Acute Magnetic Resonance Imaging Predictors of Chronic Motor Function and Tissue Sparing in Rat Cervical Spinal Cord Injury

Seung-Yi Lee
Medical College of Wisconsin

Brian D. Schmit
Marquette University, brian.schmit@marquette.edu

Shekar N. Kurpad
Medical College of Wisconsin

Matthew D. Budde
Medical College of Wisconsin

Follow this and additional works at: https://epublications.marquette.edu/bioengin_fac

Recommended Citation
https://epublications.marquette.edu/bioengin_fac/653
Acute Magnetic Resonance Imaging Predictors of Chronic Motor Function and Tissue Sparing in Rat Cervical Spinal Cord Injury

Seung-Yi Lee  
Clement J. Zablocki VA Medical Center, VA Medical Center-Research 151, Milwaukee, WI

Brian D. Schmit  
Department of Biomedical Engineering, Marquette University and Medical College of Wisconsin, Milwaukee, Wisconsin

Shekar N. Kurpad  
Department of Neurosurgery, Medical College of Wisconsin, Milwaukee, Wisconsin

Matthew D. Budde  
Department of Neurosurgery, Medical College of Wisconsin, Milwaukee, Wisconsin
Abstract
Predicting functional outcomes from spinal cord injury (SCI) at the acute setting is important for patient management. This work investigated the relationship of early magnetic resonance imaging (MRI) biomarkers in a rat model of cervical contusion SCI with long-term functional outcome and tissue sparing. Forty rats with contusion injury at C5 at either the spinal cord midline (bilateral) or over the lateral cord (unilateral) were examined using in vivo multi-modal quantitative MRI at 1 day post-injury. The extent of T2-weighted hyperintensity reflecting edema was greater in the bilateral model compared with the unilateral injury. Diffusion tensor imaging (DTI) exhibited microscopic damage in similar regions of the cord as reductions in fractional anisotropy (FA) and mean diffusivity (MD), but DTI parameter maps were also confounded by the presence of vasogenic edema that locally increased FA and MD. In comparison, filtered diffusion-weighted imaging (fDWI) more clearly delineated the location of acute axonal damage without effects of vasogenic edema. Pairwise correlation analysis revealed that 28-day motor functional outcomes were most strongly associated with the extent of edema (R = −0.69). Principal component analysis identified close associations of motor functional score with tissue sparing, the extent of edema, lesion area, and injury type (unilateral or bilateral). Among the diffusion MRI parameters, lesion areas measured with fDWI had the strongest association with functional outcome (R = −0.41). Voxelwise correlation analysis identified a locus of white matter damage associated with function in the dorsal white matter, although this was likely driven by variance across the two injury patterns (unilateral and bilateral injury). Nonetheless, correlation with motor function within the damaged region found in the voxelwise analysis outperformed morphological lesion area measurement as a predictor of chronic function. Collectively, this study characterized anatomical and diffusion MRI signatures of acute SCI at cervical spine and their association with chronic functional outcomes and histological results.

Introduction
Magnetic resonance imaging (MRI) is a powerful tool to non-invasively assess the consequences of primary spinal cord injury (SCI) and its progression. Hemorrhage and edema are the two most frequently used clinical MRI markers in acute SCI detected by T2-weighted and T2*-weighted imaging, respectively, which are generally associated with the degree of damage. Greater structural damage, hemorrhage, and edema are repeatedly reported to be associated with worse sensorimotor function, but their prognostic power is limited. Correspondingly, the relationships between hemorrhage or edema at an acute stage and long-term outcomes have been evaluated, but their prediction of long-term patient outcome is limited because of uncontrolled variables in clinical studies, such as initial injury severity, time of imaging, patient's age, and injury site. Therefore, quantifying the degree of effects by hemorrhage and edema on neurological function may provoke the need for a treatment that can further improve outcomes.

Axonal injury is another imaging signature that has shown to more accurately predict recovery after SCI. Both clinical and pre-clinical studies have shown that in MRI, diffusion-weighted imaging (DWI) has a high sensitivity to the axonal disruption occurring acutely after SCI. However, the interpretation of the resulting parameters such as fractional anisotropy (FA) or mean diffusivity (MD) is often complicated by multiple underlying pathologies, including axonal injury that occurs concomitant with edema. In the spinal cord, a filtered DWI (fDWI) approach has been described in which a diffusion gradient applied perpendicular to the cord (i.e., filter) suppresses extracellular signals and cerebrospinal fluid (CSF).
Because water within the intra-axonal space is confined by membranes along the fiber cross section, its signal is less attenuated. A subsequent diffusion gradient parallel to the cord measures intra-axonal diffusivity parallel to the cord \((fADC_{||})\), which is reduced acutely after injury due to axonal beading. This spinal cord-optimized diffusion encoding scheme enhances specificity for axonal injury in a rat model of thoracic SCI, which achieves reliable prediction of long-term functional outcomes.\(^{16-18}\)

