Wallerian degeneration after spinal cord lesions in cats detected with diffusion tensor imaging

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A B S T R A C T

One goal of in vivo neuroimaging is the detection of neurodegenerative processes and anatomical reorganizations after spinal cord (SC) injury. Non-invasive examination of white matter fibers in the living SC can be conducted using magnetic resonance diffusion-weighted imaging. However, this technique is challenging at the spinal level due to the small cross-sectional size of the cord and the presence of physiological motion and susceptibility artifacts. In this study, we acquired in vivo high angular resolution diffusion imaging (HARDI) data at 3T in cats submitted to partial SC injury. Cats were imaged before, 3 and 21 days after injury. Spatial resolution was enhanced to 1.5×1.5×1 mm3 using super-resolution technique and distortions were corrected using the reversed gradient method. Tractography-derived regions of interest were generated in the dorsal, ventral, right and left quadrants, to evaluate diffusion tensor imaging (DTI) and Q-Ball imaging metrics with regards to their sensitivity in detecting primary and secondary lesions. A three-way ANOVA tested the effect of session (intact, D3, D21), cross-sectional region (left, right, dorsal and ventral) and rostrocaudal location. Significant effect of session was found for FA (P<0.001), GFA (P<0.05) and radial diffusivity (P<0.001). Post-hoc paired T-test corrected for multiple comparisons showed significant changes at the lesion epicenter (P<0.005). More interestingly, significant changes were also found several centimeters from the lesion epicenter at both 3 and 21 days. This decrease was specific to the type of fibers, i.e., rostrally to the lesion on the dorsal aspect of the cord and caudally to the lesion ipsilaterally, suggesting the detection of Wallerian degeneration.

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Introduction

Spinal cord (SC) injury has a significant impact on the quality of life as it can lead to motor and sensory deficits through the disruption of several descending and ascending spinal and supraspinal pathways. Both the severity of the deficit and the successful rehabilitation process depend on the type and number of axonal tracts that have been altered, as well as changes in the intrinsic properties of the SC (Rossignol, 2006). The study of chronic partial lesions in the SC of cats may help understand these plastic changes by defining the relative importance of spinal and supraspinal mechanisms (Barrière et al., 2010; Barrière et al., 2008). However, traumatic lesions can at time produce unpredictable secondary damage of adjacent pathways, therefore altering the potential for locomotor function recovery. Wallerian degeneration can occur in the distal portion of cut axons when they are disconnected from their cell bodies, resulting in axon demyelination and necrosis (Beirowski et al., 2005; Waller, 1850). This degeneration can occur rostrally to the lesion in ascending tracts and caudally to the lesion in descending tracts. As this pathological event may occur within hours following injury, it may be used as an early marker for injured tract following acute SC injury (Mac Donald et al., 2007). It is therefore crucial to assess the extent of the lesion in vivo.

Diffusion-weighted (DW) magnetic resonance imaging (MRI) has raised much interest due to its specificity to white matter tissue (Beaulieu, 2002) and the possibility to map axonal architecture via fiber tractography (Mori and van Zijl, 2002). Using metrics derived from diffusion tensor imaging (DTI) (Basser and Pierpaoli, 1996), water diffusion can be characterized in the healthy and pathological white matter. Previous studies have notably shown that fractional anisotropy (FA) is sensitive to axon degeneration (Agosta et al., 2005; Beaulieu, 2002; Becerra et al., 1995; Budde et al., 2007; Cohen-Adad et al., 2008a; DeBoy et al., 2007; Deo et al., 2006; Ford et al., 1994; Fraidakis et al., 1998; Guleria et al., 2008; Kim et al., 2006; Lindberg et al., 2007; Mac Donald et al., 2007; Moller et al., 2007; Nevo et al., 2001; Schwartz et al., 2005; Stanisz et al., 2001; Sun et al., 2008; Thomalla et al., 2004; Valsasina et al., 2007; Werring et al., 2000) and more...
specifically, axial and radial diffusivities are respectively sensitive to axon injury and demyelination (Budde et al., 2007, 2008, 2009; DeBoy et al., 2007; Hofling et al., 2009; Kim et al., 2007; Mac Donald et al., 2007; Song et al., 2002; Xie et al., 2010; Zhang et al., 2009).

Although providing clinically-relevant quantitative metrics, DTI is limited in representing complex diffusion scheme (e.g., in presence of crossing fibers) and other reconstruction methods based on high angular resolution diffusion imaging (HARDI) provide more accurate visualization of diffusion profile (Tuch, 2004). Recently, Q-Ball Imaging (QBI) enabled the detection of subtle anatomical features of the SC that were not seen with DTI (Cohen-Adad et al., 2008b; Lundell et al., 2009). QBI has also been applied to the injured SC, demonstrating its ability to detect directional abnormalities (Barmpoutis et al., 2009; Cohen-Adad et al., 2009a). Metrics derived from QBI may therefore provide useful markers of diffusion characteristics in the healthy and injured SC.

