Dynamic correlation of diffusion tensor imaging and neurological function scores in beagles with spinal cord injury

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Abstract
Exploring the relationship between different structure of the spinal cord and functional assessment after spinal cord injury is important. Quantitative diffusion tensor imaging can provide information about the microstructure of nerve tissue and can quantify the pathological damage of spinal cord white matter and gray matter. In this study, a custom-designed spinal cord contusion-impactor was used to damage the T10 spinal cord of beagles. Diffusion tensor imaging was used to observe changes in the whole spinal cord, white matter, and gray matter. The Texas Spinal Cord Injury Score was used to assess changes in neurological function at 3 hours, 24 hours, 6 weeks, and 12 weeks after injury. With time, fractional anisotropy values after spinal cord injury showed a downward trend, and the apparent diffusion coefficient, mean diffusivity, and radial diffusivity first decreased and then increased. The apparent diffusion-coefficient value was highly associated with the Texas Spinal Cord Injury Score for the whole spinal cord (R = 0.919, P = 0.027), white matter (R = 0.932, P = 0.021), and gray matter (R = 0.882, P = 0.048). Additionally, the other parameters had almost no correlation with the score (P > 0.05). In conclusion, the highest and most significant correlation between diffusion parameters and neurological function was the apparent diffusion-coefficient value for white matter, indicating that it could be used to predict the recovery of neurological function accurately after spinal cord injury.

Key Words: nerve regeneration; spinal cord injury; diffusion tensor imaging; fractional anisotropy; apparent diffusion coefficient; white matter; gray matter; Texas Spinal Cord Injury Score; beagles; neural regeneration

Introduction
Spinal cord injury (SCI) can potentially cause catastrophic damage to the central nervous system (Jirjis et al., 2013) and is disabling (Chang et al., 2010), causing motor and sensory deficits, autonomic dysfunction, limb paresis, proprioceptive ataxia, urinary and fecal incontinence, and respiratory compromise (Beal et al., 2001; Cerda-Gonzalez and Olby, 2006; Kang et al., 2018). Together, these problems result in long-term disability and have a great impact on patients, family, and society (Koskinen et al., 2013). Exploring the relationship between each structure of the spinal cord and functional assessments is helpful for diagnosing SCI, be-
cause rehabilitation management and functional recovery after SCI are primarily affected by the severity and location (spinal cord segment) of the injury (Kirshblum et al., 2007).

In animal models of SCI, neurological function is generally evaluated using standard scales. The Basso, Beattie, and Bresnahan scale, the Basso mouse scale, and the Texas Spinal Cord Injury Score (TSCIS) have been used in rats, mice, and dogs, respectively. Reports have suggested that a dog model of clinical SCI can be compared with SCI in humans in terms of mechanisms of injury, pathology, outcome, classification, and functional monitoring. This model is considered an ideal translational model between rodent experiments and human clinical trials (Jeffery et al., 2006; Boekhoff et al., 2012). The TSCIS for dogs was designed to individually evaluate gait, postural reactions, and nociception based on data that could be routinely collected during a neurological examination (Levine et al., 2009). However, this method for qualitative functional assessment has some limitations, because it depends on dogs’ condition and operators’ subjective information. Further, differentiating between SCI and additional injury to the peripheral nerves and musculoskeletal system is difficult (Shields et al., 2006; Tolonen et al., 2007), and the TSCIS is inadequate for reliably assessing the effects of therapy on the injured spinal cord (Dietz and Curt, 2006).

Conventional MRI is suitable for assessing damage to spinal cord tissue (Scholtes et al., 2006) and is the best method for showing traumatic compression of the spinal cord (Miyajiri et al., 2007). However, conventional MRI has low sensitivity for diffusion abnormalities in gray and white matter, thereby limiting any correlations with neurological status (Bakshi et al., 2008). Diffusion tensor imaging (DTI) is a relatively new imaging method based on the diffusion of water molecules in tissues. The major DTI parameters are the apparent diffusion coefficient (ADC), fractional anisotropy (FA), mean diffusivity (MD), and radial diffusivity (RD). Quantitative DTI parameters can also provide information about tissue microstructure within the nervous system (Beaulieu, 2002). DTI has been shown to have potential for quantifying white matter and gray matter pathology in the spinal cord and has been successfully applied to multiple sclerosis, spondylothetic myelopathy, and SCI (Budzik et al., 2011; Petersen et al., 2012; Jones et al., 2013; Oh et al., 2013). As in other studies (Olby et al., 2001; Kim et al., 2010; Lindberg et al., 2016), the DTI parameters correlated with neurological assessments at typical time points after SCI. Therefore, the status and severity of SCI can be diagnosed and predicted by quantitative DTI analysis to ensure better rehabilitation strategies and treatment protocols. While these studies only focused on one time point or one structure, and to the best of our knowledge, few studies have focused on the dynamic relationship between DTI parameters of different structures and TSCIS in dogs.

