THE EFFECT OF IMPACT VELOCITY ON ACUTE SPINAL CORD INJURY

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Abstract

Spinal cord injury is a devastating and catastrophic occurrence and effective comprehensive treatments have not yet been discovered. Contusion models of spinal cord injury are used in the evaluation of drug therapies and neuroprotection strategies and should mimic human injury. Existing models have focused on the significance of displacement and force as parameters of injury while the importance of impact velocity remains contentious. The objectives of this study were to install and calibrate a spinal cord contusion device, establish a protocol for its operation and to use the device to examine the effect of impact velocity on mechanical injury to the spinal cord using clinically relevant velocities.

Contusion injuries were created using a modified Spinal Cord Injury Research System (Stokes et al. 1992). Male, Sprague-Dawley rats (210-320g) were injured at the T10 level. Deeply anaesthetized animals (n=31) were injured at slow (3mm/s) and fast (300mm/s) impact velocities to a depth of 1mm and then immediately sacrificed to assess the primary lesion. Sham control animals experienced the surgical procedure but were removed from the device without injury and sacrificed. The mechanical parameters of injury were assessed and Young’s moduli were estimated for the injury groups. Cord sections were stained with haematoxylin and eosin (H&E) and the SMI32 antibody to non-phosphorylated neurofilament and the injury responses in the grey and white matter were analyzed.

The results showed that the volume of haemorrhage in the white matter was a function of impact velocity (fast=0.61 mm$^3$, slow=0.24 mm$^3$, p=0.013) while the total haemorrhage volume (fast=1.51 mm$^3$, slow=1.21 mm$^3$, p=0.22) showed no difference. The SMI32 reactivity showed a significant relationship between impact velocity and axonal damage (p=0.013). Complete axonal disruption was evident in the fast injury group around the injury epicentre. Post-hoc analyses revealed a significant difference between the fast and slow/control groups in the lateral-ventral white matter (p=0.001) and ventral white matter (p=0.035) but no observed effect between slow and control groups. Damage to the grey matter, as reflected by haemorrhage volume was similar between the slow and fast groups (fast = 0.89 mm$^3$, slow = 0.97 mm$^3$) however analysis of grey matter reactivity to the SMI 32 antibody showed a significant effect of velocity (p = 0.03). A post-hoc analysis revealed significant differences between the fast and slow impact groups immediately (p = 0.04) and 1.5mm (p = 0.05) caudal to the injury site but rostral to the injury site the difference was not significant (p = 0.07). The mechanical response of the spinal cord to contusion injury demonstrated Young’s moduli of 76 kPa for the slow injury and 298 kPa for the fast injury.

We conclude that impact velocity has an effect on the magnitude of injury within the white matter during spinal cord injury and an effect on the amount of neuronal damage in the grey matter. The extent of haemorrhage in the grey matter appeared independent of impact velocity. The mechanical response of the tissue to injury showed a four-fold increase in the elastic modulus between the slow and fast groups. These results help isolate the extent of primary mechanical damage in SCI and will enable human injury to be more accurately modeled by utilizing clinically relevant impact velocities.
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1 Introduction

1.1 Motivation for the study

Traumatic spinal cord injury (SCI) affects over 16,000 people every year in the United States [1, 2] and an additional 1,200 people in Canada [3]. Injuries range from minor motor and sensory impairments to complete ventilator dependent quadriplegia. In addition to those surviving with injury it is estimated that approximately 50% of spinal cord injured patients die before reaching the hospital [2, 4]. Spinal cord injury invokes major emotional, physical and economic costs both on the patient and the economy as a whole. Improving the functional outcome for patients with spinal cord injury not only increases their quality of life but also improves their economic outlook and decreases the demand on the medical system.

There is no effective treatment for spinal cord injury. Recent attempts have been made to implement methylprednisolone as a standard treatment for spinal cord injury; however its efficacy is debatable and may in fact be harmful if treatment is initiated too long after the initial injury [5, 6]. Improvements in care for spinal cord injured patients have been primarily in the area of systemic care, such as regulation of heart rate, blood pressure and oxygen levels [7]. The effectiveness of decompression surgery is debatable [8-11] and spine stabilization helps to minimize future damage but does not improve the injury. Despite the efforts of hundreds of researchers no one has found a way to repair the human spinal cord once it has been damaged.

It is thought that part of the reason for the lack of successful treatment initiatives is because the problem of spinal cord injury is still not completely understood. The effects of varied displacement and force applied to the spinal cord during experimental injury have been well explored; however, the effects of other variables, such as velocity and duration of impact, have not been fully evaluated and are generally overlooked during the experimental evaluation process for new treatments. There are indications that impact velocity will have a significant effect on injury and should be a consideration in models of traumatic injury.
1.2 Current state of research

Standardized animal models of traumatic spinal cord injury have been established to provide a consistent injury on which to evaluate experimental treatments. There are a variety of models which range from simple blade transection of the cord to complex impact devices that control different mechanical parameters and use a number of sensors to observe the cord impact. Each model has purpose in the study of spinal cord injury; however the contusion devices create injuries that are best representative of the neuropathological response seen in human injury [12]. These contusion injury models are best suited for the evaluation of experimental therapies and the characterization of injury responses to varied loading regimes.

Contusion models of traumatic spinal cord injury have focused primarily on the importance of displacement and force as parameters of injury. Relationships have been established between the displacement of the spinal cord and impact force during injury and the resulting tissue damage and functional deficits [13-40]. Increased impact depth has resulted in increased tissue and functional losses [13, 19, 21, 40-43] with severe injuries being observed in animals with greater than 75 percent cord compression [40-42]. A similar trend has been demonstrated when force was the control parameter of injury [18, 21, 44, 45].

Few researchers have considered the effect of impact velocity [15, 18, 21, 40, 43, 44, 46, 47]. The scarce information that is available demonstrates conflicting results regarding the significance of impact velocity. Some research, particularly that using high impact velocity (v > 0.5 m/s), has demonstrated a strong correlation between impact velocity and histological measures, functional deficits and ionic concentration changes [15, 40, 46]. There is a paucity of information relating impact velocity to spinal cord injury at moderate speeds (v < 0.5 m/s) with the few existing studies reporting weak or non-existent correlations [18, 21, 44, 47]. However, those moderate speeds are likely to be most representative of a clinical injury as demonstrated by cadaveric models of human injury [48-50].
1.3 Anatomy and Physiology

1.3.1 The Human Spine

The human spine is composed of 33 vertebrae (Figure 1.1). These vertebrae comprise five distinct regions of the spinal column: the cervical spine, the thoracic spine, the lumbar spine, the sacrum and the coccyx. The vertebrae are identified by numbers cranially (head) to caudally (tail) in each region of the spinal column. The cervical spine consists of seven vertebrae (C1-C7), which shape the neck and act to support the head. Twelve vertebrae make up the thoracic spine (T1-T12). Each vertebra acts as the posterior anchor for a pair of ribs forming the torso. The lumbar spine contains five vertebrae (L1-L5), which form the lower back and are designed to carry the weight of the head, neck, trunk and upper limbs. The sacrum consists of five fused vertebrae (S1-S5) which interface with the pelvis through the sacroiliac joint and facilitate the transmission of load from the spine to the legs. The coccyx, formed by three to five fused vertebrae (Co1-Co5), is a point of fixation for a number of pelvic muscles and ligaments. Between adjacent vertebrae are intervertebral discs. The cartilaginous tissue of the discs provides flexibility to the spine and acts as a shock absorber and evenly distributes load across the surface of the vertebral bodies.

The vertebrae vary in form and function along the length of the column but do exhibit several fundamental structural similarities between the cervical, thoracic and lumbar regions. Each vertebra can be divided into two distinct

![Figure 1.1: Organization of vertebrae in the spinal column (Adapted from Cramer and Darby [51]).](image)
portions, the vertebral body and the vertebral arch (Figure 1.2). The vertebral body is the large anterior portion of the vertebra that acts primarily to support the spinal loads. The vertebral bodies increase in transverse diameter from C2-L3. The vertebral arch is composed of several bony protuberances which act to protect the spinal cord, determine spinal movement and provide attachment points for spinal muscles and ligaments. The vertebral foramen is the channel through the vertebrae bounded by the vertebral body on the anterior side, the pedicles on the lateral sides and the lamina on the posterior side. This bony channel contains the spinal cord.

![Figure 1.2: Typical vertebra. A) Superior view. B) Lateral view.](image)

**1.3.2 The Spinal Cord**

**1.3.2.1 General Organization**

The spinal cord is the reflex centre and conduction pathway between the body and the brain. The cord, like the spinal column, is divided into five regions. However, due to the rate of growth of the spinal column and spinal cord during foetal development, the lower regions of the spinal cord do not correspond with the same regions in the spinal column. The conus medullaris, or end of the cord, corresponds to the L2 vertebral level in an adult, thus the spinal cord occupies approximately the superior two-thirds of the vertebral canal. The remainder of the canal is filled with the cauda equina which is the bundle of nerve roots traveling from the spinal cord to their corresponding vertebrae where they exit the canal. There are two enlargements in the spinal cord at the cervical and lumbar levels. These enlargements correspond to innervation of the limbs. The cervical enlargement corresponds
to spinal cord levels C4-T1 and innervates the upper limbs. The lumbosacral enlargement corresponds to spinal cord levels T11-L1 and innervates the lower limbs.

There are 31 pairs of spinal nerves attached to the spinal cord: eight cervical, twelve thoracic, five lumbar, five sacral and 1 coccygeal. The nerves are identified by their position relative to a vertebra. The cervical spinal nerves are numbered 1-8 with each nerve protruding above the vertebra of the same number. As there is no C8 vertebra, spinal nerve C8 protrudes between vertebrae C7 and T1. Subsequently, all remaining spinal roots are labelled corresponding to the vertebra directly above the level of protrusion. The roots of the spinal cord are composed of both dorsal and ventral roots emerging from the dorsal (towards the back) and ventral (towards the front) surfaces of the spinal cord respectively (Figure 1.3). The dorsal roots contain afferent (sensory) fibres while the ventral roots contain efferent (motor) fibres.

Figure 1.3: Spinal cord, spinal nerves and spinal meninges (Adapted from Moore and Dalley [52]).

The spinal cord is protected by three membrane layers: the dura mater, arachnoid mater and pia mater, collectively called the spinal meninges (Figure 1.3). The pia mater directly
surrounds the spinal cord such that it is indistinguishable from the cord itself. The pia
wrapped spinal cord is suspended by the denticulate ligament in a channel of cerebrospinal
fluid (CSF). This ligament bridges the gap between the pia mater and arachnoid and dura
mater. The arachnoid mater is not attached to the dura mater but is held tightly against it due
to the pressure of the CSF in the subarachnoid space. The dura mater is the tough outermost
membrane surrounding the spinal cord. The dura mater forms a dural sac, a long tubular
sheath in the vertebral canal. This sheath is anchored rostrally (towards the head) to the
margin of the foramen magnum of the skull and caudally to the coccyx by the terminal filum.

The spinal cord is composed of grey and white matter. The cord is organized such that the
grey matter is an ‘H’ shaped structure in the centre of the cord and the white matter
surrounds it (Figure 1.3). The grey matter contains neuronal cell bodies and acts as the signal
processing centre of the spinal cord. The white matter contains axons, which act to transmit
signals between cell bodies in the brain, brain stem and spinal cord, across levels within the
spinal cord, and between the spinal cord and the body. Sensory information is transmitted to
the cord via the dorsal roots and is primarily carried in the dorsal portion of the spinal cord
while motor information is processed and transmitted in the ventral cord and ventral nerve
roots. A dominant feature of the ventral cord surface is the ventral median fissure (Figure
1.4). This contains connective tissue of the pia matter and the anterior spinal artery. The
ventral and dorsal sides of the cord can be distinguished by their central furrows as the dorsal
median sulcus is much shallower than the ventral median fissure.

The internal structure of the spinal cord has a variable organization along its length. The
grey matter can be divided into dorsal and ventral horns, with a lateral horn present in the
thoracic and upper lumbar portion of the spinal cord (Figure 1.4). The dorsal horns primarily
carry the cell bodies of the axons contained in the ascending fasciculi and process signals
relating to sensation. The ventral horns carry motor neurons which supply the innervation
for the skeletal muscles. Cell bodies of sympathetic system neurons which innervate several
organs, glands and other systems are carried in the lateral columns. The sympathetic system
is known as the “flee or fight” system and unconsciously activates the body for emergency
situations [52]. The grey matter has been further subdivided into Rexed Laminae, to describe
the types of the cell bodies distributed in the grey matter. The functional groupings of cell bodies within the grey matter are repeated in the axonal organization of the white matter.

The geometry of the white matter is described by symmetrical, pie shaped thirds. Each segment is called a funiculus. The dorsal funiculus is bounded by the dorsal grey horn and the dorsal median sulcus and is comprised of the medial gracile fasciculus and the more lateral cuneate fasciculus. Below the mid-thoracic level, the gracile fasciculus comprises the entire dorsal funiculus. The lateral funiculus is bounded by the dorsal grey horn at the dorsal edge but has no clear demarcation with the ventral funiculus at the ventral border. The lateral funiculus is subdivided into the dorsolateral and ventrolateral segments. The ventral funiculus occupies the remainder of the white matter. The ventral white commissure is a small portion of white matter located immediately adjacent to the central canal. It is here that axonal fibres decussate (cross). It is the decussation of fibres that facilitates the communication between one side of the brain or spinal cord and the opposite side of the body.

The white matter of the spinal cord is further organized into tract structures (Figure 1.6). The different tracts carry different functional signals. The largest descending tract is the corticospinal tract and it is of fundamental importance in motor function. This tract connects the body to contralateral (opposite side) portions of the frontal and parietal lobes of the brain; thereby facilitating voluntary, especially skilful, motor activity. In humans this tract is located primarily in the dorsolateral funiculus, (i.e. lateral corticospinal tract) with a small component in the ventral funiculus, (i.e. ventral corticospinal tract). The corticospinal tract is
a relatively new evolutionary development and found only in mammals, with the greatest development in humans. The other major tracts in the cord include: the ascending tracts in the dorsal funiculus (i.e. cuneate and gracile fasciculi) which transmit information regarding discriminative sensation, pressure, vibration and conscious proprioception (the awareness of movement and the position of joints in the limbs); the ascending spinocerebellar tracts in the dorso- and ventrolateral funiculus which carry unconscious proprioceptive information; and the ascending anterolateral system in the ventrolateral portion of the cord (i.e. spinothalamic, spinoreticular and spinotectal tracts) which carries nociceptive (pain), temperature and light touch information. Understanding the tract organization of the spinal cord allows for clinicians to offer prognosis on functional impairment by correlating the location of injury within the cord to functions carried in those tracts.

Figure 1.5 The white matter organization of the human spinal cord. The dorsolateral and ventrolateral funiculi combine to make the lateral funiculus
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1.3.2.2 Blood Supply to the Spinal Cord

The blood supply to the spinal cord arises from two sources: 1) vertebral arteries which give rise to the anterior and posterior spinal arteries and 2) radicular (medullary feeder) arteries which enter the spinal canal at various levels, originating from intercostal branches of the thoracic aorta and lumbar branches of the abdominal aorta and anastomose (connect) with the spinal arteries [53]. The number and location of radicular arteries supplying the spinal cord is highly variable. The anterior spinal artery receives from 2-17 feeder arteries and the posterior spinal artery receives 6-25 feeder arteries [54]. While the dorsal medullary feeder arteries are more numerous than the ventral ones, they are smaller. The constancy of blood flow to and within the spinal cord is assured by the redundancy of supply from the feeder arteries and the availability of alternate routes.

The anterior and posterior spinal arteries run the length of the spinal cord. The anterior artery arises from the vertebral arteries and runs caudally along the ventral median fissure. The two
posterior spinal arteries are branches from either the vertebral artery or the posterior inferior
cerebellar artery. There are few direct connections between the anterior and posterior spinal
arteries; these are provided by arterioles on the surface of the cord. The three spinal arteries
anastomose on the surface of the conus medularis.

Blood flow into the spinal cord arises from sulcal branches initiating from the anterior spinal
artery. The sulcal branches alternate right and left sides and average 2-12 arteries for each
centimetre of the ventral longitudinal arterial trunk. There is a greater density of arteries at
the cervical and lumbosacral enlargements of the spinal cord than at the thoracic level where
the grey columns are smaller. The sulcal branches supply the anterior 2/3 of the spinal cord,
particularly the ventral grey horns, a portion of the dorsal grey horns and the ventral and
lateral white funiculi. The remainder of the grey horns and the dorsal funiculus of white
matter are supplied by the posterior spinal arteries.

Arteries that penetrate the spinal cord are end arteries that give rise to a portion of the
medullary capillary bed. Capillaries are less numerous in the white matter than in the grey
matter, leading to greater blood flow in the grey matter. Furthermore, the orientation of the
white matter capillaries differs from that of the grey matter and may be a contributing factor
to their different responses to traumatic injury [54].

Injury to the spinal cord can disrupt the blood-brain barrier. The blood-brain barrier is
formed by tight junctions between capillary endothelial cells. These cells regulate the
transport of glucose, amino acids and other substances from the blood to neurons and
neuroglia. The blood-brain barrier also restricts the delivery of most therapeutics from the
blood stream into the neural tissue [53]. Injury to the spinal cord that disrupts the blood-
brain barrier allows numerous substances into the cord which would normally be excluded.
Following injury the barrier may remain disrupted for weeks allowing a continuous stream of
foreign particles into the spinal cord tissue[53]. The flood of foreign matter into the neural
tissue may assist in worsening the injury.
1.3.2.3 **Cellular Structure of the Nervous System**

The spinal cord is composed of two types of cells, neuronal and glial. The neuron is the main functional unit within the cord and is comprised of a cell body, several dendrites, synapses and a single axon (Figure 1.7). The cell body is the trophic (nutrition) centre of the neuron. It contains a nucleus and the principal protein synthesizing machinery, as indicated by the presence of ribosomes, RNA and Nissl substance. Damaged neurons often demonstrate a dispersal of Nissl substance. The cell bodies and dendrites both receive input and integrate information. The axon transmits information over long distances and synapses communicate with target cells. There are three types of glia cells with distinct functions for the nervous system: astrocytes, oligodendrocytes and microglia.

The neuronal cell body is located in the grey matter and is a signal generator and processor. The axon is a cable-like extension of the cell and is the communication pathway between cells. The axon is wrapped with myelin sheaths and this construct forms the nerve fibre. The axon, surrounding myelin and oligodendrocytes comprise most of the white matter of the cord. The axons connecting the brainstem with the grey matter of the spinal cord in the lumbar region can be up to 1.0m in length. The amount of white matter is greatest at the cervical level as all ascending and descending axons pass through this level. As nerve fibres synapse on the spinal cord the number of

*Figure 1.7: Spinal cord neuronal cell structure.*
remaining fibres is diminished and the cross sectional area of white matter is decreased. The tracts of the spinal cord are groups of fibres that travel together (throughout the central nervous system) with similar origin and termination points. Fasciculi of the cord are fibres which travel together.

Dendrites receive information from other neurons via synaptic terminals and conduct signals along their length. Axons and dendrites are thought to behave as cable conductors and their conduction velocities are dictated by their size and length. The dendrites have limited protein synthesis capabilities and the axons have even less [55]; therefore both the dendrites and axons are dependent on intracellular transport of the proteins produced in the cell body for survival.

1.3.2.4 Neurofilaments

The cytoskeleton of the neuron is similar to that of other cells. It is composed of three primary elements: microtubules, neurofilaments and microfilaments. Microfilaments are the smallest cytoskeletal elements at 4-7 nm in diameter [56]. They are made of actin filaments (43 kilodaltons (kd)) which are prominent in motile areas of neurons (growth cones of axons and dendrites) [55]. Microtubules are polymer chains of tubulin proteins and are the largest cytoskeletal elements with a diameter of 20-25nm [56]. Both axons and dendrites contain microtubules, which mediate the rapid phase of intracellular transport [55]. Neurofilaments are the main intermediate filaments of the neuronal cytoskeleton and are the most abundant cytoskeletal structures in large diameter axons [57]. They are 10 nm in diameter and are comprised of three different neurofilament proteins: light, medium and heavy. Light neurofilament protein (66kd) forms the core structure which is similar to intermediate filaments in other cell types [58]. Medium and heavy neurofilament proteins form the sidearms and have apparent molecular weights of 140kd and 220kd respectively. The sidearms are thought to participate in interactions between neurofilaments and other elements of the cytoskeleton, particularly microtubules [55].

Structural support for the axon is provided by the neurofilaments. The side arms of the medium and heavy neurofilaments are thought to assist this support by maintaining
interfilament distance [59]. In addition they appear to regulate axonal calibre and thus influence the related property of nerve conduction [57]. The correlation of axonal diameter to neurofilament number persists during axonal degeneration and regeneration [60]. Furthermore, changes in neurofilament transport correlate temporally with alterations in the calibre of axons in regenerating nerves [61].

The insight of observing changes in neurofilaments following injury was initiated by research into traumatic brain injury (TBI). Neurofilaments in brain tissue demonstrated immediate disruption upon traumatic brain injury [62]. Within 15 minutes a focal increase in immunoreactivity in disrupted axons has been observed and at the electron microscope level, the immunoreactive subunits appeared to lose their linear alignment to the long axis of the axon [62]. The misalignment of the neurofilament network may have caused misalignment of the microtubules which created impaired axoplasmic transport [62]. Disruptions in axoplasmic transport are thought to restrict sufficient proteins from reaching the axon and may lead to permanent axonal damage.

Observing damage to neurofilaments in the spinal cord was a logical progression based upon the results found in TBI research and the similarities of spinal cord and brain tissues. Initial observations showed neurofilaments and microtubules accumulated in greater numbers in the axon following injury [63]. More recent work has found contradictory results, demonstrating that neurofilaments decreased in number and density at 15 minutes post injury as seen by electron microscopy [64]. Furthermore, a loss of neurofilament proteins as observed by slab and tube gel techniques demonstrated a decrease in proteins at 15 minutes post injury and a substantial decrease in proteins by 30 minutes [64]. Immunostaining for light neurofilament demonstrated immunoreactive, swollen axons at 4 hours after compression and these swollen axons increased in number and size when measured 1 day and 4 days after injury [65].

Changes in neurofilament behaviour following spinal cord injury have also been demonstrated in human patients. Patients with a terminal illness were given cordotomies (severing of the spinothalamic tract by partial transection of the cord) for the relief of intractable pain [66]. High levels of immunoreactivity in axonal swellings was reported in
all the cordotomy cases [66]. Positive staining was also seen in the neuronal cell bodies and the anterior horn and Clarke's column, which was not seen in healthy tissue [66]. These results show that changes in neurofilament reactivity are seen in both experimental models and clinical cases of spinal cord injury. Observing these changes provides insight into the effect of spinal cord injury on the structure of axons.

1.3.2.5 Myelin

Axons in the spinal cord are wrapped in concentric double layers of a plasma membrane called myelin. Myelin is created by oligodendrocytes in the spinal cord. The myelin structure is segmented and is organized in tandem segments along the length of the axon with each myelinated segment arising from another oligodendrocyte (Figure 1.8). Gaps between each of the myelinated segments are called nodes of Ranvier and provide the basis for expedient conduction along the axon.

The myelin sheath plays a role in regulating the speed of conduction along the axon. Myelin acts as an insulator, limiting current leakage and it acts as a capacitor, minimizing the charges necessary to depolarize the axonal membrane. This results in saltatory conduction where the action potential jumps from node to node along the axon. Saltatory conduction increases the speed of signal conduction as compared with non-myelinated fibres of the same size [67]. Furthermore, myelinated fibres can conduct impulses at higher frequencies and consume less energy per impulse than non-myelinated fibres [68].

Demyelination of the axon as a result of spinal cord injury or disease leads to a number of conduction abnormalities including decreased conduction velocity [69], temporal dispersion.
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of impulses [70], conduction blockage [71] and abnormal interactions with nearby fibres [72]. Disruption and loss of myelin following injury has been tracked structurally using luxol fast or toluidine blue staining techniques [13-16, 73-78] and electron microscopy [17, 63, 64, 74, 75, 79-88]. Banik et al. observed vesiculation of the myelin sheath within an hour of experimental injury in rats with sloughing of the sheath into the extracellular space beginning 12 hours after injury using electron microscopy (Figure 1.9) [88]. The progression of myelin damage following experimental spinal cord injury has been observed by several researchers and is generally characterized by a swelling of the axolemma (inner cytoplasmic wrapping surrounding the axon) [84], followed by gaps between the axolemma and the myelin sheath [83, 84], then vesiculation and rupture of the myelin [80, 83, 88], invagination of the myelin into the axon [83] and finally the presence of macrophages within the myelin sheath remnants [84].