Most animal studies that seek to image diffuse axonal injury were conducted in rodents using high field scanner either ex vivo (DeBoy et al., 2007; Ford et al., 1994; Krzyzak et al., 2005; Schwartz et al., 1999, 2005) or in vivo using surface coils (Kim et al., 2007) or implanted coils to increase the signal-to-noise ratio (SNR) (Bilgen et al., 2001; Deo et al., 2006; Elshafey et al., 2002; Fenyes and Narayana, 1999; Madi et al., 2005). Although these studies thoroughly demonstrated the benefits of DW-MRI in models of white matter pathology, it remains crucial to show the feasibility of reproducing these results in standard clinical systems. Translating DW-MRI to clinical imaging setup is hampered by the difficulty to image the SC, notably due to its small size relative to the brain, physiological motions (respiration, cardiac, cerebrospinal fluid pulsation) (Clark et al., 2000; Kharbanda et al., 2006), partial volume effects and susceptibility-related distortions in nearby inter-vertebral disks and lungs (Cohen-Adad et al., 2008a; Voss et al., 2006).

Here we conducted a longitudinal imaging study in cats with hemisections targeting the left side of the SC thus disrupting ascending and descending fibers mainly on one side. Cats were imaged in the intact state, in the early acute phase (3 days after hemisection) and in the sub-acute phase (21 days). We employed advanced HARDI acquisition and processing methods on a 3T clinical system. We used super-resolution (Greenspan et al., 2002), parallel imaging (Griswold et al., 2002) and distortion correction (Cohen-Adad et al., 2009b) methods to reconstruct fiber tracts in various sub-quadrants of the SC with minimum susceptibility distortions. We introduced a method to conduct quantitative tractography at the group level based on other works (Van Hecke et al., 2008). We evaluated DTI and QBI metrics in the dorsal, ventral, right and left quadrants with respect to their sensitivity to detect primary and secondary lesions. We hypothesized that HARDI metrics could detect changes at the epicenter as well as rostrally and caudally to the site of the lesion.

**Material and methods**

**Animal preparation**

Experiments were conducted on adult cats (N = 12, nine females). All procedures followed a protocol approved by the Ethics Committee at the Université de Montréal, according to the Canadian Guide for the Care and Use of Experimental Animals. The well being of the cats was monitored daily and verified regularly by a veterinarian. Cats were housed in large individual cages (104 × 76 × 94 cm) with food and water ad libitum.

Surgery for spinal lesions were performed under general anesthesia and aseptic conditions. Animals were first subcutaneously injected with an analgesic (Anafen 2 mg/kg) and pre-medicated (Atravet 0.1 mg/kg, Glycopyrrolate 0.01 mg/kg, Ketamine 10 mg/kg; intramuscular administration). Cats were then intubated and maintained with gaseous anesthesia (Isoflurane 2% in a mixture of 95% O2 and 5% CO2). Heart rate and respiration were monitored throughout the surgery. Before the end of the surgery, an analgesic (Buprenorphine 0.01 mg/kg) was administered subcutaneously. Additionally, a patch of Fentanyl (25 μg/h) was applied on the skin for 5 days to alleviate pain. At the end of the surgery, animals were placed in an incubator until they regained consciousness after surgery and housed in their spacious individual cages with food and water. As a prophylactic measure, an oral antibiotic (Cephatab or Apo-cephalex, 100 mg/day) was given for 10 days following each surgery.

A spinal hemisection was done on the left side of the SC at day 0 (D0) as described in previous works (Barrière et al., 2010; Barrière et al., 2008). A low thoracic vertebra was exposed (T10) and a laminectomy was performed to approach the SC dorsally. A small incision of the dura was first made and a few drops of a local anesthetic (Xylocaine, 2%) were put on the surface of the exposed SC. Anesthetic was then injected directly into the targeted segment to reduce brisk movements during the actual section. Hemisection on the left side was achieved with a micro-knife under microscope visualization. An absorbable hemostat (Surgicel, oxidized regenerated cellulose) was used to fill the lesioned area so as to prevent axonal re-growth through the gap. The wound was then closed in anatomic layers.

**MRI acquisition**

Cats were imaged at the intact state as well as 3 days (D3) and 21 days (D21) following injury to study the evolution of the lesion and the presence of secondary injuries. Images were acquired on a 3T scanner (TIM Trio, Siemens Healthcare) using a combination of two phased array coils (6 of the 24 elements of the spine array and the 12-channel body matrix). RF transmission was performed using the body coil integrated into the magnet bore. Cats were positioned feet first lateral left. Fast low angle sequence (TR/TE=7.3/3.6 ms, flip angle=20°) was first run in all three orientations with 0.5 × 0.5 mm² in-plane spatial resolution to enable accurate slice prescription for subsequent scans.