The majority of pre-clinical studies evaluating MRI biomarkers have examined SCI in the thoracic cord, but injury to the cervical cord is more predominant in human SCI. Further, there are clear differences in neuroanatomy and structure-function relationships between the cervical and thoracic cord. For example, cervical cord has a larger gray to white matter ratio and is similarly more vascularized and susceptible to hemorrhage upon trauma than thoracic cord.\(^{19}\) Cervical SCI causes a greater degree of functional impairment than thoracic SCI. A more complete understanding of the pathophysiology of injury in rodent cervical SCI models is necessary and, in particular, it is important to determine whether MRI biomarkers examined in the thoracic cord are similarly effective in cervical injury. In this study, we aimed to quantitatively evaluate the relationships between MRI markers with neurological outcomes and immunohistochemistry in a rat model of cervical injury of both midline (bilateral) and lateral (unilateral) contusion SCI.

**Methods**

**Animals and injury model**

All procedures were approved by the Institutional Care and Use Committees of the Clement J. Zablocki VA Medical Center and the Medical College of Wisconsin. Forty Sprague-Dawley rats (275–300 g) were used for either unilateral \((n = 20)\) or bilateral \((n = 20)\) contusion cervical SCI with equal numbers of male and female within each group. Rats were prepared for surgery at spinal cord level C5 as described previously.\(^{20}\) A 10-g rod with a 1.5-mm diameter tip was dropped from 25 mm using the NYU-MASCIC impactor. The tip was positioned over the spinal cord midline for a bilateral injury and 2 mm lateral to the midline for the unilateral injury. Post-injury care followed guidelines for post-SCI care\(^ {21}\) including frequent monitoring, free access to food and water, additional fluid therapy, and carprofen administration for 3 days post-injury.

Rats were euthanized at 4 weeks post-injury with an intraperitoneal injection of sodium pentobarbital (100 mg/kg body weight) followed by intracardiac perfusion and fixation with 10% formalin in phosphate-buffered saline (PBS). The dissected spinal column was immersed in formalin for 48 h and transferred to storage in PBS for ex vivo MRI.

**Behavioral assessments**

Forelimb locomotor assessment scale (FLAS)\(^{22}\) and modified Basso, Beattie, and Bresnahan (mBBB)\(^{23}\) locomotor scale were conducted at 2 days post-injury (dpi) and weekly beginning at 7 dpi with the terminal assessments performed at 28 dpi. For mBBB, left and right hindlimbs were scored separately up to 19 points. Particularly, forelimb-hindlimb coordination was defined as a one-to-one correspondence between forelimb and ipsilateral hindlimb.\(^ {23}\) Locomotion was filmed while the rats freely walked in an open field. The left and right limb scores were scored separately, by two blinded raters, and averaged for final analysis.
Magnetic resonance imaging

*In vivo* MRI scans were performed at 1 dpi using a Bruker BioSpec 9.4T system. Animals were anesthetized with 4% isoflurane and placed supine in a commercial 38-mm diameter Litz volume coil. Isoflurane was maintained between 1.5% and 2.5% during the scans with respiratory rate and core temperature maintained within physiological ranges.

$T_2$-weighted images were acquired using a rapid acquisition relaxation (RARE) sequence with number of excitations ($NEX$) = 2, rare factor = 4 separately in the sagittal and axial two-dimensional (2D) planes. For sagittal imaging, repetition time (TR)/echo time (TE) = 2000/19, 57, 95 msec, field of view (FOV) = 30 × 30 mm, matrix = 256 × 256, slice thickness = 0.5 mm. For axial imaging, TR/TE = 2000/20, 59, 99 msec, NEX = 2, FOV = 22 × 22 mm, matrix = 170 × 170, slice thickness = 1 mm. $T_2^*$-weighted imaging used a three-dimensional (3D) multi-echo gradient echo with TR/TE = 65/2.5 msec, echo spacing = 2.9 msec, number of echoes = 6, FA = 14 degrees, $NEX$ = 2, FOV = 22 × 14 × 11 mm, and matrix = 64 × 64 × 48, and respiratory gating. For DTI, an axial four-segment echo planer image (EPI) was acquired with TR/TE = 1800/28 msec, NEX = 4, FOV = 22 × 22 mm, matrix = 96 × 96, slice thickness = 1 mm, $b$ = 800 sec/mm$^2$, 15 directions, and 3 non-diffusion weighted ($b$ = 0) acquisitions. A second DWI acquisition was performed using the same parameters but with a filtered-DWI method, which uses a high-strength diffusion gradient ($b$ = 2000 sec/mm$^2$) perpendicular to the cord (left-right) with separate diffusion encoding parallel to the cord ($b$ = 0-1000 sec/mm$^2$ in increments of 250).

*Ex vivo* MRI was acquired for spared tissue area and lesion volume measurements. The excised cord was cut to include the cervical and upper thoracic segments (3 cm), and it was inserted into a plastic tube (0.7 mm diameter) filled with PBS buffer at pH 7.4. $T_2$-weighted imaging was acquired using a 3D isotropic, variable flip angle RARE with parameters: TR/TE = 4000/5.6 msec, FOV = 50 × 30 × 30 mm, matrix = 320 × 192 × 192, rare factor = 96, and 16 repetitions. The flip angle schedule was simulated previously and yielded an effective TE of 33.6 msec. An inversion recovery pulse (TI = 1500 msec) was used to selectively null the signal of PBS buffer. Total imaging time was 6 h and 50 min for up to seven samples simultaneously.