Figure 1.9 A schematic cross section of healthy (A) and injured (B) axons (Derived from Banik [88] and Anthes [83]).

Immunohistochemical and immunocytochemical techniques have been employed more recently to track changes in myelin and oligodendrocyte proteins following spinal cord injury [64, 88-93]. Central nervous system myelin is comprised of 50% proteolipid protein (PLP), 30% myelin basic protein (MBP) and 5-15% peripheral nervous system myelin. Experimental spinal cord injury models have shown decreasing levels of MBP and PLP.
following injury. Myelin protein concentrations have been shown to be normal for the first two hours following injury and then to gradually decrease over several days, stabilizing months after injury [64, 88, 89, 91, 93].

Buss et al [90] observed decreases in levels of MBP in human spinal cord injury. The progression of myelin degradation occurred at a much slower rate in the human, with no observable changes for patients who survived 2-14 days post-injury. Small decreases in the amount of labelling were seen for patients surviving 24 days to 4 months; however, there were still many intact myelin sheathes. Complete loss of MBP signal was observed only for a patient surviving 8 years post-injury. These observations suggest tracking of MBP does not accurately reflect the immediate structural changes seen by staining and electron microscopy but is useful in the longer term evaluation of spinal cord injury.

1.3.2.6 Neurophysiology of the spinal cord

The conduction of signals throughout the spinal cord is primarily dependent upon the concentrations of sodium and potassium ions on either side of the axonal membrane. Each axon is surrounded by a membrane which acts to control the transmission of molecules into and out of the axon. Embedded within the membrane are channels which selectively allow inorganic ions to enter and exit the cell. Each of the primary ions, sodium, potassium, calcium and chlorine, have their own channels. The concentrations of the different ions on either side of the membrane result in an electrical potential across the membrane at rest, with the inside of the cell at -70mV with respect to the outside. Some of the channels remain continuously open while others are voltage-gated and open or close based upon changes in the electrical potential across the membrane. The neuron can be excited by a variety of chemical or physical stimuli which results in a 10 to 15 mV decrease in the membrane potential (called depolarization). This change triggers an opening of voltage-gated channels, across the membrane and results in a surge of positive sodium ions into the cell. This influx results in a transient change in charge across the membrane to +40mV. Once triggered, the influx is self-propagated along the axon, as the change in membrane potential results in nearby voltage gated channels being opened, a similar ionic influx occurring and more channels being opened. The voltage-gated sodium channels are only present in the axolemma at the
nodes of Ranvier. The action potential is forced to jump from node to node along the axon. Myelin acts to insulate the portions of the axon between nodes, encouraging this saltatory signal conduction. The speed of signal transmission is greatly increased due to this method of conduction.

Changes in the concentrations of ions either intracellularly or extracellularly are used to demonstrate increased permeability of the axonal membrane following injury to the spinal cord. While the axon may appear normal under electron microscopy, abnormal concentrations of ions within or surrounding the axon are indicative of that axon having lost the ability to conduct signals along its length.

1.4 Spinal Cord Injury

Spinal cord injury (SCI) is a devastating and catastrophic occurrence. It occurs in approximately 16,000 new patients in the United States [1, 2] and 1,200 in Canada each year [3, 94]. The causes of injury range from motor vehicle accidents and violence to recreational and work place activities. The life expectancy of a patient who survives the first year after injury is approximately 10 years less than the general population for those with paraplegia and 20 years less for people with quadriplegia [95]. There are in an estimated 250,000 – 400,000 people currently living with chronic injuries in the United States. These injuries cost the United States economy an estimated $9.73 billion annually [96]. Currently, there is no treatment that can improve or reverse the effect of the injury once it has occurred.

A number of studies have attempted to quantify the incidence of injury, the population of patients with chronic injury and the fatality rate associated with injury. The results of these studies are extrapolated to generate the national statistics as there have yet to be any nationwide studies. A review of spinal cord injury cases in Olmsted County, Minnesota from 1975-1981 showed an incidence of 71 injuries per million [4]. That study included all SCI victims including those who died before reaching hospital. A study reviewing rates of spinal cord injury in Ontario showed an incidence of 37.2 per million people, not including those who died at the scene [3]. The global incidence of spinal cord injury was reported to be 15 to 40 cases per million in 2001 [4]. Unless otherwise noted the incidence reported in most
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studies reflects only of those patients who reached hospital alive. The mortality rate of spinal cord injuries has been reported to be 48.3% [2].

The causes of spinal cord injury vary between countries and between urban and rural areas. Globally, traffic accidents are responsible for greater than 50 per cent of all injuries [4]. In the United States the second leading cause of injury is violence (29%) followed by falls (22%) and then recreational activities (8%) [97]. In Canada the causes of injury were primarily accidental falls (43%) and motor vehicle accidents (42.8%) with violence being a very minor cause of injury (2.2%) [3]. Spinal cord injury is predominately an affliction of the young male. The median age of injury is 32 [97] and 81 per cent of victims are male [95].

The physical costs of spinal cord injury are well understood and documented, however the economic costs of SCI are also important both to the individual and to society. As healthcare systems are forced to prioritize spending initiatives it is important to understand the socioeconomic impact of treatment in addition to the medical benefits [98]. An extensive study was undertaken by the Bureau of Economic Research at Rutgers University to determine the prevalence of SCI in the United States and the direct and indirect costs of injury [99]. The direct costs of medical treatment and housing modifications as well the indirect cost of lost wages and homecare services were evaluated in the study. The lifetime costs for a representative individual with complete quadriplegia injured at age 27 was $1 million, whereas lifetime costs for a paraplegic injured at age 33 was $500,000. When these costs were aggregated over the entire SCI population the total cost per year, in 1988 dollars was $5.6 billion. If all cases of SCI in 1988 and beyond were prevented this would result in savings of over $250 billion in the United States alone [98].

Improvement in the functional outcome of patients after spinal cord injury is beneficial for both people with SCI and society as a whole. Research has demonstrated that greater residual function following injury resulted in an increased probability of returning to work, 38.6% for paraplegics versus 27% for quadriplegics, as well as greater earning potential, $9, 612 annually for paraplegics versus $6,014 for quadriplegics [100]. Enhanced treatment
would likely provide economic benefits in the form of fewer days of hospitalization and a reduced need for a practitioner or personal assistance services throughout the recovery process.

Several treatment alternatives have been proposed and researched in recent years yet no treatment has demonstrated global efficacy in the management of spinal cord injury. Current medical treatment of spinal cord injury may include methylprednisolone, however recent reviews have reported insufficient evidence to support its use as a remedy for spinal cord injury and furthermore, it may exacerbate secondary complications associated with injury [5, 6]. Patients with significant residual compression of the spinal cord upon presentation to a clinical facility may be recommended for early (< 8 hrs post injury) decompression and stabilization surgery; however, significant neurological improvement as a result of early surgery has not been demonstrated in a clinical setting [8-11]. There is conflicting evidence supporting the effectiveness of decompression of the spinal cord either through surgery or closed reduction at any time post injury however some patients have shown neurological improvement [9, 10]. For the most part, once the initial impact to the cord has occurred there is no treatment to reverse its effect, only methods to assist in minimizing the progression of injury.

Spinal cord injury is temporal in nature and progresses through a series of phases throughout the hours, weeks and months following injury. Spinal cord injury is initiated by a physical disturbance of the cord called the acute or primary injury. The mechanical insult immediately triggers a cascade of cellular and immune system responses, known as the secondary injury, which continue to worsen the injury over the course of subsequent days and weeks. The degradation eventually stabilizes after several months at which time the injury is described as a chronic injury. The initial injury to the spinal cord and spine is the primary determinant of neurological outcome [11] and there continues to be no effective treatment alternative to reverse or minimize the functional deficits associated with injury.
1.4.1 Primary Injury

Spinal cord injury results from the vertebral column becoming mechanically unstable due to the destruction of its structural elements and the subsequent deformation of the spinal cord. Damage to the vertebral column can result from a variety of injury mechanisms. The predominant direction of loading during injury to the spinal cord in the human is compressive [101-105]. The direct mechanical compression of the cord results from displacement of fractured vertebrae and/or discs protruding in the spinal canal.

Description and classification of spinal column and spinal cord injury mechanisms and their relationship to neurological damage has been the focus of many researchers [101, 106-112]. Some mechanisms of vertebral column injury are more likely to cause spinal cord injury than others. The column injuries showing the greatest incidence of cord damage are fracture dislocations and burst fractures (Table 1.1). Furthermore, only 20 percent of cases involve isolated SCI, the remainder include other systemic injuries which exacerbate secondary injury and complicate medical treatment.

<table>
<thead>
<tr>
<th>Injury</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>Minor Fracture</td>
<td>10%</td>
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<tr>
<td>Fracture Dislocation</td>
<td>40%</td>
</tr>
<tr>
<td>Dislocation</td>
<td>5%</td>
</tr>
<tr>
<td>Burst Fracture</td>
<td>30%</td>
</tr>
<tr>
<td>SCI without radiological evidence</td>
<td>15%</td>
</tr>
</tbody>
</table>

A significant finding of the study of injury mechanisms showed that neurological impairment was associated with both the direction and degree of dislocation in the fracture dislocation injury [101]. The direction of dislocation affected the severity of neurological deficit, with 81.6% of anterior dislocations being complete injuries while 58.3% of posterior dislocations were complete. In addition, as the degree of dislocation increased so did the neurological deficit. In contrast to the previous study, reviews of thoracolumbar burst fracture injuries showed no correlation between the amount of canal compromise at clinical admission and neural deficit either at the time of admission or during long-term follow-up [113, 114]. Furthermore, CT and MRI images taken on clinical admission did not correlate with the
transient injury that occurred at the time of the mechanical impact [115]. These results suggest that the primary injury plays an important role in determining the neurological impairment of a patient, while residual compression may be less significant.

1.4.2 Secondary Injury

The primary mechanical insult to the spinal cord triggers a cascade of cellular responses that continue to degrade the spinal cord tissue for several days and weeks following injury. This degradation is known as secondary injury. The concept of secondary injury was first hypothesized by Allen when he observed that drainage of the posttraumatic haematomyelia (haemorrhage in the spinal cord) resulted in improved neurological function in dogs following spinal cord injury [116]. He further postulated that there was a noxious effect on the spinal cord resulting from the presence of hemorrhagic necrosis that caused further damage to the spinal cord [117]. Subsequent studies of spinal cord injury have uncovered several possible pathophysiological mechanisms of continued posttraumatic destruction of spinal cord tissue (Table 1.2) [118]. The significance of each mechanism and the sequence with which they occur following injury has not been clearly established.

Table 1.2: Secondary injury mechanisms [118]

<table>
<thead>
<tr>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Vascular changes</td>
</tr>
<tr>
<td>Loss of autoregulation</td>
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<tr>
<td>Systemic hypotension</td>
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<tr>
<td>Haemorrhage</td>
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<tr>
<td>Loss of microcirculation</td>
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<tr>
<td>Reduction in blood flow</td>
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<tr>
<td>Vasospasm</td>
</tr>
<tr>
<td>Thrombosis</td>
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<tr>
<td>Electrolyte changes</td>
</tr>
<tr>
<td>Increased intracellular calcium</td>
</tr>
<tr>
<td>Increased extracellular potassium</td>
</tr>
<tr>
<td>Increased sodium permeability</td>
</tr>
<tr>
<td>Biochemical changes</td>
</tr>
<tr>
<td>Neurotransmitter accumulation</td>
</tr>
<tr>
<td>Catecholamines</td>
</tr>
<tr>
<td>Excitotoxic amino acids</td>
</tr>
<tr>
<td>Arachidonic acid release</td>
</tr>
<tr>
<td>Free-radical production</td>
</tr>
<tr>
<td>Eicosanoid production</td>
</tr>
<tr>
<td>Prostaglandins</td>
</tr>
<tr>
<td>Lipid peroxidation</td>
</tr>
<tr>
<td>Endogenous opioids</td>
</tr>
<tr>
<td>Oedema</td>
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<tr>
<td>Loss of energy metabolism</td>
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<tr>
<td>Decreased adenosine triphosphate production</td>
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</table>
The development of experimental treatments for spinal cord injury has focussed on mitigating single or multiple elements of secondary injury. Calcium channel blockers [119], neurotrophic factors [120, 121], and membrane repair compounds [122] have all demonstrated laboratory success in minimizing secondary injury but have yet to demonstrate clinical efficacy. A thorough understanding of both primary injury and the continued cellular cascade of destruction are prerequisites to the establishment of clinically effective treatment modalities.

1.4.3 Models of Spinal Cord Injury

Animal models have played a valuable role in increasing our understanding of SCI and have provided a base from which to test therapeutic strategies. Several models have been developed in animals to simulate aspects of human SCI. Clip compression injuries have been developed to observe the effect on the spinal cord of enduring compression without sudden contusion [123] however; this method does not include the recording of mechanical parameters of injury and circumferential compression is not representative of the human injury scenario. Transection models of SCI have been useful for evaluating the success of regeneration strategies [124] as well as identifying locations of spinal tracts within the cord but transection injuries are rarely seen clinically. It is the contusion model established by Allen [116] which gives a better representation of the blunt traumatic injury commonly seen in humans and the subsequent neuropathologic climate after injury [12].

Allen developed a controlled weight drop model of SCI in animals. There are several devices currently in use which are modifications of this original weight drop model but record the magnitude of injury using displacement and force sensors to obtain mechanical parameters of injury. The primary limitation of the weight-drop technique is that the systems are driven by gravity and therefore independent manipulation of the mechanical input parameters is not possible. The range of injury parameters available with this method is far less than that available using a force or displacement controlled device. A variety of injury systems have been developed which offer direct control of the mechanical parameters of injury by using motors, linear actuators, pistons and other electronic devices to generate the impact.
The new contusion animal models are important for characterizing the mechanics of SCI and their relationship to damage. The behaviour of the cord during initial impact can be evaluated by tracking injury parameters such as displacement, velocity and force. The cellular response following impact leading to secondary injury can be evaluated using histopathological techniques. Function can be evaluated through the use of standardized outcome measures. While the primary purpose for the development of these animal models of SCI was to establish a consistent injury on which to test experimental therapies and regeneration strategies [43], these models also provide significant insight into the physical mechanisms through which spinal cord injuries occur. The use of animal models to understand the relationship between physical injury parameters, cellular damage and functional outcome also has application in both injury prevention and management.

1.4.3.1 Establishing a relationship between impact and injury

Several investigators have demonstrated significant relationships between the magnitude of impact and histological measures of tissue damage[13-39]. Researchers have used a number of parameters to describe the mechanics of experimental injury. The most commonly researched parameter is cord displacement. Several authors have demonstrated a relationship between the amount of cord displacement and the resulting histological and functional damage [13, 19, 21, 28, 30, 40-42, 125]. A rat contusion model of SCI demonstrated increases in the lesion length, lesion volume and a decrease in the percent tissue spared with increased impact depth [13, 21, 41]. Functional outcome also correlated to depth of impact. In rat contusion models a moderate injury occurred for impacts ranging from 0.95 – 1.1mm [13, 41] and 40-60% of canal diameter [19, 42, 43].

Some investigators have used force to define the magnitude of injury instead of displacement [18, 21, 44, 45]. Others have observed force as a variable in their experimentation [13, 30, 41]. All of the authors report a correlation between impact force, lesion size and functional outcomes. A force of 2-2.5 N has been observed to cause moderate functional deficits in rats [16, 41, 45]. It is debatable whether force or displacement provides a better descriptor of injury and which should be used as a control parameter in contusion injury. There are demonstrated correlations between impact force and displacement [13, 21, 45]. Variability in
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correlations from 30-50% [18, 21] reflect biological variability of the tissue [21], failure to properly stabilize the spinal column [126], variability in the site and size of the laminectomy [33] and failure to set and reproduce a baseline from which to generate the injury [30, 126].

Other variables have also been used to describe the mechanics of spinal cord injury including impact velocity, energy, impulse, power. Energy, impulse and power have all been shown to correlate with lesion volume and long-term functional recovery [13, 18, 44, 127-129] however, these variables are derived from the controlled displacement and/or force. Several studies have shown a linear correlation between the drop height of a constant mass and lesion size and residual function [17, 20, 116, 127-133]. The increased drop height is analogous to increased impact energy for a constant mass [127, 128]. The rate at which the impact is delivered to the cord has not been studied as thoroughly as displacement or force but there are indications that it may significantly affect the magnitude of injury.

1.5 Impact Velocity and Spinal Cord injury

1.5.1 Clinical Understanding of Impact Velocity

Traumatic injury, including burst fracture and fracture dislocation, results from a sudden, transient, contusive impact to the spinal cord that generates neurological impairment. Disruptions in neurological function may also occur in chronic, degenerative conditions such as spinal stenosis, where the spinal canal slowly narrows and applies pressure to the spinal cord. Patients with spinal stenosis often have significant cord compromise (>45%) [134] before experiencing any neurological symptoms; however patients with traumatic spinal cord injury occurring at greater velocities often show compromise of function with lesser amounts of cord displacement. It is difficult to know the effect of impact velocity to the spinal cord in clinical cases, as this information is impossible to extract from radiographs taken several hours after the initial injury. Several studies have demonstrated that the amount of canal occlusion observed by radiograph in the clinic is significantly different and not correlated to the transient injury experienced by the cord during the burst fracture injury [50, 115, 135]. It is hypothesized that the majority of neurological damage to the spinal cord occurs during the
transient portion of the burst fracture injury as residual canal compromise of up to 40-50% has been shown not to affect neurologic recovery [114].

1.5.1.1 Mechanical Parameters of a Burst Fracture Injury

Contusion models of spinal cord injury closely mimic the injury cascade and neurological outcomes seen clinically in human injuries. Understanding the relationship between modes of injury and the impact on the spinal cord is important to produce a representative model of human injury. A limited number of studies using cadaveric models of burst fracture injury have attempted to quantify both the magnitude of canal occlusion [49, 50, 111, 115, 135-137] and the velocity [48-50] at which bone fragments impinge on the spinal cord. A range of techniques has been used to measure the changes within the spinal canal during injury, immediately following impact and after unloading the specimen. Transient canal occlusion during a burst fracture injury has been reported to be 30-71% in the cadaveric model [50, 111, 135, 136]. Cadaveric models have further demonstrated residual canal occlusion following a burst fracture to be 18-47% [50, 135]. The use of a high-speed video system to image canal occlusion during the burst fracture process has demonstrated the strain rate at which the bovine canal is occluded to be 110 s\(^{-1}\) [48]. This is equivalent to an impact velocity of 1.7m/s in the human. Other studies that measured the amount of canal occlusion using strain gauges reported the velocity at which the canal was occluded to be 1.5m/s [49] and 1.87 m/s [50]. The results demonstrated a consistency in the rate at which the spinal cord would be impinged during a burst fracture injury, independent of the methodology and energy used to generate the burst fracture.

1.5.2 Engineering Knowledge: Material properties of the spinal cord

Several works have demonstrated a nonlinear relationship between force and displacement in the mammalian spinal cord [29, 31, 138-144]. This is characteristic of all soft tissues in the body. At low strains the spinal cord demonstrates nearly linear elastic properties however with the higher strains representative of injury the material response is nonlinear. In addition to the nonlinear stiffness properties the cord demonstrates rate dependent characteristics, indicating that the relationship between force and displacement also depends on the rate at which the load is applied [141].
The spinal cord is a non-linear viscoelastic material. This means that the strain rate and the duration of displacement affect the stress response and the strength properties of the cord [145]. Viscoelasticity is characterized by three primary features; creep, when a body continues to deform under a constant load; relaxation, when a body is strained and maintained at a constant strain and the induced stresses decrease with time; hysteresis, when a body subjected to cyclic loading shows a different stress-strain behaviour during the loading process than the unloading process [146]. These primary features all include an element of time on the behaviour of viscoelastic tissues. Furthermore, viscoelastic materials demonstrate strain rate dependence, when a body shows different stress-strain behaviours as a result of different loading rates. Understanding the viscoelastic nature of the spinal cord has implications in injury. A small displacement of the cord at a high speed may result in significantly more damage than a large displacement at a lower speed.

A variety of models have described the biomechanics of the spinal cord and its rate dependent material characteristics during loading. Several tensile experiments have been performed on in vivo and in vitro animal and human tissue to determine material characteristics of the spinal cord, however none of them have attempted to compare strain rate with material failure or histological outcome [31, 138, 141, 142, 147]. The time dependent nature of human spinal cord material properties has been examined however it was not correlated with either histological or functional outcome measures of injury [144].

Bilston and Thibault showed unexpected differences between the effect of loading rates on residual stress in the cord after the tissue was allowed to relax [144]. It is generally expected that during the relaxation behaviour eventually the tissue will relax to a consistent level of stress independent of the strain rate at loading; however, Bilston and Thibault observed greater residual stress in cords loaded at a higher rate. This finding has important clinical implications; for example, the decision to perform decompression surgery on a trauma victim is made only on the amount of residual compression seen in a radiograph upon presentation to a clinic. The cord behaviour suggests that the loading history of the tissue has an effect on the continued stress experienced by the cord and this suggests that two patients presenting
with identical amounts of compression on the spinal cord may in actuality have very different injuries.

The time dependent material properties of the spinal cord hold true for both live and dead tissue. However, the behaviour of the cord has been shown to be far stiffer after death, demonstrating a 150% increase in pseudo Young's Modulus 1.5 hours after death [141]. Furthermore, the stress during the relaxation phase of a stress relaxation test, for dead tissue, was 70 percent higher than that of a live animal. This suggests that in order to have an accurate representation of the tissue response to loading, it must be evaluated in living tissue.

In addition to characterizing the behaviour of the complete spinal cord, work has been done to address the relative differences in physical behaviour of the grey and white matter of spinal cords. Ozawa et al. evaluated the stiffness of the grey and white matter of a rabbit spinal cord and found there was no significant difference in their behaviour [143]. Ichihara et al. demonstrated that for the bovine cord under tension, the grey matter was more rigid and fragile than the white matter for high strain (>0.1) but found similar behaviour in both tissues for low strain levels (<0.1) [142]. Identifying differences in material properties of the grey and white matter may be significant for understanding the locations of maximum stress within the cord when it is impacted and structures most susceptible to damage [148]. Differences in material properties and tissue tolerances could account for such things as the preferential destruction of grey matter. Understanding the mechanical behaviour of the cord is a fundamental step in comprehending spinal cord injury.

1.5.3 Animal models of Impact Velocity

Animal models of contusion injury have produced contradictory results regarding the effect of impact velocity on lesion development and functional recovery. A few authors have reported a strong correlation between velocity, lesion size, function and ionic concentrations [15, 21, 40, 43, 46] while others report a weak or non-existent correlation [18, 42, 44].

Those reporting strong correlations were applying impact velocities greater than 0.5m/s to the spinal cord. In small animal models these impact velocities correlated to strain rates of
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250-1200 s⁻¹, or 2-12 times greater than that seen in the model of human burst fracture injury. Correlations between impact speed, a depth-speed product, tissue damage and SEP were established by Kearney et al. using a pneumatic indenter for impact speeds ranging from 1.5-6m/s [40]. Lesion volume and function, observed 21 days following injury, were correlated to impact velocity by Seki for velocities ranging from 1-3m/s [15]. Kwo et al. used rats to demonstrate a linear correlation between changes in total calcium concentration and impact velocity for velocities between 0.5 and 1.2 m/s [46]. Observations correlating impact velocity to lesion size and function were measured as early as 4-6 hours post-injury [40, 46] and continued to be observed at 24 hours [43] and 21 days [15].

