 day (c, d) old rats. Reproduced with permission from Reference (58).
APPLICATIONS OF DIFFUSION MRI FOR STUDYING SC MICROSTRUCTURE AND PATHOLOGIES

The majority of the diffusion MRI studies of the SC reported in the last two decades were used (a) to test new diffusion methodologies for their ability to provide microstructural information, (b) to study the origin of the diffusion anisotropy of water in neuronal tissues, (c) to characterize the microstructural changes associated with SC normal maturation and (d) to study SC structural changes due to pathologies and treatments. In the following sections, we review, for space reasons, applications that demonstrate these facets only in some SC pathologies. The application part of the review focuses on dysmyelination and demyelination in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS) as well as on amyotrophic lateral sclerosis (ALS) and traumatic SCI.

Diffusion MRI of normal and dysmyelinated SCs: from the origin of diffusion anisotropy to detailed microstructural information

Despite the fact that the diffusion anisotropy of water molecules in the WM of the SC was unequivocally documented in 1990 (14), the origin of this anisotropy in neuronal tissues has been the subject of some debate (80). The discussion began after Beaulieu and Allen demonstrated that similar diffusion anisotropies are observed for water in myelinated and non-myelinated nerves (81,82), negating the early intuitive view that myelin is either the major or the only contributor to this observed anisotropy. This was further corroborated when significant diffusion anisotropy was observed in the newborn brain, lacking myelin altogether (83).

Before we go into more detail, it is important to note that a diffusion MR experiment is indeed a filtering experiment that may be affected not only by the diffusion time and the echo time used but also by the diffusion weighting used to collect the data. The fact that the myelin membrane, the axonal membrane, and the neurofilaments (NFs) and microtubules of the cytoskeleton in axons all have the same directionality (i.e. the same macroscopic or ensemble anisotropy) makes the determination of the relative contribution of each of these structural features to the observed diffusion anisotropy of water in neuronal tissues more difficult. To address this issue, diffusion MR experiments were performed on different myelinated and non-myelinated nerves, some specifically manipulated. Based on data from these studies, in which the microtubules were depolarized with vinblastine (81,82), and on the findings from study of axons from giant squid (84), it was concluded that the NFs, the microtubules, and the myelin have only a limited effect on the observed anisotropy (81,82,84). Note, however, that only the linear part (b values of up to 1000 s mm$^{-2}$) of the signal decay was analyzed in these studies, which were performed on non-mammalian species. In addition, some reduction in the ADC values (about 10–30%) was found after exposure to vinblastine, which was attributed to the difference in freshness of the treated or non-treated nerves (81).

Some important insights regarding the contributions of the axonal and myelin membranes to the observed diffusion anisotropy in mammals were obtained from diffusion MRI studies performed on the SCs of different species (22,85–87). For example, QSI indices obtained when diffusion is measured perpendicular to the main axis of the SC demonstrate that for three-day-old rat SC, which lacks myelin, no WM/GM contrast is observed (Fig. 3B, C). Following normal maturation, however, which is accompanied by myelination, a significant contrast is observed between the WM and GM (Fig. 3B, C). The data clearly indicate that the origin of this contrast is the dramatic decrease in the radial mean displacement of water in the WM regions of the SC upon maturation (58, Figure 3D). This study suggests that the myelin is in fact a restricting barrier for water diffusion. Note, however, that in this study a much higher diffusion weighting ($D_{max} = 9.6 \times 10^4$ s$^{-2}$ mm$^{-2}$) and more importantly longer diffusion times (150 ms) were used compared with those in which the microtubules were analyzed after depolarization with vinblastine (81,82). These data demonstrate that QSI not only is suitable for studying normal and abnormally maturing but should be equally useful for studying neurodegeneration and other WM-associated disorders, as shown below.

To address further the relative importance of the axonal and the myelin membranes to the observed diffusion anisotropy in the WM, SCs (22,85–87) and brains (88–93) of genetic models of demyelination and dysmyelination were studied. Gulani et al. used DTI to study myelin-deficient (md) rat SCs ex vivo and found a reduced, but still significant, anisotropy in the md SCs as compared with their age-matched controls, despite the fact that in the md SC most of the myelin is lost (22). Based on these results, which were obtained with $b$ values as high as 1750 s$^{-2}$ and diffusion time of 14 ms, it was concluded that myelin does affect the water anisotropy, but that it is not a prerequisite for finding this diffusion anisotropy. It should be noted that in this study only a dimensionless parameter reminiscent of the FA was reported (22). It is clear, however, that the FA and similar parameters may miss the effect of a specific insult if, for example, this insult affects both axial and radial diffusivities similarly. Clearly, for evaluating the effect of myelin on water diffusivity in neuronal tissues in general and in the SC in particular, it is better to compute separately the RD and the AD, known to be sensitive to myelin abnormality and axonal damage, respectively (94). This was further corroborated when the Cohen’s group studied the md SC by high b value QSI (85,86). In these studies, both axial and radial rmsds were found to be larger in the md SCs as compared with their age-matched controls. In these cases, however, the FA was not statistically different from that of controls when diffusion times of up 50 ms were used (85). In contrast, when the diffusion time was increased to 150 ms and above, the differences between the radial diffusivities of the md SCs were dramatically lower than those of control SCs, resulting in a statistically significant difference in the computed FAs of the md and the control groups (86).

In many of the dysmyelination models, the lifespan of the animals is short and ends before full maturation is reached (85,86). Recently, Anaby et al. (87) used Long Evans shaker (les) rats, which can be kept alive for several months (95), to address this issue. QSI was used to compute the FAs and the radial and axial rmsds for the SCs of 20-, 33-, and 180-day-old les and control rats at different diffusion times. They found that the radial rmsd is the best index to detect the les pathology (87). Increases in the axial and radial rmsds were observed in the les SCs relative to the controls, making the QSI-FA less informative than the rmsds. Here again, the differences in the radial rmsds were larger at longer diffusion times for all age groups studied, as shown in Figure 4 (87). These results are consistent with the view that myelin

represents a real barrier to water diffusion and hence affects the diffusion characteristics and to some extent the FA in a diffusion-time-dependent manner. This suggests that the terms “apparent fractional anisotropy” and “apparent rmsd” are more appropriate (87). In addition, this study, which enabled following both normal maturation and maturation in the progress of dysmyelination (87), showed that in the les SCs at the age of 33 days one observes minimal radial rmsd, implying that at the age of 33 days the les SCs are more myelinated than the SCs of 20- and 180-day-old les rats. Thus, the authors suggested that this is probably the manifestation of the competition between myelination and dysmyelination processes occurring during maturation in the les SCs.

The SC contains well-defined areas of both WM and GM. Since the WM of the SC is relatively coherently organized, it is not surprising that the SC was used to test new approaches for obtaining microstructural information in neuronal tissues. The ability of QSI to provide detailed microstructural information non-invasively was presented by the Wehrli group. In this study QSI was used to measure the average AxD with a precision of a fraction of micrometer (Fig. 5A). Under the experimental conditions used, there is a very good agreement between