MRI analysis

Edema and hemorrhage length were obtained from sagittal $T_2$-weighted and $T_2^*$-weighted images, respectively, using manual measurements along the rostral-caudal direction. Similarly, edema and hemorrhage were manually circumscribed on the axial images to obtain cross-sectional area. For DTI, images were fit to the tensor model using FSL to obtain FA, MD, axial diffusivity (AD), and radial diffusivity (RD). For fDWI, images were fit to a mono-exponential decay considering only the parallel diffusion b-values to obtain fADC$_{||}$, filtered apparent diffusivity parallel to the spinal cord. To create regions of interest (ROIs), perpendicular-weighted images were converted to signal-to-noise (SNR) maps by dividing by the standard deviation from a noise region, and whole cord masks were created by thresholding at SNR ≥6. These resulting binary masks were applied to both DTI and fDWI parameter maps to obtain whole-cord mean values. Further, the DWI-detected lesion area was manually delineated on the color composite fDWI images. For voxelwise analysis, DWI parameter maps were spatially registered to a common coordinate frame based on the digitized Paxinos rat spinal cord atlas. Briefly, the steps included manual placement of landmarks within the cord at each vertebral level followed by the automated steps of straightening the cord and rigid plus non-linear warping to the
template using the spinal cord toolbox\textsuperscript{27} implemented as an automated nipype pipeline.\textsuperscript{28} The raw data and source codes are available in a public repository.\textsuperscript{29}

On \textit{ex vivo} MRI, the central cavity and spinal cord tissue were manually delineated. Lesion volume was quantified as the 3D volume of the central cavity. Spared tissue was quantified as the smallest cross-sectional area of spinal cord tissue, which occurred at the lesion epicenter.

\textbf{Immunohistochemistry procedure and analysis}

Following \textit{ex vivo} MRI, the cords were cut into 4-mm blocks, processed, and embedded in paraffin. Transverse, 10-μm thick sections were cut from the lesion site at the level with the least spared tissue using \textit{ex vivo} MRI for guidance. Sections were stained to identify white matter axons using SMI-31 (Biolegend, phosphorylated neurofilament H antibody) and imaged with a confocal microscope at a resolution of 570 nm. The area of spared white matter tissue was measured using a custom MATLAB script in which pixels were thresholded after manually cropping out gray matter. The number of SMI-31 stained axons within the white matter were counted using \textit{Threshold} (1.57–4.46%) and \textit{Analyze Particles} (size = 100–700 pixels, circularity = 0.3–1) in ImageJ (version 1.52k) and quantified as total counts.

\textbf{Statistical analysis}

Stata (StataCorp, 2011), Prism (9.0.0), and MATLAB (R2017b) were used for statistical analysis. Means of parameters by injury group (unilateral and bilateral) were compared using Mann-Whitney U tests adjusted by the two-stage step-up method of Benjamini and colleagues,\textsuperscript{29} and statistical significance is reported as q, reflecting the adjusted \( p \)-value. Similarly, the variance of each parameter was compared using an F-test\textsuperscript{30} and its significance is reported as q-value. To quantify relationships between each pair of variables, a Spearman's rank correlation was performed. For values including binary variables (sex and injury type), a Point-Biserial correlation was used. To examine the interrelationships between the full set of all variables, principal component analysis (PCA) was used. PCA is an unsupervised data reduction method that was used to visualize the major sources of variation in this multi-dimensional dataset without introducing inherent bias. A total of 19 variables were used, including injury type (unilateral or bilateral) and sex as binary variables. The variables were normalized and used as an input for PCA.

Six animals were excluded due to one or more missing data in imaging parameters or histology (final \( n = 34 \)). For voxelwise statistical analysis of DWI parameter maps, a model including FLAS scores as regressors was used with a permutation-based multiple comparisons correction implemented in FSL.\textsuperscript{31,32} To directly compare voxelwise ROIs results with lesion area (fDWI) as outcome predictors, lesion area (fDWI) and the ROI values from the significant voxels were included as regressors in a single, multiple linear regression model.

\textbf{Results}

\textbf{Cervical injury and locomotor outcomes}

Overall, the C5 contusion injury resulted in mild but persistent behavioral impairments with typical forelimb movement dysfunction in the digits, wrists, and elbows. Animals with unilateral injury typically exhibited dysfunction on the ipsilateral side of the body. Bilateral contusion injury produced significantly more severe impairment than the unilateral injury when measured by both FLAS and mBBB during the entire experiment (Fig. 1, Table 1). Most functional recovery occurred within the first week after injury.
and plateaued after 2 weeks. During the 4 weeks of testing, forelimb motor function recovered by 21 points on a 64-point scale, whereas mBBB increased by 2.3 points on a 21-point scale (n = 40). The animals regained ability to walk and support weight on their hindlimbs in the first few days. Persistent impairment included lack of full utility of wrist, flaccid digits, and occasional forelimb limping. Bladder function remained normal throughout the experiment in all rats.