Experiments observing intermediate impact velocities have failed to note significant effects of velocity or have suffered from questionable test methodology which makes the results uncertain. Work by Bresnahan et al. evaluated a range of impact velocities between 0.08 and 0.54 m/s and found a significant relationship between velocity, lesion size and function, however displacement was altered simultaneously with velocity during their testing and therefore made it impossible to determine if the observed effects were truly a result of impact velocity, displacement or a combination thereof [21]. Anderson created contusion injuries using impact velocities of 0.6, 3, 10 m/s but failed to observe a significant correlation between velocity and SEP latency 4 hours following injury [42]. This contradicts the findings of Kearney, who found a significant relationship between velocity and SEP latency at 4 hours post-injury [40]. Anderson did observe a trend towards greater hemorrhagic necrosis with increased impact velocity but did not quantify the results or determine statistical significance [42]. Weak and non-existent correlations were observed by Noyes for contusions with impact velocities ranging from 0.05 – 0.5 m/s [18, 44]. Because of the viscoelastic nature of spinal cord behaviour, it is important to remember that impact velocity cannot be considered in isolation. Increased impact velocity for a fixed displacement results in a greater peak force and also affects impulse, energy and power applied to the spinal cord during injury.
1.5.4 Cellular Models of Impact Velocity

Recent literature described tests that were performed to determine the effect of strain and strain rate on cultured central nervous system (CNS) cells and the resulting physiological damage. It has been observed that under high strain rates, axon membranes become more permeable and that increases in intracellular calcium ion (Ca\(^{2+}\)) concentration reflect that permeability. Cargill and Thibault reported an exponential relationship between Ca\(^{2+}\) and strain rate at high strain rates (>10 /s) for cultured cells [149]. At low strain rates (<0.1 /s), Ca\(^{2+}\) levels increased minimally and were independent of strain rate. Similar results were reported by LaPlaca et al. in cultured NTera-2 neurons with peak and average Ca\(^{2+}\) concentrations exhibiting strain rate and peak stress dependence [150, 151]. These results further support the importance of impact velocity in the discussion of spinal cord injury models.

1.6 Objectives

The primary objective of the proposed study was to compare how the impact velocity of a contusion injury affected the microstructure of the spinal cord with regard to haemorrhage, grey and white matter structural disruption and neurofilament de-phosphorylation using an in vivo rat model of injury with impact velocities of 3 mm/s and 300 mm/s.

To accomplish the primary objective, a series of secondary objectives were established:

- To assemble, calibrate and validate a spinal cord contusion device from Ohio State University and determine the repeatability of its impact;
- To establish testing procedures and protocols to optimize the use of the contusion device for a variety of experiments;
- To complete a series of primary injuries using the contusion device;
- To analyze the injury outcomes using traditional histological techniques for gross tissue morphology and more precise immunohistochemical techniques for specific structures;
- To create a foundation of common ground to facilitate collaboration between the Departments of Orthopaedics and Mechanical Engineering (i.e. Division of
Orthopaedic Engineering Research) and Zoology (i.e. International Collaboration on Repair Discoveries) and to allow the pursuit of new research directions by utilizing the strengths of each laboratory.

1.7 Project scope

This project focused on the effect of impact velocity on elements of the spinal cord in a rat contusion model. The study was limited to two impact velocities (3mm/s and 300mm/s), selected to be two orders of magnitude apart, allowing for a more likely possibility of observing velocity effects. The fast impact velocity was chosen to represent the strain rate at which the spinal canal was observed to occlude in cadaveric models of burst fracture injury. The level of injury was confined to T10. The effects of different levels of injury were not explored. The amount of cord displacement and the duration of displacement were held constant throughout the experiments to isolate the effect of impact velocity. It is thought that an interaction between velocity and displacement may occur in spinal cord injury. That was not examined in this thesis.

Survival of the animals was limited to less than 2 minutes post injury. This allowed for the isolation of the mechanical effects of injury from any secondary effects. The animals remained under anaesthesia for the entire duration of the protocol and therefore no functional evaluation could be performed. The histological evaluation of the lesion was restricted to haemorrhage, neurofilament de-phosphorylation and myelin structure. A number of the common methods for histological evaluation were not available for use as they are ineffective in measuring changes in the cord until several hours after the injury.

The goal in researching acute spinal cord injury (SCI) is to understand, and eventually influence, the factors that govern a patient's functional outcome. This research project constitutes one aspect of a long-term vision within the Divisions of Orthopaedic Engineering Research and Spine, to enhance our knowledge of injury mechanisms of the spinal cord. Knowledge of how the cord is injured will enable us to develop a clinically relevant experimental model. In the laboratory setting a consistent type of injury can be created on
which treatment strategies can be tested. This is critically important to assess novel therapies such as promising drug treatments or rehabilitation protocols.
2. Methods

2.1. Injury System Selection

Controlled spinal cord injuries have been created in animals since Allen developed the weight drop method in 1911 [116]. As technologies have become more advanced it has allowed the research community to more accurately create and observe spinal cord injuries in animals. In recent years particular attention has been paid to controlling and observing the mechanical parameters of injury. Often one parameter is selected as the control parameter and the others may or may not be observed during the course of injury. Several contusion devices have been designed with the express purpose of controlling and observing mechanical parameters of injury.

Several modifications of the weight drop method have since been developed; the most standardized and widely used is the New York University (NYU) impactor. The magnitude of the injury delivered by the weight drop model is characterized by the product of the mass of the weight dropped on the cord and the vertical distance it travelled, giving a gram-centimetre product. The correlation of the gram-centimetre product with the magnitude of injury has been repudiated [127, 128]; however correlations have been established between the height of the weight dropped and injury when the drop mass is constant [17, 116, 129-133].

The gram-centimetre product does not convert directly to standard mechanical parameters. Varying weight and height combinations for a constant g-cm product have differing effects on biomechanical parameters such as displacement, energy, impulse and velocity and therefore make it difficult to compare the results with tests using biomechanical parameters [127, 128]. Indirect control of impact velocity during experimentation was achieved by selecting different drop heights for a constant mass [46], however it was impossible to separate the control of the mechanical parameters, so changes in drop height also resulted in changes of impact depth and force. The impact velocity was predicted by using the conservation of energy principle and assuming negligible energy dissipation by friction or heat (Equations 1 and 2). A recent iteration of the weight drop model incorporated two
potentiometers to measure the displacements of the spinal cord and spinal column [43], but did not include apparatus for measuring the force applied to the spinal cord. The displacement and velocity corresponding to the weight drop injuries were correlated to the height of the dropped weight. The impact velocity demonstrated a second order polynomial relationship ($r^2 > 0.99$) between drop height and impact velocity for a constant mass, as predicted by the conservation of energy principle. A linear correlation ($r^2 = 0.72$) was shown for cord compression versus impact height. Using the weight drop contusion model allows the examination of impact intensity and its relationship with injury however it is difficult to relate these results to the biomechanical parameters of injury.

$$mgh = \frac{1}{2}mv^2 \quad \text{Equation 1}$$

$$v = \sqrt{2gh} \quad \text{Equation 2}$$

Where:
- $m =$ mass of dropped weight [g]
- $g =$ gravitational constant [9.81 m/s$^2$]
- $h =$ height of weight dropped [m]
- $v =$ velocity of dropped weight at impact [m/s]

Another contusion device was developed by Anderson which used a pneumatic cylinder to create a contusion injury in the spinal cord [19]. This device controlled for cord displacement by offsetting the impactor tip a known displacement from the spinal cord and controlled velocity by altering the air pressure delivered to the cylinder. There were no sensors to record the actual displacement, velocity or force experienced by the spinal cord during the injury process.

A further iteration of the contusion device developed by Anderson used an electromagnetic shaker to generate the injury stroke and involved a closed-loop feed-back control system [18]. The electromagnetic device was further optimized to eliminate the need for the feed-back control system by using a manually set mechanical stop to determine the maximum displacement of the impactor [152]. This electromagnetic injury device has been deemed the OSU impactor, the Electromechanical Spinal Cord Injury Device (ESCID) and the Spinal
Cord Injury Research System (SCIRS) at different intervals in its development and will be referred to as the SCIRS for the remainder of this thesis. The impact velocity and the duration of impact were also controllable through a user interface. A non-contacting displacement transducer recorded the movement. Loading on the spinal cord was recorded by an impedance head, which measured total force and acceleration of the impactor. The acceleration measure was used to calculate and subtract inertial forces from the total force to give net force acting on the spinal cord.

The most recent spinal cord contusion system, named the Infinite Horizon spinal cord injury device (Precision Systems and Instrumentation, Lexington, KY), was described by Scheff et al. and featured a force controlled stepper motor to generate the spinal cord injury [45]. The control program dictated that the impactor was withdrawn immediately upon equalling or exceeding the desired force; therefore no variation in dwell time was possible. The stepper motor technology limited the maximum achievable velocity of the system to 130mm/s. A linear encoder recorded the displacement of the impactor and a force transducer measured the applied load, providing a thoroughly defined injury.

The Spinal Cord Injury Research System provided several benefits over other models of contusion injury. For the purpose of evaluating the effect of mechanical parameters on spinal cord injury in the rat; 1) it allowed for input of the desired magnitude, rate and duration of injury to generate a tightly controlled and repeatable injury, 2) it recorded both the force and displacement applied to the spinal cord during injury so the injury could be fully defined and compared for a number of mechanical parameters, 3) the use of an electromagnetic shaker provided very repeatable and reliable system performance for the desired slow and fast loading regimes.

2.2. Spinal Cord Injury Research System (SCIRS)

The SCIRS (Ohio State University, Columbus, OH) was designed to generate repeatable spinal cord injuries that could be characterized by their mechanical parameters of impact. The system was designed to generate a range of standardized, moderate contusion injuries in rats on which to test the effectiveness of experimental therapies. The system provided the
opportunity to tightly control the displacement and velocity of injury and the displacement, force and acceleration transducer allowed the impacts to be fully described by their mechanical parameters. While not its intended use, the system allowed for exploration of the effects of varied mechanical parameters on spinal cord contusion injury. The key operating principles of the system were the rigid stabilization of the animal during impact and the delivery of a repeatable and well defined mechanical injury.

The rigidity of the system was provided by the stage on which all components were mounted (Figure 2.1). An electromagnetic shaker that included the impactor for contusion the cord was positioned in a trunion which was mounted to a linear screw slide. The shaker assembly was vertically mounted to the rigid base of the SCIRS via a two foot tall, four inch square aluminium pillar. The pillar was connected to a large aluminium plate approximately two feet by two feet square. A two-axis machine tool table was attached to the aluminium plate and allowed for horizontal positioning of the animal beneath the impactor tip. The mass of the tool table helped to isolate the system from vibration. Attached to the tool table was the spinal frame in which the animals were suspended. The spinal frame linked to the Allis clamps which clamped to the animals' vertebrae. A hoist and scale were positioned beneath the animals in the spinal frame.

The mechanical impact was delivered by a methylmethacrylate impactor tip which was attached to the electromagnetic shaker via a rod assembly. The delivered impact was characterized by sensors mounted in line with the impactor tip (Figure 2.2). The rod assembly was comprised of two small aluminium tubes, an impedance head, a plastic plate, a threaded rod and several nuts. The first aluminium tube was threaded at both ends and connected the impactor tip to the impedance head transducer. Above the impedance head was a second threaded aluminium tube connector which connected to a 3 inch long threaded rod. A methylmethacrylate plate with a slot was mounted on the threaded rod and was sandwiched between two nuts to secure it in place. The plate extended two inches from the rod and had a one inch square, flat aluminium piece secured to it. The aluminium piece acted as the target for the non-contacting displacement transducer. The head sensing portion of the transducer was mounted to the trunion assembly of the electromagnetic shaker. The threaded
rod then passed through a slot in a brass plate. The brass plate was connected to the shaker assembly and acted as the mechanical stop. Above the brass plate a plastic nut was secured on the threaded rod and provided the impact interface with the mechanical stop. The threaded rod then screwed into the mounting hole of the moving portion of the electromagnetic shaker.

Figure 2.1 Schematic diagram of the Spinal Cord Injury Research System.
The operation of the system was displacement controlled and allowed the user to input the desired displacement parameters for the injury including impact velocity, depth of impact and duration of impact. The system used an open-loop control system to generate the pattern of injury. Without feedback on the position of the impactor during motion the correlation between the input parameters and actual system output was unreliable (Figure 2.3). Furthermore, as a result of the lack of feedback on the control signal a mechanical stop was required to restrict the indenter to a fixed depth of impact to ensure accurate and repeatable injuries [152]. The open loop control system reduced the number of required input parameters, as compared with a closed-loop system and was expected to make the system more accessible to a broader range of users [152]. A personal computer using a Windows
based operating environment ran the provided software which coordinated both the control of the injury system and the recording of data from the transducers.

Figure 2.3 The input signal and resulting output displacement for a representative specimen. The input values set would have resulted in a curve similar to the blue lines. The resultant system performance is shown in red. The placement of the mechanical stop is indicated by the green line.

The user interface of the SCIRS offered four modes of operation: displacement calibration, force calibration, touch and hit. The displacement calibration mode was used to record the voltage output from the displacement transducer for five seconds. During this time the user manually displaced the impactor one millimetre and the system recorded the change in transducer signal. The change could be measured on screen and was entered as the voltage calibration of the transducer. The force calibration mode was used to establish the mass adjustment value for the system. The program generated a 60 Hz, 0.64V signal for five seconds. This mode records from the displacement transducer and either from the force or acceleration transducers (depending on the electrical connection). The peak-to-peak voltage was measured for the force curve and the calibration was then repeated by running the same sequence after disconnecting the cable for the force transducer and connecting the acceleration cable to the terminal block. Using the peak-to-peak voltages of force and acceleration then allowed the user to approximate a mass adjustment value for the suspended mass. The touch mode initiated a small amplitude signal used to indicate contact with the
spinal cord and establish a consistent baseline for injury. A continuous 60Hz, 0.04V signal was generated by the touch mode. The amplitude of the signal decreased with initial contact with the dura mater and then increased as greater force was applied to the spinal cord. Once the desired preload was applied to the cord the touch signal was stopped. The hit mode generated the impact to the spinal cord based upon the user inputs of slope (millivolts/second), peak voltage (volts) and dwell time (seconds). A standard retraction sequence was preset in the control program and withdrew the impactor tip 0.5 mm beyond the injury baseline and held the tip retracted for three seconds before returning to the zero position.

The injury sequence for the SCIRS relied on the touch and hit modes of operation. The anaesthetized animal with exposed dural surface was secured in the spinal frame of the SCIRS system via Allis clamps on the vertebrae immediately rostral and caudal to the injury site. The rigid clamping of the animal below the impactor tip ensured the impactor motion was representative of the amount of spinal cord compression. The frame was mounted on the two-axis machine tool table which allowed for horizontal positioning of the anaesthetized animal with handwheel-driven lead screws. The impactor was mounted to a vertical linear slide which was also adjustable with a manual lead screw. The touch signal was initiated and assisted in the vertical positioning of the impactor tip by indicating contact with the spinal cord as the impactor was manually lowered. The contact with the spinal cord was monitored with an oscilloscope. The impactor tip was positioned to establish the desired preload. The touch signal was stopped. Next the hit signal was initiated. The displacement, force and acceleration transducers recorded the parameters of injury at 8000 Hz. During the retraction phase of the hit signal the impactor tip was manually raised. The animal was then unclamped from the system.

2.2.1. Key Components of the SCIRS

The SCIRS was developed around an electromagnetic shaker (Model V203/S Ling Dynamic Systems Inc., Yalesville, CT) that used input signals to control the rate and duration of displacement of the system. The shaker used an electromagnetic field generated by a fixed centre pole magnet and a controlled current flowing through a moving coil to generate the driving motion of the impactor rod. The current was generated from an amplified signal
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The motion of the system and its interaction with the spinal cord were recorded by a non-contacting displacement transducer (Model KD2300, Kaman Instrumentation Corp., Colorado Springs, CO) and a force/acceleration impedance transducer (Wilcoxon Research Inc. Model Z11, Gaithersburg, MD). The displacement transducer used induction technology to measure changes in the proximity of a conducting surface to the sensor face without requiring contact with the sensor. This removed weight and movement restrictions from the vibrating armature. The linearity of the sensor performance was dependent upon the maintenance of a minimum offset (0.5mm) of the conducting surface from the sensing face [154]. Furthermore, contact between the conduction surface and sensing face could have damaged the sensor. The impedance transducer used a piezoelectric material to measure both force and acceleration of the impactor. To measure force the impedance transducer was mounted in series with the impact tip and vibrator. This resulted in inertial forces, due to the suspended mass of the impactor tip below the impedance head, being seen by the force transducer. The accelerometer allowed for the calculation of the inertial forces and they were subtracted from the measured force to provide the actual force applied to the spinal cord. Signals from the transducers were amplified, scaled and filtered by the signal conditioners (displacement – Kaman Corp M/nKD2300-4SB with 15 VDC dual power supply, impedance head – Signal Conditioner Model 133, Endevco Corp, Capistrano, CA).

The SCIRS was anchored on a custom designed stage. The purpose of the stage was to secure the spinal cord in one place, under the mechanical vibrator. The base supported a two-axis machine tool table, which allowed accurate positioning of the spinal cord beneath the impactor tip (Figure 2.4). The spinal frame was secured to the machine tool table and had connectors that allowed for adjustment of the horizontal components of the frame. The
frame supported two cinch clamps which locked down the Allis clamps. The Allis clamps grasped the vertebral elements of each rat. The impactor rod assembly was connected to the electromagnetic shaker which was mounted to a linear slide. The single axis, screw-driven ball slide (Model #NT03-601, Edmund Optics, Barrington, NJ) was mounted on the stage arm. This allowed manual vertical placement of the vibrator assembly.

The impact to the spinal cord was delivered via the methylmethacrylate impactor tip (Figure 2.2). The impactor tip was shaped like the frustrum of a cone with a two millimetre flat surface that interacted with the spinal cord. The flat surface had chamfered edges to lessen the possibility of concentrated stresses or laceration of the tissue by the sharp edges. The tip threaded to an aluminium tube which connected to a threaded stud on the impedance head.

2.3. Assembly, Calibration and Validation of the SCIRS

The SCIRS was provided with the majority of the hardware assembled in units. These were pieced together and the required electrical connections for the system were completed. Connections were established by comparing the features of each connector and outlet to determine pairings and using logical deduction. No wiring diagrams or assembly directions
were included with the SCIR system. Before powering on the entire system each transducer was checked for electrical continuity.

The performance of each transducer was evaluated and the sensors were calibrated as necessary. The calibration process for the displacement transducer consisted of using spacers to displace the metal plate away from the sensing face a fixed distance and adjusting the output of the sensor using the signal conditioner settings. The spacers were used to establish a repeatable offset that was also measured using a micrometer to ensure accuracy. A minimum offset of 0.5mm was required for this sensor to operate correctly. In addition, contact between the sensing face and the conducting target could have damaged the sensor. The retraction portion of the control curve moved the impactor (and conducting target) an additional 0.5mm opposite towards the sensing face. Therefore a minimum offset of 1mm was selected to ensure the sensor could accurately record the position of the conducting face. A desired sensitivity was selected for the sensor (i.e. 1 V/mm) and the output of the sensor was forced to the desired calibration curve by adjusting a series of resistors in the signal conditioning system. Adjusting the resistors changed the slope, linearity and y-intercept of the output curve. Following the full scale calibration procedure outlined in the manual, the zero (y-intercept), gain (slope) and linearity settings were systematically adjusted until the sensor response was accurate across the measurement range (Figure 2.5). The sensor provided was unable to supply satisfactory linearity for the 1V/mm sensitivity and, by direction of the manufacturer, was calibrated for a sensitivity of 0.5V/mm. The calibration data of the sensor showed a sensitivity of 0.504 V/mm and an accuracy of ± 1% for a displacement of 2mm (Figure 2.6). In the operating range of zero to one millimetre the sensor showed greater accuracy. The calibration of the displacement transducer was limited by the 0.01mm resolution of the micrometer used to measure the displacement.
Figure 2.5 Calibration procedure for the displacement transducer [154]

Figure 2.6 The output of the calibration procedure for the displacement transducer. The output of the transducer was adjusted to match the desired resolution of 0.5V/mm.

The impedance head transducer was supplied factory calibrated and did not require a recalibrating procedure. The output of the transducer was validated by suspending known masses below the impedance head and vibrating the system using a 0.4V 60Hz sinusoidal signal. Several masses were suspended below the impedance head (Figure 2.7). The voltage signals for force and acceleration were recorded for each mass. The peak-to-peak voltage was measured for each signal from the output curves (Figure 2.8). The apparent mass of the specimen below the transducer was calculated based upon the ratio of the force signal voltage...
to the acceleration signal voltage (Equation 3). However, for that equation to be satisfied the ratio of the force and acceleration signals, with no mass suspended below the transducer, needed to be known. To avoid causing damage to the impedance head, the aluminium tube attached to the head was not removed. Therefore the mass of the aluminium tube and the ratio of force and acceleration signals for the unloaded case were calculated based upon the measurements of the force and acceleration ratio for the other loading cases (Equation 4). There was a small amount of variability in these numbers and therefore a mean value was assumed for the validation procedure (Table 2.1)

Figure 2.7 The validation procedure for the impedance head transducer. The impedance head is shown in cross-section to show the internal mass being vibrated. The internal mass correlated to the $E_{Fca}$ and $E_{Aceal}$ used in the calculations of apparent mass. The impedance head is also shown with various weight configurations. A constant acceleration was used during the calibration procedure and a range of masses were suspended below the impedance head. The output from the force and acceleration transducers was recorded.
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Figure 2.8 The output voltages for the force (A) and acceleration (B) signals from the impedance head transducer for a suspended mass of 2.8 grams. The peak-to-peak voltage amplitudes were recorded from the force and acceleration signals and used in the calculation of the apparent mass below the transducer.

\[
\frac{E_F}{E_A} \times \frac{E_{Acal}}{E_{Fcal}} = W_x \quad \text{(Equation 3)}
\]

\[
m + x = \frac{E_F}{E_A} \times y \quad \text{(Equation 4)}
\]

Where:

- \(E_F\) = force voltage with the apparent weight [V]
- \(E_A\) = acceleration voltage with apparent weight [V]
- \(E_{Fcal}\) = force voltage for unloaded sensor [V]
- \(E_{Acal}\) = acceleration voltage for unloaded sensor [V]
- \(W_x\) = apparent weight suspended below sensor [g]
- \(m\) = known suspended mass [g]
- \(x\) = unknown mass of the aluminium tube [g]
- \(y\) = unknown quantity \((E_{Acal}/E_{Fcal})\)

Solved for \(x\) and \(y\) using values of \(m\) and \(E_F\) and \(E_A\) measured from the calibration experiments.
Table 2.1: Validation of the impedance head

<table>
<thead>
<tr>
<th>Suspended Mass</th>
<th>Calculated Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.92g</td>
<td>6.01g</td>
</tr>
<tr>
<td>7.08g</td>
<td>7.21g</td>
</tr>
<tr>
<td>9.30g</td>
<td>9.42g</td>
</tr>
<tr>
<td>11.54g</td>
<td>11.25g</td>
</tr>
<tr>
<td>13.77g</td>
<td>13.29g</td>
</tr>
</tbody>
</table>

The force calibration protocol within the control program was used for the validation procedure. This calibration protocol allowed only the recording of either the force or acceleration signals but not both simultaneously. Therefore the small error in the validation numbers may be a result of small differences in the force or acceleration values, as two tests had to be run for each mass and the results compared. During the initial system verification procedures, substantial drift was noted in the impedance head signals. This was attributed to the effects of surrounding instrumentation. A high pass filter was added to the signal conditioning protocol and successfully eliminated the signal drift.

2.4. Establishment of Impact Parameters

The purpose of this study was to observe the effect of impact velocity on the spinal cord immediately following injury in a rat model. It was desired that this study use impact parameters representative of those expected for a traumatic injury to the spinal cord. Furthermore, as the effect of impact velocity on injury was not previously conclusively demonstrated, a range of variables was desired that would ensure the observation of an effect if one existed. Finally, it was determined that the depth of impact should remain constant, to eliminate any confounding effects on the injury, and should also be representative of a moderate injury so that the influence of impact velocity could be observed.

A review of the literature revealed very little mention of the velocity at which traumatic injury occurs in the human spinal cord. Wilcox et al. used a bovine model of a human burst fracture injury and used high speed video to determined a peak rate of canal occlusion to be $110 \text{ sec}^{-1}$ [48]. Assuming an average human spinal canal diameter of 15.5mm [134] this would result in an impact velocity of 1.7m/s. This value is further supported by Oxland [49] who observed spinal canal occlusion, as a result of a burst fracture injury, to occur at 1.5m/s.
It was assumed that the spinal cord viscoelastic response would be consistent between rat and human tissue. Therefore, using the strain rate of $110^{-sec}$, for a rat spinal canal with diameter 3.0 millimetres [155], the required impact velocity would be 330 mm/s.

