High Resolution Quantitative MRI in a Non-Surgical Model of Spinal Cord Injury

by

Wendy Kathleen Oakden

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Medical Biophysics
University of Toronto

© Copyright by Wendy Oakden 2015
Abstract

Magnetic resonance imaging (MRI) is very sensitive to the presence of damage resulting from injury or disease, but often lacks specificity. Quantitative MRI can significantly increase the specificity in the presence of pathology but must be validated, often using an animal model, for each type of injury or disease. In the case of spinal cord injury (SCI) most models are difficult to image, either due to the location of the injury, or as a result of damage to surrounding tissues resulting from invasive surgical procedures. This thesis describes a non-surgical model of rat SCI which uses MR guided focused ultrasound and microbubbles to create an injury the cervical spinal cord which is optimal for performing quantitative MRI, and compares it with other models of SCI using MRI, histology, and immunohistochemistry. It also describes the difficulties encountered when implementing the quantitative T₂ (qT₂) MR sequence at the very high resolution required to image the rat spinal cord, the limitations on the qT₂ sequence due to the presence of diffusion, and how the effects of diffusion were minimized. Using the new SCI model and qT₂ sequence, qT₂ and diffusion data were acquired at 24 hours, 1 week, and 2 weeks following SCI, and the quantitative MRI parameters were correlated with histology. The increased specificity gained using quantitative MRI will increase the information available at each timepoint, reducing both the variability and cost of longitudinal studies aimed at developing treatments for SCI.
Acknowledgements

I would like to thank the following people for getting me through this in one piece:

Dr. Greg Stanisz, my supervisor, who has always known when to harass me for not working hard enough, and when to send me home for a break. Or give me a new project. Or just stop by for chocolate.

Dr. Bojana Stefanovic & Dr. Sandra Black, for being such encouraging and supportive committee members.

The MRIgFUS spinal cord team who helped me to get this project off the ground, especially Meaghan O’Reilly who did all the focused ultrasound, Isabelle Aubert & Cari Whyne for their encouragement and editing skills, Margarete Akens for her animal expertise, and finally Jacek Kwiecien for stepping in and teaching me everything I know about histology.

Lab members and other Sunnybrook folk, past and present, who have made this such a fun place to work, especially Eve Lake, Rafal Janik, Nicole Fichtner, Sharon Portnoy, Kim Desmond, Colleen Bailey, Adrienne Dorr, Sofia Chavez, Lucy Kershaw, Firas Moosvi, Anna Mlynarczyk, Sadie Yancey, Norm Robert, James Odegaard, Lynsie Thomason & Margaret Koletar.

But mostly I need to thank my partner, Sky Roy, who has encouraged, supported, and put up with me every step of the way, and my daughter Elli who is always ready with hugs when I need them.
Table of Contents

Acknowledgements .............................................................................................................. iii

Table of Contents .............................................................................................................. iv

List of Abbreviations ........................................................................................................ viii

List of Tables ..................................................................................................................... x

List of Figures ................................................................................................................... xi

Chapter 1 – Introduction ................................................................................................. 1

1.1 SCI in Humans ............................................................................................................. 1

1.2 Models of SCI .............................................................................................................. 4

1.2.1 Animal Models of SCI: ........................................................................................ 4

1.2.2 Invasive models: .................................................................................................... 5

1.2.3 Closed spinal column injuries .............................................................................. 8

1.2.4 Disadvantages of Current Models ....................................................................... 8

1.3 Pathophysiology of Spinal Cord Injury ..................................................................... 9

1.4 Application of MRI to SCI ....................................................................................... 11

1.4.1 Quantitative MRI Methods .................................................................................. 14

1.5 Objective of Current Work ......................................................................................... 17

1.5.1 Use of Focused Ultrasound to Create a Model of Spinal Cord Injury ................ 18

1.5.2 Quantitative Imaging of SCI ................................................................................. 19

1.6 List of Contributions ................................................................................................. 22

Chapter 2 - A Non-Surgical Model of Cervical Spinal Cord Injury Induced with Focused Ultrasound and Microbubbles .................................................................................. 23

2.1 Introduction ............................................................................................................... 23

2.2 Methods ...................................................................................................................... 25

2.2.1 Ethical Considerations ......................................................................................... 25
2.2.2 Experimental Overview .......................................................... 25
2.2.3 Induction of Focused Ultrasound Injury .................................... 27
2.2.4 Behavioural Assessment .......................................................... 28
2.2.5 MRI .......................................................... 29
2.2.6 Histology .............................................................................. 29
2.2.7 Data analysis ........................................................................ 30
2.3 Results ...................................................................................... 31
2.3.1 Behavioural Assessment Following FUS .................................... 31
2.3.2 Histology .............................................................................. 32
2.3.3 MRI .................................................................................... 35
2.4 Discussion ................................................................................ 38
2.4.1 Reproducibility and injury tolerance ........................................ 38
2.4.2 Histology Assessment ............................................................... 39
2.4.3 MRI Assessment ................................................................... 39
2.4.4 Applicability ......................................................................... 40
2.5 Conclusions .............................................................................. 41

Chapter 3 - Effects of diffusion on high resolution quantitative $T_2$ MRI 42
3.1 Introduction .............................................................................. 42
3.2 Calculating the Effects of Diffusion on $T_2$ .................................... 44
3.2.1 Effect of diffusion on measured $T_2$ ....................................... 45
3.2.2 The Effects of Readout Gradients: ........................................ 46
3.3.3 Spoiler Gradients: ................................................................. 47
3.3.4 Phase Encoding Gradients ..................................................... 48
3.4 Simulations: ............................................................................. 49
3.4.1 Effect of Resolution ............................................................... 49
4.3.4 Correlations with Histology ................................................................. 77
4.4 Discussion ................................................................................................. 80
4.5 Conclusions ............................................................................................... 83
Chapter 5 - Conclusions and Future Directions .............................................. 84
5.1 Summary ..................................................................................................... 84
5.2 Future improvements to the FUS SCI model ............................................ 85
5.3 Further improvements to the qT₂ sequence ............................................. 85
5.4 Conclusions ............................................................................................... 86
References ........................................................................................................ 87
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI</td>
<td>spinal cord injury</td>
</tr>
<tr>
<td>ASIA</td>
<td>American Spinal Injury Association</td>
</tr>
<tr>
<td>NYU</td>
<td>New York University</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>LFB</td>
<td>luxol fast blue</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>GFAP</td>
<td>glial fibrillary acidic protein</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>haematoxalin &amp; eosin</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>γ</td>
<td>Larmor frequency</td>
</tr>
<tr>
<td>B₀</td>
<td>magnetic field strength</td>
</tr>
<tr>
<td>RF</td>
<td>radio frequency</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>PD</td>
<td>proton density</td>
</tr>
<tr>
<td>MT</td>
<td>magnetization transfer</td>
</tr>
<tr>
<td>Gd</td>
<td>gadolinium</td>
</tr>
<tr>
<td>DWI</td>
<td>diffusion weighted imaging</td>
</tr>
<tr>
<td>DTI</td>
<td>diffusion tensor imaging</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional MRI</td>
</tr>
<tr>
<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</td>
</tr>
<tr>
<td>λ₁</td>
<td>axial diffusivity</td>
</tr>
<tr>
<td>λ⊥</td>
<td>radial diffusivity</td>
</tr>
<tr>
<td>SNR</td>
<td>signal to noise ratio</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>MTR</td>
<td>magnetization transfer ratio</td>
</tr>
<tr>
<td>qT₂</td>
<td>quantitative T₂</td>
</tr>
<tr>
<td>MWF</td>
<td>myelin water fraction</td>
</tr>
<tr>
<td>FUS</td>
<td>focused ultrasound</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>I/E</td>
<td>intra/extracellular</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>D-V</td>
<td>dorsal-to-ventral</td>
</tr>
<tr>
<td>L-R</td>
<td>left-to-right</td>
</tr>
<tr>
<td>R</td>
<td>Pearson’s product moment correlation-coefficient</td>
</tr>
<tr>
<td>BSCB</td>
<td>blood-spinal cord barrier</td>
</tr>
<tr>
<td>MSME</td>
<td>multi-slice multi-echo</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
</tr>
</tbody>
</table>
$\Delta x$ linear voxel size

$\Delta z$ slice thickness

$D$ apparent diffusion coefficient

$G$ gradient strength

$\delta$ gradient duration

$\Delta t$ time between gradients

$b_{qT2}$ per echo b-value

$T2_{app}$ apparent T2 value

$G_x$ readout gradient

$\text{BW}$ bandwidth

$\text{FOV}$ field of view

$T_s$ total sampling time

$\delta_{\text{ref}}$ gradient refocusing time

$\text{NNLS}$ non-negative least squares

$\text{MnCl}_2$ manganese chloride

$\text{WM}$ white matter

$\text{DC}$ dorsal column

$\text{LC}$ lateral column

$\text{VC}$ ventral column

$\text{GM}$ gray matter

$\text{DH}$ dorsal horn

$\text{VH}$ ventral horn

$\text{VS}\ T_2$ very short $T_2$

$\text{VL}\ T2$ very long $T_2$

$\text{MW}$ myelin water

$H$ hemorrhage

$V$ vacuolation

$E$ edema

$V/E$ vacuolation and/or edema

$M$ macrophages

$\text{A-GM}$ acute gray matter

$\text{A-WM}$ acute white matter

$\text{C-GM}$ chronic gray matter
List of Tables

Table 2-1 Summary of experimental design and injury severity .................................................. 27
Table 4-1 Range of $T_1$ values for the different components in the $T_1$ distribution for both white and gray matter. .................................................................................................................. 69
Table 4-2 Summary of MRI and histopathology timepoints, injury severity, and number of injured vs.
normal appearing ROIs for both acute and chronic groups. ........................................................................... 70
Table 4-3 Correlation ($\rho$) between MRI parameters and Histology in acute GM/WM (24 hours).
Statistically significant correlations are shown in bold ............................................................................ 77
Table 4-4 Correlation ($\rho$) between MRI parameters and Histology in chronic GM/WM (2 weeks).
Statistically significant correlations are shown in bold ............................................................................ 77
Table 4-5 Mean and standard deviations for statistically significant correlations. ................................. 78
List of Figures

Figure 1-1. Cross-section of the human spinal cord and vertebrae. ........................................ 2
Figure 1-2. Histology sections from normal and injured spinal cord ........................................ 10
Figure 1-3. Excitation and refocusing pulses used in the qT2 sequence .................................. 20
Figure 1-4. Sample T2 spectrum from normal white matter ............................................... 21
Figure 2-1. MRI of rat spinal cord used for treatment planning ........................................... 26
Figure 2-2. Photograph of rat, dorsally recumbent on the treatment sled ............................... 26
Figure 2-3. Sagittal Gd enhanced T1-weighted MRI to assess extent of primary injury .......... 28
Figure 2-4. MR visible signal changes due to FUS treatment ............................................. 31
Figure 2-5. MRI and histology of acute FUS injury ......................................................... 33
Figure 2-6. MRI and histology of chronic FUS injury ....................................................... 34
Figure 2-7. Areas of signal abnormality in spinal cord for acute and chronic groups ............ 37
Figure 3-1. RF, spoiler and readout gradients used in a qT2 sequence ................................ 45
Figure 3-2. Relationship between Apparent T2 and Linear Voxel Size ................................ 50
Figure 3-3. Effect of SNR on MWF .................................................................................... 51
Figure 3-4. Effect of decreasing spoilers on T2 distribution ............................................... 52
Figure 3-5. Actual T2 (a) and resolution (b) vs measured T2 in MnCl2 phantoms ............... 56
Figure 3-6. Diffusion parallel and perpendicular to the rat spinal cord ................................. 57
Figure 3-7. Effect of diffusion on SNR ................................................................................ 60
Figure 4-1. Spinal cord ROIs ................................................................................................. 66
Figure 4-2. Example T2 distributions for normal and injured white matter ....................... 68
Figure 4-3. Acute qT2 and histology ................................................................................... 72
Figure 4-4. Chronic qT2 & Histology .................................................................................. 74
Figure 4-5. T2 values for I/E water peaks in normal control WM and GM ROIs ................. 75
Figure 4-6. Histogram summary of T2 distributions .............................................................. 76
Figure 4-7. Luxol fast blue histology and perpendicular diffusivity maps ............................ 79
Chapter 1 – Introduction

This thesis describes the application of quantitative T₂ (qT₂) magnetic resonance imaging (MRI) to spinal cord injury (SCI) in a rat model. It covers the development of a non-surgical model of SCI induced using a combination of focused ultrasound (FUS) and microbubbles which is optimized for MRI. It also examines the difficulties involved with implementing the qT₂ sequence at the high resolution required to image SCI in a rat, the impact of diffusion on qT₂ measurements, and the modifications required to minimize these effects. Finally the correlations between quantitative MR parameters and histopathology in this model of SCI are examined.

1.1 SCI in Humans

Acute traumatic SCI affects approximately 14,000 people in North America annually (Hadley, 2000), including 2,200 in Canada (“Spinal Cord Injury Canada Facts,” 2014). The most common cause is motor vehicle accidents which account for approximately 50% of all traumatic SCIs. Other causes include falls, sports, and violence (Ho et al., 2007). SCI results in deficits in ambulatory, sensory, and autonomic function which depend on the neurological level and extent of injury (Fehlings & Baptiste, 2005).

The spinal cord plays an important role in sensory processing, motor control, and autonomic function. In addition to relaying information to and from the brain, the spinal cord is also involved in the initial processing of much of this information. The function of the spinal cord is strongly dependent on its connection to the brain and ceases to respond normally if this connection is damaged or severed (Nógrádi & Vrbová, 2000).

The normal anatomy of the spine is usually described by dividing the spine into cervical, thoracic, lumbar and sacral sections, with 7 cervical, 12 thoracic, and 5 lumbar vertebrae (Fig. 1-1). The sacrum is located below the lumbar spine and is part of the pelvis. The spinal cord, which resides within the spine, is segmented in terms of the nerve roots which exit from the cord between each vertebrae, resulting in 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal segments. Each spinal nerve innervates a single dermatome (Nolte, 2002).
Figure 1-1. Cross-section of the human spinal cord and vertebrae.

Damage to the spinal cord affects the nerves below the level at which the damage occurs. The level of SCI is defined relative to the segment closest to the brain at which damage has occurred, with 55% of all SCI occurring at the cervical level (C1-C8), 15% at the thoracic level (T1-T11), 15% at the thoracolumbar level (T11-L2), and 15% at the lumbosacral level (L2-S5) (Sekhon & Fehlings, 2001). Injuries are described as being 'incomplete' when some degree of sensation or motor function remains below the level of the injury, and 'complete' when both feeling and motor control are lost.

The American Spinal Injury Association (ASIA) motor score, is considered the reference standard for neurologic examination of individuals with SCI (Hadley et al., 2013). Ten key muscles have been selected, five in the upper limb and five in the lower limb, each representing a single myotome from C5 through T1 and L2 through S1, to allow the precise level of motor impairment to be identified. The muscles were chosen to be testable with the patient supine and with minimal movement of the spinal column. Likewise,
key sensory points, representing each sensory dermatome from C2 through S4-5, allow the sensitivity of each to the sensation of light touch and pin prick to be assessed (Ho et al., 2007). The ASIA score is then defined for both motor and sensory function as the highest level at which impairment is detected, for each side of the body, as well as the degree of completeness, with A being a complete injury, and B-D being decreasing degrees of incomplete injuries, and E describing neurologically normal subjects (Hadley, 2002).

The higher the level of injury, the greater the resulting disability, and the more expensive the treatment. The mean length of stay for the SCI patients in acute care is 18 days, followed by 45 days of rehabilitation (Priebe et al., 2007). Damage to the cervical spine can result in tetraplegia, paralysis of both the arm and legs. In the case of high tetraplegia, the patient may also require the assistance of a ventilator for breathing. Regulation of heart rate, blood pressure, and body temperature can also be impaired. The trunk and abdomen are controlled by nerves leaving the spinal cord at the thoracic level, while legs and feet are controlled at the lumbar level. Finally, bladder and bowel control as well as sexual function occur at the sacral level. As the level of injury decreases, so does the cost of treatment, however incomplete motor function at any level still costs well over $100,000 to treat during the first year. In addition to the cost of the initial treatment, there are also lifelong expenses to modify the home and vehicle to accommodate the patient, and recurring costs for medical equipment, supplies and personal equipment which range from ~$15,000/year for someone with incomplete motor function to more than $100,000 for someone with high tetraplegia (Priebe et al., 2007).

Reduced mobility and the need for assistance in performing basic functions of everyday life are not the only concerns. Secondary complications following SCI include pressure ulcers, pneumonia and other respiratory complications, and genitourinary problems (Chiodo et al., 2007). Increasing the mobility and independence of a patient with SCI would have a dramatic impact both in terms of monetary cost and improvement in quality of life. This may be accomplished either by preventing damage, post-injury therapy or through the use of regenerative treatments.

Standard care following acute SCI includes stabilization in order to prevent additional trauma, maintenance of mean arterial blood pressure at 85-90 mm Hg to improve spinal cord perfusion, and either closed reduction or surgical decompression to reduce pressure on the spinal cord (Hadley & Walters, 2013). There are currently no pharmacological therapies recommended for acute SCI (Hurlbert et al., 2013). Methylprednisolone, the standard of care for many years (Bracken et al., 1990, 1997; Fehlings, 2001;
Wuernser et al., 2007), is no longer recommended (Hadley & Walters, 2013; Hurlbert & Hamilton, 2008; Hurlbert et al., 2013).

There is a pressing need for the development of treatments for SCI, either to prevent additional damage resulting from the secondary injury mechanisms like inflammation and vascular dysfunction, or to regenerate axons and restore function. Before any potential therapies can be brought to clinical trials, they must be validated in an animal model of SCI. The list of existing animal models is quite extensive and includes many different species and methods of injury (Aldskogius, 2013).

1.2 Models of SCI

There are many primary mechanisms of acute human SCI including compression, impact, distraction, and laceration. The two main goals of an SCI model are to understand biological response of tissue to injury and the pathophysiology involved and to test potential therapies. To be useful, a model has to reproduce some aspects of the human injury, as well as offer advantages over direct clinical observation (Blight, 2000). Models of SCI can either mimic the primary mechanisms of human injury, or permit examination of particular aspects of SCI pathology. In addition to the type of injury, the choice of animal also affects mechanical aspects of damage and recovery, and responses on a cellular level.

1.2.1 Animal Models of SCI:

A variety of different animals have been used over the years to model SCI, with a weight-drop injury described in a dog model by Allen, as early as 1911. A summary of spinal cord contusion models by Young (2002) describes SCI induced in rabbits (Ferraro, 1927) and then in primates by several different groups beginning in the late 1960s (Albin, White, Acosta-Rua, & Yashon, 1968; Black, Shepard, Jr, & Markowitz, 1979). In the 1970s the most commonly used animals were dogs (Koozekanani, Vise, Hashemi, & McGhee, 1976) and cats (Senter & Venes, 1979), and by the 1980s the feline model had come to dominate the field. Pigs (Lee et al., 2013) and sheep (Yeo, Payne, Hinwood, & Kidman, 1975) have also been used as models.

In the mid 80s a weight-drop device similar to that used in the feline model was developed for use with rats (Noble & Wrathall, 1985), and much of the work shifted to rodents largely because of cost and ethical considerations (Young, 2002). Mice are also interesting because of their transgenic potential which allows the impact of particular genes or pathways to be investigated (Kwon, Oxland, & Tetzlaff, 2002). However, mice exhibit different inflammatory responses and the secondary injury develops very differently without the cavity formation seen in other species (Inman, Guth, & Steward, 2002). Rats are readily available, and the morphological, biochemical, and functional changes that occur after SCI are similar to those seen in
humans (Fleming et al. 2006; McTigue et al. 2000; Metz et al. 2000; Norenberg et al. 2004). Therefore nowadays rats are the most commonly used. A variety of different methods for injury induction are described below involving mechanical, chemical, and radiation induced damage to the spinal cord.

1.2.2 Invasive models:

Invasive models of SCI involve surgical exposure of the spinal cord via laminectomy at the site where the injury is to be created. Once the spinal cord has been exposed, there are several different ways in which the injury can be induced. The most common are contusion or compression, and transection injuries which are described below, along with chemically mediated and distraction/dislocation injuries.

While a laminectomy is typically required to produce these transection injuries, techniques have been developed for both transection (Guertin, 2008), and compression models (Sheng et al., 2004) which do not require a laminectomy, although these do not appear to be widely used.

1.2.2.1 Contusion Injuries

Contusion and compression injury models are thought to most accurately mimic the consequences of the most common types of human injury (Kwon et al., 2002). The first well-described contusion model involves dropping a weight from a known height down a vented guide tube positioned perpendicular to the exposed spinal cord, or onto an impounder plate resting on the cord (Allen, 1911). Variations in impounder plates, and resistance encountered within the guide tube made this injury mode difficult to reproduce reliably (Onifer, Rabchevsky, & Scheff, 2007).

The New York University weight-drop device (NYU impactor model) was designed to help standardize contusion injury models (Gruner, 1992). This model involves dropping a 10g rod onto the exposed dorsal surface of the spinal cord from a range of different heights. The spine is clamped above and below the lesion to reduce motion of the spinal column during impact. The impact velocity of the rod, the distance of cord compression, the cord compression rate, and the dynamic force applied to the cord are all measured by the device (Kwon et al., 2002).

Ohio State University developed a computer feedback-controlled electromechanical impactor (Bresnahan, Beattie, Todd, & Noyes, 1987; Noyes, 1987). To eliminate a source of variation, the impactor is actively withdrawn following impact to prevent bouncing back onto the cord. Both the force transmitted to the spinal cord and the cord displacement are measured, identifying situations where the impactor hits adjacent tissue.
Finally, the commercially available Infinite Horizon impactor (Scheff, Rabchevsky, Fugaccia, Main, & Lumpp, 2003) senses the surface of the exposed spinal cord and continues to displace spinal tissue until it attains a predetermined force, at which point the impactor tip immediately withdraws.

While the NYU Impactor is the most commonly used device, all three are able to create reproducible injuries with a range of injury severity (Kwon et al., 2002; Scheff et al., 2003).

1.2.2.2 Compression (Aneurysm clip or calibrated forceps)

Rather than the single, rapid blow to the spinal cord delivered by an impactor device, clip compression simulates the combination of impact and persistent compression typical of burst fractures and fracture-dislocations (Fehlings & Tator, 1995). Following a laminectomy to expose the spinal cord, different severities of injury can be created by adjusting the closing force of the aneurism clip and the duration of compression (Kwon et al., 2002).

As an alternative to the use of aneurysm clips which compress the spinal cord with a known force, calibrated forceps can be used to compress the cord to a predetermined thickness (Blight, 1991). While the use of a consistent force produces different amounts of compression depending on the mechanical properties of the tissue, compression to a consistent thickness results in different amounts of damage depending on the size of the cord (Blight, 1991).

1.2.2.3 Laceration/Transection

While transection or laceration injuries are less commonly seen in humans, they are ideal models for studying regeneration since in a complete transection there are no surviving axons immediately following injury, and so it becomes easier to confirm axonal regeneration (Kwon et al., 2002). Complete transections are quite reproducible, but result only in a single degree of injury.