Table 1. Forelimb and Hindlimb Motor Function of All Animals (unilateral, n = 20; bilateral, n = 20)

<table>
<thead>
<tr>
<th>Days post-Injury (dpi)</th>
<th>FLAS</th>
<th>mBBB</th>
<th>q-value</th>
<th>FLAS</th>
<th>mBBB</th>
<th>q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unilateral</td>
<td>Bilateral</td>
<td></td>
<td>Unilateral</td>
<td>Bilateral</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45 ± 13.4</td>
<td>23 ± 15.6</td>
<td>0.000057</td>
<td>12.0 ± 3.5</td>
<td>9.8 ± 1.6</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>7</td>
<td>55 ± 5.6</td>
<td>42 ± 8.6</td>
<td>0.000001</td>
<td>14.0 ± 2.4</td>
<td>10.9 ± 0.6</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>14</td>
<td>57 ± 4.0</td>
<td>47 ± 6.2</td>
<td>0.000001</td>
<td>14.5 ± 2.2</td>
<td>11.3 ± 0.6</td>
<td>0.000013</td>
</tr>
<tr>
<td>21</td>
<td>58 ± 4.3</td>
<td>48 ± 5.7</td>
<td>0.000001</td>
<td>14.4 ± 1.8</td>
<td>11.5 ± 0.7</td>
<td>0.000061</td>
</tr>
<tr>
<td>28</td>
<td>59 ± 3.5</td>
<td>50 ± 6.6</td>
<td>0.000002</td>
<td>14.5 ± 1.9</td>
<td>11.8 ± 1.1</td>
<td>0.004513</td>
</tr>
</tbody>
</table>

Lower score indicates worse impairment. Full score is 64 for forelimb locomotor assessment scale (FLAS) and 21 for the modified Basso, Beattie, Bresnahan (mBBB) locomotor rating scale. Unilateral and bilateral comparisons at all time-points were significant (q < 0.05).

The FLAS reflects a composite of nine subscores across separate functional domains, and the variance in the nine subscores was evaluated across all time-points using PCA. Two components captured 80% of
the variance. Movement of shoulder, elbow, wrist, digit, paw placement, and forward locomotion activity were weighted similarly on PC1 (−0.9 to −0.68), except body position (−0.40) and trunk stability (0.23; see Supplementary Table S1). On PC2, coordination, body posture, and trunk stability weighted more than other subscores. The results reflect that variance in the total score was not driven by certain subscores and that the total score was an appropriate reflection of the variance in the FLAS measurements during the recovery period.

Qualitative MRI results

Multi-modal MRI was performed to reveal hemorrhage, edema, and axonal integrity after cervical SCI. Images from representative animals are shown for the unilateral and bilateral injuries (Fig. 2). Edema as assessed by T2-weighted hyperintensity was more focal in unilateral SCI and more diffuse in bilateral SCI. Hemorrhage, identified on T2*-weighted imaging, typically revealed hemorrhagic foci smaller in size than edema that were occasionally scattered instead of being in a single continuous spot and typically occurred in the gray matter or gray-white matter interface. Hemorrhage was evident in most animals with unilateral (15/20) or bilateral (16/20) injury at 1 dpi, although there was no evidence of overt hemorrhage from either the posterior or anterior spinal arteries. On diffusion images (Fig. 2, DTI and fDWI), the extent of injury appeared more focal than on T2-weighted images. In particular, MD maps revealed a region with lower MD with adjacent regions of increased MD. Similar patterns were evident on AD maps. On fADC∥ maps, the extent of the lesion was clear with decreased fADC∥ in the white matter, with no evidence of increased values adjacent to the lesion and no contamination from CSF. Color-coded composite images (DWI∥/fADC∥) provide high contrast between injured and non-injured tissue in both the unilateral and bilateral injury model.

FIG. 2. MRI in acute cervical spinal cord contusion injury (1 day post-injury). In a unilateral injury at the lesion epicenter, edema is reflected in T2w hyperintensities, with a small degree of hemorrhage in the T2*w hypointensity. A similar degree of edema occurs in bilateral injury, but there was no hemorrhage in this animal. A decrease in MD is present in both injuries, with an adjacent increase in MD suggestive of vasogenic edema. In the composite filtered DW image, the extent of white matter damage is highlighted with tract-specific differences in the location damage between the two injury types. AD, axial diffusivity; FA, fractional anisotropy; fADC, filtered apparent diffusion coefficient; fDWI, filtered diffusion-weighted imaging; MD, mean diffusivity; MRI, magnetic resonance imaging; T2w, T2-weighted; T2*w, T2*-weighted.

Ex vivo axial MRI was acquired to provide reliable measures of tissue sparing (Fig. 3, T2-weighted Ax) and to further aid locating the injury for immunohistology section preparation. With slice-matching as close
as possible, the shape and location of the lesion between MRI and histology was qualitatively similar for both unilateral and bilateral injuries.