Anatomical scans consisted of a sagittal T2-weighted turbo spin echo (TSE) sequence (TR/TE=9200/71 ms, flip angle=142°, 0.75 × 0.75 × 1 mm³, 256 × 256 matrix, bandwidth=205 Hz/pixel, 11 averaging), an axial proton density weighted TSE sequence (TR/TE=8990/10 ms, flip angle=150°, 0.36 × 0.36 × 2 mm³, 512 × 512 matrix, bandwidth=205 Hz/pixel, 11 averaging) and a sagittal T1-weighted magnetization prepared rapid gradient-echo (MPRAGE) sequence (TI/TR/TE=900/2300/4 ms, flip angle=9°, 0.75 × 0.75 × 1 mm³, 256 × 256 matrix, bandwidth=200 Hz/pixel).

DW data were acquired using a twice-refocusing single-shot spin echo EPI sequence (Reese et al., 2003). To minimize susceptibility artifacts, parallel acquisition using GRAPPA (GeneRalized Autocalibrating Partially Parallel Acquisition) was employed with R=3 acceleration factor in the phase encoding direction (A-P). Respiratory gating was employed to minimize motion artifacts (acquisition on the expiration phase). Other parameters were: sagittal orientation, 10 slices (no gap), spatial resolution=1.5 × 1.5 × 2 mm³, TR/TE=9500/109 ms, 128 × 128 matrix, bandwidth=1562 Hz/pix, echo spacing=0.78 ms, 64 diffusion-encoding directions, b-value=1000 s/mm², 5 repetitions, acquisition time ~30 min (depending on respiratory rate). Although cardiac-gating was shown to reduce ghosting artifacts in DW-MRI (Summers et al., 2006), the relatively high cardiac frequency in cats (~2 Hz) compared to that in humans (~1 Hz), the sometimes irregular cardiac frequency due to the anesthetic state and the presence of respiratory gating made it difficult to apply this technique within a reasonable scan time for anesthetized animals. In addition to the DW images, two series of b=0 images were acquired (10 repetitions each): one with the same phase encoding as for the DW images (A-P, referred to as EPI+), and the other with phase encoding rotated by 180° (P-A, referred to as EPI−). These two additional scans were subsequently used to correct residual
susceptibility-related distortions (see Processing section). The first series of DW images was then repeated with slices shifted by 1 mm through plane. This was achieved to enhance spatial resolution using super-resolution technique and therefore reach $1.5 \times 1.5 \times 1$ mm$^3$ voxel size as previously described (Cohen-Adad et al., 2009a). Overall the total DW acquisition was about 1 hour long.

Processing

Data processing consisted of individual analyses (for each cat and each session) and group analyses (for each session). One dataset from the ‘intact cat’ session and three datasets from the D21 session were discarded due to poor image quality (spiking artifacts) or problems related to anesthesia.

Individual analysis

DW images were first corrected for motion. For each series (with and without 1-mm shift), all repetitions were registered to the first repetition using $b=0$ images to compute the transformation matrix. Registration was done with FSL FLIRT (Jenkinson et al., 2002), using a 3-D rigid-body transformation matrix (6 degrees of freedom) with normalized correlation as cost function. Following motion correction, DW data were averaged across repetitions. Similar steps were conducted for the EPI+ (phase encoding A-P, or “blips up”) and EPI− (phase encoding P-A, or “blips down”) scans.

The super-resolution technique consisted in acquiring an additional set of HARDI data by shifting the slices in the Z direction by 1 mm (corresponding to half the size of a voxel in the Z dimension). For reconstructing the super-resolution dataset (at 1 mm slice thickness instead of 2 mm), we “merged” the first and the second dataset together. A new volume was thus generated by interleaving alternately one slice from the first dataset and one slice from the other dataset. Simple interleaving method was chosen in place of more advanced reconstruction technique (Greenspan et al., 2002) due to the high level of noise in DW images and the presence of small image artifact that sometimes occurred in only one of the two datasets, rendering reconstruction methods less robust.

Susceptibility-related distortions were corrected using the reversed gradients method as implemented in (Voss et al., 2006). The advantage of the reversed-gradient method is its robustness to the manifold nature of B0 inhomogeneities in the spinal region. Namely, rapid phase variations occur at the intervertebral disks interface (order of $1-5$ voxels) and slow phase variations occur close to the lungs (order of $100$ voxels). Although other methods exist, the reversed-gradient method was shown to be appropriate for DW-MRI of the spinal cord (Cohen-Adad et al., 2009b). Two series of $b=0$ images (EPI+ and EPI−) were acquired with the same slice prescription and parameters as that ones from HARDI data, but with the phase encoding reversed from each other (A-P and P-A). Then, geometric and intensity correction fields were estimated and subsequently applied to diffusion-weighted EPI. Fig. S1 (supplementary material) illustrates the efficiency of the distortion correction method for a representative sagittal EPI slice.