Partial transection, where tracts of the spinal cord are cut selectively, is more difficult to reproduce, and the results are easier to misinterpret as there are surviving axons following injury (Ramer, Harper, & Bradbury, 2000). The lesions produced can be comparatively mild, making postoperative care easier and also allowing for comparison with the uninjured contralateral side of the cord (Kwon et al., 2002).

1.2.2.4 Distraction & Dislocation

In addition to compression, contusion, and laceration, trauma can also occur when the spinal cord is stretched (Breig, 1970) or sheared at the dislocation between two vertebrae (Sekhon & Fehlings, 2001). To mimic distraction and dislocation injuries vertebral facets at the C4-5 level are surgically removed, and
clamps are secured to pairs of vertebrae above (C3 & C4) and below (C5 & C6) the level of intended injury. For the fracture dislocation model, dorsal ligaments are also removed, and the lower pair of clamped vertebrae are then dislocated dorsally by 2-4 mm and returned to their original positions. For a distraction injury, the upper pair of vertebrae are clamped at a 15° angle, and then the lower pair are distracted caudally by ~4 mm. Vertebral stabilization and pain management are required following this injury (Choo et al., 2007).

1.2.2.5 Chemically induced SCI

Rather than applying a mechanical force to the spinal cord, chemicals can be used to model specific aspects of the secondary injury cascade such as ischemia, neuronal death, oligodendrocyte death, inflammation, and demyelination. These chemicals can be applied topically to the exposed spinal cord, using micro-injections directly into the spinal cord, or injected intravenously. Irradiation with either X-rays or visible light (with the addition of photo-sensitive dyes), can be used to control the amount of damage (Onifer et al., 2007).

Specific examples of chemically mediated injury include:

- IV injection or topical application of photosensitive rose bengal (Watson, Prado, Dietrich, Ginsberg, & Green, 1986) followed by irradiation of exposed vertebrae to produce vascular thrombosis. The duration of irradiation can be used to produce different grades of damage.
- Microinjection of the vasoconstrictor, endothelin, naturally occurring in spinal cord following trauma, that induces ischemia (Salzman et al., 1996).
- Microinjections of Zymosan, a yeast particulate which activates resident microglia and macrophages, causes inflammation and leads to locomotor dysfunction and pathology similar to that seen following traumatic SCI (Popovich et al., 2002).
- Intra-spinal microinjection of lysolecithin which causes demyelination (Hall, 1972) followed by remyelination (Jeffery & Blakemore, 1995).
- Topical application of kainate or NMDA to the dorsal spinal cord causes excitotoxicity in neurons (Ikonomidou, Qin Qin, Labruyere, & Olney, 1996).

While none of these models resembles SCI in humans, they permit some of the secondary processes involved in damage to the spinal cord to be examined independently of one another.
1.2.3 Closed spinal column injuries

1.2.3.1 Subdural Inflatable Balloon
This model uses a subdural balloon catheter to produce a compression injury. A laminectomy is performed at the thoracic level, the dura opened and a balloon, designed for endovascular occlusion of intracranial vascular malformations, introduced into the sub-arachnoid space and moved rostrally up to one or two vertebral levels above the laminectomy. The balloon is then rapidly inflated with varying volumes of saline (10 to 100 µl) and left in place for durations from 1 to 10 min. (Martin et al., 1992). The balloon can be inflated with contrast medium, allowing exact identification of its intradural position using either computed tomography (CT) or MRI.

While a laminectomy is required to create this injury, the dura and spinal canal remain closed at the lesion site itself. This minimizes the fibroadhesive scar which may penetrate the spinal tissue, interfere with post-traumatic regeneration of axons, and hamper further experimental procedures such as delayed grafting.

1.2.3.2 Radiation induced SCI
Radiation is an obvious way to damage the spinal cord non-surgically. Models of radiation-induced SCI are used extensively to study the side-effects of radiation therapy for the treatment of cancer (Mastaglia, McDonald, Watson, & Yogendran, 1976; Medin et al., 2011; Powers, Beck, Gillette, Gould, & LeCouter, 1992). While radiation does injure the cord, it takes months to years for clinical signs of an injury to appear, and the condition is progressive and fatal (Delattre et al., 1988; Mastaglia et al., 1976; Medin et al., 2011). The length of time required for the injury to develop, and the unique mechanisms involved make this a poor model of traumatic SCI.

1.2.4 Disadvantages of Current Models
With advances in technology, the reproducibility of contusion and compression models has improved dramatically. Most of these models however have been developed in the thoracic spine, while over 50% of human injuries occur in the cervical spine (Sekhon & Fehlings, 2001). Moving a surgical model of SCI from thoracic to cervical spine requires significant effort and is sometimes quite difficult as the cervical spine is larger with less space between the spine and spinal cord. This makes clip compression more cumbersome, and alters the tissue parameters involved in contusion model assessment (Nicole Forgione, personal communication). In addition, at higher levels SCI becomes more severe as it involves more bodily functions. Respiratory deficit results in a high mortality rate (Baussart, Stamegna, Polentes, Tadié, & Gauthier, 2006), loss of bladder control requires manual bladder expression (Scheff et al., 2003), and
animals suffering from forelimb paralysis will require hand feeding and subcutaneous fluids (Pearse et al., 2005). Unilateral rather than bilateral injury models can be advantageous as function can be maintained by the undamaged half of the spinal cord, which is both easier on the animal, and on the personnel involved in post-surgical care.

Most human SCI are 'closed' injuries, without an open wound of the cord or dura matter (Ramer et al., 2000), while most SCI models involve a laminectomy which is the removal of the dorsal portion of the vertebral bone called the lamina. Laminectomies can cause deformation of the spinal column, impair biomechanics, and increase the risks of infection, vascularity and degenerative processes (Guertin, 2008; Seitz, Aglow, & Heber-katz, 2002; Sheng et al., 2004). This can affect locomotion and functional recovery which is a problem for assessing treatment (Guertin, 2008), and spinal cord deformation prevents linear alignment of the cord, making MRI studies more difficult to perform.

Other discrepancies also exist between human injury and animal models. In addition to being under anesthesia during injury induction, experimental animals are not subjected to the multiple other drugs as traumatized patients typically are (Kwon et al., 2002).

No single model manages to simulate all of the complex biomechanical stresses of distraction, compression, bending, and shear to which the human spinal cord is subjected during trauma (Kwon et al., 2002). Whatever injury model is used, it is essential that the injury be reproducible. The injury location, extent, and degree must ideally be precisely defined to permit testing of potential therapies. Finally, the ability to create unilateral injury will reduce the mortality as well as the post-surgical care.

1.3 Pathophysiology of SCI

It is widely accepted that SCI is bi-phasic, where the primary mechanical injury, caused by forces including compression, contusion, shear, distraction and dislocation, is followed by a secondary injury mechanism (Bartholdi & Schwab, 1996; Ramer et al., 2000; Rowland, Hawryluk, Kwon, & Fehlings, 2008; Sekhon & Fehlings, 2001; Tator & Fehlings, 1991; Tator, 1995). These secondary mechanisms cause damage over a period of hours to weeks following the initial injury. They include vascular dysfunction, edema, ischemia, inflammation, excitotoxicity, electrolyte shifts, free radical production, and apoptosis (Rowland et al., 2008).

The immediate effects of the injury include the traumatic severing of axons, necrotic and apoptotic cell death. This is rapidly followed by edema of the spinal cord and hemorrhage, primarily in the central gray
matter, accompanied by necrotic cell death. Petechial hemorrhage in the surrounding white matter is also common, and the combination of swelling and hemorrhage can result in ischemia. Microglial cells are activated, and proinflammatory cytokines tumor necrosis factor alpha (TNFα) and interleukin 1 beta (IL-1β) are released (Rowland et al., 2008). There is also swelling of individual axons and traumatic demyelination (Kakulas, 2004). Typically a portion of white matter is preserved, mainly at the periphery of the spinal cord (Kakulas, 1999). Both edema and hemorrhage can be observed using hematoxilin and eosin (H&E) staining (Ross, Gordon, & Pawlina, 2003). Luxol fast blue (LFB) can be used to visualize myelin, and immunohistochemistry is used to detect various inflammatory cytokines (Ross et al., 2003). Figure 1-2 shows some sample histology sections from normal and injured spinal cord (Norenberg, Smith, & Marcillo, 2004).

Figure 1-2. Histology sections from normal and injured human spinal cords.
(A) Cross section of normal spinal cord stained with luxol fast blue and periodic-acid Schiff (LFB-PAS).
(B) Solid cord injury showing loss of the normal architecture and myelin loss (LFB-PAS). (C) Contusion/cavity in which the cord has been largely replaced by a cyst (H&E).
(D) Laceration injury in which the pial surface has been breached (H&E).
(E) Massive compression showing fragmentation, distortion and hemorrhage (H&E).
Bar =2 mm. Figure from (Norenberg et al., 2004)


By the 2 week point, edema is resolved and the blood-spinal cord barrier (BSCB) is re-established. The central area of hemorrhagic necrosis is replaced by a cyst which is often multilocular. Macrophages infiltrate the spinal cord and remove debris. Astrocytes at the periphery of the lesion proliferate and form a gliotic
scar around the lesion which is correlated with an increase in glial fibrillary acidic protein (GFAP). This scar is a barrier to axonal regeneration and prevents macrophages from causing additional damage to healthy neural tissue (Kakulas, 2004; Rowland et al., 2008). Cysts are easily observed on H&E or Nissl stained sections, although the size of the cystic cavity is often overestimated as cell debris is lost during tissue preparation (Weber et al., 2006). Immunohistochemistry can be used to identify astrocytes by staining for GFAP (Farooque, Badonic, Olsson, & Holtz, 1995), and macrophages by staining for CD68 (van den Berg, Döpp, & Dijkstra, 2001).

The glial scar continues to mature and stabilize over the months following injury. There is often some axonal sprouting from 3 weeks to 8 months following injury which does not produce significant functional recovery. Eventually the lesion becomes more or less static, although Wallerian degeneration of axons continues, and syringomyelia can develop over a period of years following injury (Kakulas, 1999, 2004; Rowland et al., 2008).

As it is impossible to assess SCI in vivo using histology, other imaging techniques are required.

### 1.4 Application of MRI to SCI

The Report of the National Institute on Disability and Rehabilitation Research Spinal Cord Injury Measures Meeting, held in 2006, recommended that MRI be used for evaluation of the spinal cord after injury (Lammertse et al., 2007). CT and plain radiography should be used to assess the bony anatomy of the spine in patients with SCI, while MRI can be used to identify the location of the SCI, demonstrate the degree of spinal cord compression, and identify regions of hemorrhage/contusion, edema, and spinal cord disruption (Lammertse et al., 2007). The expert consensus in the report was that T₁-weighted, T₂-weighted, and T₂*-weighted anatomical magnetic resonance (MR) images be acquired.

The source of the MRI signal in the human body is protons, primarily those in the nucleus of the hydrogen atom. In the presence of a large magnetic field, the protons spins precess about this field, resulting in a net magnetization. The frequency at which these spins precess is called Larmor frequency, and is a function of both gyromagnetic ratio (γ) of the spin and the strength of the magnetic field (B₀). This magnetization is difficult to detect as it is much smaller than B₀, so radio frequency (RF) radiation at the Larmor frequency is used to rotate the polarization of the individual protons and tip the net magnetic moment into the transverse plane. This rotating magnetic moment will induce an oscillating current in nearby coils of wire which can be detected and used to generate images.
Each proton generates its own very small magnetic field, resulting in an overall magnetization which varies in space depending on the physical distribution of protons, and the rotation and translational motion of nearby protons. The rate at which the proton spins fall out of phase with one another, causing the signal to decay is mainly dependent on the correlation time between neighbouring protons, and is characterized by $T_2$. $T_1$ characterizes the rate at which the magnetization returns to equilibrium along the main magnetic field and becomes available to be re-excited. $T_1$ relaxation depends on rotational and translation motion occurring at the Larmor frequency.

$T_1$ and $T_2$ relaxation times are inherent tissue properties which result from interactions between hydrogen protons, and are affected by the molecules to which these hydrogen protons belong. $T_2$ is particularly sensitive to alterations in tissue structure due to damage or disease, as these often affect the structure of the underlying tissue. For example, the signal intensity on a $T_2$-weighted image increases as the free water content of the tissue increases provided that the proton density (PD) of the tissue is not simultaneously decreasing.

Tissue-dependent differences in $T_1$ and $T_2$ can be used to generate contrast in MR images by adjusting sequence timing parameters. An MR image typically requires many repetitions of a pulse sequence involving an RF excitation, echo time (TE) delay, and repetition time (TR) delay which determine the relative contributions of PD, $T_1$, and $T_2$ to the signal intensity in the resulting image. In addition there are many other contrast mechanisms including magnetic field inhomogeneities which generate $T_2^*$ contrast, gradient magnetic fields which can be used to generate diffusion-weighted images, and off-resonance RF which can be used to generate magnetization transfer (MT) contrast. Exogenous contrast agents such as gadolinium (Gd) can also be injected, increasing the $T_1$ contrast.

As a result of its sensitivity to small alterations in the tissue water environment, MRI is very sensitive to the pathological changes resulting from injury. Morphological changes are quite obvious, with fractures, spinal cord compression and other deformations easily observed (Kulkarni, Bondurant, Rose, & Narayana, 1988), but altered signal intensity within the gray and white matter of the spinal cord can be more difficult to interpret. Initial studies looking at correlations between patterns of signal changes on MRI (Kulkarni et al., 1988), were followed by animal studies which attempted to replicate the MRI signal changes and correlate them with histopathology (Weirich et al., 1990). During the acute stages of SCI, hemorrhage appears hypointense on $T_2$-weighted imaging as the iron present in deoxyhemoglobin and in methemoglobin in intact erythrocytes shortens $T_2$ relaxation times, as does hemosiderin. Hyperintense signal on $T_2$-weighted
imaging was found to be correlated with edema formation, and degeneration of myelin in white matter, likely due to the increased water content of edematous tissue (Weirich et al., 1990). A common finding is a combination of both hemorrhage and edema, which presents as a hypointense centre with hyperintense periphery (Bondurant, Cotler, Kulkarni, McArdle, & Harris, 1990; Kulkarni et al., 1988). The presence of hemorrhage is correlated with poor clinical outcomes (Bondurant et al., 1990; Kulkarni et al., 1988). Further studies have shown that these patterns change over time, and should be interpreted with regards to the length of time since injury as the underlying pathology evolves (Ohta, Fujimura, Nakamura, Watanabe, & Yato, 1999).

As MRI hardware continues to improve, more subtle changes to the spinal cord tissue have been noticed. A commonly-described feature of SCI is the loss of gray/white matter contrast. Unlike in the brain, where the main source of gray/white matter contrast is myelin (Koenig, 1991; Whittall et al., 1997), in the spinal cord there is also a difference in spin density between gray and white matter (Narayana, Fenyes, & Zacharopoulos, 1999). It has been suggested that this loss of contrast is due to accumulation of edema in the white matter (Narayana, Abbe, Liu, & Johnston, 1999). With increasing resolution it can be appreciated that the hyperintense regions are initially scattered throughout the cord, and hyperintense signal and cord swelling are highly correlated (Mihai et al., 2008). There are many studies which examine the longitudinal changes in signal patterns, as well as the size of these abnormalities in animal models and humans (Mihai et al., 2008; Narayana, Grill, Chacko, & Vang, 2004; Sundberg, Herrera, & Narayana, 2010). In these longitudinal studies, an initial loss of gray/white matter contrast on both T₁ and T₂ was observed along with swelling of the spinal cord and hyperintensity on T₂-weighted imaging at 24 hours. By 1 week the swelling had decreased, gray/white matter contrast had returned, and T₂-weighted images of the lesion showed a hypointense core with a hyperintense rim. The swelling continued to decrease and by 2 weeks post-injury the hypointense region had grown and the hyperintense rim had shrunk, while the overall volume of the spinal cord had decreased. Similar patterns were observed in human studies (Boldin et al., 2006; Miyanji, Furlan, Aarabi, Arnold, & Fehlings, 2007).

These anatomical imaging methods have been designed to maximize the contrast between normal and pathological spinal cord to better visualize the extent of SCI. In spite of their sensitivity to pathological changes, they are not specific to a given type of pathology. For example, hyperintensity on a T₂-weighted image can indicate either edema or demyelination. Especially when there are co-occurring pathologies such as hemorrhage and edema which have opposite effects on the T₂ signal, there will likely be regions where
one pathology masks the presence of the other. Quantitative MRI techniques can be used to improve specificity and obtain additional information about the health of the spinal cord.

Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) are sensitive to the degree and direction of water diffusion in tissue. The amount of diffusion is decreased by acute ischemia, and increased in the presence of a cystic cavity, while the direction of diffusion is strongly affected by the presence of intact axons in normal white matter. Functional MRI (fMRI) is sensitive to $T_2^*$ changes resulting from different levels of blood oxygenation in active spinal cord regions and is an indirect measure of neuronal activity. Magnetic resonance spectroscopy (MRS) is used to measure metabolites including N-acetyl aspartate (NAA) which is considered to be a marker of axonal integrity, and lactate which is elevated in cerebrospinal fluid (CSF) following SCI (Anderson, Prockop, Means, & Hartley, 1976).

1.4.1 Quantitative MRI Methods

1.4.1.1 Diffusion Weighted & Diffusion Tensor Imaging

DWI uses magnetic field gradients to sensitize the MR signal to the diffusion of water along a particular direction. Diffusion tensor imaging combines a series of DW images which are all sensitive to diffusion along different directions, to determine both the magnitude and direction of diffusion within a particular voxel (Mori & Zhang, 2006). White matter is composed primarily of myelinated axons in which diffusion is fastest parallel to the axons. Axonal membranes provide restriction to the diffusion; therefore diffusion measured in the direction perpendicular to the axons is much slower. A variety of parameters can be calculated based on the diffusion tensor including the fractional anisotropy (FA) which ranges from 0 (isotropic diffusion) to 1 (unidirectional diffusion); axial diffusivity ($\lambda_{||}$) measures diffusion parallel; and radial diffusivity ($\lambda_{\perp}$) which measures diffusion perpendicular, to the white matter tracts. These measures are altered by the changes to tissue microstructure following SCI and are often used as measures of axonal integrity (Ellingson, Schmit, Ulmer, & Kurpad, 2008).

Longitudinal DTI studies in rats following SCI have shown that FA is decreased, indicating that the tissue had become disrupted and that the diffusion of water is no longer highly restricted by the structure of the white matter (Deo, Grill, Hasan, & Narayana, 2006; Sundberg et al., 2010). Several studies have also linked increasing values of $\lambda_{\perp}$ with loss of myelin following SCI (Kozlowski, Raj, et al., 2008; Song et al., 2002, 2005), suggesting that changes in $\lambda_{||}$ and $\lambda_{\perp}$ can be used to differentially detect myelin and axonal abnormalities following SCI (Kim et al., 2007), albeit these results remain controversial.
Human studies have demonstrated that DW images can be more sensitive to structural changes than T1-weighted images, especially at very early stages of SCI with hyperintensity on DWI indicating decreased diffusivity (Sagiuchi, Tachibana, Endo, & Hayakawa, 2002). However, T1-weighted imaging appears to be equally sensitive within the first 24 hours of injury (Pouw et al., 2012). Decreased signal on DWI also provides an early indication of cystic formation following SCI (Bammer & Fazekas, 2003). More recent work has demonstrated that DWI is able to differentiate cytotoxic from vasogenic oedema (J. S. Zhang & Huan, 2014), and that decreased FA correlates with neurological impairment (Chang, Jung, Yoo, & Hyun, 2010; Koskinen et al., 2014; Petersen et al., 2012).

1.4.1.2 fMRI

fMRI relies on repeatedly scanning the same regions of the brain or spinal cord while a subject alternately rests or is subjected to some sort of stimulus which often involves performing a task. Animal experiments generally involve electrical stimulation of a specific body part, while human subjects can perform a variety of cognitive, motor, and visual tasks. Statistical correlations are calculated to determine which regions of the brain or spinal cord are responding to the stimulus. fMRI of the spinal cord is particularly difficult due to its small size as well as respiratory and cardiac motion (Harel & Strittmatter, 2008).

fMRI in humans with SCI has been used in the brain to examine brain plasticity following SCI (Shoham, Halgren, Maynard, & Normann, 2001) and during therapy (Winchester et al., 2005). fMRI has also been used to map function within the injured spinal cord, demonstrating response to stimulus which is sometimes independent of the subject's perception (Stroman et al., 2004; Stroman, Tomanek, Krause, Frankenstein, & Malisz, 2002).

In animal models fMRI has been used in the brain, where it is very useful in assessing sensory function following SCI, which can be difficult to monitor in animals (Hofstetter, Schweinhardt, Klason, Olson, & Spenger, 2003), as well as investigating brain plasticity and reorganization (Hofstetter et al., 2003; Ramu, Bockhorst, Grill, Mogatadakala, & Narayana, 2007). fMRI of the spinal cord itself has been used to investigate the process of reorganization of sensory pathways in the lumbar spinal cord following a rostral injury at the thoracic level (Endo, Spenger, Westman, Tominaga, & Olson, 2008).

1.4.1.3 MR Spectroscopy

Very few studies of spinal cord MRS have been conducted. It is difficult to perform as the signal arising from protons associated with water molecules is several orders of magnitude larger than that from the protons associated with metabolites (Cooke, Blamire, Manners, Styles, & Rajagopalan, 2004). To increase
the signal to noise ratio (SNR), MRS is typically performed using a single-voxel approach, with a voxel size on the order of 2-3 mL. It is very sensitive to magnetic field inhomogeneities which occur at the interface between bone and tissue, and motion artefacts arising from both cardiac and respiratory motion, making the spinal cord a very difficult place to perform MRS (Cooke et al., 2004).

The advantage of proton MRS is its ability to detect the presence of cerebral metabolites including NAA which is a marker of axonal integrity, and lactate which is indicative of hypoxia (Marliani, Clementi, Albini-Riccioli, Agati, & Leonardi, 2007). Phosphorus spectroscopy can also be used to measure pH and phosphate metabolism including adenosine triphosphate (ATP) which provides information about the local rate of energy metabolism (Vink, Noble, Knoblach, Bendall, & Faden, 1989). Both increased lactate and altered energy metabolism have been observed in rabbit spinal cord following SCI (Vink et al., 1989).

MRS has been performed in the cervical spinal cord in patients with chronic whiplash injury, where decreased NAA relative to creatine was observed, suggestive of axonal damage (Elliott, Pedler, Cowin, Sterling, & McMahon, 2012). MRS has also been used in human brain following SCI to examine the effect of neuropathic pain in thalamus where NAA was shown to be negatively correlated with pain intensity (Pattany et al., 2002). In the motor cortex, increased NAA was observed following functional recovery from injury (Puri et al., 1998).

### 1.4.1.4 Magnetization Transfer

Magnetization transfer (MT) is sensitive to the presence of myelin (Stanisz, Kecojevic, Bronskill, & Henkelman, 1999), and changes in myelination (Odrobina, Lam, Pun, Midha, & Stanisz, 2005). It is not possible to image myelin directly using conventional MRI. The $T_2$ of the protons bound to macromolecules, particularly the lipids in the myelin sheath, is too short (~10µs). MT works by saturating the magnetization of these bound protons using an off-resonance RF pulse which does not directly affect the water protons. Then the exchange of magnetization between the macromolecular and water protons causes a decreased signal in the water protons, which is observed as a decreased signal on MRI relative to that acquired without the off-resonance RF pulse. The magnetization transfer ratio (MTR) is simply the relative difference in signal intensity with and without MT. MTR of the cervical spinal cord is able to distinguish between SCI patients and controls (Cohen-Adad, Buchbinder, & Oaklander, 2012).

