**Supporting Materials**

**Methods***Computational Simulations*

Monte Carlo simulations were performed to assess the effects of the diffusion filter pulse b-value on relative signal from intra- and extra-axonal compartments using the Camino toolkit (1). The geometry consisted of impermeable, aligned cylinders with a gamma distribution of diameters (2) and an intracellular volume fraction of 0.62, which was equal to the restricted fraction derived from the rat spinal cord white matter. The timing and strength of the diffusion gradients were identical to the *in vivo* PGSE sequence, with diffusion gradients applied perpendicular to the cylinders at b-values ranging from 0 to 4000 s/mm2. The signal was obtained separately from the intra- and extracellular spins and was expressed as a fraction of the combined signal normalized to the non-diffusion weighted signal.

*DDE-PRESS Model Fitting*

To evaluate the fitting accuracy of the different signal models, the corrected Akaike’s Information Criterion (AICc) was calculated as a measure of model performance according to the equation:

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|  |  | [4] |

where k is the number of parameters, L is the likelihood for the function, and n is the number of data points, with lower values indicating improved goodness-of-fit (3).

**Results**

*Computational Simulations*

Monte Carlo diffusion simulations in non-exchanging cylinders were consistent with the experimental results (Fig. 2B). With diffusion weighting and geometrical factors similar to the experimental data, the fraction of the total signal originating from the intracellular (restricting) space did not substantially change with b-values above 2000 s/mm2 (Sup. Fig. S1).

*DTI Region of Interest Analysis*

As described in the main text, several different ROI analysis were performed on the DTI data, including a whole-cord manual ROI and a perpendicular-weighted ROI masked with either the PRESS voxel geometry or the whole cord. Additionally, the weighted, whole-cord ROI was applied to either the DW images prior to DTI estimation, or to the DTI parameter maps after voxelwise estimation. The results of the regression analysis with injury severity are shown below in the table to highlight how different ROI methods can induce different results. Briefly, perpendicular-weighted ROIs considerable improve the sensitivity to injury severity for all measures expect RD (as expected, since its contribution to the DTI results are minimized with perpendicular weighting). Moreover, the difference between PRESS masking and whole-cord masking are minor, particularly for AD, suggesting the non-cord signals do not significantly contribute to the sensitivity of the DTI results. Overall, these results demonstrate that ROI analysis of DTI data as a marker of injury severity is heavily influenced by the methodology used, which has important implications to maximize sensitivity and ensure consistency across studies.

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| Supporting Table S1. Regression with Severity: R2 (p-value) | | | | |  | |  | |  | |  |
| **Perp-Weighted** | **Mask** | **DTI Fit** | **FA** | **AD** | | **MD** | | **RD** | |
| No | Cord | Voxelwise | 0.31 (0.010) | 0.14 (0.102) | | 0.00 (0.814) | | 0.15 (0.088) | |
| Yes | PRESS | ROI signal | 0.41 (0.002) | 0.44 (0.001) | | 0.10 (0.163) | | 0.01 (0.592) | |
| Yes | Cord | ROI signal | 0.60 (<0.001) | 0.56 (<0.001) | | 0.34 (0.006) | | 0.05 (0.328) | |
| Yes | Cord | Voxelwise | 0.55 (<0.001) | 0.57 (<0.001) | | 0.33 (0.007) | | 0.00 (0.766) | |

**References**

1. Hall MG, Alexander DC. Convergence and parameter choice for Monte-Carlo simulations of diffusion MRI. IEEE Trans Med Imaging 2009;28(9):1354-1364.

2. Skinner NP, Kurpad SN, Schmit BD, Budde MD. Detecting Acute Nervous System Injury with Advanced Diffusion Weighted MRI: A Simulation and Sensitivity Analysis. NMR Biomed 2015;28(11):1489-1506.

3. Cavanaugh JE. Unifying the Derivations for the Akaike and Corrected Akaike Information Criteria. Statist Probab Lett 1997;33(2):201-208.

**Figures**

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**Supporting Figure S1.** A simulated PGSE experiment in aligned axons further emphasized that increasing the b-value greater than 2000 s/mm2 does not substantially improve the selective attenuation of the extra-axonal component.

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**Supporting Figure S2. Real versus Magnitude Spectra Integration.** The absolute signals in the real and magnitude signals differ, but the normalized signal are equivalent, with the magnitude signal exhibiting less noise at the highest b-values.

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**Supporting Figure S3. DDE-PRESS Model Fitting.** In single sham animal (left) and in all sham animals (right), the biexponential model of magnitude-valued data provided the best goodness-of-fit, compared to either the monoexponential or kurtosis diffusion models, as evidenced by the lower Akaike’s Information Criterion (AICc), which accounts for the number of parameters in the model.