A fast impact velocity was selected based upon the extrapolation of experimental data for a rat sized cord. An impact velocity of 300mm/s was chosen to represent the traumatic loading rate. To ensure the likelihood of an observed effect for this preliminary exploration of impact velocity, a slow impact was chosen to be two orders of magnitude less than the fast impact group. The slow impact velocity was chosen to be 3mm/s.

To make sure the effect of impact velocity was isolated from the effect of other impact parameters, consistent depth of injury and duration of injury were selected. Review of the existing literature determined that a moderate injury using the SCIRS was likely to occur at an impact depth of 1mm [13, 41]. The peak displacement was instantaneous. There was no pause in motion at the peak displacement to apply any residual compression.

2.5. SCIRS Modifications

The desired mechanical parameters for injury were not within the operable range of the SCIRS as it was originally designed. In addition, design flaws and omissions were observed, particularly in the standardization and repeatability of the system. Modifications were made to the system to increase the range of impact velocities that could be generated however the injury parameters remained at the extreme limits of the system capabilities even with the modifications (Figure 2.9).

During the preliminary animal studies it was noted that the accurate placement of the animal beneath the impactor tip was difficult. It was observed that the screw drive of the linear slide (Model NT03-601, Edmund Industrial Optics) was not straight. This resulted in the impactor tip having a helical path while being lowered, making it very difficult to accurately align it with the animal. Review of the specifications for the ball slide indicated a maximum vertical load capacity of 0.5kg [156] while the mass of the vibrator and mounting trunion was 3.17kg [153]. The linear ball slide was replaced with a more robust model (Model A40 5” travel,
Velmex Inc., Bloomfield, NY) having a vertical load capacity of 22kg [157]. This ensured the vibrator and mounting assembly was easily and accurately adjustable during the injury procedure. To assist in the accurate positioning of the impactor tip above the rat spinal cord a stereoscopic microscope (Model MZ-6, Leica Microsystems Canada Inc, Richmond Hill, ON) was added to the SCIR system.

Rigid clamping of the animal in the spinal frame beneath the impactor was important for minimizing variability within the system. In previous studies, the animal was secured in the Allis clamps and then the hoist supporting the animal would be lowered to an arbitrary point to allow for most of the animal’s weight to be carried by the clamps and eliminate spinal column movement resulting from breathing artefacts [13, 16, 41, 152, 158]. To increase the...
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repeatability of the procedure, a scale (Model i1200, My Weigh Canada, Vancouver, BC) was introduced to standardize the percent body weight being carried by the clamps while minimizing the effect of breathing artefact, which would have influenced the measurement of low level forces on the cord [16, 21].

Measurement of forces acting on the spinal cord during low speed impacts could not be achieved with the impedance transducer. The recording of forces by the impedance head required a minimum frequency of motion of 10 Hz (20 mm/s for a displacement of 1mm) [159]. Therefore a second force transducer was introduced (100N load cell, Model 31, Sensotec, Columbus, OH) (Figure 2.10). This sensor was strain gauge-based and capable of measuring slow speed and static loads. The additional mass of the second force transducer to the impactor rod assembly limited the system performance at higher velocities and therefore was included in the system only for the slow velocity impacts. The software to acquire data from the load cell and the signal conditioning system were previously installed on a separate computer. To avoid the complications of reconfiguring the hardware and software, the entire computer system was integrated with the SCIR system. The data acquisition of the two systems was then coordinated with the use of a trigger signal generated by the SCIR system control program.
The minimum achievable impact speed was further restricted by the data acquisition protocol in the control program. The original code used a sampling frequency of 8000Hz. This restricted the maximum number of data points to 64000 before the buffer was filled. The minimum velocity at which the system could operate was 30mm/s. The sampling rate for the slow impact tests was adjusted to 80 Hz in the control code. This allowed the system to perform at an impact velocity of 3mm/s.

2.6. Experimental Protocol

The experimental protocol (Figure 2.11) was based upon the strengths of several previous studies in the field of spinal cord injury models. As knowledge in the area of spinal cord injury has increased a number of variables have been identified which may influence the model’s results. This protocol was established to maximize control over those variables while maintaining the efficiency of the system.

![Experimental Protocol Diagram]

Figure 2.11: Experimental Protocol for the study.
2.6.1. Specimens

A number of biological variables are known to have an effect on the outcome of spinal cord injury. The specimens for this study were selected to control the most dominant variables and to isolate the effect of impact velocity on injury. Variables known to significantly affect the outcome of spinal cord injury include the strain of rat used [36], the size of the spinal cord [160] and the gender of the animal [161].

This study used 31 male, Sprague-Dawley rats (South Campus Animal Facility, University of British Columbia, Vancouver, BC) with a mean body weight of 266g (sd =22g). Male rats were chosen for the study as males are the more common victims of spinal cord injury and hormone differences between genders have been demonstrated to affect the outcome of injury [161]. The Sprague-Dawley strain was selected as they have been used in previous similar studies [13, 16, 41, 158, 162], and because the micro-surgeon performing the experiments had extensive experience handling that strain [163-165]. The weight of an animal has been shown to correlate with cord size within the same gender [160]. Therefore a small range of animal body weights was used to ensure consistency of the size of the spinal cord.

The animals were divided into three groups for the study. The first group was a sham control group (n=9). These animals experienced the surgical procedure, were mounted in the SCIR system and received only a touch force on their spinal cords before being sacrificed. The second group was the slow impact group (n=12). These animals received an injury with an impact velocity of 3mm/s. The fast impact group were injured with a velocity of 300mm/s (n=10). Animals with a force trace indicative of slipping in the clamps during the impact or complications in the surgical procedure were removed from their respective groups prior to data analysis. Deviations in the tissue handling protocols following injury also resulted in that animal being removed from the study.

The use of animals for these experiments was approved by the University of British Columbia Animal Care Committee, as adhering to the regulations of the Canadian Council on Animal Care (CCAC).
2.6.2. Pre-test procedures

The time-dependent nature of the injury process required that validation of the SCIR system be performed prior to the initiation of the surgical procedure. A number of settings had to be established and validated to ensure the desired impact would be delivered. The accuracy of the calibration of the non-contacting, inductive displacement transducer depended upon the initial offset between the sensor and metal plate. To ensure repeatable and accurate measurement by the displacement transducer the calibration was validated prior to each testing day. A custom-coded program was used to standardize and improve the efficiency of the calibration procedure. The displacement calibration was then validated in the SCIR system control program by moving the impacter a known distance (1mm) and recording the output voltage. The mechanical stop was then placed at the desired impact displacement (i.e. 1mm). A series of impacts were run with the indenter suspended in the air (air hits). These air hits were used to confirm that the output of the impacter corresponded to the desired injury protocol.

The force output was used to establish the mass adjustment value. The value was selected to minimize the forces seen when the impacter tip was unloaded. This adjustment, multiplied by the acceleration, compensated for the inertial forces seen by the transducer due to the motion of the mass of the aluminum tube and impactor tip suspended below the transducer.

Finally, the touch signal was generated and the oscilloscope was activated to ensure an accurate display of the touch force on the screen. The surgical procedure was initiated following the successful completion of the pre-test system validation.

2.6.3. Surgical Exposure of the Dura Mater

A standardized procedure was established to ensure consistent and stable exposure of the dura mater of the animal. The surgeries were performed by a microsurgery specialist (J. Liu). Each animal was weighed to ascertain the required dosage of anaesthetic. An intraperitoneal injection of ketamine hydrochloride (80mg/kg) (Bimed-MTC, Cambridge, ON) and xylazine hydrochloride (10 mg/kg) (Bayer Inc, Etobicoke, ON) was give to each animal. Ketamine provided both anaesthetic and analgesic effects however it also increased
muscle tone in the animals [166]; therefore, xylazine was given as a muscle relaxant. Once sedated the backs of the animals were shaved and swabbed with iodine to prepare for surgical exposure. The animals were then allowed to stabilize for 10 minutes in the anaesthetized state before surgery was initiated.

The dural surface exposure surgery was performed with the animal secured in a stereotactic frame (Figure 2.12). Traction was applied to the tail of the animal to help straighten the spinal column and provide stability during the surgical procedure. Following traction application, the animal was allowed to stabilize its respiration for two minutes before surgery continued. A two centimetre incision was made through the musculature of the back to expose the spinal column at T9-T11. The ribs of the animal were used to landmark the T11 vertebrae. Rongeurs cut pieces from the lamina of the T10 vertebra to create a two millimetre diameter, partial laminectomy at the T10 level. The size of the laminectomy was confirmed with a two millimetre diameter wood dowel. Gelfoam (Johnson & Johnson Inc.) pieces were used to absorb bleeding at the laminectomy site to ensure a clear view of the exposed cord for accurate alignment with the impactor tip.

Figure 2.12: The surgery to expose the dorsal surface of the dural at the T10 level for the contusion injury.
2.6.4. Injury Procedure

Following surgical exposure of the dural surface of the cord the animal was removed from the stereotactic frame. The animal was moved to the SCIRS system where it was held in the spinal frame using Allis clamps to grip the T9 and T11 vertebrae while a hoist supported the rat’s body weight (Figure 2.13). Once the vertebrae were secured, the hoist was lowered until a scale on the hoist registered half the body weight of the animal. The 50% value fully eliminated respiratory artefacts from the touch signal but provided sufficient support so that animals would not fall from the clamps. The spinal frame was mounted on an x-y table that allowed for the position of the animal under the impactor to be adjusted for proper alignment.

![Figure 2.13 Animal clamped in the spinal frame of the SCIR system](image_url)

The touch force signal was initiated once the animal was securely fastened in the clamps. The impactor tip was lowered towards the dural surface using the z-slide. As the impactor was being lowered the impactor tip and rat were observed through the stereoscopic microscope to ensure proper alignment with the laminectomized vertebra. The amplitude of
the touch force was observed by a second operator on the oscilloscope screen. An abrupt change in the shape or magnitude of the touch force indicated contact with tissue other than the spinal cord. The impactor tip would then be retracted and the alignment adjusted. A clean touch on the spinal cord was indicated by a small decrease in force amplitude (caused by attenuation of the vibrations by the cerebral spinal fluid) followed by a steady increase in signal amplitude to the desired touch force of 0.015 N (1.5 kdyne). The sham control group was removed from the device following the touch force being applied to the spinal cord.

The touch force established a baseline from which to generate the injury protocol. The touch signal was stopped once the desired force level was established. The injury sequence was immediately initiated. For the slow impact group a one millimetre impact occurred at a rate of 3mm/s. The fast injury was to a depth of one millimetre at a rate of 300mm/s. The impact parameters in the control program did not directly correlate with impact velocity or peak displacement and were established by trial and error (Table 2.2). In both injury scenarios that impactor tip was immediately withdrawn from the spinal cord once the maximum depth of impact was achieved. The impactor was withdrawn an additional 0.5mm from the cord surface allowing time for the impactor tip to be manually raised and to ensure no residual contact with the cord surface following injury.

<table>
<thead>
<tr>
<th>Injury Group</th>
<th>Peak Voltage (V)</th>
<th>Slope (V/s)</th>
<th>Dwell Time (ms)</th>
<th>Mass Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>1.3</td>
<td>0.0037</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>Fast</td>
<td>4.0</td>
<td>1.0</td>
<td>3</td>
<td>0.05</td>
</tr>
</tbody>
</table>

2.6.5. Sacrifice and Tissue Harvest

The injured animals were immediately removed from the impact device and prepared for euthanasia. All of the animals were sacrificed less than two minutes after the impact was applied to the cord. The animals were intracardially perfused first with 120 millilitres of buffered saline followed by 250 millilitres of 4% paraformaldehyde (PF) in phosphate buffered saline (PBS) to preserve the cord tissue.
A 1.5 centimetre segment of the spinal cord, including the impact center, was harvested from each animal immediately following perfusion. The cord was dissected free from the dura and pia mater and nerve roots were cut near the cord surface to release the cord from the canal. The harvested cord sections were bathed in 4% PF solution overnight to ensure complete fixation of the tissue.

2.6.6. Tissue Preparation and Sectioning

Following preservation of the tissue with PF, the segments were then bathed in a series of sucrose solutions to protect the tissue from forming ice crystals and fracturing during the freezing process. Each segment was immersed overnight in a 12% sucrose bath, then transferred to an 18% sucrose bath for a second night and finally to a 24% sucrose bath for a third night. The sucrose protected tissue was mounted to blotting paper backings (for identification and ease of mounting) using embedding medium (Tissue-Tek, Sakura Finetek USA, Torrance, Ca), and flash frozen using an isopentane bath (British Drug House) surrounded by dry ice. The blocks of tissue were stored at -80°C until they were to be sectioned. Each segment of spinal cord tissue was sectioned longitudinally in sagittal planes into 20 μm thick pieces using a cryostat (Microm, Mikrom Instruments Inc, San Marcos, Ca) (Figure 2.13).

Lateral sectioning allowed for clear observation of the impact site on the cord and the linearly oriented axons. Each slice of tissue was serially placed on a set of 6 slides (Superfrost, Fisher Scientific, Houston, TX) resulting in a single slide having sections at 120 μm intervals representing the entire cord. The slides were then returned to the -80°C freezer until histological processing.
2.6.7. Histology

Histological methods provided an opportunity to observe specific features of the damage to the cord. Many of the techniques traditionally used for observing injury in neurological tissue were not appropriate for this study, as they rely on measures of immunological response to injury and require sufficient time after injury for that immune response to occur. Targeting strategies for this study focused on techniques which emphasized elements of short-term mechanical damage in the spinal cord. The rupture and haemorrhage of blood vessels within the spinal cord is one of the first indicators of mechanical injury [104, 130, 167-170]. It was also thought that the mechanical damage to the cord would be reflected by the disruption of neurofilament sidearms as they provide the mechanical structure of the axons [55].

2.6.7.1. Haematoxylin and Eosin Staining

Haematoxylin and Eosin (H&E) stain was used to emphasize areas of haemorrhage within the cord. Haematoxylin and Eosin is often used in studies of spinal cord injury and highlights areas of haemorrhage as well as neuronal cell bodies in neurological tissue [15, 28, 40, 42, 131, 133, 171].

The process of H&E staining involved a series of dye baths and washes to control the colour saturation of the tissue. The frozen tissue was thawed for this process. The procedure was standardized by using a motorized dipping device which moved the tissue along the line of baths, dipping and removing as necessary. The timing for each bath was controlled by altering the size of each bathing tray. A single bath tray resulted in 20 seconds of tissue submersion. Water baths were connected to a free-flowing water source which ensured the baths remained clean. The tissue began by passing under a heating element for one minute, which acted to thaw the tissue and assist in securing the sections to the slides prior to the bath process. The tissue went through a series of two xylene (Fisher Scientific, Ottawa, ON) baths and three ethanol (Fisher Scientific, Ottawa, ON) baths. A single water bath followed. The first dye was the haematoxylin (Fisher Scientific, Ottawa, ON) stain. There were seven baths of stain, to ensure sufficient dye coverage of the tissue. This was followed by a water bath to remove excess dye. A single 0.37% acid (hydrochloric acid, Fisher Scientific,
Ottawa, ON) and alcohol (70% ethanol, Fisher Scientific, Ottawa, ON) bath acted to differentiate the stain in the tissue. This was followed by an additional water bath and then a 0.015M lithium carbonate (Fisher Scientific, Ottawa, ON) bath. The lithium was rinsed in another water bath. The second series of dye baths contained the 2% eosin (Fisher Scientific, Ottawa, ON) stain. Four baths were sufficient for adequate staining of the tissue. The dye was rinsed in a water bath. The tissue was then dehydrated by going through a series of three alcohol baths, two xylene baths and resting at the end of the process in a large xylene bath until mounting. Acrytol (Surgipath Canada Inc, Winnipeg, MN), a methylmethacrylate mounting medium was used and the slides were covered with a glass coverslip to preserve and protect the tissue.

2.6.8. Immunohistochemistry

The key principle of immunohistochemistry relies on the affinity and binding of an antibody to its antigen. A series of reactions are then built off this primary link to allow for visualization of the presence of the bound antibody. The technique described here is the indirect method of labelling and requires the use of two antibodies. The direct method of labelling involves the conjugation of the antibody with a chromophore and may destroy some of that antibody's reactivity in the process. The indirect method is more sensitive than the direct method and has the advantage of using a more generic, commercially available reagent [172].

The first links of the immunolabelling process use the reactivity of antibodies to antigens. The initial step of the labelling process is the selection of an antibody that specifically targets the desired antigen (Figure 2.15). In this study the desired antigen is the one specific to dephosphorylated heavy neurofilaments. The antibody which targets the antigen of interest is called the primary antibody (i.e. SMI-32). The primary antibody is usually developed in a different species (i.e. mouse) than the tissue to be analyzed (i.e. rat) to avoid cross-reactivity between the subsequent secondary antibody and the endogenous immunoglobulins in the tissue. The next link is a secondary antibody which is specific to the immunoglobulin of the primary antibody species (i.e. anti-mouse) (Figure 2.16). The secondary antibody, in this case, is biotinylated (conjugated with biotin).
The biotin molecule provides the link for the avidin in next stage of the process. The avidin molecule has a very high affinity for binding with biotin. The avidin: biotinylated enzyme complex (ABC) increases the sensitivity of signal detection by up to forty times [173]. The ABC is a macromolecular structure made up of several avidin molecules and biotinylated peroxidase enzymes. The structure of the ABC molecule has not been defined, however it is assumed there is at least one free binding site on an avidin molecule within the structure and this site links to the biotin molecule on the secondary antibody [173]. The peroxidase enzyme portion of the ABC molecule provides the basis for visualization. Without the amplifying step, there would be one enzyme molecule for each antibody, however with the ABC complex there are several enzyme molecules linked to each antibody. The final step to develop the visual reaction is the use of 3-3' diaminobenzidine (DAB). The oxidation reaction of DAB is catalyzed by the peroxidase enzymes and results in the formation of a chromogenic (coloured) product. The chromogens can be visualized with light microscopy.
Figure 2.17 The third step involves amplification of the signal with the ABC macromolecular complex. The complex structure of the ABC molecule links several peroxidase enzymes to each antibody resulting in better sensitivity to the presence of the antigen [173].

Figure 2.18 Finally an oxidation reaction with an enzyme substrate results in a visible chromogenic product. The interaction of the substrate with the ABC complex results in a large amount of visual product being developed for each labelled antigen [173].

2.6.8.1. Immunolabelling for Neurofilament Sidearm Disruption

The targeting and tracing of neurofilaments (NF) in the spinal cord could be achieved with a variety of techniques. Traditional staining techniques have relied on reduced silver methods such as Bodian or Bielschosky [55]. More recently, a number of immunohistochemical techniques have been developed to target NF proteins. Antibodies have been developed to label specific NF proteins such as the light (NF-L), medium (NF-M) or heavy (NF-H) [57, 58, 62, 174, 175]. Furthermore, there are antibodies which can target phosphorylated or nonphosphorylated NF-H indicating the presence or absence of sidearms on the protein structure [176-178]. The SMI-32 antibody (Sternberger Monoclonals, Lutherville, MD), which targets a nonphosphorylated epitope on the heavy neurofilament protein, was selected to observe the effects of mechanical loading on the sidearm structures and determine axonal damage. Observing the disruption of neurofilament sidearms was considered an indication of structural damage to the axon as the sidearms provide the mechanical structure for the axons [55].
Immunohistochemical techniques require careful adherence to, and revision of, the recommended procedures based upon the initial results found for the specific tissue being processed. The appropriate concentration of antibody was evaluated using several samples of tissue. The recommended concentration of 1:1000 proved too strong and 1:10,000 too weak. For this study the protocol for the SMI-32 antibody was determined to be most effective for a concentration of 1:5000.

Targeting of the neurofilament de-phosphorylization protein was achieved using standard immunocytotoxic techniques. The slides were removed from -80°C storage immediately before the immuno protocol began. The slides were thawed on a heating tray for fifteen minutes. This assisted in the adherence of the tissue to the slides for the remainder of the immuno process. The area containing tissue on the slides was outlined using a liquid blocking pen (LB-630 Liquid Blocker, Kiyota International Inc, Elk Grove Village, IL). This ensured that a minimum amount of fluid was required to cover the tissue. Large baths, like those used in the H&E staining technique, are prohibitively expensive for antibody use. The tissue was then rehydrated in a 0.01 percent PBS solution for five minutes. This further ensured the tissue sections remained mounted to the slides.

While the tissue was being rehydrated, a 0.3 percent solution of hydrogen peroxide (H₂O₂) was prepared in 95 percent methanol (MeOH). The tissue was soaked in a coplin jar on a shaker table for 30 minutes in the H₂O₂. This step acted to block any endogenous peroxidase activity in the tissue which is common when blood enzymes are present. This would have lead to abnormally high background staining and might have caused problems in distinguishing positively labelled axons from the remaining tissue.

The tissue was then rinsed in a series of 0.01M PBS baths (3 x 5 minutes). The primary antibody was made in a 1:5000 concentration using 1μL of SMI-32 (Sternberger Monoclonals) and 5000 μL of 0.01M PBS with triton. The inclusion of triton increased the membrane permeability and allowed for more complete labelling of the tissue. The slides were incubated in the primary anti-body overnight at room temperature.
On the second day, the slides were rinsed in a 0.01M PBS solution (3 x 5 minutes). They were then incubated for 30 minutes in 1:10 solution of normal donkey serum (Jackson ImmunoResearch Laboratories Inc, West Grove, PA) in PBS with triton. The donkey serum acted to minimize background labelling by blocking a tissue interaction with the secondary antibody. The donkey serum was removed and the slides were incubated in the biotinylated secondary antibody (1:500 donkey anti-mouse IgG, Jackson ImmunoResearch Laboratories Inc, West Grove, PA) for one hour. The biotinylated secondary antibody provided the link between the primary antibody and the signal amplifying steps. The slides were rinsed in PBS (3 x 5 minutes) and then covered with an avidin-biotin complex (ABC, Vector Laboratories Canada Inc, Burlington, ON) and incubated for 30 minutes. The avidin-biotin complex amplified the signal from the labelled antigens. Following incubation the slides were rinsed in PBS (3 x 5 minutes). The labelling was exposed using diaminobenzidine (DAB) complex with the option nickel step (Vector Laboratories Canada Inc., Burlington, ON) and allowed to react until saturation (8-10 minutes). Excess DAB was removed and the slides were rinsed in distilled water (3 x 5 minutes).

To prepare the slides for coverslip mounting, the sections were dehydrated in a series of three baths: 2 minutes in isopropynol, 2 minutes in toluene, and 2 minutes in toluene. The coverslips were then mounted using Entellan (EM Scientific) and allowed to dry overnight.

2.6.9. Image Capture and Measurement

A Zeiss Axioplan II microscope was used to analyze specimens. Areas of haemorrhage in the H&E stained tissue were observed with a 5 times objective lens, total magnification of 50 times. This allowed for visualization and quantification of the macroscopic spread of haemorrhage within the spinal cord. Slides stained with H&E were photographed in RGB 24 bit colour using a Retiga Exi digital capture camera (Q-Imaging Burnaby, BC) and RGB filter (RBG slider, Q-Imaging, together with Northern Eclipse software (Northern Eclipse 6.0 Empix Imaging Inc). Digital images were montaged by pixel matching in Adobe Photoshop 6.0 to create a complete picture of the injury site and avoid duplicate counting of positively stained areas.
Haemorrhage areas were measured by outlining areas labelled red with H&E staining (Figure 2.19). An LCD tablet (Cintiq 15X, Wacom Technology Corporation, Vancouver, WA) was used to ensure accurate tracing of the haemorrhage areas and increase the efficiency of the image analysis. The areas of haemorrhage were calculated based upon the outlined areas using image analysis software (Northern Eclipse 6.0 Empix Imaging Inc.). Haemorrhage areas in each of the grey matter and white matter were observed separately and a total injury area was also recorded based upon the sum of the grey and white matter haemorrhage areas. The area at the epicentre of injury was recorded. The impact epicentre was determined to be located on the slide with the greatest total haemorrhage area. The area measurements from each segment of cord tissue were combined using the frustrum of a cone equation (Equation 5) to determine a volume measure of haemorrhage within the grey and the white matter of the spinal cord for each animal (Figure 2.19) [13, 21, 41, 127, 128, 131].

\[
V = \frac{1}{3} h \left[ B_1 + B_2 + (B_1 \times B_2)^{\frac{1}{3}} \right]
\]

Equation 5

Where:

- \( V \) = volume of haemorrhage between two segments [\( \text{mm}^3 \)]
- \( B_1 \) = base area of haemorrhage [\( \text{mm}^2 \)]
- \( B_2 \) = area of haemorrhage of the next tissue segment [\( \text{mm}^2 \)]
- \( h \) = distance interval between the two measured areas of haemorrhage [mm]

Slides labelled with the nonphosphorylated neurofilament antibody were image captured at 200 times magnification for ease of observation of the reactive axons. Greyscale, 18 bit images were taken for these slides, as the labelling was monochromatic. Greyscale images resulted in smaller image files which reduced the time required for processing compared with the colour images.
Figure 2.19: Tracing method for selecting areas to be measured. Combined sections of haematoxylin and eosin stained tissue were used to generate a volume measure of haemorrhage.