### 1.4.1.5 Quantitative $T_2$

Quantitative $T_2$ ($qT_2$) is another technique which is sensitive to the presence of myelin. In white matter a multiexponential fit of the $T_2$ decay curve reveals the presence of multiple water components relaxing with
different $T_2$ relaxation times (MacKay et al., 1994, 2006; Stanisz et al., 1999; Whittall et al., 1997).

Because of its proximity to macromolecules, the water trapped in the myelin sheath has a significantly shorter $T_2$ than either intra- or extra-cellular water. The myelin water fraction (MWF), calculated from the $T_2$ distribution, is a surrogate measure of myelin and has been shown to be significantly correlated with the amount of myelin in various brain regions (Whittall et al., 1997) and can be used to identify demyelination in multiple sclerosis lesions (Moore et al., 2000) as well as a compartment with longer than normal $T_2$ in the white matter of patients with phenylketonuria (Sirrs et al., 2007). It has also been used to look at white matter in healthy human spinal cord (Minty, Bjarnason, Laule, & MacKay, 2009) and to measure myelin in both healthy and injured rat spinal cord (Dula, Gochberg, Valentine, Valentine, & Does, 2010; Kozlowski, Liu, Yung, & Tetzlaff, 2008; Kozlowski et al., 2008).

### 1.5 Objective of Current Work

The goal of this work was to evaluate the effectiveness of $qT_2$ as a technique for monitoring MRI-visible changes in the spinal cord over a period of days to weeks following injury. To do this, it was necessary to develop a model of SCI which was more amenable to imaging, especially quantitative imaging which is extremely sensitive to motion and magnetic field inhomogeneities, as well as requiring higher SNR than conventional anatomical imaging. It was also necessary to modify the $qT_2$ pulse sequence to avoid a resolution dependent decrease in measured $T_2$ due to diffusion.

Most models of SCI are highly invasive, requiring a laminectomy prior to induction of the injury, while most human injuries occur within a closed spinal column. Laminectomy is a very invasive procedure requiring trained personnel and antibiotic treatment to prevent infection. Even in the absence of damage to the spinal cord, laminectomy alone can result in epidural fibrosis (Kasimcan, Bakar, Aktaş, Alhan, & Yılmaz, 2011; Türkdoğan et al., 2014), compressing or stretching associated nerve roots and causing pain, which can interfere with behavioural studies of SCI (Kosta, Kojundžić, Sapunar, & Sapunar, 2009). Finally, laminectomy can also cause spinal deformities (Kosta et al., 2009) which create problems for MR imaging as sagittal images are difficult to acquire, and coronal images can require multiple acquisitions, if the spine is not straight.

Chapter 2 of this thesis describes a novel, non-surgical model of SCI in the rat, created using focused ultrasound (FUS) in combination with microbubbles. The non-surgical nature of the injury eliminates problems associated with laminectomy, while targeting the cervical spinal cord minimizes motion artefacts due to breathing and cardiac motion. In addition, the cervical spinal cord is slightly larger in diameter than
the thoracic or lumbar cord, increasing the amount of tissue available for imaging. MRI guidance was used to place the injury very precisely, allowing a unilateral injury which minimized functional deficits in breathing and urination. This is desirable from both an ethical and economical point of view. Chapter 3 describes the issues that were encountered when implementing qT₂ at the very high resolution required for imaging the rat spinal cord. The large imaging gradients increased the effects of diffusion, resulting in a resolution-dependent decrease in apparent T₂. Strategies for minimizing these effects are described, as are the limits on resolution for accurate T₂ quantification and some of the artefacts that can arise. Chapter 4 describes the results of using qT₂ in the FUS and microbubble model of SCI, and the interpretations of different patterns observed in the T₂ distributions based on histology results. This model proved to be well suited for quantitative MRI studies of SCI as there was no deformation of the spinal cord, no subdural hematomas, and the unilateral nature of the injury left the opposite half of the spinal cord available in the imaging plane for comparison although it could not necessarily be considered to be uninjured. Finally Chapter 5 discusses the future directions of this work. These include improvements to the FUS model of SCI, and applications of qT₂ in the study of SCI.

1.5.1 Use of Focused Ultrasound to Create a Model of SCI

FUS was first investigated as a method for ablating tissue non-invasively in the brain in the early 1940s (Lynn, Zwemer, Chick, & Miller, 1942). The use of a curved, rather than flat transducer means that the ultrasonic energy can enter the body without significant absorption until the beam reaches the focal point (Lynn et al., 1942). MRI-guided FUS induced thermal ablation is being used clinically to treat a variety of cancers including liver, prostate, and bone as well as uterine fibroids (Jolesz & McDannold, 2008; Jolesz, 2009). In spite of problems due to scattering and strong acoustic absorption by the skull, advances in technology including FUS systems with over 1000 transducers, have made it possible to treat essential tremor by thermally ablating a small region of the brain (Elias et al., 2013; Lipsman et al., 2013). The amount of power required to thermally ablate regions of the brain in these patients is very high (300-1250W), which can cause problems if the treatment target is located too close to the bone (McDannold, Park, Mei, Zadicario, & Jolesz, 2010). The addition of microbubbles can reduce the ultrasound power required by more than an order of magnitude (Huang, Vykhodtseva, & Hynynen, 2013; McDannold, Vykhodtseva, & Hynynen, 2006a; Tran, Seo, Hall, Fowlkes, & Cain, 2003), which allows lesions to be created much closer to bony structures such as the skull or spine.

Intravascular microbubbles such as Definity (Lantheus Medical Imaging) can nucleate inertial cavitation, which occurs when the microbubbles are exposed to ultrasound energy at a high enough frequency (Miller
Inertial cavitation is a violent phenomenon occurring as a bubble collapses which produces momentary high temperatures at the bubble core, extremely high pressure shock waves, and liquid microjets which damage surrounding tissue (Miller & Thomas, 1995; Mitragotri, 2005; Tran et al., 2003). The combination of FUS and microbubbles is being investigated as a method for opening the blood-brain-barrier (BBB) (O’Reilly & Hynynen, 2012b), where it can be used to deliver chemotherapy to brain tumors (Treat et al., 2007), or increase the amount of anti-Ab antibody in the brain in order to reduce Ab accumulation in Alzheimer’s disease (Jordão et al., 2010).

Chapter 2 describes the MR-guided FUS-induced SCI in a group of 19 rats. This includes conventional T2-weighted MRI, estimates of lesion size, and behavioural assessments of these injuries for up to 2 weeks following injury. Histology using H&E and LFB as well as immunohistochemistry looking at CD68 and GFAP is obtained at 24 hours and 2 weeks following injury. The model is compared with other models of SCI in the rat and is observed to produce lesions similar to those produced with contusion models of SCI.

1.5.2 Quantitative Imaging of SCI

As described in Section 1.3, MRI provides excellent soft tissue contrast and can allow visualization of the injury. T2-weighted imaging is particularly sensitive to changes in the spinal cord following injury; however, it is not specific. Following SCI it is necessary to diagnose the extent of hemorrhage, edema, cord compression, demyelination, and laceration or transection (Lammertse et al., 2007). On T2-weighted imaging it is possible to identify regions of hyper- or hypo-intensity, or regions where the contrast between gray and white matter is lost. While this is very useful for identifying the location and estimating the extent of damage, it does not provide a definitive diagnosis. Hemorrhage and edema have opposite effects on T2 with hemorrhage decreasing the signal intensity while edema increases the signal intensity. This will result in one type of pathology either obscuring or masking the presence of the other, and making the injury more difficult to diagnose. In addition the mechanism involved in the loss of gray/white matter contrast on MRI is not well understood. Given the sensitivity of T2-weighted images to changes in the spinal cord following SCI, it was hypothesized that quantitative measures of T2 would increase this sensitivity.

Quantitative T2 is a technique which examines the entire T2 decay curve instead of acquiring a single T2-weighted image. The advantage of this approach is that the T2 values of different compartments can be separated (MacKay et al., 1994, 2006; Stanisz et al., 1999; Whittall et al., 1997). This technique is typically applied in white matter where the water trapped within the myelin sheath has a much shorter T2 than that of intra/extracellular water.
A typical qT$_2$ sequence consists of a 90° excitation pulse followed by a train of 180° refocusing pulses (Fig. 1-3).

**Figure 1-3.** Excitation and refocusing pulses used in the qT$_2$ sequence. An echo is acquired following each refocusing pulse.

An image is acquired at each echo time, and then a T$_2$ decay curve is acquired by calculating the average signal over a region of interest (ROI) or single voxel from each image. The decay curve is typically fit using a non-negative least squares algorithm, described in more detail in Chapter 3, to generate a T$_2$ distribution. In white matter, this distribution typically consists of a MW peak with T$_2$ ranging from 10-50 ms, an intra/extracellular (I/E) water peak with relaxation time of 70-90 ms, and a CSF peak with relaxation time ~2 s (MacKay et al., 2006). A sample T$_2$ distribution is shown in Figure 1-4.

Measurements of MWF have been correlated with myelin in healthy white matter (MacKay et al., 1994; Whittall et al., 1997), in demyelinating diseases such as multiple sclerosis (Laule et al., 2008; Whittall et al., 2002), and in demyelination following SCI (Kozlowski, Raj, et al., 2008). An increase in the T$_2$ of the I/E water peak has been correlated with inflammation (Odrobina et al., 2005).
In the process of implementing the qT$_2$ sequence at a high enough resolution to investigate the rat spinal cord which is only 4 mm across at its widest, we realized that the T$_2$ relaxation values obtained were significantly lower than anticipated, and were in fact dependent on the resolution. The cause of this decrease in T$_2$ was due to cumulative signal decrease resulting from diffusion effects. qT$_2$ is typically performed at resolutions of 1 mm in plane, and the imaging gradients required to achieve this relatively low resolution are not strong enough to induce noticeable effects on the signal. However at resolutions required to image the rat spinal cord, which is 4 mm across at its widest, the effect began to have a significant impact on the measured T$_2$. This has a minimal effect on the quantification of myelin which is what most other studies use qT$_2$ for, however we were interested in the T$_2$ value of the intra/extracellular water as it is indicative of inflammation which we expected to see in SCI. Chapter 3 describes a diffusion-minimized qT$_2$ sequence which has minimal impact on T$_2$ values seen in vivo for resolutions up to 0.2 mm in plane. In Chapter 4 the results of applying this qT$_2$ sequence in an MR-compatible model of SCI are discussed.
1.6 List of Contributions

The following is a list of papers and presentations directly relating to this thesis.

**Published Papers**


**Submitted Papers**


**Conference Oral Presentations**


**Invited Oral Presentations**

Chapter 2 - A Non-Surgical Model of Cervical Spinal Cord Injury Induced with Focused Ultrasound and Microbubbles

This section describes the procedure used to create the MR-guided focused ultrasound (FUS) induced spinal cord injury (SCI) in a group of 19 rats. They are followed for up to 2 weeks with qualitative MRI, and behavioural assessments. Histology using H&E, luxol fast blue (LFB) to look at myelin, as well as immunohistochemistry looking for macrophages and astrocytes is also described at both 24 hours and 2 weeks post-injury. This model is compared with other models of SCI in the rat and is observed to generate lesions similar to those produced with contusion models of SCI. This work has been published in the Journal of Neuroscience Methods (Oakden et al., 2014).

2.1 Introduction

It is widely accepted that acute SCI is bi-phasic, where the primary mechanical injury, caused by forces including compression, contusion, shear, distraction and dislocation, is followed by a secondary injury mechanism. (Bartholdi & Schwab, 1996; Ramer et al., 2000; Rowland et al., 2008; Sekhon & Fehlings, 2001; Tator & Fehlings, 1991; Tator, 1995). As the primary injury has already occurred, the target of most therapies is to prevent damage resulting from the secondary injury mechanisms which transpire over a period of hours to weeks following the initial insult. These secondary mechanisms include vascular dysfunction, edema, ischemia, inflammation, excitotoxicity, electrolyte shifts, free radical production, and apoptosis. There have been at least nine randomized prospective controlled clinical trials of neuroprotective pharmacological agents in acute SCI, however none have demonstrated convincing neurological benefits in spite of promising results in pre-clinical studies (Rowland et al., 2008; Tator, 2006). It has been suggested that due to the heterogeneity of human SCI, treatments should be tested in a range of pre-clinical models prior to clinical trials (Schwab et al., 2006). Regenerative therapies are also a subject of active investigation as the adult central nervous system (CNS) has a limited ability to regenerate following injury (Rowland et al., 2008).

Animal models of SCI improve the understanding of the various mechanisms involved in the secondary phase of SCI, and permit the testing of potential treatments. The most commonly used animal models of SCI involve surgical exposure of the dorsal spinal cord followed by contusion, compression, or transection of the cord (Bligh, 2000; Ramer et al., 2000). Rats are frequently used due to their size and availability (Onifer et al., 2007) and pathology similar to that seen in human SCI (Fleming et al., 2006). While
contusion and compression are the most prevalent injury mechanisms seen in human SCI (Norenberg et al., 2004; Sekhon & Fehlings, 2001), human injuries typically occur in a closed vertebral system (Ramer et al., 2000). From an experimental perspective, the high level of invasiveness of these injury models often requires significant post-operative care and can limit post-operative imaging, as the surgical incision site can interfere with coil placement for MRI during the acute phase of SCI. Conventional MRI permits the qualitative assessment of SCI non-invasively in vivo, and provides a better estimate of lesion volume than histology (Ditor et al., 2008). Quantitative techniques such as diffusion tensor imaging, magnetization transfer, and quantitative T2 provide additional information (Dula et al., 2010; Kozlowski, Raj, et al., 2008). The strengths of histological evaluation include the characterization of tissue damage at the cellular level.

A precise, non-surgical model that can selectively target identified regions of the spinal cord would allow for robust image based evaluation of both acute and chronic phases of SCI. While it is possible to injure the cord non-surgically using radiation, it takes months to years for clinical signs of an injury to appear, and the condition is progressive and fatal (Delattre et al., 1988; Mastaglia et al., 1976; Medin et al., 2011). The length of time required for the injury to develop, and the unique mechanisms involved make radiation induced spinal cord damage a poor model for treatments targeting traumatic SCI. An alternate, less-invasive intervention that can rapidly induce clinically relevant repeatable neural injury patterns may be focused ultrasound (FUS), which has been used to thermally induce lesions in the brain. FUS has been used to thermally ablate regions of the brain, treating conditions such as essential tremor (Elias et al., 2013; Lipsman et al., 2013) and chronic pain (E. Martin, Jeanmonod, Morel, Zadicario, & Werner, 2009). At low power, FUS and microbubbles have been used to facilitate local drug delivery to the brain in animal models through transient opening of the blood-brain-barrier (BBB) without damage to critical neural structures (Hynynen, McDannold, Vykhodtseva, & Jolesz, 2001; McDannold, Arvanitis, Vykhodtseva, & Livingstone, 2012). At slightly higher power, FUS with microbubbles can produce targeted mechanical lesions in neural tissue while avoiding bone heating (McDannold et al., 2006a). While FUS and microbubbles do not replicate the injury mechanisms involved in human SCI, microbubbles have a mechanical impact on the microvasculature (McDannold, Vykhodtseva, & Hynynen, 2006b), and vascular injury is known to play a key role in both primary and secondary damage in SCI (Nelson, Gertz, Rennels, Ducker, & Blaumanis, 1977; Tator & Fehlings, 1991). As such, lesions produced by FUS and microbubbles may represent a potential approach for non-surgical MR-guided targeted damage to the spinal cord.
The goals of this work were twofold. First, to determine whether FUS and microbubbles can induce reproducible and localized non-surgical cervical SCI in a rat. Second, to assess the clinical relevance of the resultant injury pattern using behavioural analysis, MRI, and histopathology.

2.2 Methods

2.2.1 Ethical Considerations
All animal procedures were conducted with the approval of the Animal Care Committee of Sunnybrook Research Institute and in compliance with the guidelines established by the Canadian Council on Animal Care and the Animals for Research Act of Ontario.

2.2.2 Experimental Overview
Injuries were induced using MR guided FUS, targeting the right side of the cervical spinal cord, starting just below C1 and extending 6 mm caudally. Six spots were treated at 1 mm spacing. A schematic of the FUS transducer, with the sagittal MRI of the rat spinal cord used for targeting the treatment is shown in Figure 2-1, while a photograph of the rat on the treatment sled, positioned above the FUS transducer is shown in Figure 2-2.
Figure 2-1. MRI of rat spinal cord used for treatment planning. White dots represent treatment targets. Scale bar is 5 mm.

Figure 2-2. Photograph of rat, dorsally recumbent on the treatment sled. The sled is located on top of the water bath containing the FUS transducer.
Follow-up MRI took place at 24 hours for all animals. Ten animals (acute group) were sacrificed immediately following the 24 hour MRI session. The remaining 9 animals (chronic group) were imaged again at 1 week and 2 weeks. Three of these animals with no MR-visible abnormalities were sacrificed at 1 week, while the remaining 6 animals were sacrificed at 2 weeks post injury.

### Table 2-1 Summary of experimental design and injury severity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Animals</th>
<th>Follow-up MRI</th>
<th>Histology</th>
<th>Injury Severity (assessed using Histology)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hours</td>
<td>1 week</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Acute</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

### 2.2.3 Induction of Focused Ultrasound Injury

Nineteen male Wistar rats (Charles River Canada) weighing 250-400g were used in this study. They were anesthetized with a mixture of ketamine (40-50 mg/kg) and xylazine (10 mg/kg) delivered via intramuscular injection in the hind leg. The hair was removed from the neck and back using clippers and depilatory cream. A 22G angio-catheter was placed in the tail vein for delivery of microbubbles and the MRI contrast agent. The animals were placed dorsally recumbent on a plexiglass sled designed to be moved between the 7 T MRI and the ultrasound delivery system. The animals’ heads were supported with an upwards tilt to straighten the spine in the target region. Following the acquisition of T1-weighted images for treatment localization, the sled (Fig 2.2) was moved to a three-axis positioner operationally similar to that described by Chopra et al. (2009). The ultrasound transducer was located on a positioning arm in a water bath below the animal. The water bath was coupled to the animal using a sealed water pack built into the sled. Ultrasound coupling gel was applied between the water pack and the animal. Ultrasound was generated using a 1.114 MHz, spherically focused transducer (Aperture = 7 cm, F-number = 0.8), driven using a function generator and RF power-amplifier. The applied RF-power was measured with a power meter constructed in-house, connected to the controlling computer. Sonications consisted of 10 ms ultrasound bursts at a repetition rate of 0.5 Hz, for a total of 5 minutes. The acoustic power during the burst was set to 1.3 W for the first animal, and 1.6 W for the remaining animals as this power level was well-tolerated. Definity microbubbles (0.02-0.04 ml/kg, Lantheus Medical Imaging) were injected into the tail vein catheter at the start of the sonication, followed by saline (0.5 ml). During each sonication, 6
spots were treated at 1 mm spacing to produce a band of damage in the right side of the cervical spine immediately below the level of C1. Following sonication the sled was moved back to the 7 T MRI and Gd contrast-enhanced (Omniscan, 0.2 ml/kg, GE Healthcare) T₁-weighted images (Fig. 2-3) were acquired to assess the extent of BSCB disruption and potential primary injury.

Figure 2-3. Sagittal Gd enhanced T₁-weighted MRI to assess extent of primary injury. A hyperintensity can be observed in the spinal cord between the C1 and C2 vertebrae, resulting from extravasation of the contrast agent. Scale bar is 5 mm.

Two animals received a second sonication as the first did not result in Gd signal enhancement in the spinal cord. Following FUS the rats were given a subcutaneous injection of meloxicam as an analgesic (Metacam, 2 mg/kg, Boehringer Ingelheim, Canada).

2.2.4 Behavioural Assessment
Open field locomotion was evaluated by a trained veterinarian at 24 hours post-FUS for all animals, then again at 3 days, 1 week, and 2 weeks post-FUS for the chronic group. Animals displaying abnormal locomotion were tested further using a flexion reflex test (Tupper & Wallace, 1980). The flexion test involves pinching the paw of the affected limb. If the spinal cord is intact, the animal will move its paw away.
2.2.5 MRI

All MRI experiments were conducted on a 7 T horizontal bore Avance BioSpec 70/30 scanner (Bruker BioSpin, Ettlingen, Germany) using an 8 cm inner diameter volume coil for RF transmission, either the same coil or a rat brain coil array to receive, and a 20 cm inner diameter gradient insert coil with a maximum gradient amplitude of 668 mT/m (Bruker BioSpin, Ettlingen, Germany).

2.2.5.1 MRI for targeting and assessment of FUS injury induction

On the day of FUS injury induction, images were acquired in both the sagittal plane and in an oblique view perpendicular to the sagittal plane parallel to the spinal cord using a T₁-weighted fast spin echo sequence with 2 echoes, effective TE of 10 ms, TR = 500 ms, 5x5 cm field of view, 150x150 matrix, 1 mm slice thickness, 3 averages, and 75 kHz bandwidth. These images were acquired before FUS for targeting and immediately following FUS for treatment assessment with a gadolinium contrast agent (Omniscan, 0.2 ml/kg, GE Healthcare).

2.2.5.2 MRI for injury assessment

Follow-up MRI, was performed in vivo at various time-points following FUS (Table 1) under 2% isoflurane anesthetic. Images were acquired in both coronal and sagittal planes using a T₂-weighted fast spin echo sequence with 8 echoes, effective TE 35 ms, TR 2500 ms, 3x4 cm field of view, 200x266 matrix, 1 mm slice thickness, 4 averages, 50 kHz bandwidth.

In addition, ex vivo images were acquired following formalin fixation of the spinal cord tissue en bloc. These images were used to guide slicing of spinal cords into segments and permit alignment of MRI and histology. Sagittal T₂-weighted images were collected using a fast spin echo sequence with 16 echoes, effective TE 62 ms, TR 2000 ms, 1.6x4.4 cm field of view, 160x440 matrix, 1 mm slice thickness, 16 averages, 50 kHz bandwidth. Coronal T₂-weighted images were acquired using a fast spin echo sequence with 16 echoes, effective TE 66 ms, TR 2000 ms, 1.4x1.4 cm field of view, 140x140 matrix, 1 mm slice thickness, 60 averages, 50 kHz bandwidth.