We hypothesized that different structural attributes of the spinal cord would correlate differently with functional assessment. This study aimed to investigate dynamic correlation between DTI parameters of different spinal cord structure and functional assessment after SCI in dogs.

### Materials and Methods

#### Ethics statement

All animal procedures were approved by the Experimental Animal Center of Capital Medical University of China (approval No. AEEI-2015-055). The study was conducted according to the ethical rules of the Animal Experiments and Experimental Animal Welfare Committee.

#### Animals

Seven healthy female beagles weighing 10.0 ± 0.5 kg and aged 2.0 ± 0.5 years were used in the study (Beijing Marshall Biotechnology Co., Ltd., Beijing, China). All beagles were allowed free access to food and water. To fulfill the inclusion criteria, no dog had clinical signs of an underlying disorder of the spinal cord and all dogs had normal spinal cords as assessed by standard MRI protocols under anesthesia. Each dog had a maximum ethology score on the new TSCIS. Exclusion criteria were as follows: any suspicion of a neurological disorder affecting the spinal cord, a history of spinal cord disease, or a previous spinal cord surgery.

#### Establishment of the SCI model

Dogs were anesthetized via intraperitoneal administration of 2.5% pentobarbital sodium (Item No: P11011, Merck, Darmstadt, Germany) at a dose of 125 mg/kg and intramuscular administration of xylazine hydrochloride (Dunhua Shengda Animal Pharmaceutical Co., Ltd., Dunhua, Jilin Province, China) at a dose of 0.1 mL/kg, and then fixed on the operating table in the prone position. An intraoperative antimicrobial (cefoxitin sodium) was intravenously administered when the spinal cord was surgically decompressed (Webb et al., 2010). Skin preparation was performed for a 30 cm-long 15 cm-wide rectangular area centered on the 10th thoracic vertebra (T10). After conventional betadine and alcohol disinfection, a 6-cm longitudinal incision was made around the midpoint of the T10 spinous process in a sterile environment. The adipose layer and the fascia were then carefully cut and the spinous process and the adjacent muscles were bluntly dissected and provided adequate hemostasis. The T9, T10, and T11 spinal processes and the interspinal ligaments between them were cut and the vertebral laminas of both T10 and part of T9 and T11, were uncovered to expose approximately 1 cm of the spinal cord, as described previously (Dong et al., 2016).

In the current study, a new spinal cord contusion impactor for large animals was designed based on the principles of the MASCIS Impactor Method (Young, 2002) (Patent No. ZL 2016 2 0915673.4). The spine of each animal was fixed with a spinous process clip to center the T10 spinal cord under the head of the 20-g falling hammer. The device was first calibrated by first dropping the hammer until it nearly contacted the spinal dura. The hammer was then lifted to 25 cm and released in free fall to induce SCI with the fshalling phenomenon. The impact velocity, force, displacement, and dwell time were the same for each dog. Immediately following the injury, the wounds were irrigated with saline, and adequate hemostasis was applied. A piece of gelatin sponge...
was used to cover the injured spine and muscle layers were sutured together (Liu et al., 2018) (Figure 1).

Hydrogen peroxide and betadine were used to clean the surgical site, which was bound with sterile gauze. Once the dogs regained consciousness, pregabalin (25 mg/kg; Pfizer Manufacturing Deutschland GmbH, Betriebstätte Freiburg) was administered orally every day for 1 week.

All dogs were fed with enteral nutritional suspension (100 mL/10 kg; twice a day; Nutricia Pharmaceutical Co., Ltd., The Netherlands) for 1 week post-injury and allowed free access to food and water every day. The urinary catheter was pulled when reflex-bladder emptying returned, which was typically within 2–3 weeks after injury.