The pictures were histogram stretched in Adobe Photoshop 6.0. The histogram stretch technique maximized the intensity resolution in the image and allowed for clearer distinction between positively labelled areas. To provide maximum contrast in an image it was important that the image used as much of the available greyscale as possible. This was best achieved by using optimum lighting and imaging techniques. However this still resulted in only a portion of the available greyscale range being used. The histogram stretch technique involved observing a histogram plot of the distribution of pixels across the spectrum of available grey values (Figure 2.20). If the image did not fill the range of values, the image was stretched from its original distribution along the scale to the full range. This intensified values at the light and dark extremes of the image and provided greater sensitivity to relative differences in pixel intensities across the entire image. The histogram stretch resulted in greater image contrast without changing the comparative results.
The density of positively labelled axons was measured using the thresholding feature in Northern Eclipse 6.0. A greyscale threshold value was determined for each series of images (representing a single tissue section) as the lighting, image capture exposure time, intensity of staining and presence of abnormal light or dark areas affected the threshold value. The specific threshold value did not affect the results as all the measurements were normalized to a density measurement in an uninjured area on the same tissue segment. Once the positively labelled axons were highlighted by thresholding, a selection box was traced around the area of interest to exclude counting of reactivity in the grey matter or the background of the image (Figure 2.21). The size of the box was maximized to select the most white matter in the image without selecting non-reactive or grey matter. Deviations in the box size did not affect the measurement of reactivity if the box included at least fifty percent of the area of interest. The box size could not be standardized as the geometry of white and grey matter was variable in each specimen.
To observe changes in the presence of the dephosphorylated neurofilament epitope across the spinal cord at the level of injury, images of the ventral and lateral white matter were captured at the epicentre of each segment at 240 micrometer lateral increments. The amount of immunoreactivity is expressed as the percentage of positively labelled axons (as selected by the thresholding technique) versus the mask area as the size of the ventral columns varied for each tissue segment the mask area was variable. The percent of reactivity at the epicentre was normalized to the percent of reactivity at an uninjured portion of tissue on the same segment.

Figure 2.21 Thresholding and selection of the axons of interest. Red indicates axons that have been selected by the thresholding technique. The blue box is a manual trace to select the axons to measure.

Damage to the grey matter of the spinal cord was observed at the epicentre of injury. A similar technique to the white matter density measurement was used to evaluate the reactivity of the SMI-32 antibody in the grey matter. Images were taken at constant intervals longitudinally along the tissue section representing the epicentre of injury. On each image a central band of tissue was selected as the measurement of interest to provide a longitudinal sampling of reactivity at set intervals. The individual images were montaged to generate pictures that spanned the longitudinal spread of reactivity. Reactivity to the dephosphorylated NF-H antibody in grey matter was greater than that in the white matter as
NF are inherently dephosphorylated in the cell bodies and dendrites. The changes in the density of reactivity therefore reflected further dephosphorylation of axons, dendrites and cell bodies as well as a compaction of the cytoplasm with the cell bodies.

2.7. Analysis of the Effect of Impact Velocity on Injury

The observation of damage in the spinal cord following impact was done both qualitatively and quantitatively. The quantitative analysis used direct measuring techniques to minimize subjectivity in the measures and allow for duplication of the procedures in other laboratories for comparison purposes. Statistical evaluation of the effect of impact velocity on the measures of injury was performed to allow for conclusive statements to be made regarding the results.

2.7.1. Mechanical Parameters of Injury

The means and standard deviations were calculated for the peak values of the observed mechanical parameters of injury. The peak values for the fast impacts were determined to occur before contact with the mechanical stop to avoid noise artefact being incorporated into the measurements. These values provided a quantitative measure of the accuracy and repeatability of the test procedure. The standard deviation of the observed force values reflected the biological and systemic variability in the testing protocol. Derived values for velocity, impulse, power and energy for each impact were also calculated. The velocity was derived from the displacement-time data by taking the slopes from linear regression analyses over ranges of five data points. The impulse was calculated by integrating the force data with time. Multiplying the force and velocity data provided an estimate of the power for the injury. The energy was a summation of the product of average force and incremental displacement values for every two data points. Peak values for each parameter were chosen to be the greatest value occurring before contact with the mechanical stop. The means and standard deviations of the peak values characterize the injury protocols.

The force and displacement behaviour of the spinal cord during the loading portion of the impact regime was used to calculate approximate tissue stiffnesses and pseudo-Young’s modulus of the spinal cord for each specimen. The tissue stiffness was calculated from the
The linear slope of the force-displacement curves for each specimen and assumed a linear elastic deformation (Equation 6).

Two techniques were used to approximate the Young’s moduli for each specimen. Both techniques had inherent assumptions that were not fully satisfied by the mechanics of the contusion injury generated here. Therefore solutions for both methods were obtained and compared.

The first method for calculating Young’s modulus was based upon the equation derived by Hayes et al. for a single phase, continuous elastic material (Equation 7). The equation was based upon the Boussinesq problem of determining the stress and displacement in an elastic half-space (axisymmetric) indented by a rigid indenter. While this technique of converting indentation stiffness to a Young’s modulus is primarily used in analysis of cartilage properties, the solution is not dependent upon specific material characteristics but on geometry and boundary conditions of the sample. Values for the theoretical scaling function (κ) were dependent upon the indenter radius (a), the depth of indentation (h) and Poisson’s ratio. Several authors have derived tabulated values for κ, showing consistency across alternative theoretical solutions [179-182]. The value of κ used in the calculations of spinal cord elastic modulus were extrapolated from the tabulated values of Zhang et al [182] as he included the effect of friction as a result of greater compression ratios in the calculation of κ (Figure 2.22). The tabulated values did not provide results for a/h = 0.5 as in this study. The required κ value was obtained by interpolating between the results for a/h = 0.6 and a/h = 0.4. A κ value of 3.03 was used in this study.

The second method of calculating Young’s modulus used a finite element model of similar geometry to generate a constant to describe the relationship between the observed material stiffness and the Young’s modulus (Equation 8) [183]. The theory behind the geometric constant was developed for ex-vivo tissue samples but is applicable to any homogenous elastic tissue. The derived constant depends on the boundary conditions and geometry of the finite element model. A linear elastic finite element model describing the compression of a human spinal cord was recently described [184]. While the model geometry did not match
that of the rat contusion injury case, the relative proportions of the key elements were the same. The ratios of the impactor diameter to the spinal cord diameter both in the model and in this study were 0.5. The finite element model used a Young’s modulus of 0.26MPa and found a tissue stiffness of 2.1 N/mm. Therefore the geometric constant based on the finite element model was 0.124.

\[
\frac{F}{w_0} = S \quad \text{Equation 6}
\]

\[
\frac{F(1 - \nu^2)}{2\sigma w_0 \kappa} = E \quad \text{Equation 7}
\]

Where:
- \( F \) = force at instant prior to contacting the mechanical stop [N]
- \( w_0 \) = indentation depth into the cord [mm]
- \( S \) = stiffness of the spinal cord [N/mm]
- \( \nu \) = Poisson’s ratio
- \( a \) = radius of the indenter tip [mm]
- \( \kappa \) = theoretical scaling function
- \( E \) = Young’s modulus [MPa]

Figure 2.22 Theoretical \( K \) values, extrapolated to compression ratio of current experiment [182].
The effect of impact velocity on acute spinal cord injury

Chapter 2 Methods

\[ E = kS \quad \text{Equation 8} \]

Where:

\begin{align*}
E & = \text{Young's modulus [MPa]} \\
k & = \text{geometric constant [mm]} \\
S & = \text{stiffness [N/mm]} 
\end{align*}

2.7.2. Statistical Analysis

Statistical analyses were performed using a commercial software package (Statistica Release 5.1, StatSoft Inc, Tulsa, OK). A complete summary of the output from each analysis is presented in Appendix A.

2.7.2.1. Statistical Methods for Analysis of H&E Results

Evaluating the results of the volumetric and area measures of haemorrhage in the spinal cord as a function of impact velocity required comparison between only the fast and slow impact groups. A one-way ANOVA could be used to analyze the effect of impact velocity on haemorrhage; however, the control group could be excluded from the analysis if there was no observable haemorrhage in any of those specimens. In that case, a parametric, unpaired t-test could be used to determine statistical significance. As there was a measure of damage for three haemorrhage volume measures (total haemorrhage, haemorrhage in the white matter and haemorrhage in the grey matter) a Bonferroni adjustment [185] was applied to the p-value used to determine significance. This assisted in eliminating the chance of detecting a false positive as a result of multiple measures. Therefore a p-value of 0.017 was considered significant. The suitability of parametric techniques for the analysis of this data was confirmed with an analysis of the normality of the data using the Shapiro-Wilk statistic.
2.7.2.2. Statistical Analysis of Neurofilament Damage

The statistical analysis of damaged axons measured around the epicentre of injury in the white and grey matter was performed using a one-way MANOVA. Measures of positive labelling for the non-phosphorylated neurofilament antibody were taken at several locations within the same spinal cord. The analysis of damage distribution within the white matter was grouped by locations in the ventral portion of the spinal cord and by lateral symmetry across the spinal cord (Figure 2.23). This reduced the number of observed groups and allowed for a more powerful statistical observation to be made. The measure of damage at each location could not be considered an independent variable and therefore an analysis was required that could account for the within subject variance in the results. A two-way, repeated measures ANOVA could not be used as the data did not satisfy the sphericity requirement for a repeated measures analysis [186]. Sphericity required that the correlation between the data of all the dependent variable groups was equal and that each group had equal variance. The one-way MANOVA technique accounted for the repeated measures of the observed data but the underlying assumptions of the technique only required that there was equality of variance across all data groups. For the analysis of equal sized sample groups the MANOVA is a very robust technique and deviations of group data from the equal variance assumption has little effect on the results [186].

Post-hoc analyses were performed for findings of statistical significance for the main effect. The presence of an effect of the independent variable on each dependent variable was observed through univariate F-tests. To observe what the effect of each independent variable
was on the dependent variable Newman-Keuls tests were performed for each dependent variable. Effects associated with p-values of less than 0.05 were considered significant.
3 Results

3.1 The SCIR system

The clamping system used in the SCIRS proved insufficient for securing three animals in this study. The results of those animals could not be included in the analysis. The displacement and force acting to compress the each spinal cord could not be defined as movement of the spinal column was not properly constrained. Three other animals were also removed from the analysis due to other complications in the injury process including tissue damage during sampling sectioning, misfiring of the impactor and an improperly sized partial laminectomy. The analysis that follows was based upon the remaining animals (n=8 per group).

3.2 Parameters of Injury

Six mechanical parameters were observed and derived for each contusion injury (Appendix B). Force and displacement were directly observed while velocity, impulse, power and energy were derived from the force and displacement data (Figure 3.1 and Figure 3.2). The small variability in the mechanical parameters observed during the injury process reflected the accuracy and repeatability of the injury protocol (Table 3.1). The depth of impact showed very repeatable results within each velocity group. Between group variation was slightly higher with the fast group showing a greater mean impact depth than the slow group (1.10 mm, sd = 0.08 mm versus 0.90 mm, sd = 0.03 mm). Greater variability was seen in the peak forces experienced in each impact scenario. The fast impact group had a mean peak force of 2.37N (sd = 0.30N) while the slow impact group had a mean peak force of 0.54N (sd = 0.05N).

The derived variables showed slightly greater variation than the observed parameters (Table 3.2). The velocities showed less consistency than the impact depth and reflected the noise generated from differentiating the displacement data. The fast impact group had a mean impact velocity of 307 mm/s (sd = 5.8 mm/s, CV=1.9%) while the slow impact group had a velocity of 2.69 mm/s (sd = 0.43 mm/s, CV=16%). The peak impulse applied to the cord during the impact was greater in the slow group (0.117 ± 0.016 N.s) than it was in the fast...
group (0.0053 ± 0.0007 N.s). The instantaneous power delivered to the cord peaked at 0.65 W (sd = 0.074 W) for the fast group and 0.0011 W (sd = 0.00011 W) in the slow group. The mean peak energy applied to the cord for the fast group was $1.4 \times 10^{-3}$ J (sd = $2.1 \times 10^{-4}$ J) and for the slow group was $2.6 \times 10^{-4}$ J (sd = $1.7 \times 10^{-5}$ J). The mean values of the derived parameters for the fast impacts showed 12-16% variability and 7-16% for the slow impacts. When the data was sub-grouped and analyzed for repeatability by testing day there was generally less variability in the means for each parameter.

Table 3.1 Impact parameters of injury for the fast and slow impact groups.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Group</th>
<th>Displacement (mm)</th>
<th>Velocity (mm/s)</th>
<th>Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS15</td>
<td>Fast</td>
<td>1.03</td>
<td>302</td>
<td>2.29</td>
</tr>
<tr>
<td>CS16</td>
<td>Fast</td>
<td>1.04</td>
<td>303</td>
<td>2.09</td>
</tr>
<tr>
<td>CS17</td>
<td>Fast</td>
<td>1.03</td>
<td>298</td>
<td>2.16</td>
</tr>
<tr>
<td>CS29</td>
<td>Fast</td>
<td>1.14</td>
<td>306</td>
<td>2.32</td>
</tr>
<tr>
<td>CS30</td>
<td>Fast</td>
<td>1.16</td>
<td>315</td>
<td>2.99</td>
</tr>
<tr>
<td>CS31</td>
<td>Fast</td>
<td>1.17</td>
<td>311</td>
<td>2.27</td>
</tr>
<tr>
<td>CS32</td>
<td>Fast</td>
<td>1.21</td>
<td>312</td>
<td>2.62</td>
</tr>
<tr>
<td>CS34</td>
<td>Fast</td>
<td>1.23</td>
<td>309</td>
<td>2.22</td>
</tr>
<tr>
<td>CS20</td>
<td>Slow</td>
<td>0.91</td>
<td>3.11</td>
<td>0.60</td>
</tr>
<tr>
<td>CS21</td>
<td>Slow</td>
<td>0.92</td>
<td>3.02</td>
<td>0.54</td>
</tr>
<tr>
<td>CS22</td>
<td>Slow</td>
<td>0.95</td>
<td>3.17</td>
<td>0.45</td>
</tr>
<tr>
<td>CS23</td>
<td>Slow</td>
<td>0.90</td>
<td>3.05</td>
<td>0.49</td>
</tr>
<tr>
<td>CS37</td>
<td>Slow</td>
<td>0.89</td>
<td>2.29</td>
<td>0.56</td>
</tr>
<tr>
<td>CS39</td>
<td>Slow</td>
<td>0.86</td>
<td>2.26</td>
<td>0.59</td>
</tr>
<tr>
<td>CS42</td>
<td>Slow</td>
<td>0.85</td>
<td>2.32</td>
<td>0.54</td>
</tr>
<tr>
<td>CS43</td>
<td>Slow</td>
<td>0.86</td>
<td>2.26</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Figure 3.1 Example output of the SCIRS displacement and force sensors and the derived parameters for a slow impact.
Figure 3.2 Example output of the SCIRS displacement and force sensors and the derived parameters for a fast impact.
Table 3.2 The coefficients of variance (percent) for the mechanical parameters of injury as they correspond to the entire injury group and injury groups on each testing day.

<table>
<thead>
<tr>
<th>Group</th>
<th>Displacement</th>
<th>Force</th>
<th>Velocity</th>
<th>Impulse</th>
<th>Power</th>
<th>Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>7.2</td>
<td>13.0</td>
<td>1.9</td>
<td>12.6</td>
<td>11.4</td>
<td>15.8</td>
</tr>
<tr>
<td>Fast Day 1</td>
<td>0.6</td>
<td>14.7</td>
<td>0.9</td>
<td>6.6</td>
<td>6.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Fast Day 2</td>
<td>3.0</td>
<td>13.0</td>
<td>1.1</td>
<td>11.1</td>
<td>10.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Slow</td>
<td>3.8</td>
<td>9.2</td>
<td>16.1</td>
<td>13.6</td>
<td>10.2</td>
<td>6.9</td>
</tr>
<tr>
<td>Slow Day 1</td>
<td>2.4</td>
<td>12.5</td>
<td>2.2</td>
<td>4.4</td>
<td>6.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Slow Day 2</td>
<td>2.2</td>
<td>4.2</td>
<td>1.3</td>
<td>6.3</td>
<td>5.5</td>
<td>10.2</td>
</tr>
</tbody>
</table>

The spinal cord showed a nearly linear response to loading for the injury protocols (Figure 3.3 and Figure 3.4). The stiffness characteristics of the spinal cord varied significantly with impact velocity (p < 0.01). At the low impact velocity the spinal cord showed a mean stiffness of 0.61 N/mm (sd = 0.07 N/mm) while the fast impact group showed a mean stiffness of 2.4 N/mm (sd = 0.28 N/mm). The approximation of the Young’s modulus (E) based upon the theory of indentation testing resulted in an elastic modulus of 76 kPa (sd = 9 kPa) for the slow impact group and 298 kPa (sd = 34 kPa) for the fast impact group. The spinal cord showed a fourfold increase in stiffness response with increased impact velocity. The elastic moduli calculated using the finite element approximated geometric constant resulted in $E = 75.5 \text{ kPa} (sd = 9 \text{ kPa})$ for the slow impact group and $E = 298 \text{ kPa} (sd = 34 \text{ kPa})$. 
The effect of strain rate on acute spinal cord injury

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Figure 3.3 The tissue stiffness response for the slow impact group. The load-displacement curves show a nearly linear behaviour.

Figure 3.4 The tissue stiffness response for the fast impact group. The load-displacement curves show a nearly linear behaviour.
3.3 Haemorrhage in the Spinal Cord Following Injury

The distribution of the haemorrhage volumes across the spinal cord showed significant differences as a function of impact velocity (Table 3.3). The sham control animals showed no haemorrhage in the spinal cord after the touch force. The fast and slow impact groups showed substantially more damage. The total volume of haemorrhage in the spinal cord for the two different impact velocities was not significantly different (p = 0.22). The fast impact group had a mean haemorrhage volume of 1.50 mm$^3$ (sd = 0.4 mm$^3$) versus the slow impact group with a mean total haemorrhage volume of 1.21 mm$^3$ (sd = 0.5 mm$^3$). The haemorrhage volumes within the white matter of the spinal cords were significantly different for the two impacts (p=0.013). The slow impact group showed very little haemorrhage (0.24 ± 0.1 mm$^3$) in the white matter, while the fast group showed significantly more haemorrhage (0.61 ± 0.3 mm$^3$) The volume of haemorrhage occurring in the grey matter was also not significantly different for the two impact velocities (fast = 0.89 ± 0.18 mm$^3$, slow = 0.97 ± 0.4 mm$^3$ p= 0.62). Tests to verify the normality and homogeneity of the data demonstrated that all but one of the groups were normally distributed and all groups satisfied the homogeneity requirement (Appendix A).

Table 3.3 Volume of haemorrhage for each specimen in the fast and slow impact groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Specimen</th>
<th>Haemorrhage Volume Grey Matter (mm$^3$)</th>
<th>Haemorrhage Volume White Matter (mm$^3$)</th>
<th>Haemorrhage Volume Total (mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast 15</td>
<td></td>
<td>0.76</td>
<td>0.93</td>
<td>1.69</td>
</tr>
<tr>
<td>Fast 16</td>
<td></td>
<td>1.02</td>
<td>0.38</td>
<td>1.40</td>
</tr>
<tr>
<td>Fast 17</td>
<td></td>
<td>0.91</td>
<td>0.43</td>
<td>1.34</td>
</tr>
<tr>
<td>Fast 29</td>
<td></td>
<td>0.94</td>
<td>1.10</td>
<td>2.04</td>
</tr>
<tr>
<td>Fast 30</td>
<td></td>
<td>0.92</td>
<td>0.52</td>
<td>1.44</td>
</tr>
<tr>
<td>Fast 31</td>
<td></td>
<td>1.04</td>
<td>0.97</td>
<td>2.01</td>
</tr>
<tr>
<td>Fast 32</td>
<td></td>
<td>0.51</td>
<td>0.12</td>
<td>0.63</td>
</tr>
<tr>
<td>Fast 34</td>
<td></td>
<td>1.01</td>
<td>0.43</td>
<td>1.44</td>
</tr>
<tr>
<td>slow 20</td>
<td></td>
<td>0.96</td>
<td>0.18</td>
<td>1.15</td>
</tr>
<tr>
<td>slow 21</td>
<td></td>
<td>0.83</td>
<td>0.27</td>
<td>1.10</td>
</tr>
<tr>
<td>slow 22</td>
<td></td>
<td>0.90</td>
<td>0.11</td>
<td>1.02</td>
</tr>
<tr>
<td>slow 23</td>
<td></td>
<td>0.48</td>
<td>0.08</td>
<td>0.57</td>
</tr>
<tr>
<td>slow 35a</td>
<td></td>
<td>1.73</td>
<td>0.33</td>
<td>2.06</td>
</tr>
<tr>
<td>slow 36a</td>
<td></td>
<td>1.16</td>
<td>0.46</td>
<td>1.62</td>
</tr>
<tr>
<td>slow 37</td>
<td></td>
<td>0.56</td>
<td>0.23</td>
<td>0.79</td>
</tr>
<tr>
<td>slow 39</td>
<td></td>
<td>1.08</td>
<td>0.29</td>
<td>1.37</td>
</tr>
</tbody>
</table>
Both impact groups showed a dense concentration of haemorrhage at the location of impact, with petechial haemorrhage (small, isolated spots of haemorrhage) spreading both radially and longitudinally from the impact site (Figure 3.5). Haemorrhage in the spinal cord varied in the amount of haemorrhage as well as the distribution of haemorrhage across the cord. The fast impact group showed a more lateral spread of haemorrhage (Figure 3.6). The slow impact group showed fewer tendencies for haemorrhage to spread into the white matter but instead demonstrated a more longitudinal spread of haemorrhage within the grey matter (Figure 3.7).