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**Figure 4.** QSI of dysmyelination in les SCs. (A) Displacement maps extracted from QSI data of 33-day-old representative control SCs and les SCs at three diffusion times. Diffusion was measured perpendicular to the long axis of the SC. (B, C) Average perpendicular rmsd (rmsd⊥) values were obtained from the QSI data for WM of control (white columns) and les (black columns) SCs. Data are shown for three age groups for control and les SCs at diffusion times of (B) 22 ms and (C) 100 ms. *p < 0.05, **p < 0.005, ***p < 0.0005. Reproduced with permission from Reference (87).
the mean axon diameters (MADs) determined by QSI and the MAD extracted from histology (Fig. 5B, C) (96,97). The correlations between the MAD values extracted from QSI and histology were high. Since the resolution in a \( q \)-space diffusion MR experiment depends on the \( q_{\text{max}} \) values used, this study was performed with short gradient pulses of 0.4 ms and a system capable of producing extremely high pulse gradients about 1000 times higher than that used in clinical MRI scanners. Use of a longer \( \delta \) value (5.0 ms) resulted in the extraction of even smaller mean average axon sizes, which are even smaller than the real ones (96,97). Despite the very good correlation between AxDs extracted from QSI and histology in these studies (93,94), it is clear that such measurements have a limited clinical relevance and will be difficult to reproduce even using conventional animal MRI scanners. These studies, however, do provide a proof-of-concept regarding the ability of QSI to provide “virtual histology”. Note, however, that an MAD of 2 \( \mu m \) can in principle be measured with gradient systems capable of producing extremely high pulse gradients about 1000 times higher than that used in clinical MRI scanners. Use of a longer \( \delta \) value (5.0 ms) resulted in the extraction of even smaller mean average axon sizes, which are even smaller than the real ones (96,97). Despite the very good correlation between AxDs extracted from QSI and histology in these studies (93,94), it is clear that such measurements have a limited clinical relevance and will be difficult to reproduce even using conventional animal MRI scanners. These studies, however, do provide a proof-of-concept regarding the ability of QSI to provide “virtual histology”. Note, however, that an MAD of 2 \( \mu m \) can in principle be measured with gradient systems capable of producing gradient pulses in the range of 100–200 G cm \(^{-1} \), which can now be found in small animal MRI scanners. In general, the higher the gradient strength that can be applied to the sample the more accurate an AxD determination is to be expected, but high diffusion weighting reduces the SNR, which is always a crucial issue when MR images of SC are collected in vivo. However, it is important to note that measurement of the absolute value of axon size with an accuracy of a tenth of a micrometer is not crucial in many cases for the diagnosis of a specific pathology in the SC. To make QSI a good diagnostic tool, it is enough that upon the development of the disease a significant change in the average AxD is apparent. In addition, it should be noted that AxD determination may be even more difficult in many SC pathologies.

More recently, SCs were used to evaluate the ability of both d-PFG MR (98) and oscillating-gradient spin-echo (OGSE) (99) diffusion MR experiments to map AxDs. Mapping these parameters is of importance, since it is well known that conduction velocity of an axon is proportional to its diameter (100), and in many neurological disorders axon damage is size dependent (101). Very recently, axon size was suggested as an additional parameter to improve DTI tractography (102). Komlosh et al. used d-PFG filtered MRI, with zero mixing time, to measure the average AxD in the swine SC (98). In their study \( q_{\text{max}} \) was less than 1000 cm \(^{-1} \), and axon sizes in the range of 3.0–5.0 \( \mu m \) (Fig. 6A), which appear somewhat larger than the expectations, were found (98). OGSE is more sensitive to small axons than d-PFG due to the use of very short effective diffusion time (103), and the Gore group recently showed that it is possible to obtain very detailed microstructural information using this diffusion methodology, as depicted in Figure 6B (99).
Li et al. used $g_{\text{max}}$ of 133 G cm$^{-1}$ ($b$ values up to 2000 s mm$^{-2}$), and the intra-axonal compartment was modeled as cylinders and spheres. In contrast to most studies in the field, where water molecules in the extra-axonal space are assumed to have hindered Gaussian diffusion, Li et al. modeled the extra-axonal milieu as having a frequency-dependent diffusion (103). The five parameters extracted from this modeling, when the diffusion was measured perpendicular to the long axis of the SC, are presented in Figure 6B. The extracted parameters include the AxD, the diffusion coefficients of the intra-axonal space ($D_{\text{in}}$), the intra-axonal fraction ($f_{\text{in}}$), and the frequency-dependent diffusion coefficient of the extra-axonal space ($D_{\text{ex}}(f)$), which depends on a constant $D_{\text{exo}}$ and on $\beta_{\text{ex}}$, which is the slope of $D_{\text{ex}}(f)$ with respect to the frequency. Interestingly, using this approach the authors showed that different WM areas are characterized by different average AxDs in the range of 1.4–4.6 µm, and that there is a good correlation between AxDs extracted from MRI and histology (Fig. 6C) (99). In fact the values obtained by Xu et al. are similar to those reported by Ong et al. (96,97) and also not very different from those reported by Assaf et al. (58,59). These authors also claimed that the presented OGSE approach is suitable for studying AxDs between 1 and 6 µm (99,103).

In recent studies Shemesh et al. used non-uniform oscillating-gradient spin echo (NOGSE) to determine, through modeling, the size distribution of axons in different regions of interest (ROIs) in the rat SC and in mouse brain (104,105). The method can provide confining length with a (length)$^6$ parametric sensitivity, thus providing an efficient tool for studying micrometer-scaled cellular systems and highlighting anatomical sub-structure in the SC and the brain (104,105). The Dyrby group studied fixed monkey SCs to characterize structures at different levels of the SC (106). All these advanced diffusion MRI methods were performed on excised SC samples. The application of such demanding diffusion MRI methods in vivo is expected to be difficult, since, as stated very recently by the experts in the field, the SC is in one of the worst environments in the body for diffusion MRI (107). Nevertheless some advanced diffusion MRI methods have been applied to study the SC in vivo. For example, Cohen-Adad et al. used QBI to study cat SCs both ex vivo and in vivo (63) using a 3 T clinical scanner. The ex vivo DWI data set was collected with a $b_{\text{max}}$ of 1500 s mm$^{-2}$ and 100 encoding directions, whereas the in vivo DWI data were collected with 28 or 55 directions and $b_{\text{max}}$ was 800 s mm$^{-2}$. This study showed the feasibility of performing QBI in vivo using a 3 T clinical scanner and demonstrated the superiority of QBI versus DTI in detecting crossing fibers. Additional ex vivo QBI data were collected with $b_{\text{max}}$ values of 1000, 2000, and 3000 s mm$^{-2}$ and with exactly the same experimental parameters. This comparison showed that the FA, at least in the WM, did not change with $b_{\text{max}}$ but the generalized FA (GFA) obtained from QBI did and so also the WM/GM contrast. They also found real benefit of SS directions compared with 28 directions in the in vivo QBI acquisition.
scheme, as could be expected. Recently, Rangwala et al. demonstrated that high b value STE diffusion is feasible on SC of human subjects (108), and Grussu et al. used NODDI, very recently, to study the human SC in vivo for the first time (109). In this study (b\text{max} values of first and second shells were 711 and 2855 s mm\(^{-2}\)) NODDI maps could identify anatomical features such as GM/WM contrast and showed similar inter-subject variability to DTI. In addition, NODDI identified potential sources for DTI indices and was found to outperform DTI in terms of quality of the fit (109). Farrell et al. were the first to use QSI to study human SCs of MS patients (110), and several recent DKI studies were devoted to the involvement of the SC in MS (111–114) (see next section). Very recently, Fujiyoshi et al. demonstrated that in vivo QSI can be obtained on SCs of control and shiverer mice and common marmoset before and after induction of EAE using a 7T MRI scanner. They found that in vivo QSI is sensitive enough to depict dysmyelination, demyelination, and remyelination in these animal models (112). In addition, Duval et al. showed that AxCaliber diffusion data can be collected on the SCs of healthy subjects using a 3T scanner in about 30 min (113). There it was found that the MAD is 4.5 ± 1.1 μm (range from 3.0 μm (gracilis) to 5.9 μm (spinocerebellar tracts)). These data, however, were collected with a 30 G cm\(^{-1}\) gradient system and a 64-channel coil (113). The impact of gradient strength on diffusion MRI estimates of AxD was recently investigated (114) (on a 3 T system equipped with a 30 G cm\(^{-1}\) gradient system), showing that the highest gradient strength possible should be used to obtain a more accurate AxD (114).