Following distortion correction, fiber tractography of the whole SC was conducted using MedINRIA software (Fillard et al., 2007) with the following stopping parameters: FA threshold $=0.2$, minimum length $=10$ mm, sampling $=1$ (i.e., one fiber per voxel). We selected these parameters based on preliminary results, so that most tracts would be reconstructed despite the presence of degeneration, given that a fibrous structure would still be present, ensuring sufficient anisotropy for tracts to be reconstructed, as been suggested in Zhang et al. (2009). Note that tracts were usually stopped at the lesion epicenter. This did not affect regions distal to the lesion as we performed multi-seeded tractography, i.e., if, for example, a lesion would prevent tracts to be continuous from a region caudal to the lesion to a region rostral to the lesion, the continuity would be ensured by the rostral portion of the cord (without effective link between the caudal and the rostral areas). Of course, this would affect the epicenter of the lesion, where the tract would be interrupted. In such case, we manually extended the region of interest to cover the lesioned area, in order to perform statistics there – keeping in mind that the main goal of this article was to detect changes in DTI metrics distal from the lesion epicenter.

Reconstructed fiber bundles were then transformed into a volume representing fiber counts, and subsequently thresholded (>10 fibers) to yield a mask of the SC, similarly to what has been proposed in (Van Hecke et al., 2008). The minimum number of fiber bundles was based on preliminary results, so that “outlier” tracts (i.e., not part of the longitudinal spinal pathways) would be excluded from the analysis. The SC mask was used to generate four sub-masks using an automatic algorithm. The algorithm acted on each axial plane separately, and subdivided axial mask into the dorsal, ventral, left and right regions equally. The subdivision was done by computing the cumulative intensity along X and Y, then finding the center of the mask in both X and Y direction. The four generated segments were then rotated by 45° and the intersection between each segment and the SC mask yielded the four-quadrant mask (dorsal, ventral, left and right). Although this procedure implied that the gray matter was also included in the masks, the smallness of the cat spinal cord rendered it difficult to exclude the gray matter completely, without partial volume effect.

DTI estimation and derived metrics were obtained using FSL (Smith et al., 2004). Of all metrics computed, the fractional anisotropy (FA), the first eigenvalue (axial diffusivity, $\lambda_0$) and the average of the 2nd and 3rd eigenvalues (radial diffusivity, $\lambda_r$) were further considered for quantitative tractography.

Q-Ball orientation distribution function (ODF) was estimated voxel-wise using the method described in (Descoteaux et al., 2007). The HARDI signal was expressed by means of the spherical harmonic basis, which allows an analytical solution for the Funk-Radon transform to obtain the diffusion ODF. We used spherical harmonic decomposition of order 4 and a regularization parameter of 0.006 (Descoteaux et al., 2006). From the reconstructed ODF, we computed the generalized fractional anisotropy (GFA) (Tuch, 2004), which is a HARDI anisotropy measure similar to the more common DTI fractional anisotropy (FA) (Pierpaoli and Basser, 1996). The GFA is defined as the standard deviation divided by the root mean square of the ODF.

Group analysis

For each session (Intact, D3 and D21), metrics were quantified on each of the sub-mask (dorsal, ventral, right and left). Then, these pairs of measurements were registered across cats with respect to the lesion location (cat-dependent). To demonstrate the better sensitivity of diffusion metric for assessing white matter damage compared to more traditional measurements, T2 signal from the b = 0 image was quantified using the same masks. However, variations in B1 homogeneity and coil sensitivity prevented us from comparing absolute T2-weighted signal across sessions. Therefore, the latter signal was normalized with the mean CSF signal. To achieve this, the SC mask was dilated using a $3 \times 3 \times 3$ mm$^3$ box, then the non-dilated mask was subtracted from the dilated mask. The mean signal of the $b=0$ image from the resulting mask was then used as a normalization factor for the $b=0$ image.

Statistical analysis

Statistical analysis was done with the Matlab Statistical Toolbox (The Mathworks, MA, USA). The Gaussianity of the data was first tested using a chi-square goodness of fit to the normal distribution. This test passed for each metric and for each session ($P<0.001$). A
three-way ANOVA was then computed for each metric to test the effect of session (Intact, D3, and D21), cross-sectional region (left, right, dorsal, and ventral) and rostrocaudal region (rostral to the lesion, caudal to the lesion and at the lesion epicenter). We chose to separate the cross-sectional and the rostrocaudal categories in the ANOVA to test the global effect of laterality (left versus right) and rostrocaudal location. The ANOVA was Bonferroni-corrected for comparison between metrics (5 metrics in total). The rostral area was defined between −45 mm and −15 mm from the lesion (~T8-T10). The epicenter area was defined between −3 mm and +3 mm from the lesion (T11). The caudal area was defined between +15 mm and +45 mm from the lesion (~T13-L1). If an effect of session was found (P < 0.05), a post-hoc paired T-test was computed between sessions (including a Bonferroni correction).