2.2.6 Histology

Immediately following their final MRI session animals were deeply anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg), the chest opened and 100 IU sodium heparin injected into the left ventricle. The blood was washed out with lactated Ringer’s solution (Baxter, Canada) via the left cardiac ventricle with the outflow created by cutting the right heart auricle (Kwiecien, Blanco, Fox, Delaney, & Fletch, 2000). The rats were perfusion-fixed in 4% paraformaldehyde, pH 7.4. Spinal cords were then removed and post-fixed
in 10% buffered formalin for 48 hours and imaged using MRI (as above). Spinal cords were then sliced into transverse segments 3 mm thick, embedded in 3% agar (Shaw, Johnson, & Watson, 1983), processed in increasing concentrations of ethanol and xylene, and embedded in paraffin. Cross sections 5 and 10 µm thick were cut and mounted on glass slides. Five µm sections were stained with H&E and used for immunohistochemistry. The glass-mounted 5 µm thick sections were heated at 58 °C overnight and deparaffinised in a Target Retrieval Solution, pH buffer (DAKO) at 97 °C for 20 min in a DAKO PT Link Pre-Treatment Module for Tissue Specimens PT101 apparatus. Primary antibodies against CD68 and GFAP were obtained from DAKO Corp and used at 1:50 dilution to label macrophages and astrocytes, respectively. Primary antibodies were detected using the DAKO EnVision+Dual Link System-HRP (DAB+) kit, a system based on an HRP labelled polymer conjugated with secondary antibodies. Ten µm sections were stained with LFB for myelin and counterstained with cresyl violet. Histological analysis was performed under a Nikon Eclipse 50i microscope and areas with pathology were photographed.

2.2.7 Data analysis

Individual animals were classified as uninjured, moderately injured, or severely injured based on histology (details in section 2.3.2). Cross-sectional area of the spinal cord affected by the FUS treatment was estimated by measuring both, front-to-back and left-to-right extent of the abnormal MR signal, perpendicular to the spinal cord, and assuming the affected area was an ellipsoid (Fig. 2-4d). For the contrast enhanced images obtained immediately following FUS, front-to-back measurements were made on the sagittal images (Fig. 2-4a) and left-to-right measurements were made on the oblique longitudinal images (Fig. 2-4b) of the cord as coronal images were not available. For T₂-weighted images acquired at 24 hours, 1 week, and 2 weeks post injury, both measurements were made on the coronal images (Fig. 2-4c). Measurements were repeated 3 times per image and the average and standard deviations were calculated.

Alignment of MRI and histology images was performed by placing the boundary between 3 mm sections of spinal cord at the same location as the MR slice of interest, determined by measuring the distance from the base of the cerebellum. Within the slice, corresponding regions on MRI and histology were determined by manually aligning both images based on visible structures or the boundary of the spinal cord assuming uniform shrinkage across the cord.
Figure 2-4. MR visible signal changes due to FUS treatment. On the $T_1$-weighted sagittal (a) and oblique (b) images acquired immediately post-FUS, these changes are due to contrast agent leakage across the disrupted blood-spinal cord barrier (BSCB). On the $T_2$-weighted coronal images (c) there is no contrast agent and the changes are due to alterations in the tissue microstructure. The cross-sectional area of MR-visible changes were estimated by measuring dorsal-to-ventral (D-V) and left-to-right (L-R) extent of these changes and calculating the area of the resulting ellipsoid (d). Scale bars; a, b -5 mm, c – 1 mm.

2.3 Results

2.3.1 Behavioural Assessment Following FUS

The first animal was treated with low (1.3W) power FUS. As the higher (1.6W) power FUS was well tolerated, this power was used with the remaining 17 animals. As well, the analgesic was switched from buprenorphine (0.02mg/kg, Temgesic, Schering-Plough, Shire Park, Welwyn Garden City, U.K.) to the anti-inflammatory and analgesic drug meloxicam (2 mg/kg Metacam, Boehringer Ingelheim, Ontario, Canada) as swelling of the back of the neck and difficulty in head lifting was observed in both of the first two animals. All animals survived 24 hours in good health following the FUS injury protocol. A neurological assessment included observation of the rats, specifically regarding their ability to use all four legs when walking. Three rats developed a noticeable limb paralysis at 24 hours (rat 1: left front limb distal to the
elbow joint and lateral tarsal bones of right hind limb, rat 2: left front limb distal to carpal joint, rat 3: right front limb distal to elbow joint). When examined further using a flexion reflex test (Tupper & Wallace, 1980), all rats retained the ability to retract their limbs. Rat 3, with right front limb paralysis, died during the 24 hour MRI exam due to respiratory failure while under isoflurane anesthesia, while the other two recovered full use of their limbs within 1 week following FUS.

2.3.2 Histology

Histological analysis was performed in the spinal cord tissues collected at 24 hours, 1 week, or 2 weeks following the FUS injury (Table 1). In all cases damage to the spinal cord was confined to the cervical cord. Of the 12 injured animals, 9 had unilateral damage to the cord. The remaining 3 animals had centrally located spinal cord damage, one of which died while under anaesthesia during the follow-up MRI scan (Rat 3 mentioned above).

At 24 hours post-FUS, 2 of the 10 animals in the acute group were classified as having no injury as they had very small areas of hemorrhage in the examined cross sections of the cervical spinal cord (<200 µm diameter). Four animals were considered moderately injured, with scattered, isolated, petechial hemorrhages involving less than 50% of the cross-sectional area of the spinal cord. The remaining 4 animals were severely injured (Fig. 2-5). Scattered throughout the section, in both gray and white matter, these animals had large, multifocal, often coalescing hemorrhages encompassing or adjacent to large fibrin deposits; there was necrotizing vasculitis and areas of tissue necrosis; and there were scattered necrotic cells and scattered neutrophils (Fig. 2-5b, c). Within and around the areas of hemorrhage that often had a perivascular “cannonball” appearance, there was loss of myelin and numerous swollen axons (Fig. 2-5c). GFAP-positive astrocytes were localized in the viable tissue surrounding the injury (Fig. 2-5e, f). The anti-CD68 stain was negative in the areas of necrosis (not shown).
Figure 2-5. MRI and histology of acute FUS injury.
Coronal MR image (a) was taken from the rat in dorsal recumbency and the dorsal spinal cord [D] is on the bottom and the ventral [V] on the top of the photograph. Paraffin-embedded section at the same level of the spinal cord stained with H&E reveals a large area of necrosis delineated by arrowheads that contains multifocal haemorrhages (b). On higher magnification (c) of the area of necrosis, small blood vessels (*) are surrounded by fibrin delineated by small arrows, haemorrhages, and necrotic cells with dark pyknotic nuclei. In the area of necrosis there is a corresponding loss of the LFB stain for myelin (d) and of the GFAP stain for astrocytes (e, f). Scale bars; a -1 mm, b, d, e – 600 µm, c, f – 60 µm.

Of the animals in the chronic group, all three sacrificed at 1 week post-FUS, and two of the 6 animals sacrificed 2 weeks post-FUS had no observable injury. In the remaining four animals, irregular, cavity-like lesions were observed unilaterally in the upper area of the lateral column involving both the white matter and the adjacent areas of gray matter (Fig. 2-6d). There was corresponding loss of myelin (Fig. 2-6f) and of neural tissue. Free erythrocytes, fibrin deposits and free damaged myelin were not apparent within these cavities (Fig. 2-6e). The cavities were intensely infiltrated by a pure population of large, micro-vacuolated cells, with either round or oval, often indented nuclei, that were interpreted as macrophages (Fig. 2-6e), confirmed with a positive staining with the anti-CD68 antibody (Fig. 2-6g, h). In the neural tissue surrounding the cavities there was a 200-300 µm thick band of pronounced astrogliosis positive for GFAP (Fig. 2-6i, j). In some sections, typically at a distance of the areas of severe infiltration by macrophages there were isolated foci of hemorrhage that appeared perivascular (not shown).
Figure 2-6. MRI and histology of chronic FUS injury.
Three MR images are of the section of the cervical spinal cord from one rat injured with the FUS taken at 24 hrs (a), 1 week (b) and 2 weeks after the injury. The rat was imaged in a dorsally recumbent position therefore the dorsal spinal cord is in the bottom of the MR image [D] and the ventral is in the top [V]. The paraffin embedded sections of the same region are from the spinal cord of the rat perfused 2 weeks post-FUS. Corresponding to the unilateral area of the MR images, there is a large irregular area of cavitation delineated by arrowheads. The injury cavity contains severe infiltration by large, foamy mononuclear cells interpreted as macrophages (d, e). There is a corresponding loss of myelin (f) and positive staining of a proportion of macrophages with CD68 antibody (g, h). The macrophage-rich exudate in the injury cavity is surrounded by astrogliosis (i, j). These images demonstrate the localization of the injury to one side of the spinal cord. Scale bars; a,b,c, d, f -1 mm, e, h, j – 60 µm, g, i – 600 µm.
2.3.3 MRI

All MRI signal changes in the neural tissue were contained within the FUS treated cervical region of the spinal cord. The regions of signal abnormalities in the \( T_2 \)-weighted coronal images corresponded to the regions of damage observed on histology. In the Chronic group, 3 of the animals with no \( T_2 \)-weighted signal abnormalities were sacrificed following the 1-week MRI examination and determined to be uninjured based on histology.

Immediately following FUS the Gd-based contrast agent leaked through the BSCB causing a signal increase on \( T_1 \)-weighted MRI (Fig. 2.3). In 4 animals there was also a region of signal decrease at the centre of the region treated with FUS, 3 of these were classed as severely injured based on histology (1 at 24 hours, 2 at 2 weeks), the last as moderately injured (at 24 hours). These areas of signal decrease were considered to be part of the enhancing region for the purposes of measuring the area of FUS treatment, provided the area with decreased signal was contained within a larger region of increased signal.

Figure 2-5 shows a representative example of both MRI and histology acquired at 24 hours, from an animal with severe injury. In all 8 severely injured animals both hyperintense regions and regions with loss of gray/white matter contrast were observed. These regions were identified as having abnormal signal based on comparison with control spinal cord in a matching location. Visual comparison with histology revealed that hyperintensities corresponded to loss of myelin and edema, while loss of gray/white matter contrast corresponded to regions with scattered hemorrhage and vacuolation. In one animal there was also a region of hypointense signal on MRI at 24 hours, which was hyperintense at 1 and 2 weeks, and corresponded with a cavity on histology. Two of the 4 moderately injured, and 3 of the 7 uninjured animals displayed slight hyperintensities and loss of gray/white matter contrast at 24 hours.

Figure 2-6 shows a set of representative \( T_2 \)-weighted MR images from a single animal at 24 hours, 1 week, and 2 weeks following FUS, and the corresponding histology at 2 weeks. The area of hyperintensity seen at 24 hours had decreased in size at 1 week, and there was a dark region adjacent to or surrounded by the region of hyperintensity (Fig. 2-6b). Two weeks post-FUS the degree of hyperintensity had decreased, however the area with abnormal signal intensity was unchanged (Fig. 2-6c). Hyperintense MR signal corresponded with loss of myelin and large cavities, while hypointense signal was present in regions with macrophages containing hemosiderin (Fig. 2-6d,f). One animal displayed no hyperintensity, only loss of gray/white matter contrast at 1 week and a hypointense region at 2 weeks, which corresponded with hemosiderin containing macrophages on histology.
Figure 2-7 shows a graph of the calculated areas of abnormal signal MRI at all time points. 24 hours following FUS, all animals with severe injury on histology were observed to have a signal increase, or hyperintensity, on T2-weighted images which was larger than that seen immediately following FUS. The area of signal abnormality at 24 hours was significantly correlated with severity of injury (Pearson's R = 0.77, p=0.025). The area of Gd-enhancement following FUS was not significantly correlated, although there was a trend (Pearson's R =0.46, p=0.25). Moderate injury, characterized by scattered hemorrhage on histology, was observed only at 24 hours post-FUS (Fig. 2-7a). Areas of abnormal MR signal, which included hypo- and hyper-intensity as well as loss of gray/white matter contrast, all decreased by 1 week, at which point the presence of abnormal signal on MRI was indicative of severe injury (Fig. 2-7b).
Figure 2-7. Areas of signal abnormality in spinal cord for acute and chronic groups. Cross-sectional area of spinal cord abnormalities from contrast enhanced images immediately post-FUS, and T2-weighted images at 24 hours, 1 week, and 2 weeks post-FUS for both the acute (a) and chronic (b) groups. Classification into uninjured, moderate or severe injury occurred based on histopathology. For comparison, the cross-sectional area of the entire cord is 9-10 mm².
2.4 Discussion

FUS in combination with microbubbles was successfully used to induce SCI non-surgically in several animals. Opening of the blood-brain barrier using a combination of FUS and intravascular microbubble injection has been extensively studied in the brain (Choi, Pernot, Small, & Konofagou, 2007; Hynynen et al., 2001; McDannold et al., 2012; Nhan et al., 2013). This thesis confirms the earlier work (Wachsmuth, Chopra, & Hynynen, 2009) demonstrating a similar opening of the BSCB using MR guided FUS and microbubbles, at lower power which does not result in permanent damage to the spinal cord.

The histological changes observed at 24 hours were classified as mild, moderate, or severe based on the amount of hemorrhage present. In the groups which survived to 1 or 2 weeks only severe injury or no injury were observed. The absence of moderate injury in the chronic group likely indicated that this intermediate degree of injury, characterized by petechial hemorrhages involving less than 50% of the spinal cord, either healed or progressed to a more severe injury. Since approximately half of the animals in the chronic group appeared uninjured following the FUS treatment, there was likely a threshold of damage beyond which the SCI progressed to leukomyelitis. This would indicate that the BSCB can be opened transiently without permanently damaging the spinal cord.

2.4.1 Reproducibility and injury tolerance

Overall 42% of animals developed a severe injury with an additional 21% developing moderate injuries. Several animals were observed to have shifted laterally on the treatment sled between the initial MRI used for targeting and the post-FUS MRI which may have resulted in misalignment of the treated area.

While partial paralysis was observed in 3 animals, smaller neurologic deficits may have been observable with more sensitive behavioural tests. Overall, the FUS injury itself was very well tolerated, with an immediate mortality rate of 0% and a 24-hour mortality rate of only 5%. Post-FUS care was minimal, involving a single dose of analgesic, and animals were all able to self-sufficiently obtain food and water. Minimal evidence of discomfort was observed, and no intensive care was required following SCI. This was likely due to both the unilateral and the non-surgical nature of the injury.

More than half of the animals did develop injuries; however, reproducibility of this model requires improvement. In previous studies where the FUS treatment was conducted inside the bore of a clinical MRI, consistent localization and size of Gd-enhancement was observed (Weber-Adrian et al., 2014) with relative enhancement dependent on FUS power (Wachsmuth et al., 2009). The next generation of FUS hardware is being built to operate within the bore of the 7 T small animal MRI system, which should reduce
motion issues while permitting high image resolution. We hypothesize that greater consistency in injury localization will improve reproducibility of this injury. The ability to tune the level of damage may also be possible in the future by implementing an active treatment controller, as has been proposed for BBB disruption (O’Reilly & Hynynen, 2012a). Active control is particularly important in reducing the variability of FUS exposures when treating through the vertebral bone.

2.4.2 Histology Assessment

At 24 hours post-FUS, pathology in severely injured animals consisted mainly of hemorrhage with some tissue necrosis, neutrophils, and loss of myelin. At 2 weeks, cavity formation was observed, along with macrophage infiltration, astrogliosis and loss of myelin. These results were consistent with those reported in literature for compression/contusion injuries (Rowland et al., 2008).

2.4.3 MRI Assessment

The area of Gd-enhancement following FUS correlated with severity of injury. This likely indicates that larger areas of Gd-enhancement were the result of better targeting of the spinal cord and corresponded with more ultrasound energy being deposited in the spinal cord rather than in surrounding tissue. Small areas of Gd-enhancement, seen in some animals, likely indicated that the animal had shifted laterally while being moved from the MR scanner to the FUS treatment platform, and the FUS energy was not correctly targeted to the spinal cord. Only one case of severe injury was observed with an initial area of enhancement less than 2 mm². This particular animal appeared to have two separate regions of enhancement in the spinal cord with a gap in between, rather than a single contiguous region of enhancement. It is possible that the gap was actually an area with a sufficient accumulation of Gd that the T₂ shortening effect of the Gd counteracted the signal increase due to T₁ effects (May & Pennington, 2000), and appeared iso-intense with the surrounding tissue. The presence of a Gd-enhancing region following FUS was therefore a necessary, but not sufficient, criterion for determining the success of the injury induction.

T₁-weighted MRI contained regions of both hyperintensity and loss of gray/white matter contrast at 24 hours at the site of injury. Hyperintensities corresponded with areas on histology with loss of myelin and edema. Loss of gray/white matter contrast was indicative of regions of mixed hemorrhage and vacuolation. One animal displayed a hypointense region at 24 hours, which had become hyperintense by 1 week and corresponded to a cavity on histology at 2 weeks. This was likely an area of severe hemorrhage at 24 hours. Centrally located hypointensities began to appear at 1 week and were more pronounced by 2 weeks. At 2 weeks, hyperintensities corresponded with cavities, and hypointensities corresponded with hemosiderin
containing macrophages on histology. Loss of gray/white matter contrast was indicative of white matter vacuolation. The histological analysis performed in parallel to MR imaging demonstrated that the signal intensity on T₂-weighted MRI was representative of different underlying pathology depending on the length of time post-injury.

The area of signal abnormality on T₂-weighted MRI at 24 hours was significantly correlated with injury severity. This area was smaller than the area of contrast enhancement following FUS in most of the uninjured (6 of 7) and moderately injured (3 of 4) animals, which likely reflects areas where the BSCB was permeabilized but did not experience edema. Standard deviations of the area measurements were largest at 24 hours as the precise boundaries of signal abnormality were most difficult to determine, especially when the abnormality included loss of gray/white matter contrast.

A decrease in the area of signal abnormality on T₂-weighted MRI from 24 hours to 1 week was seen in the chronic group. This could be related to the fact that areas near the edge of the lesion were initially affected by edema (hence the hyperintensity at 24 hours) but recovered, while the centre of the lesion remained damaged.

### 2.4.4 Applicability

Mechanically induced damage resulting from the collapse of microbubbles within the blood vessels, referred to as inertial cavitation, is thought to be the primary mechanism for lesion generation in neural tissue with FUS and microbubbles (McDannold et al., 2006a). Previous histological analysis of these types of lesions suggests that FUS and microbubbles can induce both ischemic and hemorrhagic lesions (Huang et al., 2013). In brain tissue it has been observed that gray matter suffers greater damage for the same exposures than white matter, which is reasonable since gray matter is more vascular and the microbubbles are confined to the vasculature (McDannold, Zhang, Power, Jolesz, & Vykhodtseva, 2013). While this injury mechanism is very different from those that cause human SCI (Ho et al., 2007), the resulting pathology has some aspects of a contusion or compression model which attempt to mimic the mechanical events in human SCI (Blight, 2000). A mild impact or compression results in hemorrhage due to mechanical disruption of capillaries, venules, and arterioles, but induces very little gross damage to the substance of the spinal cord. Hemorrhage is seen primarily in gray matter and there is some invasion of neutrophils (Beattie, Hermann, Rogers, & Bresnahan, 2002; De Girolami, Frosch, & Tator, 2002; Hill, Beattie, & Bresnahan, 2001). The macrophage infiltration and cyst formation seen following this injury was in line with that observed in other injury models (Beattie et al., 2002; Dusart & Schwab, 1994; Hill et al., 2001). Much of the post-traumatic
tissue damage and subsequent neurological deficits which occur following SCI are due to this secondary reactive process which makes it an important target for treatment (Kwiecien, 2013; Ramer et al., 2000; Tator & Fehlings, 1991).

The injury was created in a closed vertebral system, but yielded a similar pathology to that observed in compression or contusion injuries. The non-surgical nature of the injury reduced the risk of infection and permitted imaging to be performed immediately following injury induction. MR-guidance of the injury ensured that injury location was optimal for imaging, and not too close to the heart or lungs where motion might cause artefacts. The animal procedure was only slightly more complicated than for a typical MRI experiment, making this model more accessible to researchers with imaging expertise than models requiring extensive surgical experience. Finally, the ability to create unilateral injuries, although not unique to this model, meant that respiration was unaffected and there was no overt urinary dysfunction.

While this model is unlikely to replace any of the standard models of SCI, it has been suggested that due to the heterogeneity of human SCI, treatments should be tested in a range of pre-clinical models prior to clinical trials (Schwab et al., 2006). The non-surgical nature of this model is ideal for imaging-intensive preliminary assessment of potential treatments, increasing the understanding of the mechanisms involved.

2.5 Conclusions

In this chapter a novel model of SCI was presented which was non-surgical, well-tolerated, compatible with MRI and replicated the pathology seen in contusion and compression models of SCI. Reproducibility of the generated lesions may be improved when FUS injury induction takes place inside the MR scanner and active treatment control is used. The ability to non-surgically induce localized lesions with FUS that can be monitored with MRI has potential for future studies considering SCI modeling and the development of therapeutic approaches.

Developing a model of SCI optimized for quantitative MRI was only the first step. The qT₂ sequence is used primarily in humans, and when it was first used to acquire images in the rat spinal cord it was observed that the resulting T₂ values in white matter were lower than anticipated. Further investigation revealed that the T₂ values were dependent on the resolution. The next chapter describes the mechanism behind this apparent decrease in T₂, the modified sequence used to improve qT₂ results, and explores the resolution limitations of the qT₂ sequence.
Chapter 3 - Effects of diffusion on high resolution quantitative $T_2$ MRI

This chapter discusses the relationship between resolution and apparent $T_2$ value measured using the q$T_2$ sequence. The rat spinal cord is only 4 mm across at its widest point, requiring an in-plane resolution of at least 0.2 mm/pixel in order to avoid partial volume effects as it contains regions of both gray and white matter. Alterations to the standard q$T_2$ sequence used in human brain and spinal cord were required in order to avoid substantial underestimation of $T_2$ in the rat spinal cord. A modified version of this work has been published in NMR in Biomedicine (Oakden & Stanisz, 2014).

3.1 Introduction

The highest achievable resolution in MRI is on the order of 10-100 µm, depending on the magnetic field strength, strength of the imaging gradients, and the physical properties of the tissue being imaged (Callaghan, 1993). While it is possible to image a single cell it is extremely time consuming and possible only for a very small field of view (Flint et al., 2012). Quantitative imaging methods are often used to gain information about water molecules in different compartments within a cell, or group of cells, even though the precise physical locations of these compartments cannot be determined (Harrison, Bronskill, & Henkelman, 1995; Li, Stanisz, & Henkelman, 1998; Stanisz & Henkelman, 1998; Stanisz et al., 1999). Quantitative $T_2$ (q$T_2$) is one such technique, and reveals the presence of multiple water components relaxing with different $T_2$ relaxation times (MacKay et al., 2006; Menon & Mackay, 1989; Stanisz & Henkelman, 1998; Stanisz et al., 1999; Whittall et al., 1997). This is especially informative in white matter where water trapped in the myelin sheath surrounding axons has a significantly shorter $T_2$ than either intra- or extra-cellular water (Does & Snyder, 1995; MacKay et al., 1994; Stanisz et al., 1999). The signal decay curve of healthy white matter is typically decomposed into three separate pools based on $T_2$ relaxation time. The shortest of these relaxation times (10-50 ms) is correlated with water trapped within the myelin sheath, the intermediate relaxation time (70-90 ms) is suggested to represent intra/extracellular (I/E) water, and the longest (~2 s) with CSF (MacKay et al., 2006). These values have been established in healthy human brain at 1.5 and 3 T and are generally accepted as $T_2$ is considered to be weakly dependent on magnetic field (Bottomley, 1984; Stanisz et al., 2005). Alterations in relaxation time of these pools has been shown to correlate with particular pathology such as demyelination (Laule et al., 2006, 2008; Webb, Munro, Midha, & Stanisz, 2003) or inflammation (Odrobina et al., 2005; Pun et al., 2005; Webb et al., 2003). Due to the sensitivity of $T_2$ to microstructural changes, histology is required to determine the pathologic
significance of a particular $T_2$ distribution. Once the underlying pathology is well understood, $qT_2$ can be a powerful tool for investigating a disease, as is the case with multiple sclerosis (Laule et al., 2006) or phenylketonuria (Sirrs et al., 2007). To fully explore the effects of various pathologies on $qT_2$, animal models of central nervous system disease are often required. In that case higher resolution is necessary for examining smaller structures and reducing the within-voxel heterogeneity. Unfortunately the gradients required for high resolution begin to have a noticeable effect on the characteristics of $T_2$ decay curves. High field, high resolution $qT_2$ and multi-slice multi-echo (MSME) imaging of ex-vivo human brain (Laule et al., 2008), in-vivo mouse brain (Guilfoyle, Dyakin, O’Shea, Pell, & Helpern, 2003; Kara et al., 2012) and ex-vivo rat spinal cord (Dula et al., 2010; Kozlowski, Liu, et al., 2008) all report significantly shorter $T_2$ values (ranging from 30-60 ms) than $qT_2$ images acquired in human brain at 1.5 and 3 T (80-90 ms).