**Diffusion MRI of EAE and MS**

MS is an inflammatory, demyelinating, neurodegenerative disease of the CNS with devastating consequences, for which effective treatment is still lacking (115). Therefore, patients with MS and animal models of MS such as EAE have been studied extensively using MRI (116) and diffusion MRI (117). MS pathology is complex and involves demyelination, axonal loss, and inflammation. In addition, SC lesions are observed in 30–40% of the asymptomatic patients and in about 90% of confirmed MS patients (107,118) and is a major contributor to the observed disability. Therefore, it is not surprising that diffusion MRI has been used to study the SC of both EAE animals (119) and MS patients (110,111,117).

EAE, which is the most studied experimental model of MS, shares the clinico-radiological paradox seen in MS, where MRI lesions, observed on relaxation-based MR images, do not generally correspond to the level of long-term disability or impairment documented (119). Therefore, there has been a constant search for more robust and specific MRI methods that will overcome this problem, and DTI was suggested as one possibility among other MR methods for this purpose (107,119). DeBoy et al., who studied the EAE model in rat SCs ex vivo (b\text{max} of 800 s mm\(^{-2}\)), showed that DTI indices (i.e., FA, D\(_{ax}\) and D\(_{||}\)) all detect the pathology even in remote distal sites, and that the DTI parameters correlated well with axon counts, degenerating axon counts, and SMI-31 (a marker for phosphorylated neurofilament (NF)) (120). No correlation was found between DTI indices and Luxol fast blue (LFB), a stain used to observe myelin under a light microscope. In addition, DTI parameters were found to be more sensitive to the EAE pathology than conventional T2-weighted MRI (120). Feng et al. studied the EAE model in swine SC in vivo using a clinical 3T scanner with DTI (b\text{max} 1000 s mm\(^{-2}\)) (121). Here EAE was induced by the classical method, no demyelination was observed, and a reduction in AD was reported. Interestingly, this reduction in AD correlated with anti-amyloid precursor protein positive axon counts; the correlation was statistically significant as compared with control values only after the clinical symptoms of the disease were apparent on the clinical score as seen in Figure 7A. No demyelination was observed by histology and no change in RD was recorded (121).

The Song group, who played a pivotal role in studying experimental models of different pathologies of the CNS ex vivo and in vivo using DTI (122–127), have studied SC involvement in EAE and MS (93,122,125–127). In an early study, Kim et al. showed that AD decreases due to the axonal damage associated with the EAE pathology (125). Regional elevated RD was observed in ROIs with diminished staining of myelin with LFB (125). A statistically significant increase in RD was observed at the end of the acute stage and in the chronic stages of EAE. Budde et al. grouped the in vivo DTI indices (b\text{max} of 785 s mm\(^{-2}\)) of control and EAE mice according to their clinical scores and correlated indices with staining for myelin and axonal markers (SMI-31 and myelin basic protein (MBP), respectively) and a marker for inflammation (4',6-diamidino-2-phenylindol, DAPI). The DTI–histology correlations were performed after coregistration and pixel-by-pixel comparison between DTI parameters and histological markers. The axonal, but not the radial, diffusivity correlated with the clinical score (Fig. 7B) and with SMI-31 (Fig. 7C) (127). The lack of correlation between RD and the histological markers may be due to large variations in RD indices and the inflammation that characterizes this model. It is important to note that in this study strong correlations were observed between inflammation, demyelination, and axonal damage as deduced from the staining with the different histological markers. In a more recent study Wang et al. used conventional DTI (b\text{max} of 1000 s mm\(^{-2}\)) to study in vivo the effect of FTY720 daily treatment on the development of EAE pathology in mice (93). Two doses and two administration regimes were tested and compared with sham-operated and vehicle-treated groups. One group of mice was treated with FTY720 prior to disease onset (prophylactic treatment group), and one group was treated after disease onset (therapeutic treatment group). The results depicted in Figure 8 show that the A\(_{||}\) of the sham and the FTY720-treated groups were higher than that of the vehicle-treated group and that the changes were more pronounced in mice treated prior to disease onset than in those treated therapeutically. The same trend is observed for the RD but the differences there were statistically less significant (93). Note, however, that the RD is much smaller than the AD, and the standard deviations are larger, reducing the statistical significances of these differences. The relative anisotropy was found to be higher in the groups treated prophylactically with 3 and 10 mg/kg FTY720 compared with the vehicle-treated group. In the therapeutically treated mice, however, no such statistically significant differences were observed. Interestingly, when Klawiter et al. studied MS tissues ex vivo using DTI with somewhat higher diffusion weighting (b\text{max} of 1813 mm\(^{-2}\)/s) and compared the MRI findings with histology, a correlation was observed between RD and degree of demyelination. It was then concluded that RD can be used as a surrogate marker for demyelination, whereas AD, which was also altered with axon injury, seems to be less specific (128).

Biton et al. studied the EAE swine model ex vivo by high b value QSI (129) at relatively long diffusion time (b\text{max} of 2.03 × 10\(^4\) s mm\(^{-2}\), Δ of 200 ms, and d\text{max} of 411 cm\(^{-1}\)). Both the
radial and axial rmsds, in the x- and z-direction, respectively, were measured from QSI. The QSI anisotropy index was also computed. The values of the control WM were compared with the values of ROIs identified by $T_1$- and $T_2$-weighted MRI as MS plaques and as normal-appearing white matter (NAWM) in the EAE-diseased SC. Both the rmsd and the probability for zero displacement, obtained when the diffusion was measured perpendicular to the fiber direction, were able to differentiate between the control WM and the EAE WM plaques and also between the NAWM of the EAE SC and the control WM and EAE plaques (Fig. 9). NAWM ROIs in the EAE SC were found to have intermediate QSI values. Interestingly, when an ROI in the EAE SC had an rmsd similar to that of a control SC it also appears normal on the hematoxylin and eosin (H&E), LFB, and NF images (Fig. 9). This indicates that QSI images can, indeed, be regarded as "virtual histology" (129,130). These studies showed that QSI indices are extremely sensitive to MS pathology and detect not only the plaques in EAE SCs and MS brains but also abnormalities in the NAWM of EAE SCs and MS patients (60,129,130). In addition, in MS patients, a correlation was also found between the changes in the QSI indices and the N-acetyl aspartate (NAA) levels measured using $^1$H MRS (131). QSI is also sensitive to the demyelination process that occurs in the SC as a result of chronic hypertension in spontaneous hypertensive rats (132) and has been used recently to image brain and cancerous tissues (133–135).

DTI has been used extensively to study the brains of MS patients (136,137). In the SC early work was performed using diffusion MRI by Clark et al., but these authors did not characterize the full tensor (138). Since then, more studies have been devoted to DTI in the SC of MS patients (139–146). For example, Agosta et al. demonstrated, in a longitudinal study, that FA and other parameters measureable by MRI, such as the cross-sectional area (CSA) of the SC in MS patients, correlate with increased disability (140–142). Ciccarelli and coworkers examined the SCs of MS patients by a combination of cervical cord DTI and $^1$H MRS, with the aim of verifying which of these MR indices correlate with acute disability (143). MS patients showed lower FA, lower connectivity as assessed by DTT at the lateral cortico-spinal tracts (CSTs) and at the posterior tracts, and lower total NAA levels compared with controls (143). This study evaluated the FA, the RD, the connectivity, and myo-inositol, total NAA, total choline, and total creatine levels, and employed several clinical tests including the Expanded Disability Status Scale (EDSS) test. Correlations were found, inter alia, between EDSS and the RD of the lateral CSTs. The conclusion from this study was that it will be worthwhile performing longitudinal DTI studies of MS patients (143), especially using multiparametric MRI where the different MRI parameters are correlated to clinical outcome.