FIG. 3. *Ex vivo* axial MRI and histology reveal the extent of the lesion cavity at 28 days post-injury. T2w axial MRI (left) and slice-matched sections (right) stained for neurofilaments (SMI-31) show the extent of damage in the cord for three representative spinal cords from each injury type. MRI, magnetic resonance imaging; T2w, T2-weighted.

Quantitative assessment of multi-variate MRI
Quantitative measurement of imaging parameters is shown in Figure 4, and measurements were compared between unilateral and bilateral injury (Fig. 5, Supplementary Table S2). Overall, anatomical changes were different between the two types of injury for histological spared tissue area (q = 0.012 × 10^{-3}), spared tissue area by *ex vivo* MRI (q = 0.012 × 10^{-3}), lesion volume (3D) by *ex vivo* MRI (q = 0.023 × 10^{-3}), and SMI-31 (q = 0.012 × 10^{-3}) count. Significant differences were also observed in edema length (q = 0.0005) and area (q = 1.0 × 10^{-6}), but not in hemorrhage length or area. The groups were less different from one another based on diffusion imaging parameters, with lesion area (q = 0.05 × 10^{-3}) and whole-cord FA (q = 0.035) having a significant group difference, whereas whole-cord fADC|| (q = 0.14), AD (q = 0.07), and MD (q = 0.21) were not significantly different between the two groups. Along with mean comparison, variance of each parameter was compared (Supplementary Table S2). There were no group differences in variance in any of the parameters.
FIG. 4. MRI quantification and derived parameters. Sagittal length and axial cross-sectional area of edema and hemorrhage were derived from T$_2$w and T$_2^*$w MRI, respectively. Lesion area was manually segmented on fADC composite images. Whole-cord regions of interest were semi-automatically created on DW images to derive whole-core mean fADC (shown) and DTI metrics (not shown). Spared spinal cord cross-sectional area was obtained from ex vivo MRI and histological sections. Axonal counts were also obtained from SMI-31-stained sections. Representative images from different subjects are shown to highlight each contrast. DTI, diffusion tensor imaging; fADC, filtered apparent diffusion coefficient; fDWI, filtered diffusion-weighted imaging; MRI, magnetic resonance imaging; T$_2$w, T$_2$-weighted; T$_2^*$w, T$_2^*$-weighted.
FIG. 5. Group differences between unilateral and bilateral injuries. A non-parametric t-test (Mann-Whitney U) was used with false discovery rate adjustment. *q < 0.05; ***q < 0.001; ****q < 0.0001. Spared tissue area measured by ex vivo MRI (J) and histology (L). fADC, filtered apparent diffusion coefficient; MRI, magnetic resonance imaging; Ns, not significant.

For ex vivo MRI and histology results, minimal spared tissue area measured with either MRI or histology was strongly correlated (R = 0.82) across all specimens evaluated (n = 38). Strong correlation was also observed between functional outcome (FLAS) and spared tissue area (R = −0.66), or ex vivo MRI spared tissue area (R = 0.5), or lesion volume (3D; R = −0.60).

Pairwise correlation analysis

All pairwise combinations of 19 variables were examined with a correlation matrix indicating their relationships with one another using non-parametric statistics (Fig. 6). Stronger correlations were evident within each class of the parameters compared with between different classes. For example, the two scoring scales that both assess locomotor function (FLAS and mBBB) had strong correlations with one another, ranging from R of 0.64 to 0.83 across different time-points. Further, each scale was also strongly correlated across the 2- and 28-day time-points (FLAS [R = 0.73] and mBBB [R = 0.62]). Acute MRI predictors of functional or biological outcomes varied by contrast type (Supplementary Table S3). Edema area measured with T2-weighted MRI was a strong predictor of histology-measured spared tissue area (R = −0.68), lesion area (fDWI; R = 0.70), and FLAS score at 28 dpi (R = −0.61). On the other hand, the area of hemorrhage had weaker correlation with FLAS at 28 dpi (R = −0.41). Of the DTI or fDWI metrics, lesion area measure on fADC|| images had the strongest correlation with FLAS at 28 dpi (R = −0.41). DTI parameters had low correlations with anatomical MRI markers, ex vivo MRI markers, histology parameters, and motor functional outcomes. Biological outcomes including histology and ex vivo MRI had consistently high relationships with one another. Spared tissue area and SMI-31 count were moderately correlated (R = 0.52), and spared tissue area and lesion volume (3D) measured by ex vivo imaging showed a strong negative correlation (R = −0.87). Sex had little relationship with any of the measured variables.
Principal component analysis