Cord straightening

The SC was straightened in the coronal plane for better visualization of maps derived from diffusion metrics. This was achieved using a method illustrated in Fig. S2 (supplementary material). The cord was manually delineated on the sagittal plane using about 10 landmarks, and the traced path was regularized using spline functions. Then, curved coronal slices were reconstructed using trilinear interpolation. The procedure was done using the software OsiriX (http://www.osirix-viewer.com/) and takes about 1 min to be performed. More automatic methods have been proposed (Horsfield et al., 2010; Wassermann et al., 2010), although not applied here due to the relatively small amount of images to process.

Histology

At the conclusion of the experiment, a lethal dose of pentobarbital was injected. A piece of SC segment between T8 and L1 was carefully dissected out and fixed in a 10% solution of paraformaldehyde for several weeks. The blocks were cryoprotected by successive transfers into increasing concentration (10%, 20%, and 30%) of sucrose solution in 0.1 M phosphate buffer for 72 h at 4 °C. For histological examination, the SC was frozen and 40-μm-thick coronal sections of a SC segment of 4 mm centered on the lesion were cut using a cryostat. Each section was mounted on a slide and stained with cresyl violet to evaluate the precise location and extent of the lesion. No specific histological staining for white matter degeneration was performed.

Results

Fig. 1 shows sagittal slices of b = 0, FA and FA color maps from a representative intact cat. The acquisition and processing pipeline yielded very good image quality with limited distortions. The super-resolution method enabled two-fold increase in the through-plane spatial resolution, providing coverage of about 8 voxels in the lateral direction (the SC of a cat is approximately 8 mm thick in the lateral direction, at the thoracic level).

Fig. 2a shows a reconstruction of three straightened coronal FA maps located at the dorsal, middle and ventral aspect of the SC. The thickness of reconstructed coronal slice was 1.5 mm (native resolution). One can observe a clear decrease in FA ipsilaterally, suggesting the loss of white matter tissue. This decrease also extends to the contralateral region in the dorsal aspect of the cord, since part of the

![Fig. 1. Example of seven sagittal slices from a b = 0 (a), FA (b) and FA color map (c) obtained after superresolution reconstruction and distortion correction. Almost no distortion remains, and the through-plane resolution now enables one to cover the spinal cord with about seven slices. Note that the spinal cord of a cat is approximately 8 mm thick in the left-right direction. Orientation is indicated as R: Rostral, C: Caudal, D: Dorsal and V: Ventral.](image)
lesion included the other side also as confirmed by the conventional histology (Fig. 2c). Hi-resolution proton-density (PD) TSE image and histological slices centered on the lesion are shown side-by-side (Figs. 2b and c) and confirm the lesion disrupted axons on the left side of the cord and particularly those in the dorsal funiculus (with some preservation of the ventral tracts) with an extension to the right dorsal column. Note that the poor contrast of the TSE scan only shows subtle abnormality on the dorsolateral aspect.

Q-Ball ODFs in a cat with hemisection are shown in Fig. S3 (supplementary material). ODFs show highly anisotropic profile rostrally and caudally to the lesion with main orientation along the SC axis, which is consistent with the longitudinal orientation of the main spinal white matter tracts. At the lesion location however, ODF profile is drastically modified, suggesting loss of white matter tissue and therefore loss of anisotropy.

**Quantitative tractography**

Group analysis provided quantification of diffusion metrics along the SC of cats, for the three sessions (Intact, D3 and D21). Fig. 3 shows group results of quantitative tractography of mean FA along the dorsal, ventral, right and left aspects of the SC. FA is relatively constant along the intact SC, with a mean value of $0.35 \pm 0.02$. At D3, FA is decreased at the lesion epicenter with a rostro-caudal extension of about 1 mm. At D21, FA is also decreased at lesion epicenter with slightly larger rostro-caudal extension (~1.5 mm). Interestingly, lower FA is noticeable on the dorsal aspect rostrally to the lesion for both D3 and D21 conditions (red arrows), suggesting degeneration of ascending fibers. Similarly, lower FA is noticeable on the left lateral quadrant caudally to the lesion at D3 and D21 (green arrows), suggesting degeneration of major descending fibers.

The three-way ANOVA showed significant effect of session for FA ($P < 0.001$), GFA ($P < 0.05$) and radial diffusivity ($P < 0.001$) (Table 1). Significant effect of cross-sectional location was also found for FA, radial and axial diffusivities, GFA and T2. Significant effect of rostro-caudal location was only found for FA and radial diffusivity ($P < 0.05$).