Théaudin et al. examined a small cohort of MS patients over three months for evolution of SC damage, and found that those patients who improved clinically had a significant reduction in the RD in the lesions. In the NAWM, however, these MS patients presented with reductions in ADCs, RD, and ao and increases in FA (146). Oh et al. used conventional DTI in conjunction with magnetization transfer (MT) to study a large number of MS patients with the aim of evaluating the ability of DTI and the MT ratio (MTR) to differentiate between MS patients with comparable lesion burdens and high and low disability levels (144,145). For patients with low lesion counts, FA, MD, $\lambda_\perp$, $\lambda_\parallel$, MTR, and SC...
CSa were all more abnormal in the high-disability subgroup than in the low-disability subgroup. For the MS patients with high lesion counts, FA, MD, $\lambda_\perp$, and SC CSa, but not $\lambda_\parallel$, were more abnormal in the high-disability group versus the low-disability subgroup (144). Data from this study corroborate the notion that, for MS, RD seems to be a more suitable index than $\lambda_\parallel$ to describe the disease load (144,145).

Van Hecke et al. examined with DTI, DTT, and T2 the NAWM in the SC of MS patients (139). In these DTI experiments $b_{\text{max}}$ was set to 700 s mm$^{-2}$ and 60 directions were collected (139). FA, $\lambda_\perp$, $\lambda_\parallel$, and $\lambda_\parallel/\lambda_\perp$ were computed for controls and MS patients with or without $T_2$ SC lesions. FA, $\lambda_\perp$, $\lambda_\parallel$, and $\lambda_\parallel/\lambda_\perp$ were significantly lower in the SC of MS patients with SC lesions than in control subjects. For SC of MS patients with no $T_2$ lesions, however, only the FA and $\lambda_\parallel/\lambda_\perp$ were significantly different from those of controls (139). These results suggest that the SC of MS patients may be affected by the disease even when $T_2$ lesions are not apparent. This indicates that diffusion MRI provides additional information and may be superior relative to relaxation-based MRI when analyzing the disease load in SC of MS patients (139).

The first application of QSI in human SC in vivo was reported by Farrell et al. using a 3 T MRI scanner ($b_{\text{max}}$ of 4685 s mm$^{-2}$; 31 $q$ values, two directions, $q_{\text{max}}$ of 414 cm$^{-1}$). Four MS patients were evaluated, and this analysis showed that the MS pathophysiology could be identified by the QSI indices (110). The authors commented, however, that due to the heavy diffusion weighting needed for the $q$-space analysis and the relatively long echo time in the spin-echo echo planar imaging (EPI) sequence used, this methodology, which is now increasingly used in human brain (147–149), remains challenging to perform on human SC in vivo. In addition, these authors showed that different rmsds were extracted from studies that used different $q$ values, which should not be surprising since $q_{\text{max}}$ determines the resolution of such diffusion experiments (59). However, although the QSI parameters were extracted from QSI experiments collected with different $q_{\text{max}}$ values it was possible to detect the differences between the control and MS groups. In 2015, an additional in vivo QSI study of SCs of MS patient was published (150). This study combined QSI ($q_{\text{max}}$ of $\sim$1000 cm$^{-1}$ interpolated to 2000 cm$^{-1}$) and $^1$H MRS and correlated the MR findings to five behavioral tests, some of which were specifically developed to monitor SC disability of the kind found in primary progressive MS. The aim was to explore whether MR methodologies are able to detect early spinal neurodegeneration that causes clinical...
disability. Despite the fact that no significant atrophy was measured by the $T_2$ MR images, an increase in QSI RD and a decrease in the level of several metabolites was observed. Interestingly, these MRS and QSI changes correlated with several behavior tests. For example, reduced QSI RD correlated with the Modified Ashworth Scale and vibration perception thresholds. The authors concluded that the observed relationship between the imaging indices and disability measures suggests that early SC neurodegeneration may underlie clinical impairment (145). They also suggested that MR approaches should be used in clinical trials of neuroprotective agents and stated that the predictive abilities of QSI and $^1$H MRS with regard to disability will be evaluated on the same cohort at one- and three-year time points. The quality of the data that were obtained on the human SC in this study can be seen in Figure 10 (150).

QSI is a challenging experiment to perform in SCs in vivo, but DKI, which measures the deviation from Gaussian diffusion and requires less diffusion weighting, is gaining more importance in MRI of the brain (64,65,151–153) and was recently used to study the SC in vivo (66,67). Raz et al. collected $T_2$ and DK images on a small cohort of MS patients ($n = 19$) and controls ($n = 10$) using DKI parameters collected with $b_{max}$ of 2500 s mm$^{-2}$ (66). They found $T_2$ lesions in 18 of the 19 MS patients examined. The authors found a decrease in whole SC FA and mean kurtosis. FA and GM mean kurtosis in WM were significantly decreased, and the whole SC MD increased, in the MS patients compared with controls. These preliminary results suggest that DKI of SC can be used to characterize lesions in the NAWM and GM in MS patients, thus providing complementary information to DTI (66).
An interesting recent development in the field of diffusion MRI of the CNS was the introduction of the DBSI approach by the Song group (78,79). They hypothesized that DBSI would provide diffusion indices with higher specificity to pathophysiology as compared with DTI indices. DBSI, like QSI and DKI, requires high diffusion weighting beyond the mono-Gaussian regime used by DTI. Recently, Wang et al. used DBSI ($b_{\text{max}}$ of 3000 s mm$^{-2}$) and DTI to study EAE in mice (79). They compared sham-operated mice with vehicle-treated EAE mice with EAE mice treated with Lenaldekar (LDK, 1H-indole-3-carbaldehyde quinolone-8-yl-hydrazone), an anti-leukemia drug, that had been found previously to reduce demyelination in EAE (154). LDK or vehicle treatment started on Day 26 just before the second remission. The clinical scores of the mice were taken daily for a period of 36 days. Mice were sacrificed and tissues fixed at Day 20 and Day 36. The diffusion MRI findings were compared with myelin and axonal markers (anti-MBP and SMI-31, respectively), with a marker that visualizes immune-reactive materials (goat anti-mouse IgG antibody), and with a marker for inflammation (DAPI).

Representative DBSI maps for the different groups are presented in Figure 11, and the correlation of the DTI and DBSI indices to the histological staining is shown in Figure 12 (79). This study demonstrated that the correlations between the DBSI indices and the histological markers are indeed much more statistically significant than are the correlations between the DTI indices and the histological markers (compare $R$ and $p$ values in Figure 12A-C with those in Figure 12D, E) (79). In addition, the DBSI model indeed demonstrates that $\lambda_2$ has a firm negative correlation with MBP-positive area, suggesting that DBSI RD is indeed a good marker of myelin integrity in EAE. These results also clearly demonstrate the beneficial effect of LDK treatment. The DBSI measures, the DTI-derived $\lambda_1$, and the histology for the LDK-treated group were close to those of the sham-operated mice and were significantly different from the vehicle-treated animals (79).

Wang et al. have used, very recently, the same methodology to differentiate and quantify inflammation, demyelination, and axonal injury in MS patients (155). In this study DBSI indices were compared with histological findings in autopsy MS SC specimens (155). The authors performed a detailed protocol for coregistration to assure that the analyzed DBSI ROIs corresponded to the histological ROIs analyzed. The DBSI data on the human SC specimens were obtained using 99 directions and a $b_{\text{max}}$ of 3200 s mm$^{-2}$. The results of this study are presented in Figure 13. In this study it was found that the DBSI fiber fraction index correlated very well with the areas of silver stain in the three samples (Fig. 13D, H, L). The DBSI $A_D$, a measure of axonal integrity, correlated with the area of silver stain in only one sample (Fig. 13E, I, M). Interestingly, the DBSI RD showed a negative correlation with the area of LFB staining in all three cases studied (Fig. 13F, J, N). In two of the three samples the correlations between the RD and the silver stain area are highly significant. The DBSI restricted fraction correlated with the hematoxylin stain only (Fig. 13G, K, O). These results clearly corroborate what was observed in previous QSI studies of EAE and MS (60,124,125). The authors performed a detailed protocol for coregistration to assure that the analyzed DBSI ROIs corresponded to the histological ROIs analyzed. The DBSI indices were compared with histological findings in autopsy MS SC specimens (155). Since ALS pathology comprises damage to WM, diffusion MRI has been used to attempt characterization of ALS.