In homogeneous systems, $T_2$ is well-defined and only very weakly dependent on magnetic field (Bottomley, 1984). In tissue there are microscopic field variations resulting from differing tissue susceptibilities, and paramagnetic or super-paramagnetic particles which are not completely compensated by the spin-echo and can cause a decrease in measured $T_2$ which will depend on both field strength and echo time (Brown & Fantazzini, 1993; Carr & Purcell, 1954; Stanisz et al., 2005). Increasing field strength also leads to increased SNR, allowing for higher resolution. The imaging gradients used to achieve those higher resolutions increase diffusion weighting and thus decrease the signal intensity, leading to a decrease in measured $T_2$ which only appears to be dependent on field strength (Gullmar, Haueisen, & Reichenbach, 2005; Stejskal & Tanner, 1965). On the other hand, sequences designed to measure diffusion deal with imaging gradients by calculating random motion based on the entire sequence rather than only those gradients deliberately being used to add diffusion weighting (Mattiello, Basser, & Le Bihan, 1997; Mattiello, Basser, & LeBihan, 1994). Imaging sequences, especially those with long echo trains, can suffer from a diffusion-related decrease in signal or change in contrast depending on the resolution and the tissue (Bieri, Ganter, & Scheffler, 2012; Kiselev, 2003; M Weigel, Schwenk, Kiselev, Scheffler, & Hennig, 2010; Matthias Weigel & Hennig, 2012). Understanding these changes is vitally important when interpreting images and when comparing values across studies. Finally there are sequences which aim to quantify the $T_2$ as consistently as possible, where it is necessary to both minimize the effects of diffusion and be aware of the magnitude of possible $T_2$ miscalculation. Nuclear magnetic resonance (NMR) microscopy acquires a single echo per TR, with modifications to the conventional spin echo readout prewinder to avoid underestimation of $T_2$ due to imaging gradients (Hsu et al., 1995), but this approach takes too long to be used in vivo.
The quantitative $T_2$ ($qT_2$) sequence is based on the Carr-Purcell-Meiboom-Gill (CPMG) sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958), and uses a multi-echo readout from which transverse signal decay curves can be obtained and analyzed. The CPMG sequence minimizes the effects of diffusion due to a constant background gradient (Carr & Purcell, 1954); however the additional gradients required for imaging also act as diffusion sensitizing gradients and can contribute substantially to the signal decay (Neeman, Freyer, & Sillerud, 1990; Matthias Weigel & Hennig, 2012). The effect on the $qT_2$ sequence is more significant than a simple loss of signal with time; the diffusion sensitization increases with each echo, altering the decay curve and decreasing the apparent $T_2$ values. This is similar to the effect seen in conventional MSME or turbo spin echo sequences (Bieri et al., 2012; Matthias Weigel & Hennig, 2012) but simpler to understand as the diffusion weighting in each image is constant across that image.

This chapter describes the incorporation of a fully refocused readout gradient, typically used for flow compensation (Hinks & Constable, 1994; Simonetti, Wendt, & Duerk, 1991), into the original $qT_2$ sequence first proposed by Poon and Henkelman (Poon & Henkelman, 1992), and investigates the relationship between $T_2$ underestimation and resolution for both the original and the refocused readout sequence. It also explains how to calculate the underestimation of $T_2$ resulting from both imaging and spoiler gradients, demonstrates the issues arising from anisotropic diffusion, and examines the effects on myelin water fraction estimation. In addition I discuss the distortions in the $T_2$ distribution resulting from decreasing spoiler gradient schemes, similar to the complications from nonlinear echo time spacing (Does & Gore, 2000).

3.2 Calculating the Effects of Diffusion on $T_2$

The $qT_2$ sequence consists of a 90° excitation, followed by a train of 180° refocusing pulses (Fig. 3-1). Each echo contributes to a different image, meaning that each image has its own echo time, with TE typically referring to the first echo time. Each 180° is bracketed by a pair of spoiler gradients (Fig. 3-1 a&c), required to remove stimulated echoes and out of slice magnetization, which resemble the diffusion sensitizing gradients used in a pulsed gradient spin echo experiment (Stejskal & Tanner, 1965). Imaging gradients (Fig. 3-1 b&d) can also be considered as pairs of diffusion sensitizing gradients. The parameter describing diffusion weighting (b-value) arising from the gradients bracketing a 180° pulse can be calculated using the sequence parameters, or estimated based on linear voxel size ($\Delta x$) and slice thickness ($\Delta z$).
Figure 3-1. RF, spoiler and readout gradients used in a $qT_2$ sequence. The standard $qT_2$ sequence described by Poon & Henkelman (1992) uses decreasing spoilers (a) and places the readout prewinder at the beginning of the sequence (b). The diffusion minimized $qT_2$ sequence uses equal spoilers (c) and refocuses the readout gradients after each readout (d).

3.2.1 Effect of diffusion on measured $T_2$

In the pulsed gradient spin echo sequence developed by Stejskal and Tanner (1965) the measured signal, $S$ is attenuated relative to $S_0$, the signal measured in the absence of diffusion weighting by $S = S_0 e^{-Db}$ where $D$ is the apparent diffusion coefficient, and the b-value parameter describing the diffusion weighting is given by: $b = (γGδ)^2 \left( Δ - \frac{δ}{3} \right)$, where $γ$ is the Larmor frequency, $G$ the gradient strength, $δ$ the gradient duration, and $Δ$ the time between gradients.

Assuming that the echoes are evenly spaced, let $b_i$ refer to the b value resulting from the gradients between the initial excitation and the first echo at time $TE$, and $b_i$ refer to the b value resulting from the gradients between times $TE:(i-1)$ and $TE:i$, then the signal for echo $n$ is equal to:

$$S(n) = M_0 e^{-\frac{(TE-n)}{T2}} e^{-D \sum_{i=1}^{n} b_i}$$

(3.1)
If the spoiler and imaging gradients do not change with echo number (Fig. 3-1b&c), then \( b_i \) is constant and will be referred to as \( b_{qT2} \), and the signal for echo \( n \) is equal to:

\[
S(n) = M_0 e^{-\frac{(TE \cdot n)}{T_2}} e^{-b_{qT2}Dn}
\]  

(3.2)

Omitting diffusion and fitting the signal to a single exponential gives:

\[
S(n) = M_0 e^{-\frac{(TE \cdot n)}{T_2_{app}}}
\]  

(3.3)

resulting in an apparent \( T_2 \) value, \( T_2_{app} \) which is now dependent on experimental parameters \( b \), \( TE \), and intrinsic diffusion coefficient of the tissue \( D \):

\[
\frac{1}{T_2_{app}} = \frac{1}{T_2} + \frac{b_{qT2}D}{TE}
\]  

(3.4)

### 3.2.2 The Effects of Readout Gradients:

For the standard readout (Fig. 3-1 c), the apparent \( T_2 \) is given by Eq. 3.4. The situation becomes slightly more complicated in the case of refocused readout gradients (Fig. 3-1d) where \( 2b_i = b_{i>1} \) for \( i > 1 \). Then setting \( b_i = b_{qT2} \), \( b_{i>1} = \frac{1}{2}b_{qT2} \) provides the following formula for a signal at each echo, \( S_n \)

\[
S(n) = M_0 e^{-\frac{(TE \cdot n)}{T_2}} e^{-b_{qT2}D(n-\frac{1}{2})}
\]  

(3.5)

which results in slightly modified Eq. 3.4:

\[
\frac{1}{T_2_{app}(n)} = \frac{1}{T_2} + \frac{b_{qT2}D}{TE} \left( 1 - \frac{1}{2n} \right)
\]  

(3.6)

While the apparent \( T_2 \) value is dependent on the number of echoes, \( n \), the dependence is small and can either be neglected for small values of \( b_{qT2} \), or a small gradient with \( b \)-value of \( \frac{1}{2}b_{qT2} \) may be added to the beginning of the sequence, reducing this to Eq. 3.4.

The readout gradient, \( G_x \), depends on the bandwidth (BW) and the field of view (FOV) through following formulae:

\[
G_x \delta = \frac{BW}{\gamma \cdot FOV} \cdot \delta = \frac{2\pi N}{\gamma \cdot FOV \cdot T_s} \cdot \frac{T_s}{2} = \frac{\pi}{\gamma \cdot \Delta x}
\]  

(3.8)
as BW is simply the number of data points acquired (N), divided by the total sampling time (T), and Δx is voxel size in the x direction.

The precise value of \(b_{qT^2}\) also depends on the pulse sequence timing parameters, which in turn are influenced by physical properties of the scanner such as the maximum gradient strength and slew rate. In order to place an upper bound on the value of \(b_{qT^2}\), it is necessary to estimate the diffusion time, \((Δ − \delta/3)\), where \(\delta = Ts/2\). For the standard readout (Fig. 3-1b), \(Δ = TE − Ts/2\), resulting in \((Δ − \delta/3) = TE − (2Ts)/3\). Finally T, can be expressed as a fraction of TE, say \(Ts = TE/r\), for some value of r, resulting in

\[
\frac{1}{T_{2,app}^2} \leq \frac{1}{T_2} + \left(\frac{\pi}{\Delta x}\right)^2 \left(1 - \frac{2}{3r}\right)D
\]

(3.9)

for the standard readout (Fig. 3-1 b).

Alternatively if the readout is fully refocused (Fig. 3-1d), there are two symmetric pairs of gradients between echoes, each contributing to \(b_{qT^2}\). In each pair, the readout prewinder comes immediately before the readout gradient so the diffusion time \((Δ − \delta/3)\) is replaced by \(\frac{1}{3}(δ + \delta_{ref})\) where the gradient refocusing time, \(\delta_{ref}\), is some fraction of \(δ\), say \(\frac{\delta_{ref}}{δ} \leq 1\). Again assuming that \(Ts = \frac{TE}{r}\),

\[
\frac{1}{T_{2,app}^2} \leq \frac{1}{T_2} + \left(\frac{\pi}{\Delta x}\right)^2 \left(\frac{2}{3r}\right)D
\]

(3.10)

for the fully refocused readout.

### 3.3.3 Spoiler Gradients:

Stimulated echoes are formed by a train of 3 or more RF pulses. The first pulse excites the magnetization and tips it into the x-y plane. The second RF pulse returns the magnetization to the z-direction where it experiences only \(T_1\) relaxation, and the third RF pulse returns it to the x-y plane where it forms a stimulated echo. These echoes can be formed by imperfect 180 pulses in the \(qT_2\) sequence, in which case the stimulated echo occurs at the same time as the spin echo and interferes with the measurement of \(T_2\). Imperfect refocusing pulses are inevitable when slice selective pulses are used (Crawley & Henkelman, 1987). The original sequence stipulates a decreasing spoiler gradient scheme (Fig. 3-1a) in order to remove the effects of stimulated echoes.
The main disadvantage of the decreasing spoiler gradient scheme is that the final spoiler is supposed to completely dephase magnetization across the slice, and the first spoiler is multiplied by the echo train length (ETL). For thin slices and long echo trains, this initial spoiler may be impossible to achieve. In addition, when combined with significant diffusion effects, the apparent $T_2$ decay becomes non-monoexponential.

If the spoiler gradient amplitude decreases with echo number, (Fig. 3-1a), then $T_2^{\text{app}}$ is expressed as a function of the echo number, $n$:

$$\frac{1}{T_2^{\text{app}}(n)} = \frac{1}{T_2} - \frac{D}{TE \cdot n} \sum_{i=1}^{n} b_i$$  \hspace{1cm} (3.7)

and the resulting signal decay curve can no longer be fit with a mono-exponential model.

One alternative to removing stimulated echoes using decreasing spoiler gradients is to fit for them using a technique developed by Prasloski et al. (Prasloski, Mädler, Xiang, MacKay, & Jones, 2012). Another option is to minimize them using non-selective refocusing pulses which have improved performance across the excited slice, reducing stimulated echoes, but exciting magnetization outside the slice of interest. Equally sized spoilers (Fig. 3-1c) are sufficient to spoil the out of slice signal as it is not excited by the initial slice selective excitation pulse. The size of these spoilers depends on the geometry of the sample being scanned and the coil sensitivity. Setting these spoiler gradients appropriately is a matter of trial and error as it depends on the geometry of the object being imaged as well as the behaviour of the RF pulse outside the slice of interest. Incrementing the size of the spoiler gradients until these unwanted echoes are removed requires time, so there may be a temptation to simply make them as large as possible while avoiding eddy current effects which will then increase the effects of diffusion.

### 3.3.4 Phase Encoding Gradients

Phase encoding gradients which bracket the readout, as in most multi-slice multi-echo (MSME) -style sequences, contribute diffusion weighting unevenly across the image as each phase encoding step acquires its own diffusion weighting. However they are smallest near the centre of k-space where the majority of diffusion contrast is encoded (Mattiello et al., 1997, 1994). A study of the diffusion sensitivity of MSME-style sequences, also referred to as turbo spin echo sequences, demonstrates that the phase encoding gradients account for $< 10\%$ of the total diffusion sensitivity generated by imaging gradients (Matthias Weigel & Hennig, 2012).
In the following sections the interaction between diffusion and the qT$_2$ sequence are investigated using simulations. They are then demonstrated in both phantoms and freshly excised rat spinal cord.

3.4 Simulations:

3.4.1 Effect of Resolution

To illustrate the relationship between resolution and apparent T$_2$ for the standard qT$_2$ sequence readout (Fig. 3-1b) and the refocused readout (Fig. 3-1d), the apparent T$_2$ was calculated based on Eqs. 3.9 & 3.10, using a T$_2$ of 80 ms and D of 0.8·10$^{-3}$ mm$^2$/s to approximate I/E water, and a T$_2$ of 2 s and D of 3·10$^{-3}$ mm$^2$/s to approximate CSF (Sener, 2001). The underestimation of T$_2$ is tissue dependent and increases with both T$_2$ and D. T$_2$ comparable to that of I/E water was selected to demonstrate the effects on typical neural tissue while T$_2$ of 2 s (equivalent to CSF) was selected to demonstrate the largest effect expected to arise in vivo. As the length of the sampling time relative to TE affected both sequences differently, Ts = TE/2 and Ts = TE/5 were plotted for both sequences.

Figure 3-2 shows the calculated effect of readout gradient strength on the apparent T$_2$, for both the standard (Fig. 3-1b) and refocused (Fig. 3-1d) readout gradient schemes, based on assumptions outlined above. At a resolution of 0.1 mm/voxel the standard readout resulted in <10% underestimation of T$_2$ in healthy gray and white matter; however, there was a >50% underestimation of T$_2$ for CSF. The resolution at which the refocused readout provides a significant improvement over the non-refocused readout depends on both the T$_2$ values being measured, and the rate of diffusion, and for healthy tissue occurs in the range of 0.02 to 0.2 mm/voxel. The transition from minimal error to very large error can be quite abrupt.
Figure 3.2. Relationship between Apparent T₂ and Linear Voxel Size
Relationship between apparent T₂ and linear voxel size for both the standard (black) and refocused (gray) sequences. I/E water (left) T₂ of 80 ms and D= 0.8*10⁻³ mm²/s; CSF (right) with T₂ of 2 s and D= 3*10⁻³ mm²/s. Actual T₂ is shown as a horizontal dashed line. Relationship between sampling time (Ts) and echo time (TE) affects the two sequences differently: TE / Ts = 2 (dash-dotted lines) and TE / Ts = 5 (solid lines). Note the differences in scale.

3.4.2 Myelin Water Fraction
Monte Carlo simulations to investigate the effects of varying parameters on MWF estimation were performed using MatLab (MathWorks, Natick, VA). T₂ distributions were simulated 1000 times each using a MWF of 20%, and T₂ values of 15 ms for the myelin water, and 80 ms for I/E water (MacKay et al., 2006). The idealized decay curve was calculated and adjusted for diffusion using Eq. 3.4 with apparent diffusion coefficient, D=1.0*10⁻³ mm²/s, selected to be slightly higher than typically seen in I/E water (Sener, 2001) to avoid underestimation of the effect on MWF, b₂ T₂ values of 0, 10, and 20 s/mm², TE 5 ms, and echo train length of 128. Gaussian distributed noise was generated and added to the idealized decay curve. SNR was calculated by taking the sum of all the M₀ values used for a particular decay curve, and dividing by the scaling parameter used to generate the noise. The MWF was measured by calculating the relative fraction (area under the curve) of the short T₂ component. Statistical significance of the difference resulting from b value for a given SNR was assessed using a Student's t-test with alpha of 1%

Figure 3-3 shows histograms of MWF for SNR of 100 and 500 and b₂ T₂ values of 0 (no diffusion) and 20 s/mm².
Figure 3-3. Effect of SNR on MWF
MWF histograms for $T_2$ distributions with I/E water component located at 80 ms and short component located at 15 ms. Actual MWF is 20%. Histograms of the fitted MWF are shown for SNR values of 100 (gray) and 500 (black) and b values of 0 (solid) and 20 s/mm$^2$ (dashed).

An SNR of 500 was sufficient to measure MWF accurately (mean MWF = 19±1%), in which case a large amount of diffusion weighting ($b_{qT_2}=20$ s/mm$^2$) resulted in a mean MWF of 18±1%. For an SNR of 100, already resulting in a decreased MWF of 16±3%, the effect of diffusion was more pronounced, with $b_{qT_2}=20$ s/mm$^2$ reducing the MWF estimate to 12±5%. In other words, if the MWF were already underestimated, the presence of diffusion exacerbated the situation. These differences were statistically significant for both SNR 100 and 500.

3.4.3 Decreasing Spoiler Gradient
To demonstrate the effects of decreasing spoiler gradients (Fig. 3-1a), mono-exponential and bi-exponential signal decay curves were calculated using Eq. 3.7 to satisfy the decreasing spoiler gradient sequence as described in Poon & Henkelman (Poon & Henkelman, 1992) for a 32 echo sequence.

Parameters were TE of 10 ms to adequately measure the short $T_2$ component, apparent diffusion coefficient of $0.8\cdot10^{-3}$ mm$^2$/s, $T_2$ of 80 ms for mono-exponential decay, and $T_2$s of 20 ms and 80 ms with 20/80% weighting for bi-exponential decay to approximate values seen in gray and white matter (Sener, 2001). For the non-negative least squares (NNLS) analysis an energy constraint of $\mu=20,000$ was used (see Eq. 3.11) to provide a small degree of smoothing. Refocusing pulse length $t_r=0.4$ ms, and slice thicknesses of 5 mm, 1
mm and 0.5 mm were used. Spoiler gradient durations were calculated assuming a gradient strength of \( G_s = 500 \text{ mT/m} \), to fully dephase the magnetization across the entire slice: 
\[
t_s = \frac{2\pi}{\gamma G_s \Delta z}.
\]

From Eq. 3.7 it is clear that \( T_2 \) distributions resulting from decreasing spoiler gradients are more complex in the presence of diffusion. Assuming a maximum gradient amplitude of 500 mT/m resulted in spoiler gradient durations of 0.3 ms, 1.5 ms, and 3.0 ms for slice thickness of 5 mm, 1 mm, and 0.5 mm respectively. Figure 3-4 shows the theoretical effects of decreasing spoiler gradients on the bi-exponential \( T_2 \) distribution in the absence of noise using minimal energy constraints. The average apparent \( T_2 \) decreased with slice thickness and the single well-defined I/E water peak altered its shape so that the distribution no longer appeared bi-exponential, although the MWF was unchanged. In the case of mono-exponential decay the single peak broadened substantially and asymmetrically, and the mean \( T_2 \) value decreased.

![Figure 3-4. Effect of decreasing spoilers on \( T_2 \) distribution](image)

\( T_2 \) distributions from bi-exponential transverse relaxation with short \( T_2 = 20 \text{ ms} \) containing 20% of the initial signal, and long \( T_2 \) of 80 ms, and apparent diffusion coefficient \( D = 0.8 \cdot 10^{-3} \text{ mm}^2/\text{s} \). These data were simulated assuming decreasing spoiler gradients required for a slice thickness of 5 mm (gray), 1 mm (blue-dashed) and 0.5 mm (red). Other parameters were: maximum spoiler gradient amplitude of 500 mT/m, TE of 10 ms and ETL 32.

### 3.5 Experimental Methods

All MR experiments were conducted on a 7 T horizontal bore Avance BioSpec 70/30 scanner (Bruker BioSpin, Ettlingen, Germany) using either a home-built 3cm solenoid coil for both transmit and receive or an 8cm inner diameter volume coil for transmit with a rat brain coil array, and 20cm inner diameter gradient insert coil with maximum gradient amplitude of 668 mT/m (Bruker BioSpin, Ettlingen,
Experiments were conducted on manganese chloride (MnCl₂) phantoms and freshly excised rat spinal cord.

### 3.5.1 qT₂ sequence details:
All qT₂ measurements were performed using a slice selective excitation pulse, and a composite (90,180,90) refocusing pulse with a duration of 0.36 ms and bandwidth of 4444Hz to achieve a more uniform flip angle across the slice and minimize the effect of stimulated echoes.

Two different CPMG-based qT₂ sequences were used: a fully refocused readout sequence, and an MSME-style readout sequence. For the fully refocused readout qT₂ sequence, a first order flow compensated readout gradient (Hinks & Constable, 1994; Simonetti et al., 1991) (Fig. 3-1d) replaced the standard readout (Fig. 3-1b). Equally sized spoiler gradients (Fig. 3-1c) were applied in the slice select direction, bracketing each refocusing pulse. All phase encoding gradients were rewound between echoes.

The MSME-style readout qT₂ sequence used an extended readout gradient, similar to the standard readout (Fig. 3-1b) in that the prewinder followed the excitation, with additional readout prewinder gradients turned on concurrently with the phase encoding gradients to minimize eddy currents during the readout. Otherwise the MSME-style qT₂ sequence was identical to the refocused readout qT₂ sequence.