To assess the effect of session, post-hoc paired $T$-tests were performed between sessions (Intact, D3 and D21) for metrics that showed an effect on the ANOVA (Table 2). At the lesion epicenter, FA was significantly decreased between the Intact and both the D3 and D21 conditions in the dorsal ($P < 0.05$) and left ($P < 0.005$) quadrants of the cord, whereas significant decrease was observed in the right quadrants only between the Intact and D21 conditions. The latter observation suggests an increase of lesion size between D3 and D21.

**Fig. 2**

(a): Coronal views of straightened FA maps at various depths of the spinal cord. Decrease of FA in the dorsal-left aspect at T11 is consistent with the known location of the lesion (red arrow).

(b): Axial PD-TSE with high in-plane resolution (360×360 μm) centered on the lesion. The lesion is delineated manually based on the contrast between healthy and injured tissue (dashed line).

(c): Histological slice centered on the lesion and oriented/scaled similarly to the axial TSE image. Orientation is indicated as L: Left, R: Right, C: Caudal, D: Dorsal and V: Ventral. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 3**

(a): Group results of quantitative tractography showing mean FA along the dorsal, ventral, right and left aspect of the spinal cord for the three sessions: Intact (left), D3 (middle) and D21 (right). The mean FA across quadrants is shown as a thick blue line. Standard deviation is not shown for clarity purpose. Interestingly, lower FA is noticeable on the dorsal aspect rostrally to the lesion (red arrow), and on the left aspect caudally to the lesion (green arrow). These trends are both observed at D3 and D21 and may be associated with degeneration of ascending fibers rostral to the lesion, and of descending fibers caudally to the lesion.

(b): Sagittal and axial views of the ROIs derived from tractography and used for metrics quantification along the spinal cord. Note that all quadrants included gray matter.
More interestingly, within a region 15 mm to 45 mm rostral to the lesion, FA was significantly reduced on the dorsal quadrant of the cord (P<0.005). This observation agrees with the hypothesis that at the time cats were imaged (D3 and D21), tracts in the dorsal column rostral to the lesion were affected by Wallerian degeneration therefore altering diffusion properties of the white matter at that specific location. Similarly, caudal to the lesion, FA was significantly reduced on the left lateral quadrant of the cord (P<0.005), in agreement with the degeneration of descending tracts that are mostly located in the lateral and ventral aspect of the cord.

Radial diffusivity (λ_r) was significantly increased rostrally and caudally on the left lateral quadrant of the cord between the Intact and D21 phase. Contrary to axial diffusivity, most changes were observed between the D3 and D21 (i.e. fewer changes before the early acute phase).

Results of GFA quantification shared very similar patterns to the ones observed using the FA. At the lesion epicenter, GFA was significantly reduced between the Intact and both D3 and D21 conditions in the dorsal and left quadrants of the cord, and between the Intact and D3 state in the ventral quadrant. Also, GFA was significantly reduced on the dorsal aspect rostrally to the lesion (P<0.05) and on the left quadrant caudally to the lesion (P<0.005).

Discussion

This paper presents a longitudinal study of partial SC injured cats using DTI and tractography. The method has been optimized on a clinical 3T system to achieve in vivo imaging with high spatial resolution and minimum susceptibility distortions. Quantitative tractography was conducted in various quadrants of the SC to evaluate the sensitivity of HARDI metrics to detect primary and secondary lesions. Results demonstrated significant changes several centimeters from the lesion epicenter at both 3 and 21 days after injury, suggesting the presence of Wallerian degeneration.

Reversed-gradients for correcting susceptibility distortions

In this study we only acquired b=0 images with blips up/blips down, and applied the deformation field to the DW images. It would have been possible to acquire the whole HARDI data with blips up/blips down, in order to account for diffusion gradient-related distortions, such as the ones induced by eddy currents. However, this would have required significantly longer acquisition time. Moreover, distortions caused by the diffusion gradients were minimized by the use of a twice-refocusing pulse sequence (Reese et al., 2003). Therefore, the deformation field was only derived from b=0 images to specifically correct for distortions caused by susceptibility artifacts.

Tractography-based statistics in the spinal cord

An important contribution of the present study is the introduction of advanced acquisition and processing methods to perform quantitative tractography in sub-quadrants of the cord at the group level. Combining super-resolution technique, respiratory gating, parallel imaging and distortion correction methods, we were able to acquire in vivo HARDI data at 3T using standard coils, with 1.5×1.5×1 mm³ spatial resolution and minimum susceptibility-related distortions. We introduced a semi-automatic processing pipeline with only few steps requiring manual interventions, i.e., creation of a whole-cord mask, fiber tractography (multi-seeded, therefore fully automatic) and identification of lesion location on the anatomical scans. Sub-masks were generated automatically in four quadrants of the cord, allowing one to conduct quantitative tractography at the group level, similarly to the tract-based spatial statistics (TBSS) introduced for brain images (Smith et al., 2006), although notable differences with the latter approach is the absence of non-linear registration and tract skeleton.