**Diffusion MRI in ALS**

ALS is a devastating and fatal disease, of which the origin and etiology are far from being understood, and for which there is no efficient treatment (156). ALS is characterized by loss of upper motor neurons in the cortex as well as lower motor neurons in the brainstem and the SC. The clinical presentation of ALS varies considerably, complicating early diagnosis and prognosis, so there has been a search for effective biomarkers for ALS (157,158). Since ALS pathology comprises damage to WM, diffusion MRI has been used to attempt characterization of ALS.
In addition, post-mortem tissues show that within the WM of the SC of ALS patients there is a loss of large myelinated fibers in the corticospinal tracts and ventral roots. Although several diffusion MRI studies of ALS brains and brain stems have been reported, only recently have analyses focused on SCs of ALS patients.

As a mutation in SOD1 has been observed in a small proportion of patients with familial ALS syndrome, mice overexpressing the SOD1 mutation have been used as an experimental model of ALS. Recently, the SCs of SOD1 mice were examined by diffusion MRI. Underwood et al. used conventional DTI with high in-plane resolution to study the lumbar SC of SOD1 mice in vivo at 16.4 T and found a reduction in FA in the ventral and left and right ventrolateral regions compared with wild-type mice. No statistically significant differences in FA were observed for the dorsal or dorsal lateral regions between the two groups, consistent with the fact that the ascending sensory axons are preserved in this region of the SOD1 mice. In addition, FA was slice dependent, and the MD values were found to be similar in the SOD1 and wild-type mice. A closer look at the data shows that the RD was increased in the brainstem motor nuclei and in ventrolateral areas; however, in the cervical ventrolateral areas the increase was not statistically significant. The fact that axial and radial diffusivities changed in opposite directions resulted in a statistically significantly lower FA in these areas in the SOD1 mice compared with wild-type mice. A close look at the data shows that the RD was increased in the brainstem motor nuclei and in ventrolateral areas; however, in the cervical ventrolateral areas the increase was not statistically significant. The fact that axial and radial diffusivities changed in opposite directions resulted in a statistically significantly lower FA in these areas in the SOD1 mice compared with wild-type mice. A close look at the data shows that the RD was increased in the brainstem motor nuclei and in ventrolateral areas; however, in the cervical ventrolateral areas the increase was not statistically significant. The fact that axial and radial diffusivities changed in opposite directions resulted in a statistically significantly lower FA in these areas in the SOD1 mice compared with wild-type mice.
brains of two types of SOD1 mouse in vivo with $T_2$-weighted MRI, also studied their SCs ex vivo by diffusion MRI (168). In this study, decreases in AD and FA progressed with the disease stage. These decreases correlated with axonal count in most WM regions. Interestingly, in the case of the 129Sv SOD1G93A mice the AD was reduced compared with the wild-type mice prior to onset of the disease, whereas in the C57 SOD1G93A mice this occurred only at advanced stage of the disease (168). This was the first diffusion MRI study to demonstrate the differences in the time course of the axonal degeneration of motor neurons in these two strains of SOD1 mice.

Cohen-Adad et al. studied the SC of ALS patients using DTI ($b_{\text{max}}$ of 1000 s mm$^{-2}$, 64 directions), from which the FA, AD, RD, and MD were estimated (165). The DTI results were compared with anatomical and MT-MRI and correlated to clinical disability and electrophysiological parameters (165). FA was decreased and RD was increased in the ALS SCs as compared with controls, whereas no difference was observed in the AD between the two groups, suggestive of secondary demyelination and preservation of axonal architecture. The MTR was reduced in the ALS SCs compared with controls, but only FA correlated with the disability test and the electrophysiological indices measured. These results corroborate the “dying-back” hypothesis, which suggests that motor unit loss and associated muscle function precede the death of motor neurons (160). Nair et al., who used a similar DTI protocol DTI ($b_{\text{max}}$ of 1000 s mm$^{-2}$, 30 directions) found similar results when they evaluated ALS patients (164). No significant changes were found in AD or in MD values between controls and ALS cords, whereas significant differences were observed for FA and RD, which were also shown to correlate with tapping speed (Fig. 15A, B) (164). The results corroborated the "dying-back hypothesis" and showed that the differences between the ALS and control groups increase along the cervical cord, with distal segments generally showing larger differences (Fig. 15C, D). In a more recent study, where the CSa, MTR, and DTI indices of the SCs were evaluated nearly a year after the first examination, it was concluded that atrophy and DTI metrics predicted ALS disease progression, but that CSa rate of change is a better biomarker for the disease progression than are DTI and MTR metrics (170). Clearly, there is an urgent need for more diffusion MRI studies of ALS to evaluate further the prognostic capacity of diffusion MRI in this pathology.

Diffusion MRI and DTT in traumatic SCI

Traumatic SCI affects more than two million people worldwide (171). Although some neurological function may be recovered in the first year after injury, in about 50% of traumatic SCI cases the patient is left with severe disability for life, posing immense social and economic burdens (171). Much remains unknown concerning the development of secondary damage following traumatic SCI, and the evaluation of the efficacy of new therapeutic interventions requires the development of surrogate markers for outcome. Therefore it is not surprising that, in recent years, much effort has focused on imaging of traumatic SCI (171). Diffusion MRI in general and DTI in particular have been extensively used to study traumatic SCI both ex vivo and in vivo in experimental models (124,172–199) and also in human subjects (200–212). Early work focused more on ex vivo diffusion MRI studies of traumatic SCI, but more recently in vivo studies have been conducted. The general aim of many of these diffusion MRI studies was to identify surrogate
markers that correlate with behavioral and neurological disability. Traumatic SCI, after the mechanical event that causes cell death, may trigger, in the acute stage, many secondary processes such as hypoxia, ischemia, hemorrhage, and edema, as well as GM morphological changes and axonal damage and degeneration in later stages. The relative contribution of each process may be affected by the type of the trauma and its severity. Each process may have a different effect on the diffusion characteristics of the tissue, making the goal of finding a single surrogate marker for the outcome after traumatic SCI complex.

Using conventional diffusion MRI ex vivo ($b_{\text{max}}$ of 1000 s mm$^{-2}$), Nevo et al. demonstrated that, in a contusion model of traumatic SCI in the rat, treatment with T cells specific to the antigen of MBP increases the anisotropy index as calculated from DWI compared with the non-treated group (172,173). Schwartz et al. treated injured SC with unmodified fibroblasts and with fibroblasts modified to secrete brain-derived neurotrophic factor (BDNF) and found that both tADC and the anisotropic index ($tADC/lADC$), collected 12 weeks after the hemi-section, were closer to the control values in the group treated with fibroblasts that secrete BDNF (174). This occurred...
despite the fact that the sizes of the lesions did not differ between the two groups. In this study improved lADC and anisotropic index values correlated with improved behavioral recovery.

Ex vivo DTI of injured SCs transplanted with vitrogen and fibroblasts were shown to identify the realignment of the reactive astrocytes in the GM in the vicinity of the site of injury. These astrocytes were found to orient themselves perpendicular to the WM tracts (193). DTI was also used to characterize ex vivo WM damage at 3 and 8 weeks following dorsal column (DC) transection (187). LFB and MBP staining was used to assess myelin damage, and NF-H in combination with NF/Tub staining was used to assess axonal damage. FA, lADC, tADC, and MD were calculated in different ROIs comprising bundles of parallel ascending and descending axons. Both myelin water fraction and tADC showed significant correlation with LFB staining at 3 weeks and 8 weeks post-injury. Interestingly, IADC and FA correlated significantly with NF/Tub staining at 3 weeks post-injury, but only IADC displayed significant correlation with histology at 8 weeks post-injury (187).