**MRI**

The beagles were placed in the supine position and maintained under anesthesia during the entire MRI scan with a mixture of pentobarbital sodium and xylazine hydrochloride. Scanning was performed with a Siemens 3.0T Prisma fit MRI scanner (Siemens Magnetom, Essen, Germany) with a 32 Ch Spine-cord coil. Fat-saturated sagittal T2-SPACE sequences were acquired with in-plane resolution = 0.84 mm × 0.84 mm, repetition time/echo time = 1500/142 ms, slice thickness = 0.75 mm, and ACQ matrix = 320 × 320. Axial diffusion imaging was based on single-shot EPI sequence using the following parameters: repetition time/echo time = 7300/83 ms, slice thickness = 4 mm with a 0.8 mm gap, in-plane resolution = 1.3 mm × 1.3 mm, 30 directions, b values = 800, number of excitations = 2, and an extra 4 b0 images. MRI exams were carried out pre-contusion and at 3 hours, 24 hours, 6 weeks, and 12 weeks post-injury.

**DTI preprocessing**

White matter centerlines were defined on T2W imaging using customer software (Siemens Magnetom). T2 images were segmented into whole spinal cord, gray matter, and white matter using the SpinalCord toolbox, and were then used as the masks for DTI processing. DTI image preprocessing included: (1) registration to T2W images, (2) correction for eddy current, and (3) calculation of the following DTI parameters: FA, ADC, MD, and RD. The injury epicenter was T10 and the epicenter lesion (T10) was defined as the region of interest. The parameters of the epicenter lesion were analyzed (Figure 2).

**Functional scores**

The TSCIS (Levine et al., 2009) for dogs was designed to individually evaluate gait, postural reactions, and nociception in each limb. The scoring times were preoperatively, 3 hours, 24 hours, 7 days, and then weekly until 12 weeks post-injury. The score was double-blinded (two-person scoring). The behavioral score of each dog was the double-blind average from each lower limb. Additionally, the scoring process was recorded with a high-speed camera to determine scoring accuracy.

**Statistical analysis**

The statistical analysis was performed with IBM SPSS software version 21.0 (IBM Corp., Armonk, NY, USA). Testing for normal distribution of the acquired data was performed with the Shapiro-Wilk normality test. A repeated-measures analysis of variance (ANOVA) was used to assess the changes in DTI parameters, TSCIS scores over time, and the main effects. Pearson correlation analysis was used to test for correlations between DTI parameters and the TSCIS at corresponding time points. Data are expressed as mean ± SD. P values < 0.05 were considered statistically significant.

**Results**

**DTI**

The whole spinal cord: Figure 3A shows that ADC values differed at each time point, with the epicenter value decreasing immediately after lesion induction and then increased from 3 hours to 12 weeks post-injury. The repeated-measures ANOVA showed a main effect of time on the ADC value (F = 5818.966, P < 0.001). Preoperative ADC values were significantly different from those after SCI (Figure 3A). FA decreased by 12 weeks post-injury (Figure 3B). We found a main effect of time on FA value (F = 265.183, P < 0.001). Post-SCI FA values were significantly lower than preoperative values (Figure 3B). Changes in MD and RD values over time are shown in Figure 3C and D, respectively.

White matter: Figure 4A shows that ADC values differed depending on the time, and the pattern of change was similar to that of ADC values for the whole spinal cord post-injury. We found a main effect of time on ADC value (F = 1148.028, P < 0.001). Figure 4A also shows the results of the statistical comparison between ADC values before and after SCI. Figure 4B indicates that the trend for FA values over time was similar to what was found in the whole spinal cord, with a main effect of time on FA (F = 393.797, P < 0.001). Changes over time and statistics for MD and RD values are shown in Figure 4C and D, respectively.

Gray matter: Figure 5A shows that the effect of time on ADC values was similar to what was observed for the whole spinal cord (F = 722.745, P < 0.001). Figure 5B indicates that FA values decreased by 12 weeks post-injury and the ANOVA showed that a main effect of time on FA value (F = 167.646, P < 0.001). Changes over time and statistics for MD and RD values are shown in Figure 5C and D, respectively. The P values for before/after SCI comparisons for each of the DTI parameters are shown in Additional Table 1.