Given that we reconstructed the Q-Ball ODF, it would have been possible to use the ODF instead of the diffusion tensor to perform tractography in the spinal cord. However we decided not to do that because in the spinal cord gray matter, some fibers are not oriented longitudinally, therefore in some cases the principal eigenvector of the tensor is oriented in the cross-sectional plane of the spinal cord. Tractography based on the ODF would have yielded at least two maxima: one longitudinal and one in the plane perpendicular to the cord, as been shown in (Cohen-Adad et al., 2008b). Yet, in this study, we aimed at quantifying diffusion metrics in ascending and descending

Table 1

Result of the three-way ANOVA that tested the effect of time (Intact, D3, and D21), cross-sectional region (left, right, dorsal, and ventral) and rostro-caudal region (rostral to the lesion, caudal to the lesion and at the lesion epicenter). P-values were Bonferroni-corrected for comparison between metrics. P-values > 1 have been truncated to 1.000.

<table>
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<tr>
<th>Tested effect</th>
<th>Session</th>
<th>Cross-sectional</th>
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<td>0.000</td>
<td>0.045</td>
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</table>

Table 2

Post-hoc paired T-tests between sessions: intact (I), 3 days post injury (D3) and 21 days post injury (D21) for metrics that showed a significant effect on the ANOVA. P-values were Bonferroni-corrected for comparison between sessions (and truncated to 1.000). Metrics were computed caudally, at the epicenter and rostrally to the lesion. The rostral area was defined between +15 mm and +45 mm from the lesion (~T13). Metrics were averaged in each four quadrants of the spinal cord: dorsal (D), ventral (V), left (L, ipsilateral to the lesion), and right (R). Note that the P-values displayed are truncated (not rounded) after the 3rd digit. To facilitate the reading of this table, levels of statistical significance are represented as light gray (P<0.05) or dark gray (P<0.005).

<table>
<thead>
<tr>
<th></th>
<th>Rostral (−45 to −15 mm)</th>
<th>Epicenter (−3 to 3 mm)</th>
<th>Caudal (15 to 45 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>V</td>
<td>R</td>
</tr>
<tr>
<td>FA I−D3</td>
<td>0.024</td>
<td>1.251</td>
<td>0.921</td>
</tr>
<tr>
<td>I−D21</td>
<td>0.000</td>
<td>1.287</td>
<td>0.420</td>
</tr>
<tr>
<td>D3−D21</td>
<td>0.048</td>
<td>1.000</td>
<td>1.416</td>
</tr>
<tr>
<td>λ_r I−D3</td>
<td>1.000</td>
<td>1.000</td>
<td>0.756</td>
</tr>
<tr>
<td>I−D21</td>
<td>0.465</td>
<td>0.099</td>
<td>0.165</td>
</tr>
<tr>
<td>D3−D21</td>
<td>0.87</td>
<td>0.324</td>
<td>1.000</td>
</tr>
<tr>
<td>GFA I−D3</td>
<td>0.024</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>I−D21</td>
<td>0.099</td>
<td>0.336</td>
<td>1.000</td>
</tr>
<tr>
<td>D3−D21</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>
longitudinal spinal pathway only, therefore we used the diffusion tensor as a way to filter out some non-longitudinal components. Another argument is that DTI is less sensitive to noise in the data and provide robust estimate of white matter principal direction (in regions with no or very few crossing fibers).

Anterograde (Wallerian) and retrograde degeneration

Significant differences were detected between sessions (Intact, D3 and D21) for diffusion metrics measured ipsilaterally rostral and caudal to the injury, whereas no change was detected for the CSF-normalized T2-weighted signal. This result further confirms the sensitivity of diffusion measures to axonal pathology. Previous studies have also shown diffusion changes distal to the site of the lesion (Cohen-Adad et al., 2011; Cohen-Adad et al., 2008a; DeBoy et al., 2007; Kim et al., 2007; Zhang et al., 2009), suggesting the detection of Wallerian degeneration, which occurs when axons are being separated from their cell body (Beirowski et al., 2005).

Changes in FA, GFA and radial diffusivity were mostly (but not only) detected rostral to the injury on the dorsal aspect, and caudal to the injury on the ventro-lateral aspect. This finding supports anterograde Wallerian degeneration of ascending tracts rostral to the injury and of descending tracts caudal to the injury. However, since several axonal transport mechanisms were also disrupted after spinal lesion (Perlson et al., 2010), retrograde degeneration was also expected to occur and we indeed observed significant changes in the dorsal aspect caudal to the injury and in the ventrolateral aspect rostral to the injury. It should also be noted that the specificity of metric quantification in ascending and descending tracts was hampered by partial volume effects and by the simplistic delineation of the cord in four quadrants.