PCA was performed to quantify the relationships between all measured variables across domains (Fig. 7). PCA produced four principal components with eigenvalue >1 that explained 75.1% of the total variance. PC1, PC2, PC3, and PC4 accounted for 44.1%, 14.7%, 10.1%, and 6.2% of the variance, respectively, and plots of PC1 and PC2 loadings revealed the clustering of individual variables (Fig. 7). PC1 was weighted primarily by spared tissue area (−0.79), motor functional scores (−0.80), injury type (−0.77), and size of edema (0.82). Consistent with cross-correlation analysis, motor functional scores were clustered with one another. Spared tissue area and SMI-31 count presented similar PC1 and PC2 weighting with spared tissue area measured by ex vivo imaging and motor scores. Edema length and injury type appeared clustered with positive PC1 weighting. Whole cord DTI metrics (AD, MD, FA) shared similar weights on PC1 and PC2. These metrics were orthogonal to anatomical MRI markers, histological results, ex vivo MRI markers, and motor functions, which indicates an independent relationship. Sex was a minor factor in explaining variance of PC1 (−0.06) and PC2 (−0.28).
Spatial registration and voxelwise correlations

Quantitative axial diffusion parameter maps were spatially aligned to a common coordinate space based on a rat spinal cord template. Maps averaged across either the unilateral or bilateral injury groups revealed consistent reductions in white matter FA, AD\textsubscript{DTI}, and fADC\textsubscript{||} examined qualitatively (Fig. 8A). In the unilateral injury, decreases were restricted to the left lateral white matter, as expected. In the bilateral injury, reductions were evident in the dorsal columns as well as within the more centrally located lateral and ventral white matter, consistent with larger overall lesions in the bilateral compared with unilateral injuries. In voxelwise correlation analysis with FLAS scores (Fig. 8B), portions of the dorsal white matter had a significant positive correlation with FLAS, whereas no regions of significant negative correlations were evident in any of the parameters. To further understand the relationship between voxelwise (i.e., tract-based) or whole-cord injury severity metrics from diffusion MRI and their respective ability to predict functional outcomes, a multiple linear regression analysis was performed. An ROI was obtained from the FA correlation map in voxels with significant correlation coefficients. Compared with lesion area (fDWI), which was previously evaluated in pairwise associations ($R^2 = 0.18, p = 0.004$), the ROI approach exhibited an improvement in predicting 28-day FLAS scores ($R^2 = 0.29, p = 0.0002$). Notably, a model including both values revealed that only the ROI values had a significant relationship with 28-day FLAS ($t = 2.47, p = 0.018$), whereas lesion area (fDWI) was not strongly related to outcome ($t = -0.656, p = 0.52$) when accounting for the tract-specific voxelwise results.
Discussion

This work examined cervical contusion SCI in a rat model to characterize the relationships between MRI-derived markers of hemorrhage, edema, and axonal injury in the acute stage with chronic neurological and biological outcomes. Compared with prior work in the thoracic spinal cord of the rat, the cervical injury model has greater clinical relevance because the majority of SCI occurs at the cervical level and there are clear functional and neuroanatomical differences from cervical SCI. Overall, the results demonstrated strong association between anatomical alterations (edema and tissue sparing) and chronic motor function in a single injury severity. In general, a high degree of within-domain correspondence was observed between measures that assess similar features. As expected, measures of tissue sparing from either histology or ex vivo MRI were consistent with one another. Quantitative measures of diffusion imaging in the whole cord were weakly associated with tissue sparing or chronic functional outcomes. Voxelwise correlation analysis revealed spatially localized area of higher association with functional outcomes, which was masked in the whole-cord analysis.

Neurological assessments for cervical SCI

In most studies using a cervical SCI model in rats, BBB or mBBB scores have been used to assess motor function. Although it is a well-established method, mBBB is more suited for hindlimb motor function. Several forelimb functional assessment scales have been reported, but no consensus recommendation or standard exists for cervical SCI in rodents. FLAS is more suitable for motor functional outcome evaluation after cervical SCI because it was developed in a similar contusion SCI model in rats. However, FLAS has not been widely used to evaluate motor functional outcomes after cervical SCI. Hence, in this study, mBBB and FLAS were both evaluated to assess their possible differences or similarities of locomotor recovery. As expected, there were strong correlations between
FLAS and mBBB at 2 dpi (R = 0.83) and 28 dpi (R = 0.96). Further, correlations between acute MRI parameters with FLAS at 28 dpi were remarkably similar to the correlations with mBBB at 28 dpi (Supplementary Table S3), reflecting that any differences between the two scales were relatively minor. FLAS revealed lasting impairment in paws and wrist mobility at chronic time-points. This is similarly observed in human SCI, in which patients can sit up after weeks of injury but dexterity recovery is delayed or permanently disabled.37

One major limitation of the FLAS scale is that it is not a hierarchical scoring scale like mBBB in which animals necessarily progress to better scores during recovery. Instead, it evaluates motor function in nine separate domains with the points being summed for a total score, and there is some clear non-linear behavior of the composite scores. To confirm that the total score was not heavily influenced by a limited set of domain scores, PCA was performed to examine the variability in FLAS subscores across all animals and time-points (Supplementary Table S1). This analysis revealed consistent and nearly equal weighting of almost all subscores within the first two principal components, demonstrating quantitatively that the FLAS total score captures the overall functional status of the animals. Thus, FLAS total score was used for the subsequent analyses instead of using a more granular subscore analysis.