**TSCIS**

As shown in Figure 6, in all animals, the lowest functional impairment scores (including gait, postural reflex, and nociceptive perception) for both lower extremities were obtained immediately after SCI. Over time, function loss induced by the lesion gradually recovered, showing a sharp increase between 24 hours and 3 weeks after surgery, and then appearing to stabilize. The behavioral scores at each postoperative time point were remarkably lower than those before surgery. The ANOVA showed a main effect of time on the behavioral scores (F = 210.680, P < 0.001). Preoperative TSCIS significantly differed from the TSCIS 24 hours after SCI (Figure 6).
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Correlation between DTI and TSCIS
The whole spinal cord: The correlation analysis showed that ADC value was highly correlated with TSCIS. As shown in Figure 7, the correlation between ADC and behavioral score was very high in the whole spinal cord \((R = 0.919, P = 0.027)\). In contrast, there was almost no correlation between TSCIS and FA, MD, and RD values \((P = 0.542, P = 0.286\) and \(P = 0.548\), respectively).

White matter: The correlation analysis showed that ADC value was highly correlated with TSCIS. As shown in Figure 8, the correlation between ADC and behavioral score was very high in white matter \((R = 0.932, P = 0.021)\). In contrast, there was almost no correlation between TSCIS and FA, MD, and RD values \((P = 0.338, P = 0.094\) and \(P = 0.492\), respectively).

Gray matter: The correlation analysis showed that ADC value was highly correlated with TSCIS. As shown in Figure 9, the correlation between ADC and behavioral score was high in gray matter \((R = 0.882, P = 0.048)\). In contrast, there was almost no correlation between TSCIS and FA, MD, and RD values \((P = 0.552, P = 0.572\) and \(P = 0.923\), respectively).

By analyzing the correlation between the DTI parameters and behavior, we found that the correlation between TSCIS and white matter DTI parameters was higher than that with the whole spinal cord. The correlation with the whole spinal cord DTI parameters was higher than that with gray matter.

Discussion
In this study, we observed changes in DTI parameters at the lesion site and in behavioral functional recovery after SCI. We also found correlations between DTI parameters of different structures and behavior scores. Not only was DTI a sensitive and noninvasive imaging technique that could quantitatively explore different components of the spinal cord, but it could also predict neurological function after SCI and provide an accurate diagnosis of SCI. This can be helpful for the detection and treatment of SCI and for guidance in rehabilitation programs.

DTI parameters reflected pathological changes in SCI
The ADC values decreased 3 hours after surgery and then increased 12 weeks after surgery. A similar study examined changes in ADC values from 1 to 4 weeks after SCI in rats and found that the ADC decreased (Wang et al., 2014). In other studies, ADC values after ischemic stroke were detected, and results showed that they decreased immediately after injury, but that this downward trend vanished after several hours and was replaced by an overall rise in ADC values that lasted for an extended period of time (Kucharczyk et al., 1991; Sotak, 2002). This is consistent with our results. This downward trend may be a result of cell swelling. An increase in cell radius due to swelling leads to an increased tortuosity.

All P values from the before/after (24 hours) TSCIS comparison are shown in Additional Table 2.
In addition to this mechanism, swelling also decreases the extracellular volume fraction, which decreases the contribution of extracellular diffusion to the overall signal (Hall and Alexander, 2009). ADC values have been associated with the degree of myelination (Leong et al., 2015). The ADC results suggest that both axonal injury and demyelination occur throughout the spinal cord as a result of traumatic contusion and that changes in axonal (or neurofilament) density and diameter caused by cellular swelling may also occur. Ellingson et al. (2008) suggested that axonal damage might occur slightly more quickly than damage to myelin after contusive injury. Mac Donald et al. (2007) considered that the pathological changes in the acute stage of injury are dominated by pure axonal injury, which is caused by disrupted neural filaments and accumulated organelles. This can hinder water diffusion along the axons (Povlishock, 1993; Gorrie et al., 2002; Maxwell et al., 2003). In addition, microstructural damage is often associated with edema, including cellular edema and vasogenic edema, which can further complicate the explanation for the changes in DTI parameters. At the subacute stage, demyelination was reported to be the prominent pathological change that was expected to reduce barriers to diffusion perpendicular to the fiber direction (Song et al., 2005; Mac Donald et al., 2007).