Conventional DTI performed on SCs ex vivo examined 2, 5, 15, 20, and 25 weeks after moderate spinal contusion injury showed, as expected, a decrease in IADC and MD and an increase in tADC along the entire cords (177). Interestingly the ADC distribution could be fitted to exponential functions with a half time of about 4 days (177). Jirjis et al. used ex vivo conventional DTI ($b_{\text{max}}$ value of 500 s mm$^{-2}$) to verify the ability of DTI parameters to characterize the severity of the SCI (182). They evaluated animals for 10 weeks after the contusion injury and found that the best correlation between the severity of the injury, judged by Basso–Beattie–Bresnahan score (BBBS), was with the MD extracted from DTI ($r^2 = 0.80$) (182). In a more recent study, Kelley et al. correlated ex vivo DTI ($b_{\text{max}}$ of 1000 s mm$^{-2}$) with locomotor activity (from BBBS) and histopathology in a contusion model of SCI over a range of severities of injury (Fig. 16A, B) (183). FA of the mild SCI group differed from FA of the moderate and severe SCI groups, while differences between FAs of the moderate and severe injury groups were not statistically significant (Fig. 16C). In addition, 4 weeks after the injury, positive correlations were observed between the FA at the epicenter and in caudal areas and the BBBS, as shown in Figure 16D, E. In addition FA correlated with foot position and the amount of spared tissue (183).

Ex vivo QSI was also used to study SCI in hemi-crush and dorsal root axotomy models in the rat (178,189–191). Nossin-Manor et al. demonstrated that QSI ($q_{\text{max}}$ of 1277 cm$^{-1}$, $b_{\text{max}}$ of $105 \mu m^2 s^{-1}$, $\Delta/\delta$ of 150/2) can detect the response to mild and severe hemi-crush insult at 5 days, 10 days, and 6 weeks following the hemi-crush (Fig. 17). The data clearly show that 6 weeks following a mild hemi-crush injury there is a significant spontaneous

\[ \text{Figure 14. DTI of the SCs of SOD1 mice. (A) Animal alignment in the MRI scanner with the asterisk indicating Slice 1 referred to in subsequent panels. (B) Representative FA map within the lumbar region of a WT mouse, showing the dorsal (D), ventral (V), right and left ventrolateral (VLr, VLI) and right and left dorsolateral (DLr, DLl) ROIs analyzed. (C, D) DTI identifies decreased FA values in ventral WM regions of SOD1 mice in both (C) left ventrolateral and (D) right ventrolateral ROIs. (E, F) Changes in (E) axial and (F) RD values ($\mu m^2 s^{-1}$) reflect axonal degeneration in SOD1 mice. Error bars are standard errors of the means. Reproduced with permission from Reference (167).} \]
recovery (Fig. 17C, G). Interestingly, such a recovery was not observed 6 weeks after severe injury (Fig. 17F, H). The damage index computed from the QSI images correlated with locomotor scores and footfall tests (Fig. 17I, J) (190). Farrell et al. used ex vivo QSI ($q_{\text{max}}^\perp$ of 1533 cm$^{-1}$, $b_{\text{max}}^\perp$ of 74 200 s mm$^{-1}/C_0$, $q_{\text{max}}^\parallel$ of 575 cm$^{-1}$, $b_{\text{max}}^\parallel$ of 10435 s mm$^{-1}/C_0$) to study dorsal root axotomy 3 and 30 days after injury (178). Axotomy is known to cause early axonal degeneration followed by delayed myelin damage. Farrell and co-workers measured the QSI and DKI indices parallel and perpendicular to the long axis of the SC along the DTI indices. They found that $\text{FWHM}^\perp$ increased significantly in the injured SC, whereas $\text{FWHM}^\parallel$ decreased relative to the control value. Summaries of the DTI, QSI, and DKI measures extracted 3 and 30 days post SCI are depicted in Figure 18A, B, and C, respectively. These data show that most MR measures are able to distinguish between lesioned and non-lesioned tissues, but it appears that transverse diffusion characteristics better reflect the difference between the lesions 3 days and 30 days post injury (178) (i.e. the progress in secondary damage following the injury).

Due to improvements in the methodology, diffusion MRI has been used more in recent years to study SCI in vivo in rodents. For example, Deo et al. used serial DTI ($b_{\text{max}}$ of 706 s mm$^{-2}$) to study mild SCI in a contusion model in the rat 2, 4, 6, and 8 weeks following injury using implanted coils. In this study the DTI metrics were shown to recover in a region-specific manner (176). When the DTI parameters were compared with histology, it was found that the tADC correlates with myelin in rostral and caudal regions in both the dorsal and ventral WM but not in the lateral WM (181). Surprisingly, no correlation was observed between the IADC and axonal damage in this study. The Song group demonstrated in a series of studies (124,184,185,188,195) on a graded contusion model in rodents that AD obtained from DTI in the hyper-acute stage (i.e. 3–6 h after the injury) correlates well with histology performed immediately after the imaging. In addition, it was found that the spared normal ventrolateral WM, as deduced from the AD, correlates with the Basso mouse scale (BMS) locomotor scores obtained two weeks after the insult, as shown in Figure 19. These results demonstrate that AD in the sub-acute stage reflects the axonal damage and predicts outcome two weeks later (184).

More recently it was shown that WM ROIs with $\text{AD}$ higher than 1.5 $\mu$m$^2$ ms$^{-1}/C_0$ at 3 h following the contusion injury were able to conduct action potential 4 weeks after the injury, and that this parameter in the lateral funiculus was highly predictive of locomotor function at 4 weeks (185). Very recently, Tu et al. used DTI
in vivo DTT has seldom been applied to the study of the SC in general or SCI in particular. Recently, the Okano and Nakamura groups studied by diffusion MRI the common marmosets both in vivo and ex vivo (179,180,186). An example of in vivo DTT ($b_{\text{max}}$ of 1000 s mm$^{-2}$, 12 directions) performed on the SC of common marmosets is presented in Figure 20. After providing evidence that DTT can be used to study common marmosets both ex vivo and in vivo, the same DTT methodology was used to study the damage development and the spontaneous functional recovery in a contusive SCI model (186). After establishing by comparison with RT97 and SMI-31 staining that the FA threshold for DTT should be about 0.40, the DTI-based fiber estimate ratio (DFER), which represents the ratio of percentage of spared axons relative to control, was correlated with different behavioral tests. It was found that DFER correlates with the open field rating scale, cage-climbing test, and spontaneous motor activity. Two patterns for FA and $\lambda_\parallel$ were found for the CST and DC fibers (Fig. 20A). In the CST, FA and $\lambda_\parallel$ decreased caudally to the epicenter and did not recover even 15 weeks post injury, whereas in the DCs the decreased values seen rostral to the epicenter 2 weeks after the contusion did recover partially over the next 13 weeks. $\lambda_\parallel$ values at the epicenter increased during the 15-week follow-up period (186). DTI shows disruption of the fibers at the lesion epicenter: at 15 weeks post injury the fibers of the CST are still disrupted but some fibers in the DC tract could be seen extending beyond the lesion epicenter (Fig. 20B, C). Cohen-Adad et al. studied the time course of DTI parameters (six directions, $b_{\text{max}}$ of 1700 s mm$^{-2}$, and $\lambda$ of 15 ms) from 16 h up to 30 days post-injury in unilateral L2–L4 dorsal root axotomy and compared these parameters with detailed histology (Fig. 21) (197). In this study in rats, decreases in FA and $\lambda_\parallel$ and an increase in $\lambda_\perp$ were statistically significant 3 days following the injury (Fig. 21B, C). The changes in DTI indices (i.e., $\lambda_\parallel$ and $\lambda_\perp$) correlated with the axonal and myelin damage as assessed by SMI-31 and LFB staining, respectively (Fig. 21E). All DTI measures as well as the histological stains demonstrate that the damage progressed for the entire 30 days period (Fig. 21).