### 3.5.4 qT₂ Data Analysis
The T₂ distribution was calculated from the T₂ decay curves using a non-negative least squares algorithm implemented in MatLab (The MathWorks, Nattick, VA). Energy constraints were used:

\[
\chi^2 = \sum_{i=1}^{N} \left( \sum_{j=1}^{M} A_{ij} s_j - y_i \right)^2 + \frac{1}{\mu} \sum_{j=1}^{M} s_j^2
\]

where the \( s_j \) are the fitted values, \( y_i \) are the measured values, and \( A_{ij} \) is a matrix of the form \( e^{-TE_i/T2(j)} \) for a set of 400 T₂ values logarithmically spaced between 3 ms and 5 s. \( \chi^2_{\text{min}} \) is the value obtained setting \( 1/\mu = 0 \).

Then \( \mu \) is selected such that: 1.02 \( \cdot \chi^2_{\text{min}} < \chi^2 < 1.025 \cdot \chi^2_{\text{min}} \) (Graham, Stanchev, & Bronskill, 1996).

To verify the performance of the refocusing pulses, the method developed by Prasloski et al. (Prasloski et al., 2012) was used to estimate the actual flip angle of the refocusing pulses on a voxel by voxel basis. The objective function, \( A_{ij} \), for the NNLS fitting algorithm described above was replaced by an objective function generated using the extended phase graph algorithm for 40 logarithmically spaced T₂ values ranging from 0.015 s to 2 s, T₁ of 1 s, and a train of refocusing pulses with 8 flip angles linearly spaced between 50°
and 180°. NNLS fits with no smoothing were calculated for each flip angle, back projected, and the sum of squares fit to the original decay curve was calculated. An interpolating spline was used to extract the minimum sum of squares and corresponding flip angle for each voxel in a region of interest.

### 3.5.2 Phantom Experiments:

A series of phantoms containing water doped with MnCl₂ were prepared to cover a broad range of T₂ values. Beginning with a 52.5 ± 1.6 mg/ml stock solution, a series of dilutions were made resulting in a set of phantoms doped with 52.5±1.7, 26.2±0.9, 15.0±0.5, 10.5±0.3, 8.8±0.3, and 4.4±0.1 ∙10⁻³ mg/ml MnCl₂ in sealed NMR tubes with a 5 mm inner diameter. NMR tubes were placed in a Delrin holder built to fit inside the coil and reduce susceptibility artefacts so that they could all be imaged simultaneously. Scanning was performed at room temperature (21±1 °C), measured using a thermometer placed inside the scan room.

To investigate the effect of increasing b_qT₂ at constant resolution, images were acquired at an in plane resolution of 0.40 mm using fully refocused readout gradients (Fig. 3-1d), with TE 7 ms, TR 3 s, ETL 128, 1 mm slice thickness, 25.6 mm field of view, and equally sized spoiler gradients of 200.4 mT/m (30% of max available gradient amplitude) for three different time intervals, 0.5, 1.0 and 1.5 ms, or at 334 mT/m (50% of max) for 1.5 ms resulting in b_qT₂ values of 0.6, 2.7, 8.1, and 21.8 s/mm² respectively.

To compare the impact of different readout schemes, images of the 8.8·10⁻³ mg/ml MnCl₂ phantom were acquired at in-plane resolutions of 0.05, 0.06, 0.075, 0.10, 0.20 and 0.40 mm/pixel, TE 10 ms, TR 3 s, ETL 128, 1 mm slice thickness, 12.8 x 12.8 mm² field of view, and 200.4 mT/m spoiler gradients with 1.5 ms duration. Both the refocused and MSME-style readout schemes were used.

To quantitatively analyze the data regions of interest were drawn on the resulting images and apparent T₂ values were calculated by fitting the data to Eq. 3.3 using the fmincon fitting algorithm in MatLab (The MathWorks, Nattick, VA) and then compared with predicted values calculated using Eq.3.4. Stimulated echo effects were fit according to the method of Prasloski et al. (Prasloski et al., 2012), described above.

### 3.5.3 Freshly excised spinal cord:

Spinal cord samples were obtained from three male Sprague Dawley rats (~400g). The animals were sacrificed via euthanyl injection, and a section of cervical spinal cord was removed. Images were acquired within 2 hours of sacrifice. The qT₂ sequence used a refocused readout, spoiler amplitude 334 mT/m and duration 1.5 ms (b_qT₂ = 21.5 s/mm²), TE 7 ms, TR 3 s, ETL 100, FOV 25.6 cm, 0.2 mm in-plane
resolution, 1 mm slice thickness. Images were acquired both perpendicular and parallel to the spinal cord. Regions of interest were drawn in corresponding areas of gray and white matter, and the mean decay curves were analyzed using NNLS described below. Stimulated echo effects were calculated according to the method of Prasloski et al. (Prasloski et al., 2012). I/E water T₂ was calculated as the geometric mean of the T₂ distribution between 50 and 120 ms. MWF was defined as the percentage of the T₂ distribution between 10 and 35 ms. Statistical significance of changes in T₂ and MWF were assessed using a Student's t-test with alpha of 1%.

3.6 Results

3.6.1 Effect of diffusion on measured T₂

To demonstrate the effect of increasing diffusion weighting on apparent T₂ and validate Eq. 3.4, qT₂ data were acquired in an MnCl₂ doped water phantom with five T₂ values ranging from 20 to 200 ms, and four different spoiler gradients generating b_qT₂-values ranging from 0.6 to 21.8 s/mm². Figure 3-5a) plots the actual T₂ values of the samples vs the T₂ values measured with increasing b_qT₂ values. The dashed line shows the predicted values calculated using Eq. 3.4 based on the sequence parameters, T₂ values calculated from MnCl₂ concentrations and diffusion coefficient of water at 21 ºC. The measured values were all within 2.5% of the predicted values. Fitting for stimulated echoes resulted in a calculated flip angle of 180°, indicating that no stimulated echoes were present.

Figure 3-5b) shows the relationship between resolution and apparent T₂ for various readout schemes for the MnCl₂ doped water phantom with an actual T₂ of 123 ms. The apparent T₂ at an in-plane resolution of 0.1 mm/pixel was within 5% of the calculated T₂ for the refocused readout; however, the MSME-style readout caused a greater than 10% decrease in apparent T₂ even at an in-plane resolution of 0.40 mm/pixel. At the lowest resolution, 0.40 mm/pixel, the apparent T₂ values for the refocused and MSME-style readout schemes were 122±1 ms and 108±1 ms respectively, while at the highest resolution, 0.05 mm/pixel, they decreased to 97±1 ms and 58±1 ms. Fitting for stimulated echoes resulted in a calculated flip angle of 180°, indicating that no stimulated echoes were present.
Figure 3-5. Actual T₂ (a) and resolution (b) vs measured T₂ in MnCl₂ phantoms. Data in (a) was acquired at an in-plane resolution of 0.4 mm/pixel. Actual T₂ vs measured T₂ in MnCl₂ phantoms over a range of different T₂ values. Dashed lines are fits to Eq. 3-4. Data in (b) was acquired for the MnCl₂ phantom with actual T₂ of 123 ms for both refocused readout (squares) and MSME-style readout (circles) over in-plane resolutions from 0.05 - 0.40 mm/pixel.

Figure 3-6 shows representative T₂ distributions measured in rat spinal cord for gray matter (a), and white matter (b) sensitive to diffusion both parallel (black solid line) and perpendicular (gray dashed line) to the spinal cord. Regions of interest contained approximately 15 voxels and the resulting decay curves had an SNR of ~75. Mean apparent T₂ measured in gray matter was 46±2 ms regardless of imaging orientation, while the mean long T₁ component in white matter decreased significantly from 68±3 ms to 54±1 ms when diffusion sensitivity was parallel rather than perpendicular to the spinal cord. The changes in MWF and myelin water T₂ (mean ± standard deviation) from 18±3% and 15±2 ms to 15±8% and 13±5 ms were not statistically significant. Fitting for stimulated echoes resulted in a calculated flip angle of 180°, indicating that no stimulated echoes were present. The diffusion weighting in these images was due almost entirely to the spoiler gradients and therefore most affected by through-plane diffusion which is higher in white matter when the imaging slice is oriented perpendicular to the spinal cord. The spoiler gradients resulting in $b_{q12} = 21.5 \text{ s/mm}^2$, used to generate these artefacts and illustrate the effect, were much larger than required to spoil the out of slice signal.
Figure 3-6. Diffusion parallel and perpendicular to the rat spinal cord. Rat spinal cord sensitive to diffusion parallel (solid line, c) and perpendicular (dashed line, d) to the spinal cord. The large gradients needed for micro-imaging result in an apparent decrease in $T_2$ due to diffusion. Spoiler gradients cause through-plane diffusion effects which result in significantly different $T_2$ values being measured for white matter (WM, b) depending on the orientation of the imaging plane. Orientation of the imaging plane does not affect the gray matter (GM, a) $T_2$ values as diffusion is isotropic. The shift in location of the short $T_2$ peak in WM was not statistically significant and is likely due to noise rather than the effect of diffusion. Because of the rapid decay of the myelin-associated water, only the first few data points in the $T_2$ decay curve contribute to this peak, increasing the variability seen in the $T_2$ of the short peak relative to the I/E water peak.
3.7 Discussion

CPMG sequences, considered a gold standard for the quantitative T$_2$ measurement, are insensitive to diffusion due to background gradients provided they are acquired with short echo times. However, the contributions of imaging gradients to diffusion weighting cannot be neglected (Mattiello et al., 1994) and their impact on CPMG-based sequences using low refocusing flip angles and slice selective pulses has been demonstrated (Matthias Weigel & Hennig, 2012). In this study as resolution increased even the qT$_2$ sequence, which incorporated hard 180° refocusing pulses, was observed to suffer from signal loss due to diffusion weighting caused by imaging gradients, leading to a predictable underestimation of T$_2$ relaxation time. This effect was the result of the large number of gradients used for imaging an echo train and was present regardless of technique used to analyse the data.

The degree of T$_2$ underestimation depended on the rate of diffusion and the T$_2$ value of the sample, as well as the b$_{qT2}$ parameter of the readout and spoiler gradients. Analyzing Eq. 3.4 it would seem that this underestimation was mitigated by using a longer TE; however, this was only the case when the b$_{qT2}$ value was not itself dependent on TE. Eq. 3.4 was validated in an MnCl$_2$ phantom and therefore, could be used to correct T$_2$ values if the diffusion of the sample is known. However, the resulting loss of SNR makes it preferable to minimize the diffusion sensitivity. The T$_2$ dependence means that the degree of underestimation cannot be accurately predicted until after the T$_2$ has been measured, implying that phantom experiments measuring the accuracy of T$_2$ measurement must cover the entire expected range of T$_2$ values.

The b$_{qT2}$ value resulting from readout gradients increased with image resolution. The b$_{qT2}$ values resulting from the MSME-style readouts were much larger than the corresponding b$_{qT2}$ values for the standard and refocused readouts. It is unlikely that resolutions of 1 mm/pixel and lower will generate b$_{qT2}$ values large enough to cause a noticeable decrease in T$_2$. The typical gradients used to achieve 1 mm/pixel resolution would result in a less than 1% underestimation of T$_2$ values ~100 ms, and less than 5% underestimation of T$_2$ values ~2 s. At resolutions higher than 1 mm/pixel it becomes increasingly important to use either the standard or the refocused readout as the underestimation increases exponentially with resolution. In this study, because of its high T$_2$ and diffusion coefficient, the apparent T$_2$ of CSF was the most affected of all water compartments present in healthy brain. As the apparent T$_2$ of CSF approached ~500 ms, there was an increasing possibility that it could be interpreted as I/E water with long T$_2$, a situation which has previously been linked to inflammation (Webb et al., 2003). This suggests a maximum acceptable underestimation of CSF of ~50% which occurs for the standard readout at a resolution of ~0.2 mm/pixel and for the
refocused readout at \( \sim 0.1 \) mm/pixel. To achieve \( T_2 \) measurements at higher resolution, different imaging sequences should be employed (Hsu et al., 1995).

Decreasing spoiler gradients were originally recommended by Poon and Henkelman (1992) to remove stimulated echoes resulting from imperfect refocusing pulses. They recommended against using refocused imaging gradients as these would eliminate the image artefacts generated by the stimulated echoes by overlaying them on the image, altering the measured \( T_2 \). A new post-processing method has been developed by Prasloski et al (2012) which corrects for the presence of stimulated echoes. In this thesis, fitting for the actual refocusing angle on a voxel by voxel basis resulted in flip angles of 180°, indicating that the refocusing pulses were almost perfect, and that stimulated echoes were not present. This was due to the combination of hard, composite, refocusing pulses, and the small bore animal MRI scanner used in this study.

Spoiler gradients were still required to remove out of slice magnetization arising from non-slice-selective refocusing pulses, and the effects of their \( b_{qT_2} \) values were additive with the readout gradient \( b_{qT_2} \) values. When they were applied in the slice rather than readout direction, as is typical for the \( qT_2 \) sequence, they increased the difficulty of correcting for diffusion in anisotropic tissues such as white matter. The gradient strengths can be specified in software as a percentage of the maximum available gradient, and an inexperienced user can easily set these much higher than required.

The primary purpose of most studies incorporating quantitative \( T_2 \) is to measure MWF as a measure of demyelinating diseases (Kozlowski, Raj, et al., 2008; Laule et al., 2008; Sirrs et al., 2007) rather than the actual \( T_2 \) values of the various water compartments. The \( T_2 \) of myelin water is relatively short, on the order of 10-50 ms (MacKay et al., 2006), which minimizes the impact of diffusion on this compartment; however, diffusion decreases the time available to sample the \( T_2 \) decay curve before it reaches the noise floor (Fig. 3-7a), increasing the SNR required to adequately sample the data. The SNR requirements for properly resolving components in the \( T_2 \) distribution of white matter, which are separated by a factor of three, have been described (Graham et al., 1996). However since the \( T_2 \) underestimation increases with increasing \( T_2 \), the I/E water peak decreases more than the short peak and they shift closer together (Fig. 3-7b). Peaks which are closer together require higher SNR to be properly distinguished (Fig. 3-3). Thus the SNR requirements for properly estimating MWF are significantly increased in the presence of diffusion, and are strongly affected by the \( T_2 \) values of the various water compartments. The best solution to these problems is to minimize diffusion and be aware of the increased SNR requirements for MWF estimation in the presence of shorter apparent \( T_2 \) values.
Figure 3-7. Effect of diffusion on SNR
Simulated bi-exponential decay curves (a) and corresponding $T_2$ distributions (b) for $b$-values of 0 s/mm$^2$ (solid line) and 20 s/mm$^2$ (dashed line) for apparent diffusion coefficient $D = 0.8 \cdot 10^{-3}$ mm$^2$/s and $T_2$ values of 20 ms and 80 ms with a 20/80 weighting. The threshold at which the signal becomes indistinguishable from noise is indicated for SNR values of 100 (dotted line) and 500 (dot-dashed line).

In the excised rat spinal cord the long $T_2$ component in white matter decreased from $68 \pm 3$ ms to $54 \pm 1$ ms when switching diffusion sensitivity from parallel to perpendicular orientation relative to the spinal cord. While it is possible that some of this decrease was due to a partial volume effect with gray matter, which had a much shorter $T_2$ of $46 \pm 2$ ms, a partial voluming of 50% would be required to account for the observed decrease.

Finally, the gradient strengths required to implement decreasing spoiler gradient schemes are fairly prohibitive in the case of either long echo trains or thin slices. For slices thinner than 1 mm, they can result in additional peaks in the $T_2$ distribution. This is similar to the distortions to the $T_2$ distribution which arise from nonlinear echo time spacing (Does & Gore, 2000). As decreasing spoiler gradient schemes are primarily required for slice selective refocusing pulses, which require additional slice selective gradients thus increasing the diffusion sensitivity, they should be avoided when using slices thickness less than 1 mm.
3.8 Conclusions

Diffusion weighting, arising from the increasingly powerful gradients now available, results in an underestimation of $T_2$. This effect depends very strongly on the actual $T_2$ and diffusion coefficient of the tissue being imaged. To prevent healthy tissue being mistaken for pathology, the standard $qT_2$ readout can be used up to resolutions of 0.2 mm/pixel, while the refocused readout will continue to perform accurately up to a resolution of 0.1 mm/pixel. At higher resolutions, a multi-echo readout is no longer appropriate.

In conclusion, diffusion can significantly alter the $T_2$ values obtained when working with the very large gradients required for micro-imaging, resulting in underestimation of $T_2$, and increasing the SNR required to avoid underestimating MWF. It is recommended that decreasing spoiler gradients be avoided as they increase the complexity of the $T_2$ distribution, and that the $b_{qT2}$ values arising from both readout and spoiler gradients be reported to allow improved literature comparison.
Chapter 4 - Quantitative MRI of a Non-Surgical Model of Cervical Spinal Cord Injury in the Rat

This section describes the quantitative MR measurements in the MR-guided FUS model of spinal cord injury (SCI) developed in Chapter 2. A subset of the animals used to develop the model were followed for up to 2 weeks using qT₂ and diffusion MRI. These quantitative MR measures are correlated with pathology. This work has been submitted to NMR in Biomedicine.

4.1 Introduction

Magnetic Resonance Imaging (MRI) is an important tool in the assessment of SCI as it permits the non-invasive visualization of changes to the spinal cord. Conventional T₁ and T₂ weighted imaging are commonly used to identify alterations to the vertebral canal, cord compression, and lesion extent (Boldin et al., 2006; Lammertse et al., 2007; Miyaji et al., 2007). Various patterns of abnormality observed on MR images have been identified as corresponding with particular types of spinal cord damage. Bondurant et al (1990) propose three patterns of abnormality depicted on MR images: The type I abnormality is depicted as a region of decreased signal intensity surrounded by a thin rim of high signal intensity, consistent with intraspinal hemorrhage, on T₂-weighted images. The type II pattern is depicted as a region of high signal intensity, representing cord edema, on T₂-weighted images. The type III pattern presents as a central region of hypointensity mixed with a peripheral region of high signal intensity, consistent with contusion, on T₂-weighted images. Additionally, loss of contrast between gray and white matter is considered to be indicative of edema in white matter (Bilgen, Abbe, Liu, & Narayana, 2000; Narayana, Abbe, et al., 1999).

While MRI is highly sensitive to changes in spinal cord tissue, the signal changes observed on standard MRI are not specific to the underlying pathology. The resolution of MRI typically ranges from 0.05 to 1 mm in plane, thus even the smallest voxel contains thousands if not millions of cells. It is possible to have more than one pathology present in the same MR voxel, in which case a signal decrease due to one effect could be obscured by an increase due to the other. Moreover, pathologies such as inflammation, demyelination or axonal loss increase both the average T₁ and T₂ relaxation times (McCreary et al., 2009; Merkler et al., 2005; Odrobina et al., 2005; Ohshio, Hatayama, Kaneda, Takahara, & Nagashima, 1993; Zhang, Jones, McMahon, Mori, & Calabresi, 2012) resulting in similar pattern of signal behaviour. In addition, the pathological presentation of SCI is bi-phasic, with the acute phase characterized by hemorrhages, edema and cell necrosis lasting at least 24 hours, followed by the chronic inflammatory phase with formation of a cavity
infiltrated primarily by macrophages (Hill et al., 2001; Tator & Fehlings, 1991; Weber et al., 2006). These
distinct phases, suggest that signal changes on MR images represent different pathologies at different time-
points (Bilgen et al., 2007; Falconer, Narayana, Bhattacharjee, & Liu, 1994; Miyanji et al., 2007; Oakden
et al., 2014; Weber et al., 2006).

Quantitative imaging methods can be used to gain information about water molecules in different
compartment within a cell, or group of cells, even though the precise physical locations of these
compartment cannot be determined. Quantitative T$_2$ is one such technique, and reveals the presence of
multiple water components relaxing with different T$_2$ relaxation times (MacKay et al., 1994, 2006; Stanisz
et al., 1999; Whittall et al., 1997). This is especially informative in white matter where water trapped in
the myelin sheath surrounding axons has a significantly shorter T$_2$ than either intra- or extra-cellular water.
The T$_2$ decay curve of healthy white matter can be decomposed into 3 separate pools based on T$_2$ relaxation
time. The shortest of these relaxation times (10-50 ms) is correlated with water trapped within the myelin
sheath, the intermediate relaxation time (70-90 ms) with I/E water, and the longest (~2 s) with CSF
(MacKay et al., 2006). qT$_2$ has been used in healthy spinal cord and in SCI to look at myelin (Dula et al.,
2010; Kozlowski, Liu, et al., 2008; Kozlowski, Raj, et al., 2008). In SCI, a more complicated T$_2$ pattern is
observed. Due to the sensitivity of T$_2$ to microstructural changes, histology is required to determine the
pathologic significance of a particular T$_2$ distribution. Once the underlying pathology is well understood,
qT$_2$ may provide a powerful tool for investigating a disease, as is the case with multiple sclerosis (Laule et
al., 2006).

Another technique widely used to assess SCI is diffusion tensor imaging (DTI). DTI is a quantitative
technique which measures the rate and direction of diffusion in tissue, and is very sensitive to the effects of
microscopic tissue properties including the presence and structural integrity of myelin (Norris, 2001). DTI
has been used extensively in the investigation of SCI (Deo et al., 2006; Kozlowski, Raj, et al., 2008; Loy et
al., 2007; Sundberg et al., 2010; Tu, Kim, Yin, Jakeman, & Song, 2013) where it is more sensitive in
detecting early pathological changes than conventional MRI (Lammertse et al., 2007).

The goal of this study was to probe the correlations between features observed using the full T$_2$ distribution
and histopathology in a rat model of SCI at 24 hours (during the acute phase) and at 2 weeks post-injury,
during the inflammatory phase of the SCI. DTI was also performed as it is the most commonly used
quantitative MR technique used in the imaging of SCI.
4.2 Methods

4.2.1 Animal Preparation
The induction of SCI using MR guided FUS is described in Chapter 2, section 2.2. In addition, 6 healthy control rats were imaged with MRI but did not receive FUS treatment.

4.2.2 MRI
Quantitative MRI experiments were carried out on three groups of animals: a control group of 6 healthy animals, an acute group of 8 animals (scanned and euthanized at 24 hours post-FUS), and a chronic group of 8 animals; 6 of which were scanned at 24 hours, 1 week, and 2 weeks, and euthanized at 2 weeks post-FUS; and 2 which were scanned at 24 hours, 1 week, and euthanized at 1 week post-FUS.

All MR experiments were conducted on a 7 T horizontal bore Avance BioSpec 70/30 scanner (Bruker BioSpin, Ettlingen, Germany) using an 8 cm inner diameter volume coil for transmit, either the same coil or a rat brain coil array to receive, and a 20 cm inner diameter gradient insert coil with maximum gradient amplitude of 668 mT/m (Bruker BioSpin, Ettlingen, Germany).

MRI was conducted under 2% isoflurane anesthesia. T₂-weighted fast spin echo sequence with 8 echoes, TE/TR 35/2500, 3x4 cm field of view, 200x266 matrix, 1 mm slice thickness, 4 averages, 50 kHz bandwidth was used to acquire images both coronal and sagittal to the spinal cord. The coronal slice with the greatest visible T₂-weighted signal abnormality was selected for quantitative imaging. The slices were all located between the C1 and C4 vertebrae. When no abnormalities were visible, a slice located at the caudal end of the C2 vertebra was used.