Quantification of the amount of haemorrhage area occurring at the epicentre of injury showed great variability between specimens but followed the same trend as the volume measures (Table 3.4). A strong trend in the differences in the area of haemorrhage occurring within the white matter of the spinal cord were observed (p=0.048) with the fast injury group (0.81 ± 0.80 mm$^2$) showing greater haemorrhage area than the slow injury group (0.2 ± 0.14 mm$^2$). Haemorrhage at the epicentre of injury showed no significant difference (p = 0.20) between the two groups for the total amount of haemorrhage area. The slow group had a mean total haemorrhage area of 1.30 mm$^2$ (sd = 0.55 mm$^2$) versus the fast group with 1.73 mm$^2$ (sd = 0.71 mm$^2$). The amount of haemorrhage isolated to the grey matter was larger in the slow impact group (1.10 ± 0.5 mm$^2$) than it was in the fast injury group (0.92 ± 0.25 mm$^2$) but it was not statistically significant (p= 0.38).
Figure 3.6 Area of haemorrhage at the epicenter of injury for fast impact injuries (50x magnification). Spinal cord tissue stained with H&E shows haemorrhage in red and undamaged tissue in pink. Holes in the tissue are artefacts that were created during the flash freezing and cutting of the tissue and were not due to the injury process.
Figure 3.7 Area of haemorrhage at the epicentre of injury for the slow impact group (50x magnification). Spinal cord tissue stained with H&E shows haemorrhage in red and undamaged tissue in pink. Holes in the tissue are artefacts that were created during the flash freezing and cutting of the tissue and were not due to the injury process.
Table 3.4 Haemorrhage area at the epicentre of injury for each specimen in the fast and slow impact groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Specimen</th>
<th>Haemorrhage Area Grey Matter (mm$^2$)</th>
<th>Haemorrhage Area White Matter (mm$^2$)</th>
<th>Haemorrhage Area Total Cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>15</td>
<td>0.75</td>
<td>1.79</td>
<td>2.54</td>
</tr>
<tr>
<td>Fast</td>
<td>16</td>
<td>1.04</td>
<td>0.28</td>
<td>1.32</td>
</tr>
<tr>
<td>Fast</td>
<td>17</td>
<td>1.22</td>
<td>0.12</td>
<td>1.35</td>
</tr>
<tr>
<td>Fast</td>
<td>29</td>
<td>0.77</td>
<td>1.41</td>
<td>2.17</td>
</tr>
<tr>
<td>Fast</td>
<td>30</td>
<td>1.12</td>
<td>0.29</td>
<td>1.40</td>
</tr>
<tr>
<td>Fast</td>
<td>31</td>
<td>0.73</td>
<td>2.01</td>
<td>2.74</td>
</tr>
<tr>
<td>Fast</td>
<td>32</td>
<td>0.55</td>
<td>0.07</td>
<td>0.63</td>
</tr>
<tr>
<td>Fast</td>
<td>34</td>
<td>1.19</td>
<td>0.54</td>
<td>1.72</td>
</tr>
<tr>
<td>slow</td>
<td>20</td>
<td>1.00</td>
<td>0.06</td>
<td>1.06</td>
</tr>
<tr>
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3.4 Non-phosphorylated Neurofilament Reactivity in Damaged Axons

The density of non-phosphorylated neurofilament reactivity in the axons of the spinal cord demonstrated a significant relationship between impact velocity and axonal damage ($p = 0.013$). The slow and control groups showed very little disruption of white matter integrity as a result of impact while the fast group showed substantially more damage (Figure 3.8). The density of positive axons also varied in the response to injury at different locations within the spinal cord. In the fast group there were varying degrees of complete axonal disruption as a result of impact (Figure 3.9). The axons in spinal cords injured in the slow group showed less tissue disruption than the fast group and only a slight increase in dephosphorylation (Figure 3.10). The sham control specimens showed no visible reaction to the touch force (Figure 3.11). The most severe injuries showed disruption of nearly all axons in the ventral white matter while other animals showed greater residual connections. The reactivity of axons to the SMI-32 non-phosphorylated neurofilament anti-body centered on the primary area of haemorrhage, with disrupted axons showing the greatest intensity of labelling.
The lateral-ventral white matter showed the greatest amount of axonal disruption and demonstrated a significant relationship between impact velocity and tissue injury ($p = 0.0005$). A post-hoc analysis revealed the fast group was significantly different from both the slow ($p = 0.001$) and control ($p = 0.0009$) groups and there was no difference between the slow and control groups' responses to injury ($p = 0.54$). A similar effect of velocity on injury was found for observations made in the ventral white matter immediately in front of the ventral grey horn ($p = 0.031$). The fast impact group showed a significantly different response to injury from both the slow ($p = 0.035$) or control ($p = 0.04$) groups. The slow and control groups were not different in their response to injury.
Figure 3.9 Ventral white matter at the epicentre of injury for specimens in the fast impact group. Dark coloured axons indicate positive labelling for SMI-32 non-phosphorylated neurofilament anti-body. Areas around the haemorrhage show varying degrees of complete disruption in several axons.
Figure 3.10 Ventral white matter at the epicentre of injury for specimens in the slow impact group. Dark coloured axons indicate positive labelling for SMI-32 non-phosphorylated neurofilament anti-body. Areas around the haemorrhage show some complete disruption of axons and a small increase in reactivity in axons bordering the injury epicentre.
Figure 3.11 The ventral white matter at the epicentre of injury for specimens in the sham control group. Dark coloured axons indicate positive labelling for SMI-32 non-phosphorylated neurofilament anti-body. The axons show longitudinal orientation with no disruption. Variability in axon profiles are a result of tissue sections being cut slightly off the sagittal axis.
The medial-ventral white matter was also significantly (p = 0.046) affected by impact velocity in its response to injury. The fast impact group showed an increase in positive axonal reactivity over the control group response (p = 0.05) and a trend towards increased axonal response compared to the slow injury group (p = 0.06). There was no difference between the slow and control groups' responses. There was a trend towards greater axonal injury in the lateral columns with increased impact velocity (p = 0.06).

3.5 Non-phosphorylated Neurofilament Reactivity in Damaged Grey matter

The reactivity of grey matter around the epicentre of injury showed a significant relationship between tissue damage and impact velocity (p = 0.03). The fast impact group showed greater tissue damage than either the slow or control groups in the grey matter surrounding the area of haemorrhage at the injury epicentre (Figure 3.12). The slow group did show some increased reaction to injury in tissue adjacent to the haemorrhage site as compared with the control group. The damage to cell bodies around the haemorrhage epicentre demonstrated an increase in reactivity to the SMI-32 anti-body. Upon closer inspection many of the cell bodies closest to the region of impact demonstrated a compaction of their cytoplasm. This was characterized by an intense labelling of the cell contents showing a compressed form and surrounded by a void in the tissue (Figure 3.13). Compaction of the cell bodies was primarily seen in the fast impact group although some were present in the slow group adjacent to the foremost haemorrhage area (Figure 3.14). The reactivity of the grey matter to the SMI 32 antibody in all specimens considered in the analysis is shown in Appendix C.
Figure 3.12 Mean SMI 32 reactivity in grey matter for locations in the spinal cord rostral and caudal to the epicentre of injury. Density measures were normalized to an uninjured control portion of tissue on the same specimen to eliminate the effects of tissue processing. A value of 1 represented no reaction to injury while a value of 3 indicated a 3 fold increase in positive labelling.

Figure 3.13 Neuronal cell bodies in the grey matter labelled with the SMI-32 anti-body to dephosphorylated neurofilament. A) Healthy neurons in an uninjured portion of tissue. B) Neurons showing compactions of the cytoplasm as a result of injury.
Figure 3.14 Grey matter reactivity to SMI-32 neurofilament at the epicentre of injury in the slow (A) and fast (B) impact groups. The greater reactivity to the SM32 antibody can be seen in the fast impact specimen. The intense labelling of the cell bodies is indicative of injury. The pictures are a montage of several microscope images to demonstrate the longitudinal spread of injury.
A univariate analysis observing the effect of impact velocity on tissue reactivity at each of the five locations of interest showed that velocity had a significant effect on injury response both rostral and caudal to the impact epicentre. The effect of impact velocity was more significant caudal to the impact site than rostral. The density of grey matter reactivity immediately (0.5mm) rostral to the area of haemorrhage was significantly related to impact velocity \( (p = 0.008) \) while further rostral (1.5mm) from the injury site, the effect of velocity was not significant \( (p = 0.52) \). The caudal spread of injury was also significantly related to impact velocity both 0.5mm from the epicentre of haemorrhage \( (p = 0.0003) \) and 1.5 mm \( (p = 0.03) \) from the epicentre.

A post-hoc analysis for the locations showing a significant effect of impact velocity on tissue response revealed the specific effects of fast and slow impacts on the spinal cord. Immediately rostral to the epicentre, the tissue response to the fast impact was significantly different from the control tissue \( (p= 0.006) \), but not from the slow impact group \( (p = 0.07) \). Tissue response caudal (0.5mm) to the injury epicentre also showed the fast impact group having a significant effect as compared with both the slow \( (p = 0.04) \) and control \( (p = 0.0003) \) groups. Additionally, the injury in the slow group was significantly different from the control tissue \( (p = 0.011) \). Further caudal (1.5mm) from the injury epicentre, the response to injury was similar with the fast group showing a significantly greater injury intensity than either the slow \( (p = 0.05) \) or control groups \( (p = 0.025) \). The slow group did not differ from the control group at this cord location.
4 Discussion

The quest for effective treatment methods for spinal cord injured patients relies heavily on animal models of human injury to evaluate the efficacy of the therapy before it is clinically implemented. The accuracy with which the animal model simulates the human injury condition is paramount in providing a true evaluation of the therapeutic potential of new treatments. A large majority of the current animal models do not consider impact velocity as an essential variable in spinal cord injury. Those that have evaluated the importance of velocity as a variable in spinal cord injury have found contradictory results, with some teams reporting highly significant correlations with injury [15, 21, 40, 43, 46] while others report little or no correlation [18, 42, 44]. The mechanical response of the spinal cord to loading has been characterized as viscoelastic which supports the hypothesis that rate influences the tissue behaviour [141, 144]. The results of this study demonstrated that the impact velocity of a contusion injury significantly affected the magnitude of damage seen in the spinal cord and the mechanical response of the tissue.

The contusion injury was generated using a modified Spinal Cord Injury Research System (SCIRS). The modifications established a more accurate and more repeatable injury in the animals than previously reported in the literature [41, 158, 162], increased the usable range of the device and ensured ease of use of the system. Injury protocols were established in collaboration with our animal surgeon to optimize the efficiency of the system and make sure the ethical standards for animal care were upheld. The modified contusion device was used to complete a series of primary injuries in thirty-one animals to evaluate the effect of impact velocity on mechanical damage to the spinal cord. Tissue analysis techniques were selected to measure both the gross morphology of the cord injury and the disruption of structural elements within the axons and neurons. The interdisciplinary nature of the project required input from both the engineering and biological perspectives which facilitated communication between the different fields and established a collaborative environment on which to build future endeavours.
4.1 Limitations of this study

4.1.1 Animals as a model for human injury

Rats and mice have largely replaced the use of primates, dogs and cats in contemporary spinal cord research for scientific, ethical and economic reasons [187]. The composition of the spinal cord is similar in rats and humans; both consisting of grey matter whose cell bodies carry sensory information dorsally and motor information ventrally, and white matter composed of axons that facilitate communication between cell bodies [188, 189]. Both rat and human cords demonstrate cervical and lumbosacral enlargements [188, 189] and the cytoarchitecture of the grey matter in both rats and humans demonstrates similar organization of the Rexed’s laminae (Figure 4.1) [188, 190]. However, there are also many similar biological phenomena between rat and human SCI, particularly those related to cell death, inflammation, neurite regeneration and spinal motor pattern generators [191]. In SCI both rats and humans demonstrate a relationship between electrophysiological recordings and motor function, MRI enhancement corresponded to measures of posttraumatic cavitations and MRI lesion lengths correlated with functional outcomes [102].

![Figure 4.1 The Rexed's laminae organization of the grey matter in the human (A) and rat spinal cords (B).](image)

Nevertheless, there are several limitations of studying SCI on a rat model. There are fundamental differences between the rat and human cord regarding the location of tracts within the white matter (Figure 4.2). In humans a large portion of the corticospinal tract is in the lateral funiculus [53], however in rats the tract is in the dorsal columns [192]. In the rat
the dorsolateral fasciculus is comprised primarily of the rubrospinal and spinocerebellar tracts [192], whereas in humans the rubrospinal tract is rudimentary and is believed to end at the second cervical segment [53].

An aspect of this study not germane to the clinical situation is that animal models of contusion typically involve a dorsal impact for ease of access to the cord, while clinical human injury primarily results in a ventral impact on the cord. Differences in spinal cord geometry and the location of spinal tracts within the cord may result in very different outcomes from a dorsal impact as compared to a ventral impact. Most models assume the difference is negligible as the damage is most often seen at the centre of the cord, but that assumption has not been substantiated.

Another limitation of animal models is the use of anaesthesia during the impact. This creates a different pharmacological environment than that experienced in human SCI, but is obviously unavoidable. Salzman reported a three fold increase in tissue sparing post injury when halothane was used before injury to anaesthetize rats as compared with pentobarbital and N_2O anaesthesia [193]. Furthermore, the environment in which human cord injury occurs often involves confounding injuries that also require treatment, which may be detrimental to the spinal cord.

A further limitation of animal models is the control of biological variables. While it is important to control as many independent variables as possible during an experiment to determine the effect of the variable of interest, it is achieved at the expense of accurate representation of the human population. Mills indicated significant differences in functional outcome following experimental injury for different strains of rat and concluded that genetic factors may play a role in functional recovery following SCI thus challenging the validity of a treatment tested on a single strain of rats [36].
Figure 4.2 Major tract of the spinal white matter at the mid cervical level of the A) rat [192] and B) human [53]. Ascending tracts are blue and descending tracts are red.
In order to develop suitable clinical therapies for human treatment a reliable model must be available on which they can be evaluated. While animal models of SCI do not offer the ideal representation of the equivalent human injury, they do allow insight into the cellular progression of SCI and accurate material properties in an *in vivo* environment that is not afforded by cadaveric models [43]. Animal models of SCI have traditionally been used to test experimental therapies; however these models also provide the opportunity to gain substantial knowledge about the mechanisms through which spinal cord injury occurs.

### 4.1.2 Injury protocol

Prior to the initiation of the surgical procedures on the animals the system performance was validated by running a series of repeated tests during a short time interval. The displacement of the impactor was confirmed and the velocity slope and duration of the impact were verified. Impact tests were performed using the validated input parameters. It was only after the tests were completed and the data were being analyzed that a problem was recognized. The impact depth for the slow injury groups showed a magnitude of 0.9mm instead of the desired 1mm.

Further examination of the impactor device demonstrated that the system showed a damped response when the driving signal to the electromagnetic shaker was instantly shut off. This resulted in the impactor tip having a delayed response in its return to the zero position. Upon signal shut off the impactor withdrew to within 0.15mm of the zero position and then gradually crept back to zero over the course of five minutes. During the injury process this creep was not a factor as more than five minutes elapsed between the generation of each injury. However, during the validation process at the start of the testing day, when repeated hits were run in a short time window the impactor was not allowed sufficient time to return to the zero position.

In the slow impact group the peak voltage setting and voltage slope were minimized to deliver the slowest possible impact. The peak voltage setting determined the displacement of the electromagnetic shaker. If the peak voltage setting was greater than the voltage required to travel 1mm then interaction with the mechanical stop limited the peak displacement to
1mm. During the validation procedure the settings of peak voltage and slope were altered to provide output curves of 1mm displacement at 3mm/s velocity with no dwell time at the maximum displacement. The peak voltage setting appeared to generate travel of one millimetre during the parameter validation stage as the mechanical stop was encountered with each impact. However, it is likely that on the repeated hit cycles run during the validation stage the failure of the impactor to return to the zero position resulted in an overestimation of the actual displacement being generated. The offset of the impactor due to the failure of the shaker to return to the zero position plus the driving voltage were sufficient to reach the one millimetre setting of the mechanical stop. However, when there was time for the shaker to creep fully back to the zero position between contusion injuries the peak voltage setting was not sufficient to drive the impactor a full millimetre (Figure 4.3).

Figure 4.3 The motion of the impactor tip for an impact run repeatedly during the validation phase. The initial hit sequence during validation may not have travelled the full distance to the mechanical stop; however subsequent hits did encounter the stop giving the impression that the input values were sufficient to drive the system 1.0mm. However, when the shaker had time to creep back to the zero position between contusion injuries the impact setting showed the effect of the creep behaviour in the resulting injury motion.

The high speed tests resulted in peak displacements that were slightly greater than 1.0mm. The impact velocity used in this study represented the maximum capacity of the SCIRS. At the high velocities the contact forces with the mechanical stop were on the order of 10N.
The cantilever design of the mechanical stop appeared incapable of fully restricting the motion of the impactor rod assembly and there were indications of overshoot of the 1mm displacement at these high rates. Flexion in the system was evident upon review of the displacement and force traces resulting from each impact (Figure 4.4). The point of contact with the mechanical stop, as indicated by the sudden change in the force curve, did not correspond to the peak displacement. Instead there was an additional 0.1-0.2mm of travel after the contact with the mechanical stop. This resulted in impacts greater than 1mm occurring for the fast group.

As a result of the hysteresis and flexibility in the system, the mean displacement for the slow group (0.9mm) was less than the mean displacement of the fast group (1.1mm). This situation was not ideal however we are confident that the observed differences between the groups are not due to this effect. Previous experiments using the OSU impact device indicated that there was no statistical difference in histological or functional outcomes for impacts ranging from 0.95-1.10mm in the rat [13].

![Figure 4.4 The force and displacement data for a representative specimen in the fast impact group. The force curve indicates contact with the mechanical stop occurred before the maximum displacement was achieved. The light blue lines indicate the point on the displacement curve where the mechanical stop is encountered and the peak displacement.](image)
4.1.3 Rigid clamping of the spinal column

The analysis of the cord response to injury and the mechanical parameters of impact all assumed that the spinal column of the animal was rigidly fixed in the spinal frame of the contusion system. However, a previous review of clamping methods used in the contusion device demonstrated that when the rostral and caudal spinal clamps were used to secure the spine, the motion of the spinal column was as much as twenty-six percent of the motion of the impactor [194]. The clamps used to secure the vertebrae of the animals in this study were modified Allis clamps. The Allis clamps had teeth like elements at the gripping interface as compared to the grooves on the surfaces of the spinal clamps in the previous study. These teeth elements were thought to provide a more rigid grip on the vertebrae than the spinal clamps. Therefore it is likely that the motion of the spinal column in this study was much less than twenty-six percent of impactor tip motion. Furthermore, the evaluation of column motion in the contusion system was done for animals that were fully suspended in the spinal clamps with no additional support from a base below the animal [194]. The current study used a support beneath the animal to carry fifty percent of the animal’s weight, making stabilization less reliant on the strength of the Allis clamps to secure the animal. This likely reduced further the resultant motion of the spinal column. In addition, force traces for each animal were examined post-injury for indications of slippage in the clamp system. These animals were removed from the analysis of the mechanical parameters.

While attempts were made to minimize the motion of the spinal column during impact it was thought that the clamp configuration might create some flexion in the spinal column during indentation (Figure 4.5). A finite element model of spinal cord compression with rigid fixation of the posterior elements of the vertebral bodies immediately rostral and caudal to the contusion site showed that the motion of the vertebral body anterior to the impact site moved less than one percent of the total cord motion [184]. It is therefore unlikely that flexion of the spinal column was a significant contributor to spinal column motion.
Chapter 4 Discussion

4.1.4 Estimation of a Young’s modulus

Approximated values for the Young’s modulus for each specimen were achieved using two different calculations. The moduli found using the two methods were found to be identical. The techniques and inherent assumptions used in each method were very different and both had different possible sources of error. It was surprising that these two techniques would provide identical values. The first technique used calculations traditionally used in the modelling of a large tissue specimen and a small indentor. The second technique used a finite element model to duplicate the geometry of the contusion scenario and provide a numerical constant to describe the relationship between the Young’s modulus and the tissue stiffness response.

The indentation analysis of the mechanical response of the cord to loading assumed a rigid spinal column in the calculation of a Young’s modulus from the cord contusion data. A numeric solution to the Boussinesq problem was used as an approximation to convert the indentation stiffness to a Young’s modulus [179]. The Boussinesq problem solves for the behaviour of a continuous elastic tissue on a rigid substrate. The assumption of a rigid substrate for the spinal column is not completely accurate. Furthermore, the assumption of a

Figure 4.5 A schematic diagram of the fixation technique used to stabilize the column for the contusion injury. The calculation of the Young’s modulus assumed the column provided a rigid substrate against which the cord was compressed (A). The column response to loading would likely have resulted in some flexion of the spinal column in addition to the cord compression (B).
continuous elastic substrate does not completely apply to the cord compression problem. While the cord can be assumed continuous in the longitudinal direction, the spinal cord is constrained by the vertebral pedicles in the transverse direction. Thus, modeling the problem given the transverse geometry means that it can no longer be considered one of indentation in a continuous substrate but is also neither a confined nor unconfined compression problem.

The calculation of the Young’s modulus based upon a geometric constant provided by a finite element model of spinal cord contusion also had limitations. The finite element model was developed based upon the geometry of a human cervical spinal cord. The human cord occupied only 86 percent of the transverse canal space in the model, while the rat cord occupied approximately 93 percent of the transverse canal space, therefore the human cord response would be less constrained and have a lower stiffness value than if the rat geometry was used. This would overestimate the elastic modulus of the rat cord. However, the boundary conditions applied to the spinal cord in the finite element model limited the longitudinal motion of the spinal cord resulting in a stiffer response to load and a lower value for k. This decreased of the value of the approximated Young’s modulus. It is unknown which if either of these effects had a significant effect on the value found for k. These boundary conditions did not exist in the rat contusion model.

4.1.5 Statistical analysis

The statistical techniques used to analyze the data for this study relied on the assumptions of normality and homogeneity within the data. Shapiro-Wilks’ tests were performed on each data group to analyze the normality of distribution. The fast group data for the volume of grey matter haemorrhage showed a non-normal distribution based upon the test (p = 0.03). In addition, four out of fifteen data groups in the analysis of grey matter reactivity to SMI32 antibody showed non-normal distributions. A data group was defined as the data for a velocity group at a specific location. In addition, the grey matter reactivity analysis also showed non-homogeneity within the data as evaluated by a Levene’s test (p < 0.01). However, the techniques used for the analysis of haemorrhage volume (parametric t-test) and the grey matter reactivity (one-way MANOVA) are both robust to effects of non-normal
distribution and non-homogeneity when the number of specimens are equal in each group [185, 186].

4.2 The effect of impact velocity on spinal cord injury

Impact velocity is an often overlooked parameter of spinal cord injury. A large portion of the animal models of injury do not address velocity as a critical factor in the resulting injury. Those that have considered impact velocity in their models have shown varying degrees of effect, with some reporting highly significant correlations while others report little or no correlation with injury. This study examined the effect of impact velocity on spinal cord injury using a fast velocity representative of the rate at which spinal canal occlusion occurs in a human burst fracture injury and it isolated the mechanical effects of injury to the tissue from the cellular cascade of secondary injury by immediately sacrificing the animals after impact.

The study examined in this thesis demonstrated a significant effect of impact velocity on the magnitude and pattern of damage in the spinal cord. The fast impact velocity used in the study was selected to be representative of that occurring in the human injury scenario. The range of velocities tested was similar to those examined by previous authors who reported poor or no correlation between velocity and injury [18, 21, 44]. This study overcomes some of the shortcomings of the previous studies. The animals were more rigidly secured in the spinal frame using Allis clamps on the posterior elements of the vertebra immediately rostral and caudal to the injury site. The depth of compression was kept constant in this study, while impact velocity was varied to effectively isolate the role of impact velocity in injury. The results of the current study cannot be directly compared with the previous studies of impact velocity because of differences in survival time. Our goal was to assess the primary injury while a longer-term survival component is clearly needed in the future to determine correlations between parameters of injury, immediate tissue disruption and long term lesion development and function.

The current study clearly demonstrates important and significant effects of velocity on the spinal cord. The most important finding of the current study was the effect of impact
velocity on the type of tissue damaged. The volume of haemorrhage in the white matter was significantly increased with increased impact velocity. Specific examination of white matter damage through an antibody to dephosphorylated neurofilament supported the significant effect of velocity on white matter damage and demonstrated structural disruption of the axons. The volume of haemorrhage in the grey matter did not demonstrate a dependence on impact velocity; however an analysis of damage to cell bodies and dendrites in the grey matter did show a significant effect of impact velocity. This study demonstrated that impact velocity does have significant implications in spinal cord injury and should be considered an important component of animal models of injury.

No previous study evaluating the effect of impact velocity on spinal cord injury reported differentiated tissue damage as a result of velocity. The histological measures most commonly reported were the lesion area and volume [15, 18, 21, 44]. These measures did not distinguish between lesions in the grey or white matter. Other measures of damage to the tissue included changes in ionic concentrations [16, 46], scoring [40] and qualitative descriptions [42]. The scoring criteria used by Kearney involved separate measures (0 = no damage, 1 = mild, 2 = moderate, 3 = severe) for grey matter and white matter haemorrhage as well as myelin oedema and tissue loss to establish a total score (out of 12). The values for individual damage measures were not reported.