Zhang et al. studied in vivo HARDI ($b_{\text{max}}$ of 1000 s mm$^{-2}$ and 64 directions) to evaluate the sensitivity of different SC ROIs to detect primary and secondary lesions. The FA and the RD ($\lambda_\parallel$) in lesions differed from those of control regions 3 days and 21 days post injury. Changes were found at the lesion epicenter and, more interestingly, a few centimeters away from the lesion epicenter. These changes were specific to the type of fiber, suggesting the detection of Wallerian degeneration (175). Brennan et al. studied mild and severe contusion SCI in an in vivo mouse model at very high field strengths, where the presence of residual myelin or the degree of axonal sparing and locomotor function recovery in SCI in vivo was found that for these mice, which have higher baseline diffusivity of axons, the recovery from SCI when the locomotion activity is limited is independent of the SCI when the locomotion activity is limited is independent of the presence of residual myelin or the degree of axonal loss; instead, it is connected to the degree of spared axons as reflected in $\lambda_\parallel$ (195).

DTT may appear as the method of choice for studying SCI and fiber recovery after SCI. However, due to technical difficulties, in vivo DTT has seldom been applied to the study of the SC in general or SCI in particular. Recently, the Okano and Nakamura groups studied by diffusion MRI the common marmoset ex vivo and then in vivo (179,180,186). An example of in vivo DTT ($b_{\text{max}}$ of 1000 s mm$^{-2}$, 12 directions) performed on the SC of common marmosets is presented in Figure 20. After providing evidence that DTT can be used to study common marmosets both ex vivo and in vivo, the same DTT methodology was used to study the damage development and the spontaneous functional recovery in a contusive SCI model (186). After establishing by comparison with RT97 and SMI-31 staining that the FA threshold for DTT should be about 0.40, the DTI-based fiber estimate ratio (DFER), which represents the ratio of percentage of spared axons relative to control, was correlated with different behavioral tests. It was found that DFER correlates with the open field rating scale, cage-climbing test, and spontaneous motor activity. Two patterns for FA and $\lambda_\parallel$ were found for the CST and DC fibers (Fig. 20A). In the CST, FA and $\lambda_\parallel$ decreased caudally to the epicenter and did not recover even 15 weeks post injury, whereas in the DCs the decreased values seen rostral to the epicenter 2 weeks after the contusion did recover partially over the next 13 weeks. $\lambda_\parallel$ values at the epicenter increased during the 15-week follow-up period (186). DTI shows disruption of the fibers at the lesion epicenter: at 15 weeks post injury the fibers of the CST are still disrupted but some fibers in the DC tract could be seen extending beyond the lesion epicenter (Fig. 20B, C).

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In this study, in vivo DTI data ($b_{\text{max}}$ of 1500 s mm$^{-2}$ and $\Delta$ of 14 ms) was collected prior to injury and at 2 h and 1, 3, 7, and 30 days post SCI. In addition to SC CSA, FA, MD, $\lambda_{\perp}$, and $\lambda_{\parallel}$ were determined. The main findings were that acute changes in FA are mostly driven by the reduction of $\lambda_{\parallel}$ at the impact site. A gradual increase in $\lambda_{\perp}$ continues for 30 days post SCI. The authors commented on the fact that their observation generally agrees with what is observed in humans (198). Wang et al. evaluated a different model of SCI (196) and Zhang et al. demonstrated the feasibility of studying rat SCI using a clinical MRI scanner (199).

Since an early study of SC in MS patients (206), DTI and DTT have been increasingly used to study traumatic SCI in human subjects (201–205,207–212). For example, Chang et al. examined cervical SCI in humans using DTI and DTT ($b_{\text{max}}$ of 500 s mm$^{-2}$, 25 directions) with the general aim of evaluating whether DTI indices have clinical significance beyond those of routine MRI (201). This study concluded that DTI indices do
correlate to some extent with neurological impairment (201). Cheran et al. used conventional DTI on both hemorrhagic and non-hemorrhagic cervical SCI and found a correlation between DTI indices at the injury site and American Spinal Injury Association (ASIA) motor score, but only in the non-hemorrhagic group (202).

Patients with chronic cervical SCI were studied by a combination of HARDI ($b_{\text{max}}$ 1000 s mm$^{-2}$ and 64 directions) and MT-MRI (203). Significant differences between controls and the NAWM in patients were observed (FA, $p < 0.0001$; AD and RD, $p < 0.05$). Differences between controls and the NAWM of patients were also observed for the GFA ($p < 0.0001$) and cord area ($p < 0.05$); however, no significant differences were detected for the MD or the $T_1$- or $T_2$-weighted signals. In the chronic stage, however, MRI metrics such as MTR, FA, GFA, $\lambda_1$, and atrophy, but not $\lambda_2$ or MD, correlated with clinical disability (203). When Petersen et al. correlated conventional DTI metrics, ASIA scores, and electrophysiological measures following chronic cervical SCI, they found statistically significant decreases in FA in the CST and dorsal tracts. The reduction in SC area was also statistically significant in the C2, C5, T5, and LE ROIs (209). Similar results were also observed for FA in other studies of chronic SCI (203,204,210). The most accepted approaches for treatment of SCI focus on early neuroprotection aimed to maximize prevention of secondary injury as well as methods to stimulate plasticity in the CNS. These approaches have some beneficial effects in patients with incomplete SCI but very limited beneficial effect in cases of complete SCI (213). Recently, Tabakow et al. used DTI in combination with a handful of methods to study the recovery after traumatic SCI in human after intra-spinal cell grafting (211). The patients were observed for about a year, and in two of the three treated patients FA and DTT showed some recovery that paralleled neurological recovery (211). Although this was a very small group of patients, this study demonstrates the potential of DTI/DTT and other diffusion-based methods in evaluating SCI treatment efficacy. Clearly more effort should be devoted to evaluation of the efficacy of new therapies designed to treat SC disorders in general and SCI in particular.

### Diffusion MRI and other SC pathologies

Diffusion MRI has also been used to study and characterize other pathologies and conditions in the SC both in experimental models and in human subjects. Despite the importance of these studies, these are not reviewed in the present review due to

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**Figure 18.** DTI of SCI in the rat. Analyses of dorsal root axotomy at 3 and 30 days post SCI. (A) ROI analysis of conventional DTI indices. (B) ROI analysis of the perpendicular q-space indices (⊥). (C) ROI analysis of the parallel q-space indices (∥). $n = 6$ in each group. An asterisk denotes significant difference ($p < 0.01$). Reproduced with permission from Reference (178).
space limitations. These efforts include the study and characterization of SC tumors, SC ischemia, transverse myelitis, HIV-related SC abnormalities, degenerative myelopathy, cord compression, and canal stenosis. These studies have been reviewed to some extent elsewhere (3,4,10,16,107,214–216) and the reader is advised to consult these reviews and the references cited therein to appreciate the contribution of diffusion MRI to the study of the above SC pathologies.

**SUMMARY AND FUTURE DIRECTIONS**

Because of the small cross-sectional dimensions of the SC, the SC is suitable for ex vivo MRM. In addition, the SC has a relative organized WM and GM, so it is not surprising that the SC has been used to evaluate new diffusion MRI methodologies. Therefore, we anticipate the continuation of the application of diffusion MRI (DTI and more advanced diffusion MRI methods) to study SC microstructure and microstructural changes in the SC due to maturation, degeneration, injury, and other pathologies. These should be done to develop a better understanding of the meaning and the clinical implications of the indices extracted from DTI and more importantly from more advanced diffusion MRI methodologies, which may provide more specific microstructural indices of the SC. The aim should be to correlate these indices with detailed histology to obtain a better understanding of these indices and their correlations with microstructural and pathological changes. Although it is easier to obtain detailed microstructural information in the ex vivo SC, much needs to be done to improve our ability to reliably study SC microstructure and pathologies in vivo by diffusion MRI in animals and in human subjects.