T2- and T2*-weighted MRI

Edema detected by T2-weighted imaging has high sensitivity for injury to the cord and is routinely used for clinical diagnosis. The presence of hemorrhage detected by T2*-weighted gradient echo imaging has also been consistently related to worse outcomes from SCI in a binary manner.6,38,39 Edema and hemorrhage are commonly observed consequences of SCI that are both involved in the progressive secondary injury. However, the power of quantitative metrics derived from clinical images, including lesion length and level of cord compression, to predict long-term neurological outcomes has been conflicting.8,9,40

Nonetheless, our results show that edema length measured at the acute time-point was the strongest correlate of FLAS score at 28 dpi (R = −0.69). Edema is known to progress in the first several days after injury,38,41 and the first MRI exam in patients can vary considerably from the time of injury between hours to more than a day.42 The consistent and fixed post-injury time-point in this study may reduce the effects of edema progression with time that are experienced in clinical scenarios. The bilateral impact group had a larger extent of edema and worse functional outcomes, and T2-hyperintense edema emerged as the strongest MRI metric associated with locomotor outcome in this study. The consequences of edema are well-known and contribute to the cycle of swelling and pressure and secondary injury. Similarly, despite consistent clinical findings showing that the presence of hemorrhage in the cord leads to worse outcomes in clinical SCI,43 hemorrhage size was a modest predictor of outcome (R = −0.41) in this study. In addition to the temporal evolution previously discussed, one noteworthy limitation of T2*-weighted imaging is the competing effects of hemorrhage and edema in which the hypointensity caused by hemorrhagic iron can be obscured by the T2-hyperintensity caused by extensive edema.

More accurate quantitative acquisition or analysis may be needed, such as susceptibility-weighted imaging (SWI), to enhance sensitivity to blood products. Further, it is also possible that more extensive hemorrhage is a consequence of greater injury severity, and the single injury severity within the mild to moderate range in this model did not yield extensive hemorrhagic consequences that contributed to more damage and functional outcomes.
Diffusion-weighted imaging

Spinal cord axonal injury is a key pathology related to long-term neurological outcomes in SCI. Although DTI has been widely investigated as a surrogate of axonal injury, it can be confounded by edema and the aforementioned temporal effects. Specifically, decreased MD and AD are consistently associated with the acute axonal damage in SCI that reflects cytotoxic edema and axonal beading. These are seen in Figure 2 as regions of decreased MD and AD. However, increased MD reflecting vasogenic edema was also evident in some regions adjacent to the injured portions of the cord. The fDWI method was designed to reduce the effects of vasogenic edema in the spinal cord. fADC$_{||}$ maps, which measure diffusion parallel to the cord similar to AD, did not show increased diffusivity in these same regions and despite reducing the effects of vasogenic edema on a microscopic scale. The resulting maps have a clearer depiction of the region of damage within the cord and no complicating effects of CSF along the cord periphery.

However, diffusion imaging metrics averaged across the whole-cord cross-section were weakly associated with chronic motor functional scores, noting that the lesion area extracted from fADC$_{||}$ showed the strongest correlation among the diffusion metrics ($R = -0.41$). Because fDWI has specificity for axonal injury, in the regions with T$_2$-hyperintensity and normal fDWI axons were likely structurally intact but might have been susceptible to secondary injury. This interpretation emerges from stronger correlations between fDWI and 2-dpi FLAS ($R = -0.63$) than those between fDWI and 28-dpi FLAS ($R = -0.41$). T$_2$-weighted and, to a lesser extent, FA/MD also captured some of the edema in axons that might have been vulnerable to secondary injury. Whether these axons were functionally intact is not possible to address with MRI, but those axons were likely viable and might reflect a penumbra. However, additional work is needed to confirm and clearly establish whether fDWI and T$_2$-weighted could be used to detect a penumbra.

Because whole-cord analysis has been recognized as somewhat limiting, tract-based or voxelwise correlation analysis has been advocated. The voxelwise correlation analysis in this study demonstrated increased performance in outcome prediction compared with whole-cord or lesion-size diffusion measures and revealed spatial foci. The relevance of the region identified may be in part related to its functional importance because it appears to include the rodent corticospinal tracts, but it also may be a consequence of bias introduced by the limited variability in the spatial pattern of the injury and dependance on the two injury models used. Nonetheless, the results overall confirm the importance of examining tract-specific structure-function relationships in SCI.