Previous studies that observed a significant relationship between impact velocity and injury were tested with velocities greater than 350mm/s [15, 40, 43, 46]. Kearney et al. considered impact velocity as a parameter for injury based upon the effective use of a viscous response criteria used in injury modelling of other soft tissues in the body [40]. Using a pneumatic impactor, spinal cord injuries were created in ferrets at velocities of 1500-6000mm/s. Compression levels of 25, 35, 50 and 65 percent of cord diameter were applied to the spinal cord. Injury outcome was measured by somatosensory evoked potentials (SEP) at intervals up to four hours. Tissue was transversely sectioned and stained with H&E and Toluidine blue for histological evaluation. A single histological severity score was assigned based upon the amount of myelin oedema, tissue loss, grey area haemorrhage and white area haemorrhage. They showed a weak correlation between depth of compression and functional
recovery, but a strong correlation between velocity and function. The correlation was even better for a combined velocity-compression product. The study reported no significant neuronal cell alterations which was remarkable considering the severity of the injuries applied. The histologic severity scores were significantly correlated to all of the biomechanical parameters, but correlated better with velocity than depth of compression and correlated best with the velocity-compression product. The study provided evidence for the importance of including impact velocity in models of spinal cord injury.

Following the work by Kearney et al., both Gruner and Kwo et al. considered the effect of impact velocity in their models of spinal cord injury [43, 46]. The velocities evaluated ranged from 350-1200mm/s in the studies and were controlled by varying the height of a dropped weight. While the weight drop technique allowed for control of the impact velocity, the depth of cord compression simultaneously increased with increased drop height, therefore making it difficult to separate the effects of velocity from that of contusion depth. Both groups observed changes in ionic concentrations in the spinal cord tissue as a result of injury. Kwo et al observed the effects at six hours post injury and Gruner observed them at 24 hours post injury. Changes in concentration levels of sodium [Na], potassium [K], calcium [Ca] and water [H₂O] were observed at the six hour observation and changes in the ratio of intracellular and extracellular concentrations of sodium and potassium were measured at 24 hours. At six hours post-injury linear correlations were found between impact velocity and changes in [Na], [K], and [Ca] however no correlation was found with water. Water concentration in the tissue was considered to be representative of oedema in the spinal cord. Changes in the ratio of sodium and potassium concentrations in the spinal cord at 24 hours post injury correlated best with impact velocity. The correlation was not improved when the depth of impact was included in the analysis. The results suggest that different mechanisms may be occurring in the injury process. Changes in extracellular levels of the ions suggest that the cell membranes may have increased their permeability either by mechanical disruption or failure of voltage regulated gates to the specific ions. The lack of a correlation of velocity with oedema may suggest that the controlling injury mechanism of oedema in the spinal cord may be less governed by rate and is perhaps related to a threshold strain level or that oedema is a secondary response to injury and is not directly affected by the
biomechanical parameters of injury. These studies further support the importance of impact velocity as a parameter in models of spinal cord injury. They also demonstrate that different outcome measures may better reflect the significant effect of velocity on injury.

Most recently, a mouse model of spinal cord injury demonstrated the significant effect of impact velocity [15]. Injuries were created using a constant depth of 0.25mm and velocities of 1000, 2000 and 3000 mm/s. The study showed the effect of impact velocity on the functional recovery of the animals and the chronic lesion size. Significant effects of velocity were seen in all behavioural tests on day 28 post injury with animals in the 1000mm/s group demonstrating near full recovery. The lesion volume was calculated by measuring rostral and caudal lengths of injury spread, and an anteroposterior diameter at the epicentre and assumed a biconical lesion shape. There was no distinction made between grey and white matter damage in the paper. Images presented of the injury epicentres for representative specimens from each group showed a greater involvement of white matter in the lesion for greater impact severity however this phenomenon was not reported.

The previous studies support the importance of impact velocity as a parameter for injury. However, the impact velocities studied corresponded to strain rates of 250-1200 s\(^{-1}\) or 2-12 times greater than that observed during the human burst fracture injury model [48]. The viscoelastic nature of the spinal cord is such that with increasing loading rates, the tissue becomes stiffer. With increased cord stiffness, the probability of an observed effect of velocity for a constant compression becomes more likely. Therefore, while the studies demonstrated a significant effect of impact velocity on the spinal cord, they did so for values far exceeding those likely to be experienced during a human cord injury. As the principal purpose of animal models of spinal cord injury is to accurately mimic the human injury condition, the selection of appropriate biomechanical parameters is important.

Studies evaluating velocities that were closer to the same order of magnitude as that representative of human injury found varying degrees of correlation and significance with outcome measures. Anderson used a pneumatic impact device similar to Kearney et al. to create injuries of 25, 50 and 75 percent cord compression at velocities of 600, 3000, 10000
mm/s in the ferret [42]. He used the same SEP techniques as Kearney et al. to evaluate residual function after injury and observed haemorrhage and necrosis in the cord at four hours. The results demonstrated a statistically significant relationship between SEP and depth of contusion, but did not show a significant relationship to velocity. The histological analysis suggested that velocity had an effect on the amount of haemorrhage following injury but no quantitative measures were taken to support the observation. The study suggests that impact velocity may have a greater effect on structural damage to the cord but that the magnitude of compression is a better regulator of functional outcome. However, somatosensory evoked potentials (SEP) measure sensory function and are a less reliable predictor of motor function [195]. Furthermore, in the clinical setting it has been demonstrated that SEP at the time of hospital admission did not correlate with ultimate functional recovery [196]. Meanwhile, haemorrhage has been demonstrated by several authors to correlate highly with long term functional deficits [197-199].

The studies using impact velocities most representative of the human injury range found the lowest levels of significance with impact velocity. Bresnahan et al. created injuries with velocities of 80-540mm/s for displacements of 0.38-2.48mm in rats [21]. The animals survived for 22 days post injury and were examined for functional and histological outcomes. The results demonstrated a significant correlation between impact velocity and lesion area ($r=0.74, p<0.01$). However that correlation was the weakest of all the impact parameters. The measures of functional deficits correlated with velocity through the recovery process. Significant correlations with gross motor scores (open field walking and inclined plane test) early after injury and with more sensitive motor measurements (grid walking) in the chronic phase of injury were observed but again they were the weakest of all injury parameters.

The findings of Bresnahan et al. showing velocity as the impact parameter with the weakest correlation to injury are supported by studies by Noyes [18, 44]. Noyes used impact velocities of 50-500mm/s for a range of displacements to create injuries in rats. The animals were allowed to survive 21 days before sacrifice and were examined for functional and histological outcomes. The functional deficits of the animals were measured by grid walking and were considered chronic injuries by day 21. Of the six mechanical parameters of injury
considered, velocity was the only parameter not to significantly correlate with functional recovery \(r=0.67, p>0.05\). The greatest correlation was between impact depth and functional recovery \(r=0.77, p<0.05\). Furthermore, correlation between lesion volume and the impact parameters again showed impact velocity to have the least correlation \(r=0.81\) with impact force having the greatest correlation \(r=0.93\).

While the studies at moderate impact velocities would suggest that all the other mechanical parameters of injury have more influence over the outcome of injury the results must be considered in context. The injury model used in the studies evaluating moderate impact velocities varied both impact depth and velocity simultaneously and in an apparently random fashion [18, 21, 44]. The impact parameters were then evaluated in isolation and the interactive effective of changing both velocity and depth was not examined. Furthermore, the pins through the spinal muscles, used to secure the animals under the impactor, provided very little rigidity to the spinal column and it was later demonstrated that only 57 percent of the depth of contusion actually compressed the spinal cord while the remainder acted to move the entire spinal column [194].

### 4.2.1 Primary lesions in the spinal cord

The early response of the spinal cord to contusion injury observed in this study was more severe than was expected based on previous descriptions of damage in the literature. Furthermore, the immediate inclusion of white matter in the haemorrhage area and the complete disruption of axons have not been well characterized in the literature. The few authors who have observed tissue damage in the immediate time (<20 minutes) following a contusion injury have primarily reported haemorrhage to be concentrated to the central grey matter with petechial haemorrhages seen in the white matter in the most ventral portions of the dorsal columns [17, 27, 130]. This is in contrast to the lesions observed in the current study, where extensive white matter haemorrhage was observed immediately following injury.

Previous observations of early damage following injury used a weight drop model of injury, thus direct comparison of mechanical parameters of injury could not be performed [17, 130].
However correlations of mechanical parameters with heights of a dropped weight (10g) were previously reported (Table 4.1). The parameters of the weight drop injuries were greater than those in the fast group of the current study of 1.1mm and 300mm/s. While qualitative comparisons could be made between the current and previous studies, a direct quantitative comparison was difficult as the previous studies used transverse sections of the spinal cord to quantify damage and the current study used longitudinal sections.

Table 4.1 Approximations of the mechanical parameters of injury corresponding to the height of a 10g dropped weight *represents an extrapolated value from the published data [200].

<table>
<thead>
<tr>
<th>Height (mm)</th>
<th>Compression (mm)</th>
<th>Velocity (mm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>2.04</td>
<td>681.56</td>
</tr>
<tr>
<td>50</td>
<td>2.59</td>
<td>971.51</td>
</tr>
<tr>
<td>175</td>
<td>4.02*</td>
<td>1843.68*</td>
</tr>
</tbody>
</table>

The areas of haemorrhage at the injury epicentre were reported in one study 15 minutes after the injury [130]. While the amount of haemorrhage cannot be compared, the relative percent distribution of haemorrhage across the grey and white matter gives an approximation of the tissue primarily targeted in the injuries. The distribution of haemorrhage at the injury epicentre for the 25mm weight drop showed 75 percent of the total haemorrhage to be contained in the grey matter and 24 percent in the white matter [130]. However, the study also noted that residual tissue (without haemorrhage) in the white matter 15 minutes after injury was not statistically different than the control tissue. The current study showed both the area of haemorrhage distribution at the epicentre and the volume distribution to be 62 percent in the grey matter and 38 percent in the white matter for the fast impact group which compares well with the distribution reported in the literature. The amount of haemorrhage in white matter of the current study was more extensive in the ventral white matter at the epicentre of injury as compared to the petechial haemorrhages described in the previous study [130].

The immediate response of the spinal cord to contusion injury was examined previously with both light and electron microscopy [17]. The cord response to a mild (10g x 25mm) injury
fifteen minutes after impact had extensive haemorrhage and destruction in the ventral portion of the dorsal funicular white matter and substantial haemorrhage in the central grey matter extending into the dorsal horns. The ventral horns of the grey matter also showed compacted nuclei and chromatolysis (disintegration of the Nissl substance of the cell body) which are thought to be indicators of a dying cell. The white matter in the lateral and ventral portions of the cord appeared mostly unaffected with small petechial haemorrhages and a tendency towards increased interaxonal spacing [17]. Examination with electron microscopy revealed compacted axoplasm characterized by close packing of the neurofilaments. The compaction was most prevalent in large calibre axons and some medium ones. Most small axons appeared unaffected. The cord response to a more severe injury (10g x 175mm) demonstrated more variable and extensive haemorrhage and denser axoplasm in the axons of the ventral white matter as compared with the mild injury.

The lesions observed by Rosenberg following mild and severe contusion injuries compare well with those observed for the fast impact group in this study. The haemorrhage, while concentrated in the central grey matter, also included significant portions of the white matter of the cord, particularly in the dorsal funiculi and the ventral white matter. Compacted neurons in the grey matter were observed in the current study, similar to those previously reported and are indicators of irreversibly damaged cells. The observation of compacted neurofilaments in the axons by electron microscopy in the previous study agrees well with the observations in this study of the reactivity of the cord to the antibody to dephosphorylated neurofilaments. Dephosphorylization of the neurofilaments was an indication of destruction of the sidearm structures on the neurofilaments. It is an interaction between these sidearm structures which is thought to provide the internal structure for the axons. Additionally, the SMI32 antibody used in this study targets heavy neurofilaments and those have been primarily associated with larger calibre axons [201]. A feature of the lesions found in the fast group in the current study was the complete disruption of some axons in the lateral and ventral white matter. For some animals the axonal disruption was nearly complete through the cord at the injury epicentre. Acute axotomy has generally not been reported following contusion injuries to the spinal cord without shearing of the tissue at the impactor-cord
interface [28]. The results suggest that high speed impacts may cause greater primary mechanical damage to the spinal cord than previously thought.

The discrepancies in the results found by the previous studies and those found in the current study may be explained by the methods used to describe the mechanical parameters of injury in the weight drop system. The amount of cord compression was calculated indirectly from measuring the movement of the vertebral column and the velocity of the falling weight [200]. The formula used to calculate the amount of cord compression was not described in the previous literature however the results suggest a possible error as reported compression of the spinal cord for the 25mm height is nearly seventy percent of the cord diameter of the animal. And the compressions for the 50mm height are 83 percent of cord diameter. Impacts of this magnitude would be expected to cause severe injury in the animal, if not death [13, 21, 40, 42] However the long term observations of function recovery indicated that the 25mm injury generated mild functional deficits and 50mm injury was moderate [130]. These injury characterizations have been substantiated by other work [20, 132, 133].

Another source of discrepancy in equating the mechanical parameters between the weight drop model and the electromechanical injury system is the value reported for impact velocity. The mechanics of injury generation differ between the two systems and an important distinction must be made when discussing impact velocity. In the weight drop model the velocity of the impactor is greatest in the instant before contact with the spinal cord. The impactor decelerates as it compresses the tissue until the velocity is zero (at peak displacement). The direction of motion of the impactor is then reversed. In the electromechanical system the impact velocity at the cord surface is zero. The impactor is accelerated through the spinal cord to a maximum velocity (at peak displacement) and then retracted. The differing mechanics of injury generation may account for some of the discrepancy in relating the mechanical parameters to outcome measures. However, it is likely the indirect observation of cord compression which provides the greatest source of error.
The effect of impact velocity on acute spinal cord injury  

Chapter 4 Discussion

The slow speed impact generated in this study has not previously been examined in the literature. The lesions observed for the slow impact appeared lesser in magnitude than those in the fast impact group. The haemorrhage spread in the grey matter was nearly equivalent between the two groups while the spread into the white matter was significantly less. The distribution of haemorrhage in the cord for the slow injury (83% grey matter, 17% white matter) was comparable to that observed for the 25mm x 10g injury (75% grey matter, 25% white matter) previously reported [130]. Furthermore, there was little evidence of structural disruption in either the axons or neurons around the epicentre of injury. These findings suggest important differences in the cord’s response to injury at different impact velocities.

The previous studies also examined the temporal changes to the lesion in the spinal cord following contusion injuries [17, 130]. The histopathology observed 15 minutes post injury continued to worsen throughout the course of the study for all magnitudes of impact. This suggests that the severe damage seen following the fast impact performed in this study will continue to worsen throughout the secondary and chronic phases of injury. The considerable inclusion of white matter in the primary lesion would likely have evolved into a nearly complete disruption of the axonal tracts of the white matter and as a result functional deficits would probably be nearly complete.

4.2.2 Mechanics of tissue damage

The patterns of injury observed in the current study encouraged an examination of the possible mechanics of tissue failure. At the lower impact velocity the injury pattern was concentrated in the centre of the spinal cord and lessened towards the cord edges while the fast impact group showed damage to the cord that nearly traversed the entire cord diameter. The longitudinal spread of haemorrhage in the slow impact group was similar to that of the fast impact group; however damage to the neurons and dendrites in the grey matter was greater and spread further in the fast impact group than the slow.

Examination of different stresses in the spinal cord as a result of contusion injury showed different loading mechanisms targeted different areas of the spinal cord (Figure 4.6) [24, 148]. Hydrostatic pressure increased in the tissue immediately below the piston as a result of
direct compression. Extrusion forces were generated by pressure gradients between the hydrostatic compression and the surrounding tissue. Transverse shearing stresses were concentrated at the edges of the impactor and tended to cut the tissue elements. A physical model of the spinal cord was constructed of a silastic tube filled with gelatin to allow observation of the internal response of the cord to loading [24, 25]. The cord distortion was primarily in a parabaloid form away from the impact center similar to the pattern of fluid flow in a tube (Figure 4.6B).

![Figure 4.6 A schematic of stresses in the spinal cord resulting from contusion injuries. A) Transverse cord section. B) Longitudinal cord section [24].](image)

There was a similar distribution pattern in the damage seen in the slow and fast impact velocities of this study when compared to the expected distribution of mechanical loading on the cord. The slow impact group showed a distribution of haemorrhage damage in the spinal cord spreading primarily longitudinally and being concentrated in the center of the cord. This corresponded to the pattern of extrusion stresses described by Blight [24] in the spinal cord. At the slow loading rate it is likely that the cord would respond to loading with a viscous, nearly fluid-like behaviour of the tissue. Therefore hydrostatic compression is a diminished factor as the tissue has time to flow and moves away from the areas where load is applied to create a more uniform stress distribution. A physical model of spinal cord contusion showed greatest strains occurring at the centre of the cord resulting from the viscous flow of the material [24]. In the fast impact group the longitudinal spread of damage
remained, however the transverse spread of injury was also greatly increased, nearly completely through the cord in several specimens. This pattern suggested that at the higher loading rate (and resulting higher force) the hydrostatic pressure resulting from compression was greater and caused more local damage to the tissue.

A finite element model of contusion injury to the human spinal cord predicted the greatest strains in the tissue occurring in the dorsal columns of the spinal cord immediately under the impactor (Figure 4.7) [184]. Elevated strain levels were also reported in the ventral columns and central cord. This injury pattern is similar to the patterns of hydrostatic compression and pure compression stresses seen in Blight's model. This is likely due to the fact that the material properties of the cord were assumed to be linear elastic. Therefore there is a minimal extrusion effect on the tissue. The pattern of injury seen in the finite element model better correlated with the high impact velocity loading than the slow injury group. At the high loading rate the mechanics of the spinal cord showed a linear response to loading and it is likely that the solid phase of the cord is the dominate factor in the loading response. The finite element model used a Young's modulus for the spinal cord of 0.26MPa which is very similar to the modulus found for the fast impact group of 0.30 MPa. This could account for the similarity seen in the pattern of strain distribution and tissue damage. The slow impact group showed a Young's modulus of 0.07MPa which may have resulted in different strain distributions in the finite element model. Furthermore, at the slow loading rate there is more opportunity for a fluid response to injury and the injury distribution reflected a flowing pattern.

Figure 4.7 The distribution of dorsal-ventral strains in the spinal cord resulting from a dorsal contusion impact. Peak strains are concentrated in the tissue immediately below the impactor. Elevated strains are also seen in the portion of the spinal cord below the impactor. There is minimal strain on the lateral columns [184].
It is also likely that the different types of stresses induced different injuries to the spinal cord. The extrusion stresses concentrated in the centre of the cord likely resulted in shearing of the fragile, small capillaries of the grey matter resulting in the abundant haemorrhage observed. The amount of haemorrhage in the white matter was less than that of the grey matter, likely because of the presence of fewer capillaries in the white matter [54] and the lesser extrusion stress levels [24]. The increased white matter haemorrhage seen in the fast impact group may have resulted from either an overall increase in extrusion stresses or perhaps were an effect of the increased compression and shearing stresses.

The discrepancies in damage to the neuronal structures in the grey matter between the slow and fast impact groups may further support the theory of increased hydrostatic pressure for the fast impact group. The damage seen in the cell bodies of the fast impact group may indicate compaction of the cell and a failure to return to normal. A void was seen in the tissue surrounding each of the compressed neurons and the cytoplasm appeared compacted as the density of labelling was significantly more than the uninjured tissue. The damage seen would appear to be a result of an equal force applied on all sides of the cell body which acted to compact the cytoplasm (Figure 4.8). The types and distributions of damage observed within the spinal cord in this study suggest that different mechanisms of injury may be present when different impact velocities are used to load the spinal cord.

Figure 4.8 A schematic diagram of the hypothesized mechanism for cytoplasm compaction seen extensively in the neuronal cells of the fast impact group and scarcely seen in the slow impact group.
4.3 **Performance of the modified contusion device**

The performance of the contusion device was more standardized in the current study by improvements in injury protocol. The recalibration of the displacement transducer prior to each day of testing ensured accurate reading of the impact dynamics. The introduction of a scale to standardize the clamping and unloading procedure and thus stabilize the spinal column for impact helped to eliminate the effect of a breathing artefact on the force measurement. Previous studies have reported variations in force measurement due to respiration to be as much as 0.04N. For the slow impact group in this study a breathing artefact could have distorted the force signal by as much as ten percent.

The inability of the impedance head on the SCIRS to measure forces at velocities of less than 20 mm/s has not been previously addressed. A review of the force traces from previous studies using the impedance head to measure force applied to the spinal cord demonstrated an immediate drop in the force measurement when the velocity of the impact is negligible, as it is during the dwell phase of the injury [130, 152, 162]. The impedance head provided with the system substantially limited the operable ranges of the device. The force measured did not provide an accurate reflection of the injury for either slow loading rates, or injuries with any duration of compression. The supplementation of the system with a strain gauge based force transducer dramatically expanded the system capabilities.

The results of the current study were compared to the displacement and force output measured by other researchers creating thoracic contusion injuries in a rat model (Figure 4.9). A variety of injury models were assessed and variability in the relationship between the depth of impact and force applied may be due to differences in impact velocity, dwell time, rigidity of the spinal column and the baseline from which the injury was created. The force and displacement data of the current study showed excellent agreement with some of the more recent data for contusion injuries. The peak force corresponding to the 1.1mm displacement of the fast impact velocity correlated well with the peak forces seen for a 1.0mm contusion at the T10 level using a touch force of 0.02-0.03N [41]. The cord response to loading showed good agreement with Scheff et al. who used a force controlled contusion device to generate their injury [45]. The displacements corresponding to peak forces are
slightly less than those seen in the current study and are thought to result from the absence of a preload (touch force) on the spinal cord. These results indicate the consistency of mechanical response despite different control variables.

The effect of the magnitude of touch force on the peak injury forces is evident in a review of literature using the same injury device and parameters as the current study (Figure 4.10). The injuries that showed greater force for a near equivalent displacement to the current study all used touch forces of 0.03N to establish a baseline for injury [13, 158, 162]. The current study used a touch force of 0.015N. It required nearly 0.2mm of displacement of the dural surface to establish a 0.015N touch force in this study and substantial dimpling of the dural surface was noted by examination with a stereoscopic microscope. A touch force of 0.03N was deemed excessive for this experiment.

![Figure 4.9 A comparison of force and displacement parameters from the current study with results from other studies creating thoracic contusion injury in rats.](image-url)
The results of Somerson et al. showed varying peak forces for a constant displacement [16, 194]. Both the loading rate and duration of impact were altered simultaneously in this study. The impact velocities used in the study were all the same order of magnitude and ranged from 170mm/s – 370mm/s. The resulting peak forces corresponding to the displacement showed reasonable agreement with the results of this study. The surprising result of the Somerson study showed that the greatest forces corresponded to the lowest impact velocities and the longest duration of injury. Furthermore the force curves for the longest duration group demonstrated an increase in peak force during the holding time at peak displacement. This response of cord tissue is counterintuitive to the expected behaviour of tissue relaxation under a constant load. It is thought that the increase in force towards the end of the holding cycle of impact may in fact be a pulse or respiration artefact however; the authors suggest it may be a result of pressure wave dynamics.

The results of Bresnahan et al. appeared to show a very different loading response to the other injury protocols [21]. The velocity used to generate the injury was 430mm/s and within the range of velocities used by other experiments. The difference in the load/displacement
response for that study was a result of the technique used to secure the animal for the impact. Pins through the spinal musculature were used to secure the animal instead of the more rigid Allis clamps used in the other studies. Reviews of the clamping procedures noted that sixty percent of the displacement of the impactor resulted in spinal column movement and not cord compression [194]. Therefore the net cord displacement for the study was likely closer to 1mm making the loading response comparable to those of other studies.

The effect of modifications of the contusion device on the repeatability of the generated injury is best represented by variability in the force data. The controlled parameters of displacement and velocity showed very good repeatability in the system but are independent of biological and procedural variability. The variability in the force data reflects the effects of both the biological and procedural variables. Biological variability is reduced by standardizing the size of the animal. This study showed a 6 percent coefficient of error in the body weights of the animals for a range of weights from 220g to 310g. Previous studies have reported variations from 3 percent [13, 158] in tightly controlled studies to animal weights ranging from 200-390g [41] and 250-375g [162] in other studies. The force data in this study showed a variability of 9 percent in the slow group and 13 percent in the fast impact group. The previous studies using similar levels of biological control and similar magnitudes of impact reported force data variability of 43 percent [41] and 16 percent [162]. The studies showing tighter control over animal size showed variability in force data of 8 percent [13] and 13 percent [158]. The results of this study show improved repeatability of the injury system performance compared with similar previous studies but also indicate the need to tighter control over the size of the animals used to further lessen the data variability.