In vivo diffusion MRI of the SC will always be more challenging than in the brain. However, as already mentioned, due to the structural characteristics of the SC, diffusion is potentially an extremely important contrast mechanism for studying SC microstructure, pathologies, and treatments. The WM of the SC exhibits ensemble anisotropy in addition to shape and microscopic anisotropies, and consequently is macroscopically anisotropic (Fig. 22). These characteristics imply that even...
simple diffusion MRI methods such as DTI should be sensitive to changes that might occur in the microstructure of the SC. Therefore, diffusion MRI should become a powerful technique for studying normal maturation, degeneration, and diseases of the SC as well as tissue remodelling, restoration, and regeneration that may occur spontaneously or after treatment of SCI. In addition, DTT provides a unique tool to map fibers in vivo both in experimental models and in human subjects, adding even more to the motivation of using diffusion MRI to characterize the normal and diseased SC in vivo. The SC has small cross-sectional dimensions, is prone to physiological motion, and suffers from magnetic susceptibility differences, making it a difficult organ to analyze in vivo by diffusion MRI. With the advancement of efficient fast imaging techniques such as multi-shot EPI and single shot methods that are less sensitive to susceptibility differences, parallel imaging, the development of high-field MRI scanners that provide high SNR per unit time, and the improvements in localization, gating, and motion compensation schemes, we expect to see more in vivo diffusion MRI studies of SC in animals and human subjects. In addition, there is a need for the development of more advanced hardware and software, and better coils, such as multi-channel phased-array coils, that provide higher SNR per unit time. The further development of cryoprobes and more efficient parallel imaging schemes should also increase the quality of the diffusion data that can be obtained on the

Figure 20. Time course of DTI and DTT after SCI in common marmosets. (A) Changes in the FA, $\lambda_\parallel$, and $\lambda_\perp$ values for the CST- and DC-specific ROIs at the epicenter (epi) and sites 4 mm and 8 mm rostral or caudal from the lesion epicenter (4R, 8R, 4C, 8C). FA and $\lambda_\parallel$ values of the CST at the epicenter, 4C, and 8C were significantly lower than the pre-injury values. In contrast, the FA and $\lambda_\perp$ of the DC at the epicenter, 4R, and 8R were significantly decreased after the injury and gradually recovered. $\lambda_\parallel$ and $\lambda_\perp$ increased at the epicenter of both DC and CST in the chronic phase. (B) FA color-oriented DTT of the CST and DC before SCI shows a number of tract fibers with high FA values. After SCI, DTT revealed a prominent disruption of the tract fibers in both the CST and DC tract. The FA values gradually increased during follow-up, more prominently in the DC than in the CST. (C) The scheme shows Wallerian degeneration after contusive SCI. The red and blue areas indicate corticospinal and DC tracts. Reproduced with permission from Reference (186).
It will be desirable also to tailor diffusion MRI software used to study the brain to the SC. Last, but not least, to enable routine use of diffusion MRI of the SC in the clinical world, protocols, acquisition schemes, and parameters must be standardized. A first step in this direction was the first ever SC imaging meeting, which took place recently and resulted in

Figure 21. DTI of Wallerian degeneration following SCI in the rat. (A) Definitions of ROIs on an SC slice. The yellow dashed line outlines the lesion as defined in the average $\lambda_\parallel$ image at 30 days after axotomy (ROI#1). The blue dashed line outlines the region with loss of SMI-31-stained histology at 30 days after axotomy (ROI#3). The white line outlines normal WM in the contralateral DC (ROI#2). (B, C) Time courses of FA, parallel diffusivity, and perpendicular diffusivity in (B) ROI#1 and (C) ROI#3 of the lesion compared with contralateral ROI#2. An asterisk indicates significant difference between control and injured animals ($p < 0.01$). (D) Optical density of SMI-31 and LFB in the lesion (ROI#3) and contralateral (ROI#2) WM. (E) Correlations between parallel diffusivity and optic density of SMI-31 and correlation between perpendicular diffusivity and optical density of LFB in the lesion (ROI#3) and contralateral WM (ROI#2). Reproduced with permission from Reference (197).
two manuscripts on the current state-of-the-art SC imaging (107,217). These manuscripts identified diffusion NMR as one (of five) promising potential avenue for SC MRI. There besides outlining the technological advances needed (acquisition/post-processing hardware/software) the need for networks of collaborations was spelled out. The lack of “critical mass” of laboratories and researchers devoted to SC imaging was said to affect the rate of progress of the field of SC imaging, and we hope that the current review will encourage more researchers to join the field of diffusion MRI of the SC. A recent study on the clinical utility of state-of-the-art SC MRI techniques found DTI to be the most mature in terms of clinical utility (218). There it was pointed out that the field may benefit from large studies with a priori hypotheses, standardized acquisition methods, detailed clinical data, and robust automated analysis techniques (218).

Figure 22. Diffusion modes. (A) Microscopic anisotropy arises from the boundaries of restricted compartments. (B) Compartment shape anisotropy arises from the shape of the compartment. (C) Ensemble anisotropy is due to the coherence of packing of locally anisotropic pores.

Figure 23. Schematic diagrams illustrating the effect of neuropathological changes (upper panel) on DTI indices (middle panel) and in the multi-tensor model and DTI simplification (lower panel). In the lower panel the grey ellipsoid represents the diffusion tensor profile and black drawings represent the diffusion profiles for multiple tensor representation. (a) Normal myelinated axons and the corresponding diffusion tensor and axial and radial diffusivities (λ∥ and λ⊥, respectively). The diffusion tensor is represented here by an ellipsoid and is shown in subsequent plots as a grey ellipsoid with dashed lines. (b, c) Axon and myelin injury with and without cell infiltration, respectively, and (d, e) axon and myelin injury with axonal loss, with and without cell infiltration, respectively. Reproduced with permission from References (5,72).
Most of the in vivo diffusion MRI applications in the SC reported to date have employed conventional DTI, which is not trivial to acquire reliably in SC in vivo. It is clear that DTI, which assumes that diffusion can be described by a single component exhibiting Gaussian diffusion in all pixels, has limitations that decrease its specificity. The limitations of the indices that can be extracted from DTI compared with those that might be obtained from more advanced diffusion MRI methods are depicted in Figure 23. Indeed in recent years more advanced diffusion MRI methods (i.e. HARDI and q-ball, QSI, DKI, OGSE, DBSI, DDE, NODDI, and AxCaliber), which, in principle, may provide more specific SC microstructural indices than DTI, have been described. However, most of these diffusion MRI methods require a larger data set, higher diffusion weighting, or both as compared with conventional DTI. Therefore, the acquisition of such data sets requires longer scan times than DTI, making these approaches even more difficult to use on the SC in vivo. Despite this, in recent years, the feasibility of performing many of these advanced diffusion MRI experiments in vivo on SCs in animals and even in human subjects has been demonstrated (see, for example, (63,66,67,108–113,147,148,150,151,165). In fact a glimpse of what can be anticipated in the future from in vivo diffusion MRI of the SC in human is presented in a recent study, which used AxCaliber to map SC microstructure using optimized hardware and software (113).

We therefore foresee that DTI will become a clinically routine MRI evaluation of the SC with the more advanced diffusion methods in use in neuroscience and neurological research. DTI and other diffusion MRI methods should be used to study WM-associated disorders and pathologies, maturation, degeneration, cancer, SCI in general, and traumatic SCI in particular. The emphasis should shift to analysis of treatment response and follow-up of therapy making use of an advantage of MRI, namely the ability to perform safely multiple evaluations in a short period on the same subject. Clearly, this type of application will require improvement of co-registration and alignment methods to enable observation of the same area with high accuracy. This review has focused on the application of diffusion MRI in the SC, but clearly the added value of MRI is the fact that it is a true multiparametric imaging modality even before the use of any exogenous contrast agents. It is clear that there will be added value in combining diffusion MRI techniques with other MRI modalities and with MRS. This will reveal information on the microstructural and biochemical alterations in the SC during development, disease, injury and treatment. Despite the tremendous development in diffusion MRI of the SC in recent years, coordinated efforts are needed to transform DTI and more advanced diffusion MRI methods into a more routine clinical modality.

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