The extent of tissue sparing in chronic SCI using histopathology is regularly associated with functional outcome and is often considered a gold standard for measuring the biological efficacy of interventions to complement functional outcomes. Although tissue sparing as an outcome measure has also been called into question recently, the current study identified correlation coefficients between functional outcome (FLAS) and histology spared tissue area or ex vivo MRI spared tissue area or lesion volume (3D), remarkably similar to reported values across many studies relating function with histology in SCI rodent models. Interestingly, 2D ex vivo MRI spared tissue area was highly correlated with lesion volume (3D; $R = -0.87$), sufficiently representing true 3D tissue damage. Further, spared tissue area from ex vivo MRI was strongly related to the same measures obtained from histology ($R = 0.82$), which aligns with previous studies and supports a potential role for ex vivo MRI as a surrogate for measuring tissue sparing if available.
An acute biomarker of eventual outcome in SCI offers several advantages. First, understanding a patient’s likely recovery trajectory can help inform care and rehabilitation. The relationship between acute MRI biomarkers and chronic motor function described in this study provides critical steps to plan parallel human studies of SCI that will ultimately assist in efficient and effective patient care. Particularly, this was the first work utilizing fDWI to characterize cervical SCI. Further refinement and application of fDWI to improve diagnosis and prognosis of cervical SCI is warranted. Second, more objective assessment can be provided using multi-modal MRI. Although the International Standards for Neurological Classification of Spinal Cord Injury exam is currently the standard for clinical trials, it can be challenged by patient status in the acute setting including from non-compliance, drugs, alcohol, other comorbidities, or polytrauma. MRI has the value of using multiple contrast mechanisms and spatial resolution. Although tract-based analysis is not yet common, our results suggest that it may refine assessment of imaging markers for more accurate diagnosis and prognosis. Unfortunately, it can be challenging to adopt this method in acute SCI due to its expense and logistics. With advances in MRI technology, tract-based analysis is expected to become more available, providing more accurate information about injury status.

Limitations
The primary limitation of this study is the use of a single injury severity, and similarly, the two different injury locations may have resulted in confounding or concurrent effects that are difficult to disentangle. Specifically, despite identical drop heights, the bilateral injury resulted in worse function and larger extents of damage to the cord. Interestingly, variances of unilateral and bilateral injury groups were similar, indicating that any significant correlation was not driven by one group over the other. It is possible and likely that damage to specific tracts that subserve locomotion, including the corticospinal tract, causes greater functional impairment. However, although the voxelwise analysis did identify focal effects, there was likely insufficient variability in the spatial extent of damage throughout the cord to confidently implicate tract-specific impairments. It is also possible that the biomechanical or other features that are different between white and gray matter could have contributed to the differences in severity and outcomes between the two injury types.

Further, although it was hypothesized that fDWI, as a more direct marker of axonal injury, would be more strongly correlated with function, this did not emerge from the results. First, there is a lack of baseline scores because the primary focus was on the relationship between early imaging markers and chronic motor functional outcomes. Second, the neurological assessment was limited to locomotion, whereas the impact on sensory functional outcome was not evaluated. Further, unlike thoracic SCI in the rat, the animals in this study did not appear to have bladder dysfunction and manual bladder expression was not needed. The association between acute MRI markers and other consequences of SCI important to patients such as bladder or bowel dysfunction is warranted for future studies.

Transparency, Rigor, and Reproducibility Statement
This study or analysis plan was not formally pre-registered. This was a pilot study to characterize the injury model, thus there was no a priori sample size calculation. Imaging data were labeled using codes that were not linked to subject identifying information. The entire study was conducted over a 4-month period, and all imaging data were analyzed at the same time. The time required for imaging acquisition was 40 min per subject per scan. Complete imaging parameters are presented in the Methods section. The Spearman's correlation analysis used was based on the assumptions that a pair of variables hold a
monotonic relationship. Subjects with one or more missing data points were omitted from correlation and PCA analyses. Correction for non-parametric multiple t-test was performed using the method of Benjamini and colleagues. No replication or external validation studies were performed or are planned at this time to our knowledge. Data from this study are available in a public archive at https://osf.io/s8gnz/. All software used to perform analysis are available in Mathworks (MATLAB), Python, and Spinal Cord Toolbox, with analytical code used to conduct imaging registration and analyses present in this study available in a public repository.

Conclusions
This study investigated the relationship of early MRI biomarkers with long-term functional and biological outcomes in the cervical spinal cord of the rat. The variability in chronic functional impairment between individuals was most explained by the size of edema (length and cross-sectional area), which also had strong relationships with terminal spared tissue area and lesion area (fDWI). Image registration and voxelwise correlation analysis provided additional spatial sensitivity for chronic motor function, showing a potential to improve diagnostic and prognostic value of diffusion imaging when used with tract-based analysis. These findings may serve useful in pre-clinical treatment studies aimed at the rat cervical spinal cord.

Acknowledgments
We thank Matt Runquist and Qian Yin for MRI assistance, and Natasha Wilkins for surgical assistance.

Authors' Contributions
The authors contributed to the article as follows: study conception and design: S. Lee and M. Budde; data collection: S. Lee; analysis and interpretation of results: S. Lee and M. Budde; draft manuscript preparation: S. Lee and M. Budde. All authors reviewed the results and approved the final version of the article.

Funding Information
This work was supported by grants from the National Institute of Neurological Disorders and Stroke (NS109090) and the Office of the Assistant Secretary of Defense for Health Affairs through the Spinal Cord Injury Research Program (W81XWH-19-SCIRP-IIRA) to MBD.

Author Disclosure Statement
No competing financial interests exist.

References