For FA values, the results showed that they obviously decreased in the epicenter. Some experts have applied Monte Carlo simulations to investigate four pathological changes after TBI, including axonal injury, vasogenic edema, cytotoxic edema, and demyelination. They reported that axonal...
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Damage led to decreased FA values, vasogenic edema led to decreased FA values, cytotoxic edema led to increased FA values, and demyelination led to decreased FA values (Lin et al., 2016). Axonal injury played a dominating role in the acute injury phase. Axonal swelling and petechial hemorrhages, as well as axon disconnections, might reflect direct injury (Povlishock, 1993; Maxwell et al., 1997). Damage led to a decrease in intra-axonal diffusion and a decrease in FA. Demyelination occurred mainly in the subacute period, meaning that FA values had already been trending downward, which was consistent with our results. It must be emphasized that demyelination was not the only pathological change in the subacute phase, as there may have also been axonal repair and reconstruction of the fiber bundles. This was demonstrated by the gradual recovery of neurological function over time following SCI. In a related study, re-

Figure 5 Changes in ADC, FA, MD, and RD values over time at the epicenter lesion site for gray matter.
All parameters are represented as the mean ± SD. Seven dogs were tested at each time point. *P < 0.05 (repeated-measures analysis of variance). ADC: Apparent diffusion coefficient; FA: fractional anisotropy; MD: mean diffusivity; RD: radial diffusivity.

Figure 6 Changes in TSCIS over time after spinal cord injury.
All parameters are represented as the mean ± SD. Seven dogs were tested at each time point. *P < 0.05, **P < 0.001 (repeated-measures analysis of variance). TSCIS: Texas Spinal Cord Injury Score.

Figure 7 Correlations between the ADC, FA, MD, and RD values of the whole spinal cord and TSCIS.
(A) Correlation between ADC and TSCIS (R = 0.919, P = 0.027). (B) Correlation between FA and TSCIS (R = 0.368, P = 0.542). (C) Correlation between MD and TSCIS (R = 0.599, P = 0.286). (D) Correlation between RD and TSCIS (R = 0.363, P = 0.548). ADC: Apparent diffusion coefficient; FA: fractional anisotropy; MD: mean diffusivity; RD: radial diffusivity; TSCIS: Texas Spinal Cord Injury Score.
searchers simulated the effects of DTI parameters after traumatic brain injury (TBI) based on a b value of 1000 s/mm² and a diffusion time of 50 ms. They concluded that axonal injury decreased the FA value by 17%, vasogenic edema decreased the FA value by 8%, and demyelination increased the FA value by 26% (Lin et al., 2016). Similar changes might also occur in SCI, because the spinal cord and the brain have similar white and gray matter microstructures.

Our results showed that MD and RD values both decreased 3 hours after injury and then increased 12 weeks post-injury. A related study reported that axonal injury and cytotoxic edema can lead to decreases in MD and that vasogenic edema and demyelination can lead to increases in MD. MD therefore first decreased and then increased as time passed (Lin et al., 2016). This was similar to our results. Based on a b value of 1000 s/mm² and a diffusion time of 50 ms, axonal injury caused MD values to decrease by 16%, vasogenic edema caused them to increase by 23%, cytotoxic edema to decrease by 7%, and demyelination to increase by 16% (Lin et al., 2016). As MD changes from lower than normal (acute) to above normal (subacute), a process known as “pseudo-normalization” occurs, demonstrating that the evolution of MD is complicated (Salmond et al., 2006; Rutgers et al., 2008).

Among the factors affecting RD values, axonal injury has been shown to decrease RD by 3%, vasogenic edema to increase RD by 47%, cytotoxic edema to decrease RD by 14%, and demyelination to increase RD by 42% (Lin et al., 2016).
Therefore, we can see that the sensitivity of RD values to vasogenic edema, cytotoxic edema, and demyelination was higher than that of FA and MD values. In our results, the trend in the RD curve was the largest.