\( qT_2 \) measurements were performed using a single slice sequence with slice selective excitation pulse, and a composite (90,180,90,\) refocusing pulses with a duration of 0.36 ms and bandwidth of 4444Hz bracketed by equally sized spoiler gradients of 0.33 ms and 133.5 mT/m. TE 5 ms, TR 3 s, 80 echoes, 0.2x0.2x1.0 mm³ resolution and 2 averages, lasting 20 minutes. Readout and phase encoding gradients were fully rewound between refocusing pulses to minimize diffusion effects as described in Chapter 3 (Oakden & Stanisz, 2014). Per echo b-values \( (b_{qT_2}) \) were 0.3 s/mm² for readout gradients and 0.03 s/mm² for spoiler gradients.

DTI data was acquired with a spin echo single-shot EPI readout sequence, TE 32 ms, TR 4 s, 18 diffusion-sensitizing directions (Cook, Symms, Boulby, & Alexander, 2007), and b-values of 0 and 800 s/mm², 3x3 cm FOV, 1 mm slice thickness , 4 averages, and 150x150 matrix.
To align MRI with histology sections *ex vivo* images following formalin fixation of the spinal cord tissue en bloc were also acquired. A sagittal $T_2$-weighted fast spin echo sequence was acquired with 16 echoes, TE/TR 62/2000, 1.6x4.4 cm field of view, 160x440 matrix, 1 mm slice thickness, 16 averages, and 50 kHz bandwidth. An axial $T_2$-weighted fast spin echo sequence was also obtained with 16 echoes, TE/TR 66/2000, 1.4x1.4 cm field of view, 140x140 matrix, 1 mm slice thickness, 60 averages, and 50 kHz bandwidth.

### 4.2.3 Histology

Immediately following their final MRI session animals were deeply anaesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg), the chest opened and 100 IU sodium heparin injected into the left ventricle. The blood was washed out with lactated Ringer’s solution (Baxter, Canada) via the left cardiac ventricle with the outflow created by cutting the right heart auricle (Kwiecien et al., 2000). The rats were perfusion-fixed in 4% paraformaldehyde, pH 7.4. Spinal cords were removed and post-fixed in 10% buffered formalin for 48 hours and imaged using MRI (as above). Spinal cords were then sliced into 3-4 coronal segments 3 mm thick and the *ex vivo* MRI was used to orient the cord so that the second segment would be aligned with the quantitative MR images. Segments were then embedded in 3% agar (Shaw et al., 1983), processed in increasing concentrations of ethanol and xylene, and embedded in paraffin. Cross sections 5 µm and 10 µm thick were cut and mounted on glass slides. Ten µm sections were stained with LFB for myelin and counterstained with cresyl violet. Five µm sections were stained with H&E. Histological analysis was performed by a veterinary histopathologist, under a Nikon Eclipse 50i microscope and areas corresponding to ROIs selected for quantitative MRI analysis were photographed.

### 4.2.4 Data Analysis

MRI ROIs were drawn on the 3rd echo of the $qT_2$ echo train (TE = 15 ms). Five ROIs were drawn on the injured side of the spinal cord (Fig. 4-1), in both the dorsal horn (DH) and ventral horn (VH) in gray matter and in the dorsal column (DC), lateral column (LC) and ventral column (VC) in white matter.
**Figure 4-1. Spinal cord ROIs**
ROIs drawn on the luxol fast blue section (a) and the third echo of the qT2 sequence (b). Scale bars represent 1 mm. WM regions consisted of the ventral column (VC), lateral column (LC) and dorsal column (DC), while GM regions consisted of the ventral horn (VH) and the dorsal horn (DH).

Luxol fast blue (LFB) histology sections were used to ensure that ROIs contained primarily damaged or normal appearing tissue, and to confirm the boundaries between gray and white matter (Fig. 4-1). The corresponding areas in both the H&E and LFB stained histology sections were assessed on a scale of 0 to 2 for the presence of hemorrhage, vacuolation/edema, and demyelination, with 0 indicating the absence of that pathology, 1 mild pathology (present in <25% of the ROI), and 2 severe pathology (present in >25% of the ROI). Examples of this can be seen in Figure 4-4 with no pathology in DC (Fig. 4-4 l), mild pathology present in VC and LC (Fig. 4-4 d&g), and severe pathology in both VH and DH (Fig. 4-4 e&i) ROIs. For the data acquired at 24 hours and 1 week in the chronic group, ROIs were drawn on the images acquired at 2 weeks, and then transferred to the images acquired at 1 week and 24 hours.

Alignment of MRI and histology images was performed by placing the boundary between 3 mm sections of spinal cord at the same location as the MR slice of interest, determined by measuring the distance from the base of the cerebellum. Within the slice, corresponding regions on MRI and histology were determined by manually aligning both images based on visible structures or the boundary of the spinal cord assuming uniform shrinkage across the cord.
4.2.5 qT₂ Analysis

The sum of the signal within each ROI was calculated for every echo in the qT₂ echo train to obtain a T₂ decay curve. The corresponding T₂ distributions were determined for each ROI using NNLS in MatLab (The MathWorks, Nattick, VA) with energy constraints (Whittall et al., 1997):

\[ \chi^2 = \sum_{i=1}^{N} \left( \sum_{j=1}^{M} A_{ij} s_j - y_i \right)^2 + \frac{1}{\mu} \sum_{j=1}^{M} s_j^2 \]  

(11)

where the \( s_j \) are the fitted values, \( y_i \) is the sum of the signal for the \( i^{th} \) echo, and \( A_{ij} \) is a matrix of the form \( e^{-\frac{TE_{ij}}{T_2(j)}} \) for a set of 400 possible T₂ values logarithmically spaced between 1 ms and 5 s, and TE of 5 ms corresponding to that used when acquiring the qT₂ data. \( \chi^2_{\text{min}} \) is calculated by setting \( 1/\mu = 0 \), then \( \mu \) is selected such that \( 1.02 \cdot \chi^2_{\text{min}} < \chi^2 < 1.025 \cdot \chi^2_{\text{min}} \) (Graham et al., 1996).

4.2.5.1 Classification of peaks in the T₂ distribution

Peaks in the T₂ distribution were classified as belonging to one of 5 different categories. Very short T₂ (VS T₂) peaks, myelin water (MW) (WM only), normal I/E water, long I/E water, and CSF or very long (VL) water in the cystic cavity.
Figure 4-2. Example $T_2$ distributions for normal and injured white matter
$T_2$ distributions for normal (gray dashed) and injured (black) white matter containing all
abnormalities observed in injured tissue. Gray bands indicate approximate ranges for MWF (3-
39 ms) and long I/E water (77-250 ms). Not all features were present in every injured $T_2$ ROI.

The range of $T_2$ values for normal I/E water was determined by calculating the average ± 3 standard
deviations, over all the white and gray matter ROIs in normal controls, shown in Table 4-1 below. In white
matter the range of $T_2$ values corresponding to a VS $T_2$ peak, and a MW peak was determined by first
examining the subset of $T_2$ distributions in injured spinal cord WM which had two peaks with mean $T_2$
values less than the minimum $T_2$ for I/E water. There was no overlap between these two groups, and the
boundary between VS $T_2$ and MW peaks was considered to lie between the longest mean $T_2$ in the VS $T_2$
group and the shortest mean $T_2$ in the MW group with multiple peaks. Any peak falling between these two
groups was considered to be MW if its volume fraction was less than 30%. The fractional area of each peak
was calculated with respect to the total area under the $T_2$ distribution, neglecting the VS $T_2$ peak. The area
of the VS $T_2$ peak itself was calculated with respect to the total area under the $T_2$ distribution, resulting in
total volume fractions greater than 1. Finally, the weighted average $T_2$ of the VS $T_2$ and MW peaks was
calculated, referred to as the $T_2$ of the short component.
Table 4-1 Range of $T_2$ values for the different components in the $T_2$ distribution for both white and gray matter.

<table>
<thead>
<tr>
<th></th>
<th>White Matter</th>
<th>Gray Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Short</td>
<td>&lt; 3 ms</td>
<td>&lt; 41 ms</td>
</tr>
<tr>
<td>MWF</td>
<td>3-39 ms</td>
<td>N/A</td>
</tr>
<tr>
<td>Normal I/E Water</td>
<td>40-77 ms</td>
<td>42-53 ms</td>
</tr>
<tr>
<td>Long I/E Water</td>
<td>78-250 ms</td>
<td>54-250 ms</td>
</tr>
<tr>
<td>Very Long</td>
<td>&gt;250 ms</td>
<td>&gt;250 ms</td>
</tr>
</tbody>
</table>

4.2.6 Diffusion Analysis

The diffusion tensor was calculated for each voxel using a linear least squares regression implemented in-house using MatLab (The MathWorks, Natick, VA). Axial diffusivity, $\lambda_{||} = \lambda_1$, and radial diffusivity, $\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$, were calculated from the tensor eigenvalues and averaged over all voxels within the ROI (Basser & Pierpaoli, 1996).

4.2.7 Correlations between qMR and histology

Spearman's rank correlation-coefficient ($\rho$) was calculated using MatLab between the various quantitative MR parameters and the features identified on histology for a total of 30 ROIs at each time-point. This coefficient ranges from -1 to 1, and is a non-parametric measure of the statistical dependence between two parameters, where 1 (-1) denotes total positive (negative) correlation and 0 denotes no correlation. These were calculated separately for the acute and chronic groups, as well as for gray and white matter. The following quantitative MR parameters were considered: volume fraction of the VS $T_2$ and MW peaks; $T_2$ position of the I/E water, MW, short component (weighted average of VS $T_2$ and MW $T_2$ values) and VL water peaks and finally two diffusion eigenvalues, $\lambda_{||}$, and $\lambda_{\perp}$. Histology parameters were the presence hemorrhage or hemosiderin, vacuolation and/or edema, and cystic cavity, as well as myelin appearance (normal or abnormal). Critical values for $\rho$ were determined using a significance level of 5%, and degrees of freedom based on the number of ROIs containing the histological feature in question.
4.3 Results

4.3.1 Histology

Table 4.2 shows the injury severity and number of ROIs for both acute (24 hour) and chronic (2 weeks) groups of animals. In the acute group there were 4 animals considered to be moderately injured with scattered petechial hemorrhages involving less than 50% of the cross section of the spinal cord. The other 4 animals experienced severe injury with large, multifocal, often coalescing hemorrhages, necrotizing vasculitis and areas of tissue necrosis, as well as loss of myelin and numerous swollen axons. In the group with chronic, inflammatory changes (1 or 2 weeks) there were 4 animals determined to be relatively uninjured based on histology containing small amounts of scattered vacuolation and possible edema, and 4 severely injured animals with irregular, cavity-like lesions infiltrated by macrophages. In this group, red blood cells were absent and hemosiderin was present in many of the macrophages within the cystic cavities and in the surrounding neural tissue. There was corresponding loss of myelin in the cavities and a band of demyelination in the white matter adjacent to cavities. These injuries are described in greater detail elsewhere (Oakden et al., 2014).

Table 4-2 Summary of MRI and histopathology timepoints, injury severity, and number of injured vs. normal appearing ROIs for both acute and chronic groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>MRI/Histopathology</th>
<th>Injury</th>
<th>GM ROIs</th>
<th>WM ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs 1 wk 2 wks</td>
<td>Mild/No injury</td>
<td>Severe</td>
<td>Injured</td>
</tr>
<tr>
<td>Acute</td>
<td>8/8 NA NA</td>
<td>4</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Chronic</td>
<td>8/NA 8/2 6/6</td>
<td>4</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

The ROI analysis consisted of 5 ROIs per animal, with 24 WM ROIs and 16 GM ROIs in both the acute and chronic groups. Tissue pathology in the form of hemorrhage, hemosiderin, vacuolation/edema, or cystic cavity was present in 37 of the 40 ROIs in the acute group, and in 30 of 40 ROIs in the chronic group. ROI locations and detail of the damage observed on LFB stain are shown below for a representative animal from the acute group (Fig. 4-3), and from the chronic group (Fig. 4-4).

Only one animal had three normal appearing ROIs, 3 had 2 normal appearing ROIs, and 4 had 1 normal appearing ROI. The remaining 8 animals had pathology present in all 5 ROIs examined.
Of the normal appearing GM ROIs, there was only one in the acute group (where histology was available 24 hours post-FUS), and 3 in the chronic group, out of 16 total ROIs at each group. In all 4 cases the ROI with no remarkable changes was located in the ventral horn. In WM, there were 2 normal appearing ROIs in the acute group, and 7 in the chronic group, out of a total 24 ROIs per group. Six of the 9 normal appearing ROIs were in the dorsal column, 2 in the ventral column, and one in the lateral column.

4.3.2 The Results of Quantitative T\textsubscript{2} Analysis

Figure 4-3 shows the T\textsubscript{2}-weighted image and the T\textsubscript{2} spectra for all 5 ROIs in a representative animal from the acute group alongside the LFB histology collected at 24 hours post-FUS. On T\textsubscript{2}-weighted MRI the damage appears as both hyperintensity and loss of gray/white matter contrast (Fig. 4-3a) which corresponds with a combination of hemorrhage and vacuolation/edema on histology. The qT\textsubscript{2} distributions (Fig. 4-3 c, e, g, i & k) show that there was an increase in I/E water T\textsubscript{2} in both GM (by ~30\%) and WM (by ~50\%) in addition to the appearance of a VS T\textsubscript{2} component in the GM regions. A T\textsubscript{2} distribution from a GM ROI in a healthy control animal is shown (gray dashed line) for comparison. There were no VS T\textsubscript{2} components present in the WM ROIs. There was hemorrhage present in WM, in both the ventral and dorsal columns as well as a small amount of hemorrhage and vacuolation present in the lateral column (Fig. 4-3d,h,l). The peaks in both the ventral and dorsal column distributions (Fig. 4-3 c,k) were broader than in normal tissue; however, peaks remained well separated which was typical of WM ROIs at 24 hours. A T\textsubscript{2} distribution from a WM ROI in a healthy control animal is shown (gray dashed lines) for comparison.
Figure 4-3. Acute $qT_2$ and histology.

$T_2$-weighted MRI (a) LFB histology with WM and GM ROIs (b) and obtained 24 hours post-FUS injury. White arrow indicates hyperintensity, black arrows indicate loss of gray/white matter contrast. Top two rows correspond to GM ROIs, bottom two rows to WM ROIs. $T_2$ distributions are shown (solid line) along with a normal $T_2$ distribution from healthy control animal (gray dashed) for WM (c, g, k) and GM (e, i) ROIs. 20x magnification of representative LFB histology from within each ROI are shown for WM (d, h, l) and GM (f, j) ROIs. In the ventral horn (f) hemorrhage (H) and vacuolation (V) can be observed, in the dorsal horn (j) there is hemorrhage (H) and edema (E). In WM, the ventral column (d) exhibits vacuolation (V) and hemorrhage (H), the lateral column (h) contains normal appearing myelinated axons, and in the dorsal column (l) hemorrhage (H), vacuolation (V) and edema (E) are all present. Scale bars represent 1 mm (a, b) and 50 µm (f, j, d, h, l).
Figure 4-4 shows results from a representative severely injured animal in the chronic group with histology at 2 weeks post-FUS. The T₂-weighted image obtained at 2 weeks post-FUS (Fig. 4-4a) and T₂ distributions obtained at 24 hours, 1 week and 2 weeks (Fig. 4-4 c,e,g,i,k), are shown alongside LFB histology collected at 2 weeks post-FUS (Fig. 4-4 b,d,f,h,j,l). At 24 hours the right half of the spinal cord was hyperintense, and the left side lacked gray/white contrast (not shown). At 2 weeks the gray/white matter contrast had returned, but there was a region of hypointensity corresponding with hemosiderin-containing macrophages on histology.

A cystic cavity was present in the dorsal horn, surrounded by hemosiderin containing macrophages (Fig. 4-4j). The T₂-weighted image (Fig. 4-4a) is hypointense in this region, while the T₂ distribution (Fig. 4-4i) contains both a VL T₂ component (~483 ms) and a large VS T₂ peak (33%, 3 ms). In the ventral horn (Fig. 4-4f) there are hemosiderin containing macrophages and some vacuolation. The T₂-weighted image (Fig. 4-4a) shows hypointensity corresponding to the presence of hemosiderin, as well as normal appearing tissue. The T₂ distribution contains a VS T₂ peak at all time points. The I/E water T₂ is longer than normal at 24 hours in all 5 ROIs, but has returned to normal values by 2 weeks in all but one ROI, which was typical of results in other animals. A VS T₂ component is present in both GM ROIs, which increases in size over time. VS T₂ components are also present in WM ROIs at various time points. The MW and I/E water peaks in WM are well separated at 24 hours, but they are no longer well separated at 2 weeks in both ventral and lateral columns (Fig. 4-4c&g) which contain significant vacuolation and macrophages (Fig. 4-4d&h). The dorsal column appears undamaged on histology (Fig. 4-4l) and has a normal appearing T₂ distribution at 2 weeks post-FUS, however the T₂ distribution at 24 hours contains an I/E water peak with long T₂, as well as a large VS T₂ peak (Fig. 4-4k).
Figure 4-4. Chronic qT$_2$ & Histology
T$_2$-weighted MRI (a) LFB histology with WM and GM ROIs (b) and obtained 2 weeks post-FUS injury. Top two rows correspond to GM ROIs, bottom two rows to WM ROIs. T$_2$ distributions acquired at 24 hours (dotted), 1 week (dashed) and 2 weeks (solid line) are shown for WM (c, g, k) and GM (e, i) ROIs. 20x magnification of representative LFB histology from within each ROI are shown for WM (d, h, l) and GM (f, j) ROIs.

In the ventral horn (f) hemosiderin (H) and vacuolation (V) can be observed, in the dorsal horn (j) there is hemosiderin (H) and cyst (C). In WM, the ventral column (d) exhibits vacuolation (V), the lateral column (h) exhibits vacuolation (V) and macrophages (M), and in the dorsal column (l) contains normal appearing myelinated axons.

Scale bars represent 1 mm (a, b) and 50 µm (f, j, d, h, l).
4.3.3 Classification of peaks in the $T_2$ distribution

Figure 4-5 shows the $T_2$ values for I/E water peaks in normal control WM and GM ROIs. In normal tissue I/E water $T_2$ ranges were calculated to be 39-77 ms in WM, and 41-53 ms in GM.

![Figure 4-5. $T_2$ values for I/E water peaks in normal control WM and GM ROIs. Range indicates mean ± 3 standard deviations.](image)

The boundary between VS water and myelin water $T_2$ in WM was determined to lie between 3 and 6 ms. This was based on data from ROIs containing 2 peaks with $T_2$ values < 39 ms, which consisted of 2 normal, 13 acute, and 6 chronic ROIs. There were 10 other WM ROIs which contained peaks in the range of 3-6 ms, all of which contained less than 30% of the total signal and were therefore classified as myelin water. In normal white and gray matter the VS $T_2$ peaks were small, each containing less than 6% of the total signal, while in injured spinal cord the VS $T_2$ peaks were much larger. At 24 hours 12 of the 13 WM ROIs had VS $T_2 > 25\%$, (average 35±13%), while in chronic WM the average VS $T_2$ fraction was 14±7%.

In GM there were 15 VS $T_2$ peaks at the acute, and 11 at the chronic timepoints with average signal fractions of 28±18% and 20±14% respectively.

Four ROIs did not contain peak in the range considered to correspond to I/E water (39-250 ms in WM and 41-250 ms in GM). All 4 were located in GM at the chronic timepoint and had peaks in the range of 35-40 ms, as well as VS $T_2$ peaks. Three of them also had VL $T_2$ peaks, and all contained hemosiderin on histology.

Figure 4-6 displays histograms of all these various components <150 ms for both gray and white matter. Not presented in this figure are VS $T_2$ components, 1 in WM at 376 ms and 5 in GM ranging from 287 ms to 1166 ms. In WM there are many more VS $T_2$ components at 24 hours, and an increase in the $T_2$ of the
I/E component, relative to those of the controls (Fig. 4-6a). These changes are largely resolved by 2 weeks; however, the variability in $T_2$ values remains greater than in normal controls. In GM there is no VS $T_2$ component in most control ROIs, while there is a VS component present in most of the ROIs at 24 hours, and in more than half at 2 weeks (Fig. 4-6b). In WM, there is an increase in $T_2$ of the I/E component at 24 hours (Fig. 4-6d) which is largely resolved by 2 weeks.

**Figure 4-6.** Histogram summary of $T_2$ distributions

Histogram summarizing all peaks less than 150 ms in the $T_2$ distributions for WM (a) and GM (b) in control ROIs (black), and ROIs acquired at 24 hours (gray), and at 1 or 2 weeks (white). Shaded region indicates normal $T_2$ values for WM (3 ms-77 ms) and GM (41-53 ms).
4.3.4 Correlations with Histology

To relate changes observed on quantitative MRI with pathology of SCI, Spearman's rank moment correlation coefficients ($\rho$) were calculated between features observed on $\text{qT}_2$ and DTI, and those observed on histology. Hemorrhage, hemosiderin, vacuolation or edema, myelin, and cyst were assessed for each ROI as being absent (normal), mild (present in <25% of the ROI), or severe (present in >25% of the ROI). Thresholds for statistically significant values of $\rho$ varied depending on the number of ROIs displaying particular pathology and the critical $\rho$ value for each type of pathology is shown at the bottom of each column. Table 4-3 and 4-4 contain correlations for the acute and chronic timepoints respectively.

Statistically significant correlations are shown in bold, and the mean values for each of these parameters in normal tissue, mild pathology, and severe pathology are listed in Table 4-5.

| Table 4-3 | Correlation ($\rho$) between MRI parameters and Histology in acute GM/WM (24 hours). Statistically significant correlations are shown in bold. |
|---|---|---|---|
| MRI parameter | Hemorrhage (GM/WM) | Vacuolation or Edema (GM/WM) | Myelin Loss (WM) |
| VS % | 0.82 / 0.61 | 0.61 / 0.35 | 0.04 |
| MW % | NA / -0.29 | NA / -0.29 | -0.07 |
| MW $T_2$ | NA / -0.02 | NA / -0.17 | 0.00 |
| I/EW $T_2$ | 0.80 / 0.52 | 0.75 / 0.63 | 0.46 |
| $\lambda_{||}$ | 0.19 / -0.25 | 0.11 / 0.50 | 0.37 |
| $\lambda_{\perp}$ | 0.34 / 0.06 | 0.60 / 0.64 | 0.48 |
| Critical $\rho$ | 0.51 / 0.50 | 0.60 / 0.48 | 0.88 |

| Table 4-4 | Correlation ($\rho$) between MRI parameters and Histology in chronic GM/WM (2 weeks). Statistically significant correlations are shown in bold. |
|---|---|---|---|
| MRI parameter | Hemosiderin (GM) | Vacuolation or Edema (GM/WM) | Cyst (GM) |
| VS % | 0.76 | 0.71 / 0.27 | 0.57 |
| MW % | 0.00 | NA / 0.27 | 0.00 |
| MW $T_2$ | 0.00 | NA / 0.23 | 0.00 |
| I/EW $T_2$ | -0.29 | -0.41 / 0.33 | -0.25 |
| VL $T_2$ | 0.79 | 0.47 / 0.15 | 0.98 |
| $\lambda_{||}$ | 0.21 | 0.30 / -0.10 | 0.19 |
| $\lambda_{\perp}$ | -0.33 | -0.24 / 0.13 | -0.11 |
| Critical $\rho$ | 0.75 | 0.55 / 0.44 | 0.88 |
Table 4-5 Mean and standard deviations for statistically significant correlations. MRI parameters are each listed in separate tables (VS %, I/EW $T_2$, VL $T_2$, $\lambda_1$, and $\lambda_0$). For each tissue type: gray matter (A-GM), acute white matter (A-WM) and chronic gray matter (C-GM), significantly correlated histology parameter: hemorrhage (H), vacuolation and/or edema (V/E), or cyst (C), this table lists Spearman's correlation ($\rho$), followed by the group mean and standard deviations for the normal, mild, and severe pathology groups, along with the number of ROIs falling in each group.