Another confounding effect was the possible presence of edema, hemorrhage, inflammation and excitotoxic events (Park et al., 2004; Tator and Fehlings, 1991). The timing of those secondary damages has been studied in rat models of acute SC injury, showing that ischemia can persist during the first 24 h (Sandler and Tator, 1976), edema can spread rostrally and caudally to the lesion up to 48 h (Tator and Fehlings, 1991) and glial scarring can occur up to several months (Thuret et al., 2006). It is unlikely then that the anomalies detected here are due to edema.

Early marker of white matter pathology

Although not all changes were significant, we observed a trend similar to previous studies, in which diffusion metrics were sensitive markers of the evolution of white matter pathology in a model of pericontusional traumatic axonal injury (Mac Donald et al., 2007), demyelinating lesion (DeBoy et al., 2007) and dorsal root axotomy (Zhang et al., 2009). Namely, we observed a decrease in axial diffusivity at the early acute phase (D3) and no or few changes afterwards. In contrast, we observed an increase in radial diffusivity between the early acute (D3) and the sub-acute (D21) phase, and no or few changes during the early acute phase. Finally and as expected, we observed a decrease in FA at both stages, i.e., during the early acute phase and during the sub-acute phase. As suggested by Mac Donald et al., these changes in DTI metrics may reflect the pure axonal degeneration during the early acute phase (D3), hence increased barriers reducing water diffusivity along axons (\( \lambda_{ax} \), \( \lambda_{\perp} \), \( \lambda_{s} \)) and gliosis and demyelination during the sub-acute phase (D21), hence increased water diffusivity in the plane perpendicular to axons (\( \lambda_{ax} \), \( \lambda_{\perp} \), \( \lambda_{s} \)). As shown in Loy et al. (2007), changes in diffusion signal can already occur 3 h after axonal injury.

The specificity of diffusion measurements to white matter pathology has been previously studied in animal models of de/demyelination and suggests that axial diffusivity is more specific to axonal degeneration whereas radial diffusivity is more specific to demyelination (Budde et al., 2007, 2008, 2009; DeBoy et al., 2007; Hofling et al., 2009; Kim et al., 2007; Mac Donald et al., 2007; Song et al., 2002; Xie et al., 2010; Zhang et al., 2009). Other studies however reported possible interactions between axial and radial diffusivities, thereby limiting the specificity of these measures (Herrera et al., 2008; Sun et al., 2006; Wheeler-Kingshott and Cercignani, 2009). One argument refers to the pathophysiology of axon degeneration, as this process is known to be associated with demyelination in several pathologies such as in MS (Schmierer et al., 2007) or in SC injury (Cohen-Adad et al., 2011; Zhang et al., 2009). Another argument is related to the biophysical properties of DW-MRI, in which several physical parameters can influence diffusion metrics including myelination, axonal density, axonal diameter, or orientation of fiber bundles (Beaulieu, 2002; Sen and Basser, 2005; Wheeler-Kingshott and Cercignani, 2009).

High angular resolution diffusion imaging

One original aspect of this study was the use of Q-Ball diffusion ODF to estimate anisotropy measures in the SC white matter. Results showed that like the FA, the GFA measured distal from the injury in tract-specific regions of the cord showed significant differences between the intact state and the 21st day after injury. However, drawing definite conclusions about the effectiveness of the GFA for detecting white matter abnormalities is still limited by the interpretation of the biophysical processes underlying the diffusion ODF, since contrary to the tensor model there is no direct relationship between the diffusivity and the value on the diffusion ODF. The use of non-Gaussian metrics such as the diffusion Kurtosis (Hui et al., 2008; Jensen et al., 2005) or metrics derived from multi-tensor modeling (Hosey et al., 2005; Kreher et al., 2005) or higher order tensor (Barmopouls et al., 2009; Ghosh et al., 2008) may be useful in the course of finding biomarkers for characterizing white matter pathways in the central nervous system. Notably, metrics based on the directionality of diffusion may provide new phenotypes of pathological processes in the white matter and could potentially help following the course of anatomical reorganization in the central nervous system, as suggested in recent work (Barmopouls et al., 2009; Cohen-Adad et al., 2009a). The development of efficient directionality-based metrics is part of future investigations.

Conclusion

Acquisition of high angular diffusion-weighted imaging (HARDI) data with parallel imaging, super-resolution technique, respiratory gating and post-hoc distortion correction is feasible in the in vivo spinal cord of cats using a 3T human MRI with standard coils. DTI and Q-Ball metrics are sensitive to Wallerian degeneration in chronic spinal cord injured cats, with significant changes at the epicenter and distal to the lesion. Changes seen in metrics were consistent with the known anatomy of the spinal cord and may be used for correlation with functional recovery.

Acknowledgments

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