Furthermore, signal-to-noise ratios might influence the DTI parameters. Trends in FA have been reported to be more susceptible to noise than in MD (Lin et al., 2016). Neuronal structures might affect the diffusion parameters after SCI, and studies have reported that cavity formation occurs in gray matter after SCI (Tator and Fehlings, 1991; Hu et al., 2010; Sundberg et al., 2010). Astrocytes might affect diffusion parameters because astrocytes contain water channels and play an important role in water homeostasis (Ellingson et al., 2008; Herrera et al., 2008; Wang et al., 2009). Currently, the correlation between astrocytes and DTI parameters is not very clear, but previous studies have reported that the correlations between reactive glial scar syndrome and transverse and longitudinal diffusion are statistically significant (Lu and Sun, 2003; Ellingson et al., 2008; Wang et al., 2009). Fluctuations of cerebrospinal fluid after SCI could also be an influential factor. Inflammatory factors and necrotic substances may also affect diffusion parameters. Therefore, the interpretation of the diffusion-parameter variation may be complex.

**Correlation between diffusion parameters and TSCIS**

This study analyzed the dynamic correlation between diffusion parameters and behavioral score and found differences among the parameters. To the best of our knowledge, few reports have focused on the correlation between the diffusion parameters of the different spinal cord structures and behavioral scores in dogs. The TSCIS scoring system is primarily used to assess neurological function in dogs after SCI. This method is a precise way to quantify spinal cord dysfunction. Similar to many human SCI scores, each limb can be evaluated, which allows for limb asymmetry to be recorded at the time of initial evaluation and during the recovery phase. Another advantage is its ease of use because it did not require video-based training, treadmills, or gait analysis software (Levine et al., 2009). The TSCIS motor scoring system has been shown to be a reliable method for accurately determining SCI severity in dogs with sensitivity in recovery detection. Consistent with other studies (Bresnahan et al., 1987; Behrmann et al., 1992; Basso et al., 1996; Gensel et al., 2006), here TSCIS behavior demonstrated a three-stage pattern beginning with early severe dysfunction followed by functional recovery and then a plateau. In both humans and in experimental SCI, the initial trauma could lead to an instantaneous and often complete neurological deficit below the injury level. The initial flaccid paralysis and subsequent return of reflexes observed in the hours and days after SCI have been collectively referred to clinically as “spinal shock” (Ditunno et al., 2004; Kim et al., 2010). During spinal shock, neurological exams cannot accurately predict the degree of long-term functional impairment (Little et al., 1999). Accordingly, we did not analyze the correlation between behavioral scores and DTI parameters at 3 hours and 24 hours but instead analyzed the correlations beginning 1 week after SCI.

The whole spinal cord: The results showed that the correlation between ADC and behavioral score was very high, the correlation with FA was low, the correlation with MD was moderate, and the correlation with RD was low. Chang and colleagues (2010) reported that abnormal FA levels in the cervical spinal cord were statistically significantly correlated with abnormal motor levels but not with sensory levels. Cohen-Adad and colleagues (2011) also detected a correlation between DTI parameters (FA and RD) and a combined variable consisting of the sensory and motor scores on the International Standards for Neurological Classification of Spinal Cord Injury. Furthermore, in a study by Petersen and colleagues (2012), a decrease in FA correlated with the completeness of SCI according to the AIS A scale. In a study of pediatric SCI, the clinical findings showed a statistically stronger correlation between FA and DTI values than other parameters (Mulcahey et al., 2012). These reports are consistent with some of our results. By analyzing the DTI of white matter, we know that the correlation between ADC and behavioral score was very high, the correlation with FA was moderate, the correlation with MD was high, and the correlation with RD was low. Deo et al. (2006) reported in vivo longitudinal DTI studies up to 2 months post-SCI and correlated DTI measures in selected regions of white matter to Basso, Beattie, and Bresnahan scores. A report on DTI at 3 hours post-injury indicated that long-term neurological functional recovery after SCI could be predicted in rats because spared white matter volume was the parameter that best correlated with functional outcomes (Schucht et al., 2002; McEwen and Springer, 2006). The accurate and non-invasive acute estimation of spared white matter extent correlated with chronic locomotor recovery. For the DTI parameters of gray matter in our study, the correlation between ADC and TSCIS behavioral score was high, the correlation with FA was low, the correlation with MD was low, and the correlation with RD value almost zero. Currently, there are no reports on the correlation between diffusion parameters in gray matter and behavior. We speculate that because gray matter is mainly composed of neuronal cell bodies, and cavity formation also mainly occurs in gray matter, the correlation between the diffusion parameters of gray matter and neural functional recovery is relatively poor. The correlation analysis showed that the parameters had different relationships with TSCIS. Therefore, the state of SCI can be verified by behavioral assessment and can also be reflected by changes in DTI parameters. We recommend using DTI to estimate the neurological status of SCI because it is quantitative and relatively objective. By analyzing the correlation between DTI metrics of the different spinal cord structures and TSCIS, we saw that the correlation between nerve function and diffusion parameters in white matter was generally higher than those in the whole spinal cord or gray matter. Additionally, among the diffusion parameters, only ADC values had high correlation with TSCIS. Therefore, we can say that white matter better predicts neurological function after SCI and that the highest correlation is between neurological function and the ADC value in white matter.
We therefore consider the results of this study a basis for predicting the recovery of neurological function through the analysis of ADC values in spinal white matter. Doing this will allow patients to be informed about their degree of recovery and might also reduce unnecessary financial burden. In addition, treatment can be more targeted and improve the development of rehabilitation programs, thus promoting effective recovery of patients.