<table>
<thead>
<tr>
<th>VS %</th>
<th>$\rho$</th>
<th>Normal (%)</th>
<th># of ROIs</th>
<th>Mild pathology (%)</th>
<th># of ROIs</th>
<th>Severe pathology (%)</th>
<th># of ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - GM</td>
<td>H</td>
<td>0.82</td>
<td>0 ± 1</td>
<td>(13)</td>
<td>27 ± 20</td>
<td>29 ± 17</td>
<td>(6)</td>
</tr>
<tr>
<td>A - GM</td>
<td>V/E</td>
<td>0.61</td>
<td>7.5 ± 15.9</td>
<td>(17)</td>
<td>26.1 ± 18.4</td>
<td>27.9 ± 25.7</td>
<td>(2)</td>
</tr>
<tr>
<td>A - WM</td>
<td>H</td>
<td>0.61</td>
<td>4.7 ± 12.6</td>
<td>(26)</td>
<td>18.6 ± 16.9</td>
<td>31.9 ± 11.1</td>
<td>(7)</td>
</tr>
<tr>
<td>C - GM</td>
<td>H</td>
<td>0.76</td>
<td>1.6 ± 3.1</td>
<td>(21)</td>
<td>18.6 ± 0.0</td>
<td>26.2 ± 15.7</td>
<td>(6)</td>
</tr>
<tr>
<td>C - GM</td>
<td>V/E</td>
<td>0.71</td>
<td>0.6 ± 1.4</td>
<td>(15)</td>
<td>10 ± 11</td>
<td>32.7 ± 15.3</td>
<td>(3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I/EW $T_2$</th>
<th>$\rho$</th>
<th>Normal (ms)</th>
<th># of ROIs</th>
<th>Mild pathology (ms)</th>
<th># of ROIs</th>
<th>Severe pathology (ms)</th>
<th># of ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - GM</td>
<td>H</td>
<td>0.80</td>
<td>46 ± 2</td>
<td>(13)</td>
<td>54 ± 8</td>
<td>69 ± 16</td>
<td>(6)</td>
</tr>
<tr>
<td>A - GM</td>
<td>V/E</td>
<td>0.75</td>
<td>47 ± 2</td>
<td>(17)</td>
<td>64 ± 15</td>
<td>65 ± 10</td>
<td>(2)</td>
</tr>
<tr>
<td>A - WM</td>
<td>H</td>
<td>0.52</td>
<td>64 ± 18</td>
<td>(26)</td>
<td>73 ± 13</td>
<td>92 ± 25</td>
<td>(8)</td>
</tr>
<tr>
<td>A - WM</td>
<td>V/E</td>
<td>0.63</td>
<td>61 ± 10</td>
<td>(25)</td>
<td>67 ± 13</td>
<td>100 ± 22</td>
<td>(9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VL $T_2$</th>
<th>$\rho$</th>
<th>Normal (ms)</th>
<th># of ROIs</th>
<th>Mild pathology (ms)</th>
<th># of ROIs</th>
<th>Severe pathology (ms)</th>
<th># of ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - GM</td>
<td>H</td>
<td>0.79</td>
<td>0 ± 0</td>
<td>(21)</td>
<td>394 ± 0</td>
<td>503 ± 515</td>
<td>(6)</td>
</tr>
<tr>
<td>C - GM</td>
<td>C</td>
<td>0.98</td>
<td>0 ± 0</td>
<td>(23)</td>
<td>881 ± 424</td>
<td>385 ± 139</td>
<td>(2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\lambda_\perp$</th>
<th>$\rho$</th>
<th>Normal ($\mu$m$^2$/ms)</th>
<th># of ROIs</th>
<th>Mild pathology ($\mu$m$^2$/ms)</th>
<th># of ROIs</th>
<th>Severe pathology ($\mu$m$^2$/ms)</th>
<th># of ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - WM</td>
<td>V/E</td>
<td>0.64</td>
<td>0.4 ± 0.1</td>
<td>(19)</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>(4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\lambda_\parallel$</th>
<th>$\rho$</th>
<th>Normal ($\mu$m$^2$/ms)</th>
<th># of ROIs</th>
<th>Mild pathology ($\mu$m$^2$/ms)</th>
<th># of ROIs</th>
<th>Severe pathology ($\mu$m$^2$/ms)</th>
<th># of ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - WM</td>
<td>V/E</td>
<td>0.50</td>
<td>1.9 ± 0.3</td>
<td>(19)</td>
<td>2.2 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>(4)</td>
</tr>
</tbody>
</table>

VS $T_2$ % was significantly correlated with hemorrhage in both GM ($\rho=0.82$) and WM ($\rho=0.61$) at 24 hours, and with hemosiderin in GM ($\rho=0.76$) at 2 weeks. It was not possible to calculate correlations with
hemosiderin in WM at 2 weeks as there were only 2 hemosiderin containing ROIs. VS $T_2\%$ was also correlated with vacuolation/edema in GM at 24 hours ($\rho=0.61$) and 2 weeks ($\rho=0.71$).

In both GM and WM, I/E water $T_2$ was correlated with both hemorrhage ($\rho=0.80, 0.52$) and vacuolation/edema ($\rho=0.75, 0.63$) at 24 hours. In WM at 24 hours, axial and radial diffusivity were also correlated with vacuolation/edema ($\rho = 0.50, 0.64$).

The $T_2$ position of the VL $T_2$ component in GM, which ranged from 287 ms to 1166 ms at 2 weeks, was significantly correlated with the size of the cystic cavity on histology ($\rho =0.98$). The correlation in WM could not be calculated as there was only 1 cystic cavity observed.

While none of the correlations were statistically significant between the diffusion coefficients, $\lambda_{||}$ and $\lambda_{\perp}$, and any of the histology metrics in WM at 2 weeks, Figure 4-7 shows that changes in both $\lambda_{||}$ and $\lambda_{\perp}$ can be observed in damaged areas relative to the undamaged contralateral tissue.

Figure 4-7. Luxol fast blue histology and perpendicular diffusivity maps
Luxol fast blue histology (a&b) alongside corresponding $\lambda_{\perp}$ maps (c&d) and $\lambda_{||}$ maps (e&f) for 2 animals at 2 weeks post-FUS injury. Scale bars: 0.6 mm (a&b) and 1 mm (c,d,e,f). The spot with elevated $\lambda_{\perp}$ (black arrow) corresponds to a region with hemosiderin on histology (a).
4.4 Discussion

The signal intensity on a T₂-weighted image is related to the weighted-average T₂ value of all the components in the T₂ distribution. Examining the full T₂ distribution, revealed that co-occurring pathologic features can have opposite effects on signal intensity in a T₂-weighted image, with the VS T₂ peak decreasing and the I/E water T₂ increasing the average T₂, resulting in a T₂-weighted image that underestimates the degree of damage. There was a statistically significant correlation between the presence of hemorrhage or hemosiderin on histology, and the volume fraction of the VS T₂ peak in the T₂ distribution in gray matter at both 24 hours and 2 weeks (R=0.82 & 0.76), and in white matter at 24 hours (R=0.61).

The T₂-weighted imaging results have been described in Chapter 2 (Oakden et al., 2014), and are consistent with those observed in other animal models (Bilgen et al., 2000; Mihai et al., 2008; Narayana, Abbe, et al., 1999; Narayana et al., 2004; Ohta et al., 1999; Sundberg et al., 2010) and in human studies of SCI (Bilgen et al., 2000; Bondurant et al., 1990; Narayana, Abbe, et al., 1999). At 24 hours this typically consisted of hyperintense regions and loss of gray/white contrast corresponding with edema, loss of myelin, and hemorrhage on pathology. By 2 weeks there were regions of hypointensity in addition to regions of hyperintensity, while the gray/white contrast had returned to normal; however, the histology displayed cystic cavities, hemosiderin containing macrophages and some edema. Using qT₂, the contrast changes on T₂-weighted imaging could be explained as changes in the underlying T₂ distribution. These changes in T₂ distribution could then be related to the micro-structural changes observed on histology.

More than 90% of ROIs were classified as being damaged in the acute group, where histology was acquired at 24 hours, as compared with only 75% in the chronic group where histology was acquired at either 1 or 2 weeks post-FUS. In addition there were several ROIs (9 in WM, 2 in GM), with normal T₂ distributions at 2 weeks, but which had an abnormal distribution at 24 hours, with increased I/E water T₂ and a VS T₂ peak (Fig. 4-4k&l). This indicated that abnormal T₂ distribution at 24 hours did not necessarily predict damage at 2 weeks.

A tendency for the short T₂ peak to split into two peaks has been noted in other studies of both normal and injured white matter (Menon, Rusinko, & Allen, 1992; Minty et al., 2009; Stewart, MacKay, Whittall, Moore, & Paty, 1993; Webb et al., 2003) which likely explains some of these VS T₂ peaks observed in this study; however, many of the VS T₂ peaks in both GM and WM were extremely large, in excess of 40% of the total signal. I hypothesize that the VS T₂ peak observed in this study is due to iron in hemorrhage or hemosiderin. It is known that the presence of iron affects the signal of the CPMG echo train in a manner
that is dependent on echo time, distance between iron clusters, and field strength (Ghugre, Coates, Nelson, & Wood, 2005). The strong correlation between the presence of hemorrhage or hemosiderin on histology, and the volume fraction of the VS $T_2$ peak in both GM and WM at 24 hours, and GM at 2 weeks (Tables 4-3&4-4) suggested that the VS $T_2$ peak was indicative of blood product. Hemorrhage is well known to cause a decrease in signal on $T_2$-weighted images (Bilgen et al., 2000; Bondurant et al., 1990), but the presence of a VS $T_2$ component rather than simply a uniform decrease in $T_2$ of all nearby protons suggests that by acquiring multiple images with different $T_2$-weightings it would be possible to investigate the extent of hemorrhage separately from other pathologies, such as cyst or edema, which increase the $T_2$.

Strong correlations were observed at 24 hours between I/E water $T_2$ and hemorrhage and vacuolation and/or edema in both GM and WM. No significant correlations were observed at 2 weeks; the mean I/E water $T_2$ in injured WM is lower at 2 weeks than at 24 hours (Fig. 4-6) and would require more samples (at least 30 ROIs containing vacuolation and/or edema) to achieve significance, while in GM mean I/E water is decreased relative to controls. Studies of peripheral nerve (Odrobina et al., 2005; Pun et al., 2005; Webb et al., 2003) reported an increase in I/E water in the presence of inflammation, which remains elevated at 2 weeks in cut and crush injuries of peripheral nerve. An increase in I/E water $T_2$ has also been observed in some MS brain lesions (Laule et al., 2007) where it is hypothesized to be related to an increase in extracellular water. This thesis is the first study looking at the I/E water $T_2$ in SCI. The increased I/E water $T_2$ observed in this thesis resulted in a bright signal on the corresponding $T_2$-weighted images, in agreement with other studies based on $T_2$-weighted imaging and histology in SCI (Bondurant et al., 1990; Miyanji et al., 2007; Ohta et al., 1999).

There was a very strong correlation in GM between the presence of a VL $T_2$ component on $qT_2$ and a cystic cavity on histology. In addition, every ROI containing a cyst also contained all of the other pathologies evaluated.

Edema was strongly correlated with both radial and axial diffusivity, $\lambda_{\perp}$ and $\lambda_{||}$, in WM at 24 hours. This is likely due to increased water mobility in the presence of edema. While not achieving significance due to the small number of ROIs with damaged myelin in this study, $\lambda_{\perp}$ provided the strongest correlation with myelin damage in WM at 24 hours. It is not surprising that the correlation between myelin and MW volume fraction was lower than with $\lambda_{\perp}$ as MW volume fraction measures total myelin content and does not discriminate between intact and damaged myelin (McCreary et al., 2009; Webb et al., 2003), while $\lambda_{\perp}$ increases in the presence of damaged myelin (Kim et al., 2007; Song et al., 2002; Sundberg et al., 2010).
In healthy spinal cord, gray matter is hyperintense relative to white matter on a T₂-weighted images because of higher proton density in the gray matter (Narayana, Fenyes, et al., 1999), in spite of the longer T₂ values in WM. Examining qT₂ distributions in areas with loss of gray/white contrast immediately following SCI, showed that while I/E water T₂ was increased in both GM and WM which would have led to a signal increase in both tissues, there was frequently a VS T₂ component (<41 ms) in GM, which decreased the T₂ of the GM resulting in the observed loss of contrast. The VS T₂ component was significantly correlated with the presence of hemorrhage in both GM and WM (R=0.66 & 0.60). As GM is more vascular than WM, and is often the primary site of hemorrhage in SCI (Tator & Fehlings, 1991), the decrease in T₂ due to the presence of the VS T₂ component would explain the loss of gray/white contrast, which is more commonly ascribed to edema.

At the 2 week timepoint, there were some areas which appeared quite dark on T₂-weighted MRI, but which were observed to contain cystic cavity on histology (Fig. 4-4a,b,j). There was also a component with VL T₂, most likely associated with the water inside the cyst, which was completely masked by the signal decrease arising from the presence of hemosiderin.

Understanding the possible T₂ mechanisms behind the loss of contrast suggests that multiple T₂ weighted images could be collected, to separate the signal from the very short T₂ from the longer T₂ values in I/E water and cyst, providing a better estimate of the area of the spinal cord affected. This could improve estimates of the extent of loss of gray/white matter contrast, and permit imaging of both hemosiderin deposits and cyst.

Finally this thesis shows a range of normal I/E water T₂ values of 39-77 ms in WM and 41-53 ms in GM, acquired with minimal diffusion weighting which can generate low T₂ estimates at high resolution, as described in Chapter 3 (Oakden & Stanisz, 2014). While these values are shorter than those reported in human spinal cord (MacMillan et al., 2011; Minty et al., 2009), they are in agreement with other values reported for I/E water T₂ in rat spinal cord in vivo (Dula et al., 2010; Kozlowski, Liu, et al., 2008). Failing to take this into account could result in an overestimation of MWF when selecting cutoff values for the myelin water component, as has been demonstrated in fixed tissue (Chen, Holmes, Tetzlaff, & Kozlowski, 2014).
4.5 Conclusions

While $qT_2$ has been used to measure myelin water in SCI previously (Kozlowski, Raj, et al., 2008), and the entire $T_2$ distribution examined in a murine model of chemically-induced demyelination (McCreary et al., 2009) this thesis reports for the first time that the entire $T_2$ distribution has been investigated in a realistic model of SCI. Examining the full $T_2$ distribution revealed that the presence of hemorrhage or hemosiderin was highly correlated with a $T_2$ component exhibiting VS $T_2$ ($<3$ ms in WM or $<41$ ms in GM), rather than simply decreasing the $T_2$ of all protons uniformly. Components with longer than usual $T_2$ values were also present in regions containing VS $T_2$ peaks, which would have reduced the contrast available on a $T_2$-weighted image, making the damage more difficult to observe. Acquisition of multiple images with different $T_2$-weightings could improve MRI evaluation of SCI by separating the effect of this VS $T_2$ component from the rest of the $T_2$ distribution, allowing cyst or edema to be visualized in the presence of blood products.

In addition to the finding that the VS $T_2$ component was significantly correlated with hemorrhage or hemosiderin in both GM and WM at 24 hours, and with GM at 2 weeks, at 24 hours following injury the I/E water $T_2$ was correlated with vacuolation/edema in both GM and WM in agreement with results in peripheral nerve (Webb et al., 2003) and in brain (Laule et al., 2007).

The range of intra/extracellular water $T_2$ in healthy rat spinal cord was found to be significantly shorter than in human spinal cord. Some peaks were observed in GM which were likely associated with I/E water, but which had $T_2$ values even shorter than 41 ms, the lower end of the range established in healthy GM. The possibility of I/E water with short $T_2$, in addition to the presence of the VS $T_2$ peak means that the range of myelin water $T_2$ values must be carefully selected to avoid overestimating the MWF in the presence of SCI.
Chapter 5 - Conclusions and Future Directions

5.1 Summary

The goal of this thesis was to investigate qT₂ MRI in the setting of rat SCI. T₂ is extremely sensitive to the microstructural changes involved in injury and disease, as well as to the presence of myelin, which is of particular interest in the setting of SCI and treatment.

In Chapter 2 a novel, non-surgical model of rat SCI was introduced which employed FUS and microbubbles to induce injury unilaterally to the cervical spinal cord under MR guidance. The injury was well tolerated because of its unilateral and non-surgical nature. Histological and immunohistochemical investigation of this injury demonstrated that it resembled contusion injuries with similar pathology at both the acute timepoint (24 hours following injury induction) and at 2 weeks.

The lack of laminectomy meant that the spine remained straight and easy to image, with no subdural hemorrhage to generate magnetic field inhomogeneities. The location of the injury, in the cervical spinal cord, meant that there were minimal motion artefacts, although most imaging was performed using respiratory gating. As a result this model was particularly compatible with MRI.

Following SCI it is typical to find changes on T₂-weighted MRI. A variety of changes have been observed, hypointensities considered to be indicative of hemorrhage, hyperintensities considered to be indicative of edema or cyst, and a loss of gray/white matter contrast often interpreted as mild edema. To better understand these contrast changes a qT₂ sequence was implemented to operate at the very high resolution required to image pathology in the rat spinal cord, as this sequence allows separation of the signal coming from various water compartments within a single voxel. Chapter 3 describes the effects of diffusion on the qT₂ sequence which became problematic at high resolution. As linear voxel sizes were reduced below 1 mm, the effect of diffusion due to the imaging gradients began to have a noticeable effect on the measured T₂, resulting in a resolution dependent measure of T₂. This effect was also dependent on the actual T₂ value, the diffusivity, and the echo time of the sequence. Equations for calculating the magnitude of these effects, and the changes to the standard qT₂ sequence to minimize them and acquire high quality data are described. In addition, artefacts resulting from the interaction between diffusion sensitivity and the decreasing spoiler gradient typically used to remove stimulated echoes are described.

Finally, Chapter 4 discusses the results of qT₂ imaging in the FUS model of SCI. In addition to the MW, I/E water, and very long T₂ peaks seen in the qT₂ distribution in healthy tissue, a very short T₂ component was
observed which was highly correlated with the presence of hemorrhage or hemosiderin. An increase in the T₂ of the I/E water was observed at 24 hours post-injury, and cystic cavities were correlated with the presence of a VL T₂ peak at 1-2 weeks post-injury. Multiple pathologies were often present within a single voxel. On standard T₂-weighted imaging the signal is an average over all the protons within the voxel, meaning that co-occurring pathologies could not be resolved. In spite of the limited resolution of qT₂, it was possible to detect the presence of different water compartments within the injured spinal cord.

5.2 Future improvements to the FUS SCI model

While the combination of FUS and microbubbles was able to induce severe injury unilaterally in over 50% of the animals, the reproducibility of the model presented here needs to be considerably higher before it can be used to test potential treatments. Improving the targeting by performing FUS inside the MRI will eliminate motion which can occur between the time the MRI is acquired and the FUS is performed, and which is hypothesized to account for some of the present variability in injury location and severity. An additional improvement is the implementation of an active control algorithm which uses acoustic emissions to monitor the FUS treatment similar to that which has been implemented in the brain (O’Reilly & Hynynen, 2012a).

This study relied on the detection of Gd contrast enhancement within the spinal cord in order to determine the immediate success of the FUS treatment. This is sufficient for studies which aim to permeabilize the BSCB (Weber-Adrian et al., 2014) for the purpose of drug delivery; however, it is a necessary but not sufficient condition when the goal is to create an injury. The presence of Gd also affects both qT₂ and DTI, so the use of Gd during injury induction means that quantitative MRI results cannot be obtained prior to 24 hours as the length of time to acquire quantitative images means that the amount of Gd in the tissue will be changing during the acquisition. The next study of this model should include a quantitative T₁ measurement so that optimal parameters can be calculated for T₂-weighted and T₂*-weighted acquisitions following SCI. A comparison of MT and qT₂ in this model would also be of interest, especially at later time-points, as qT₂ cannot distinguish intact myelin from myelin debris (Webb et al., 2003).

5.3 Further improvements to the qT₂ sequence

The use of composite refocusing pulses in the qT₂ sequence, which in these experiments performed so well that stimulated echoes were undetectable, meant that the qT₂ sequence was limited to a single slice. A recent improvement to the qT₂ data analysis (Prasloski et al., 2012) permits multicomponent fitting of qT₂
data in the presence of stimulated echoes, which makes multi-slice $qT_2$ imaging much more feasible, especially in human subjects.

While the resolution of $qT_2$ was limited due to the effects of diffusion as described in Chapter 3, a better understanding of the $T_2$ distribution in SCI can guide parameter selection in standard MR imaging sequences to make them more sensitive to the various pathologies present in the tissue. Instead of a single $T_2$-weighted image being used to assess SCI, a series of high resolution images with different $T_2$-weightings could be used, each optimized to be sensitive to a different water compartment, as well as a proton density weighted image, would increase the sensitivity of MRI to the pathology present in SCI. Based on the results of Chapter 4, an image which was sensitive to the very short $T_2$ values could be used to identify regions containing hemorrhage. Another set of images could be optimized to enhance the contrast between normal tissue, and damaged tissue with a long I/E water $T_2$. This would require the acquisition of proton density and quantitative $T_1$ images, but could significantly increase the specificity of the MR imaging protocol following SCI.

5.4 Conclusions

The development of an MR guided FUS model of SCI in the rat was very successful. The pathology was similar to that observed in other SCI models. The non-surgical nature of the injury meant that it was easily performed by imaging personnel. The specialized equipment required to create the injury is commercially available, and can also be used to permeabilize the BBB without causing injury. The location of injury, in the cervical spinal cord, provided the largest possible cross section of spinal cord for imaging while the unilateral nature of the injury resulted in minimal post-injury care. While some improvements in reproducibility are required before this model will allow testing of SCI treatments, it promises to provide an excellent platform for quantitative MRI of SCI and repair. Like all models of SCI which do not involve a complete transection of the spinal cord, it is very difficult to assess whether functional recovery is due to axonal regeneration. Another limitation is the requirement for MRI guidance which significantly increases the amount of time and the expense of injury induction.

While $qT_2$ requires significant improvements in speed and SNR before it can be adopted as a clinical sequence, it can contribute towards the development of clinical imaging sequences with improved contrast between healthy and damaged tissue. The increased specificity resulting from quantitative MRI will improve the information available in longitudinal studies of SCI, reducing both the number of animals and the time required to develop new treatments.
References