Study limitations
This study had some disadvantages. First, the sample size was small and sampling error was therefore large. However, we will increase the sample size and further vary the degree of SCI to expand our results in future studies. Second, the animals are alive and under anesthesia; therefore, the respiratory rate and the heart rate may affect the acquisition of MRI signals. We will increase the scanning time points to ensure objectivity and accuracy in the future.

Conclusions
This study explored dynamic changes in DTI parameters of different spinal cord structures after SCI in dogs and their correlations with TSCIS scores over time. FA values after SCI showed a downward trend, while ADC, MD and RD values decreased after SCI but then increased. The relationship of the different parameters in each structure with neural function varied. The largest and most significant correlation was between ADC values and behavior score post-SCI, which could be used to predict the recovery of neurological function accurately after SCI. These results may be significant in the implementation of rehabilitation programs and in the diagnosis and prognosis of SCI.

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Data sharing statement: Datasets analyzed during the current study are available from the corresponding author on reasonable request.

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### Additional Table 1
The P values of the each diffusion tensor image (DTI) parameters after spinal cord injury (SCI) compared with the preoperative ones

|                | 3 hours | 24 hours | 6 weeks | 12 weeks |
|----------------|---------|----------|---------|----------|
| Spinal cord    |         |          |         |          |
| Apparent diffusion coefficient (ADC) | 0.010   | 0.062    | 0.160   | 0.721    |
| Fractional anisotropy (FA)           | 0.054   | 0.026    | 0.113   | 0.002    |
| Mean diffusivity (MD)                | 0.092   | 0.884    | 0.888   | 0.147    |
| Radial diffusivity (RD)              | 0.308   | 0.506    | 0.491   | 0.043    |
| White matter                            |         |          |         |          |
| ADC                                        | 0.082   | 0.264    | 0.339   | 0.424    |
| FA                                          | 0.182   | 0.145    | 0.225   | 0.040    |
| MD                                          | 0.234   | 0.812    | 0.804   | 0.896    |
| RD                                          | 0.400   | 0.733    | 0.699   | 0.888    |
| Gray matter                                |         |          |         |          |
| ADC                                        | 0.009   | 0.045    | 0.201   | 0.512    |
| FA                                         | 0.006   | 0.005    | 0.025   | 0.001    |
| MD                                         | 0.239   | 0.348    | 0.564   | 0.076    |
| RD                                         | 0.732   | 0.063    | 0.105   | 0.008    |

### Additional Table 2
The P values of the Texas Spinal Cord Injury Score (TSCIS) after spinal cord injury (SCI) compared with the preoperative and 24 hours ones

|                | 3 hours | 24 hours | 3 weeks | 6 weeks | 12 weeks |
|----------------|---------|----------|---------|---------|----------|
| Vs. pre       | <0.001  | <0.001   | 0.178   | 0.264   | 1.000    |
| Vs. 24 hours  | 0.011   | 0.002    | 0.004   |         |          |