Contusion injuries in rats observing force and displacement responses to loading have not been reported for velocities in the same order of magnitude as the slow impact velocity tested in this study. Neither the weight drop injury system nor the Spinal Cord Injury Research System are capable of generating injuries at such low velocities without modifications.
4.4 **Structural characteristics of the spinal cord**

Impact velocity demonstrated a significant affect on the mechanical response of the spinal cord to loading. The tissue stiffness and pseudo-Young’s modulus were four times greater at the 300mm/s loading rate than at the 3mm/s rate. The structural characteristics of the spinal cord found in this study are approximations of the nearly linear elastic response to loading seen in each of the injury scenarios. Young’s moduli were calculated using two distinct methods. The first method used a correction factor for the indentation of a continuous elastic material on a rigid substrate and the second generated a correction factor based on a finite element model of similar geometry. The correction factor generated by the indentation method was 0.124. The correction factor from the finite element model was also 0.124. Despite different inherent assumptions in both calculation methods the approximations for the Young’s moduli were equal.

There have been very few reported observations of the stiffness response of the spinal cord to contusion or compression injuries. The only group to report a Young’s modulus for an in vivo compression injury was Hung et al. [202]. They calculated an instantaneous modulus during quasi-static (0.002mm/s) compression loading of the cat spinal cord. For strains ranging from zero to forty percent they found moduli of 5-10 kPa with the dural sac intact and 2-13 kPa for the cord with the dural sac removed. No preload was applied to the cords in the study and no correction factor was applied in the calculation of the Young’s modulus. A preload would have slightly increased the value found for the modulus at each strain value however; a correction factor would have slightly decreased the modulus.

The current study found moduli corresponding to peak strains of 45-50 percent to be 76 kPa for a velocity of 3mm/s and 299kPa for 300mm/s impacts. These results demonstrated a four fold increase in the cord’s elastic response for two orders of magnitude change in impact velocity. If a similar scaling factor was applied to the data from the previous study it would be expected that the cord show a 6 fold increase in the modulus to correspond with a three order of magnitude difference in loading rate. The results of the previous study would predict a modulus of 60 kPa for the slow loading rate used in the current study. This compares well to the 76kPa modulus that was observed.
Approximations for elastic moduli for the in vivo spinal cord have been calculated in several experiments for the cord under tension. A series of experiments involving in vivo models of spinal cord injury in puppies and cats demonstrated Young's moduli ranging from 255-400 kPa [138-141, 202-204] for quasi-static loading rates in the order of $10^{-3}$ mm/s and peak strains of 1.7-5 percent. Above five percent strain the tissue behaviour became non-linear and could not be described by a Young's modulus. The results of the current study showed an elastic modulus of 76 kPa for a peak strain of 45 percent and a loading rate of 3 mm/s which is lower than expected based upon the previous studies. However, the technique used to apply loads in the previous studies required that the dural sac was removed and the cerebral spinal fluid drained from around the cord. The cord was further dried to allow for adhesion of metal rings to the spinal cord. A discussion of the in vivo technique used in the previous studies suggested that drying of the spinal cord could increase the modulus by more than ten-fold [139]. However, that series of studies showed consistent moduli for the spinal cords despite some of the tests being performed with the cords kept hydrated by a saline bath [139-141, 204] and others exposed to air for the duration of the procedure [138, 203]. It is likely that these procedures resulted in elevated values being found for the spinal cord material properties.

The stiffness of the rat spinal cord in response to contusion injury was reported by one author using an earlier iteration of the Spinal Cord Injury Research System used in the current study. He found the stiffness response to loading at impact velocities of 170-340 mm/s to be consistent between the animals [194] at 2.09N/mm. That corresponded well to the stiffness of 2.27N/mm found in this study for the fast impact group. The size of the impactor tip and the average animal weights were similar between studies and therefore a comparable correction factor would be applied to the stiffness value of the previous study to calculate a Young’s modulus and the value would have been similar to the one found in this study.

### 4.5 Techniques to observe damage in the spinal cord

Several researchers have explored the primary response of the spinal cord to injury. A number of different techniques have been used to evaluate the cord’s reaction to mechanical assault and the ability to observe the specific effects appears dependent on the amount of
time between injury and observation. Clinically, the first reported effect of mechanical insult is haemorrhage within the spinal cord, indicating disruption of the blood supply to the neural tissue. Gross approximations of haemorrhage and oedema can be visualized in the human injury through the use of MR imaging. Animal models of injury permit the luxury of a more complete and accurate histological examination of the spinal cord tissue at desired intervals following injury.

The principal outcome measure for the study relied on haematoxylin and eosin (H&E) staining of the spinal cord. H&E is a traditional histological technique which provided a high contrast stain to isolate haemorrhage and cell bodies within the spinal cord. H&E has been used extensively in the evaluation haemorrhage and tissue necrosis in spinal cord injury at a variety of time points [15, 28, 40, 42, 131, 133, 171]. The use of a traditional technique allowed us to more directly compare our results with previous studies from other laboratories.

Electron microscopy has been utilized extensively in the evaluation of primary injury to the white matter of the spinal cord. This technology has allowed for research teams to examine small cytoskeletal elements of axons for damage before it is evident under light microscopy. Within fifteen minutes of impact to the spinal cord, injury has manifested itself in changes in axonal orientation [205], axonal swelling [206], myelin disruption [83], granulation of neurofilaments and microtubules [83, 207], development of nodal blebs (outward dilations of the nodal axolemma and adjacent axoplasm) [208], membrane permeability [207, 209], haemorrhage [63] and vesicular degeneration of myelin [88]. The more recent development of antibodies to specifically target damaged structures within the spinal cord allowed for observations of disrupted cytoskeletal elements without the need for electron microscopy in this study.

The outcome measure selected for this study was chosen to represent structural disruption in the spinal cord. Several antibodies have been used in the field of spinal cord research to examine the cord’s response to injury, however most rely on the accumulation of an antigen in the spinal cord for some time after injury before they can be detected. The SMI-32
antibody to dephosphorylated neurofilaments was selected because it was expected that some structural disruption would occur in the axons immediately upon impact. Phosphorylation of the neurofilaments reflects the position of the medium and heavy neurofilament protein sidearm structures. Those sidearms provided interfilament spacing within the axons. Dephosphorylation was a reflection of disruption of the structural integrity of the axon. The SMI-32 antibody has been used extensively in brain injury research and has allowed observations of differences in response to injury as early as one hour post injury [175, 210-213]. While not as common in spinal cord injury research, the SMI-32 antibody has been used on spinal cord tissue for other applications [214]. Neurofilament disruption in spinal cord injury has been observed at one hour follow-up with other microscopy techniques [64, 83]. These combined results indicated that the use of SMI32 antibody, as a measure of damage to the axonal structure, was appropriate for this study.

4.6 Techniques for quantifying tissue damage

The area tracing technique used in the analysis of haemorrhage in the spinal cord following injury has been used in several previous studies of spinal cord injury to assess area or volume of a lesion [13, 21, 41, 125, 130, 200]. The inclusion criteria for manual highlighting of injured tissue was subjective however for this study it proved a more accurate reflection of damaged tissue than a threshold technique which counted all pixels in the image of a certain colour and picked up colour artefacts throughout the uninjured portions of the tissue. Other traditional quantification techniques such as grid counting also rely on the subjective opinion of the viewer to determine the inclusion criteria for injury.

The frustrum of a cone equation used to calculate the lesion volume has been used by studies performing similar experiments and evaluations to the current study [13, 21, 41]. A more traditional stereological method of volume calculation is the Cavalieri equation which sums the measured areas of each section and multiplies the sum by the section thickness [45, 215]. The frustrum of a cone equation resulted in a slightly lower volume measure than the Cavalieri equation, with differences of one to five percent for the specimens in this study. The correlations of lesion area versus lesion volume to functional recovery have been examined [21]. It was concluded that lesion area was a better reflector of function however
the tabulated statistical analysis contained in the paper clearly showed lesion volume to better correlate with function at the point of sacrifice, particularly for the more sensitive motor measurements ($r=0.86$, $p<0.001$ versus $r=0.81$, $p<0.01$).

The methods for analysing tissue labelled with the SMI 32 antibody have been varied. The majority of authors have reported only qualitative observations of changes in neurofilament labelling and axonal geometry [175, 210, 211, 213]. Other groups have devised scoring techniques to quantify the amount of reactivity in the tissue [212]. Quantitative analysis has been performed by a few researchers and has utilized either density measures of reactive tissue [216] or a grid counting technique of individual axons [214]. The density measure used in the current study was selected because it allowed for a greater area of tissue to be analyzed than the grid counting technique. Lower magnification images could be used in the density analysis than those required for an accurate grid counting technique.

A number of factors had to be considered in the use of density as a measure of reactivity in the tissue including the level of background staining in the tissue, the thickness of the section, the specific conditions of the immunohistochemical procedures and the level of illumination of the image [217]. While these parameters were standardized as closely as possible, the use of a non-reactive normal measurement on the tissue section provided a standard normalization to eliminate the effect of the possible processing variables described.

### 4.7 Tissue damage and functional losses

Animal models of spinal cord injury have not yet been used to determine a predictive relationship between cord damage immediately following injury and chronic functional deficits. Limitations exist in the resolution capabilities of non-invasive imaging techniques that have not, to this point, allowed accurate imaging of a primary lesion in an animal model and then functional evaluation of a chronic lesion. Clinically, predicting functional outcome from early measures of spinal cord damage is a standard procedure with the amount of haemorrhage being a leading predictor of chronic functional deficits. Magnetic resonance imaging (MRI) has demonstrated a strong correlation between haemorrhage in the spinal cord in the primary stages of injury and chronic residual function [197-199]. Functional
deficits as a result of spinal cord injury correlated more closely with haemorrhage than either oedema or cord swelling [197-199]. Patients displaying haemorrhage in the spinal cord following injury had a significantly lower motor scale score (greater functional deficit) at hospital discharge than those without haemorrhage at the early time period [197]. The results of this study showed that total volume of haemorrhage in the spinal cord immediately following injury did not correlate well with the magnitude of injury. Previous animal models of contusion injury have shown the amount of haemorrhage in the spinal cord continues to increase for several hours after injury [130]. The immediate sacrifice of the animals in this study did not allow for the full extent of the haemorrhage to develop.

Animal models of injury have established a relationship between chronic lesions and functional losses. Blight et al demonstrated that as little as 5-10% axonal sparing was required for some neurological function [14, 25]. Fehlings was able to demonstrate a logarithmic relationship between the number of residual myelinated axons at the injury site and the inclined plane test results for rats experiencing a clip compression SCI at six weeks post injury [75]. He also showed that only 12% of axons needed to be spared for full functional recovery. Noble was unable to demonstrate a relationship between spared tissue and function at short-term follow-up but showed a significant linear relationship between spared tissue and function by four weeks [130]. In addition, animal models of injury have shown that the residual axons following injury have reorganized themselves to facilitate some functional recovery [218]. These results combined with the clinical link between acute spinal cord haemorrhage and chronic functional deficits suggest that the graded histological response seen in the current study would translate to graded functional deficits in the animals. This hypothesis would best be resolved with additional study using similar injury parameters and a longer survival window.

4.8 Clinical Implications

The patterns of injury in the spinal cord for the fast and slow impact groups suggest that the primary mechanical damage may distribute differently across the spinal cord dependent on the mechanism of injury. Furthermore, the impact velocity may affect which structures are damaged in the spinal cord. In this study, the slow impact group resulted predominately in a
grey matter injury to the spinal cord whereas the fast impact group showed a combination of grey matter, axonal injury and cytoskeletal disruptions (inferred from the presence of the dephosphorylated neurofilament epitope). These observations may have implications in how spinal cord injury is addressed clinically and in the development of future spinal cord injury treatments.

Current treatment methods for spinal cord injury include stabilization of respiration and cardiovascular function, spine stabilization and decompression and anti-inflammatory steroids. The selection of treatment methods is limited and the effectiveness of the treatments in restoring functional capabilities is variable. Currently, the best predictor of functional outcome for spinal cord injury is the neurological score at admission [10, 11]. While many therapies have shown promising results when evaluated on animal models of spinal cord injury, those results have failed to translate to clinical successes [5, 219]. Accurate modelling of the human injury condition is paramount to providing a foundation upon which to develop treatment strategies. The results of the current study demonstrate that impact velocity does play a significant role in the distribution and type of damage occurring in the spinal cord and that velocity needs to be included as a parameter in models of spinal cord injury.

Understanding that injury to the spinal cord varies as a result of different injury mechanisms may also provide a clearer picture of necessary treatment techniques to target these different injuries. The slow impact group in this study demonstrated a primarily grey matter injury while the fast impact injury included both grey and white matter suggests the different treatments may be warranted. In addition, understanding that significant tissue disruption results from the primary mechanical impact for high velocity injuries the urgency for early decompression surgery may be lessened as significant damage has already occurred.

As regeneration strategies and other targeted therapies come into experimental and clinical use, the understanding of damage patterns associated with specific injuries may become important for the physical direction of treatment. The fast impact injury generated in the current study demonstrated immediate disruption of the central grey matter and the dorsal
and ventral columns of white matter with little damage seen in the lateral columns. In animal models, many treatments involve direct application on or into the spinal cord [220-222]. Applying these treatments directly to the damaged tissues may accelerate the treatment effect and lessen the required dosage.

Understanding the effects of impact velocity on spinal cord injury may have value in both the comprehension of injury and the establishment of treatment protocols. Including impact velocity as a parameter in experimental models of human spinal cord injury will create a more accurate reflection of the problem at hand and provide better feedback regarding the effectiveness of developed therapies before clinical implementation. From a clinical standpoint understanding the effect of impact velocity on injury may provide for a better initial evaluation of the required treatment.
Chapter 5 Conclusions

The Spinal Cord Injury Research System was assembled, calibrated and validated to assure proper system performance. The modifications to the device improved the efficiency and ease of use as well as the repeatability and reliability of the generated injury. The operable range of the device was increased with modifications to the hardware and software to allow for the breadth of velocities desired for this study. The performance of the modified system was evaluated during the completion of a preliminary series of injuries. The experiments examined the effect of impact velocity on spinal cord injury and demonstrated a significant effect on the mechanical response of the tissue, the distribution of damage in the cord and the type of damage occurring with increased impact velocity.

The mechanical response of the spinal cord to increase impact velocity demonstrated an increase in the peak force applied to the spinal cord for a fixed displacement. The cord response to loading was nearly linear and the approximated Young's modulus was four times greater for the fast impact than the slow.

Impact velocity had a significant effect on the distribution of haemorrhage within the spinal cord. The fast impact group showed greater haemorrhage volume in the white matter following injury than the slow impact group. However, the volume of haemorrhage in the grey matter was slightly greater in the slow group than in the fast impact group and the total volume of haemorrhage did not differ between groups. The orientation of the primary haemorrhage area was predominately dorsal-ventral in the fast impact group and longitudinal in the slow impact group. The distribution and orientation of haemorrhage in the spinal cord correlated well with theoretical stress distributions for the contusion injury mechanism.

Structural disruption of the neurofilaments in the axons and neuronal cell bodies also demonstrated a significant effect of impact velocity. There was little disruption to the axons of the slow injury group but there was significant reactivity and disruption to the axons at the epicentre of injury in the fast impact group. Ventral and ventrolateral white matter showed the greatest response to injury in the fast impact group. The reactivity in the slow impact
group did not differ from the sham control group. Compaction of the neuronal cell bodies was also observed in the grey matter of the fast impact group. Areas around the primary haemorrhage showed the greatest reactivity and spread both rostral and caudal from the epicentre in the fast impact group. The slow impact group showed some increased reactivity in the grey matter over the control group in the tissue caudal to the impact epicentre.

5.1 Future Directions

The significant effect of impact velocity observed in this study provides the impetus for future research to further define the relationship between impact parameters and injury. While a relationship between impact velocity and injury has been established, a more comprehensive definition of the relationship by testing intermediate velocities is needed. By mapping a correlation between impact velocity and different outcome parameters it may be possible to establish thresholds for injury. These would be useful both in the prevention of injury and in the modelling and simulation of spinal cord injury.

To more completely define the injury model described in this study it would be beneficial to examine the long term functional and histological effects of the injury parameters used. While preliminary effects of impact velocity are evident in the tissue damage the classification of the injury requires the observation of functional deficits resulting from these injuries. This would more easily allow for the discussion of this injury protocol in the context of previous models. Additionally, understanding the temporal progression of the secondary injury from the primary mechanical disruption could suggest opportunities for targeted treatment strategies for specific tissue disruptions.

Continued modification of the SCIRS would enhance the performance range and capabilities of the device. The control system for the Ling shaker does not utilize the full functional range of the shaker. Returning the system to a closed-loop control system would eliminate the need for a mechanical stop, and reduce the noise in the force signal recorded at the peak of impact. Furthermore, adjustments to retraction speed and displacement (for models of residual compression) are not possible in the current system but would be possible with a
closed-loop control and would likely provide a more accurate model of the human injury system.

Understanding that the spinal cord is a viscoelastic tissue, an accurate model of spinal cord injury should include the parameters of velocity and tissue compression. It would be advised that this model be extended to involve alterations of impact depth systematically with impact velocity, for values representative of the magnitudes observed in human traumatic injury. This would provide a more accurate picture of the cord response to loading and provide a more solid foundation for the evaluation of experimental therapies.

The general hypothesis amongst clinicians is that it is the energy transferred to the spinal cord during an impact which is the predicting factor of long term injury. Energy is a product of the force and displacement applied to the spinal cord. The relationship between force and displacement in a given specimen is affected by the impact velocity. An interesting experiment utilizing the modified SCIRS would be to generate injuries with a constant energy value but varied combinations of force and displacement values. This would require a preliminary study to determine the resulting force magnitudes for a range of impact displacements and velocities.

Accurate values for spinal cord tissue properties are currently lacking. Indentation testing has been used in different areas of biological tissue study to establish the mechanical parameters of structures. By establishing a data set for a variety of impact velocities and depths, the mechanical response of the cord system could be better defined. Furthermore by completing the same series of experiments with the dura removed, the response of the spinal cord could be established. The structural properties of the spinal cord and surrounding meninges are required to establish accurate mathematical models of spinal cord injury.

As finite element modelling becomes a more dominant tool in the simulation of spinal cord injury it will be important to establish a technique in which to validate the relationship between stress distribution in the spinal cord and resulting tissue damage. Developing a finite element model which is representative of the rat model of contusion injury would
provide the opportunity to correlate theoretical and experimental data and demonstrate the value of computer models.

5.2 Contributions

It is expected that this project is the first step in a new research direction for the Division of Orthopaedic Engineering Research at the University of British Columbia. This work provides the fundamental knowledge of a contusion model of spinal cord injury and establishes the testing protocols and methods required to create a repeatable and reliable injury model.

The results of this study further support the need to consider impact velocity as an important parameter in models of spinal cord injury. It is anticipated that this will spur further study in this area to establish a model system of injury that incorporates impact velocity as a controlled variable. In addition, with the increasing use of finite element modelling as a method to examine mechanical parameters of spinal cord injury, this work provides data that may be useful in the validation of such models.

In the long term this research will help to establish a more accurate model of spinal cord injury by encouraging the consideration and inclusion of more variables than simply displacement and force in the discussion of spinal cord injury. A more accurate model of spinal cord injury will provide a better basis on which to test experimental therapies and may help in the identification of different mechanisms of spinal cord injury, providing the impetus to develop targeted treatment strategies.
6 References


The effect of impact velocity on acute spinal cord injury

Chapter 6 References


Appendix A – Statistical Tables

*Volume of Hemorrhage in the spinal cord*

<table>
<thead>
<tr>
<th></th>
<th>Mean Fast</th>
<th>Mean Slow</th>
<th>Std.Dev. Fast</th>
<th>Std.Dev. Slow</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
<th>F-ratio variances</th>
<th>p variances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire Cord</td>
<td>1.536</td>
<td>1.233</td>
<td>0.450</td>
<td>0.485</td>
<td>1.296</td>
<td>14</td>
<td>0.216</td>
<td>1.162</td>
<td>0.848</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>0.888</td>
<td>0.965</td>
<td>0.178</td>
<td>0.387</td>
<td>-0.511</td>
<td>14</td>
<td>0.617</td>
<td>4.758</td>
<td>0.057</td>
</tr>
<tr>
<td>White Matter</td>
<td>0.610</td>
<td>0.243</td>
<td>0.344</td>
<td>0.121</td>
<td>2.847</td>
<td>14</td>
<td>0.013</td>
<td>8.068</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Brown-Forsythe (Brn-Fors)

<table>
<thead>
<tr>
<th></th>
<th>F(1,df)</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire Cord</td>
<td>0.149</td>
<td>14</td>
<td>0.706</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>2.249</td>
<td>14</td>
<td>0.156</td>
</tr>
<tr>
<td>White Matter</td>
<td>4.032</td>
<td>14</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Figure A. 1: Haemorrhage volume in the spinal cord, white matter and grey matter following injury in the fast and slow groups. Means of each group were plotted with error bars representing one standard deviation from the mean.
Table A.1: Shapiro-Wilk statistic to evaluate the normality of distribution in the variables.

<table>
<thead>
<tr>
<th>Group</th>
<th>Haemorrhage Measure</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>Entire Cord</td>
<td>0.9287</td>
</tr>
<tr>
<td>Slow</td>
<td>Grey Matter</td>
<td>0.5443</td>
</tr>
<tr>
<td>Slow</td>
<td>White Matter</td>
<td>0.9241</td>
</tr>
<tr>
<td>Fast</td>
<td>Entire Cord</td>
<td>0.1844</td>
</tr>
<tr>
<td>Fast</td>
<td>Grey Matter</td>
<td>0.0321</td>
</tr>
<tr>
<td>Fast</td>
<td>White Matter</td>
<td>0.3401</td>
</tr>
</tbody>
</table>

**SMI-32 Antibody Reactivity in the White Matter**

Table A.2: Summary of all Effects: MANOVA

<table>
<thead>
<tr>
<th>Wilks' Lambda</th>
<th>Rao's R</th>
<th>df 1</th>
<th>df 2</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.367598295</td>
<td>2.922082424</td>
<td>8</td>
<td>36</td>
<td>0.012766</td>
</tr>
</tbody>
</table>

Table A.3: Means of each data group

<table>
<thead>
<tr>
<th></th>
<th>Lateral</th>
<th>Lateral-ventral column</th>
<th>Ventral column</th>
<th>Medial-ventral column</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAST</td>
<td>2.24938941</td>
<td>3.228126049</td>
<td>2.699218273</td>
<td>2.464192</td>
</tr>
<tr>
<td>SLOW</td>
<td>1.40808928</td>
<td>1.454990149</td>
<td>1.352710485</td>
<td>1.67095</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1.210349917</td>
<td>1.161524296</td>
<td>1.603972435</td>
<td>1.478919</td>
</tr>
</tbody>
</table>

Table A.4: Main effect of each group

<table>
<thead>
<tr>
<th></th>
<th>Mean sqr Effect</th>
<th>Mean sqr Error</th>
<th>F(df1,2)</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>LATERAL</td>
<td>2.435319424</td>
<td>0.7585302</td>
<td>3.210576534</td>
<td>0.06073</td>
</tr>
<tr>
<td>L-V Column</td>
<td>10.00130272</td>
<td>0.904016376</td>
<td>11.0631876</td>
<td>0.000523</td>
</tr>
<tr>
<td>V Column</td>
<td>4.10103941</td>
<td>1.004281044</td>
<td>4.083557606</td>
<td>0.031763</td>
</tr>
<tr>
<td>M-V Column</td>
<td>2.182494164</td>
<td>0.609864891</td>
<td>3.578651667</td>
<td>0.045983</td>
</tr>
</tbody>
</table>

Table A.5: P-values for the Newman-Keuls post hoc analysis in the ventral-lateral column.

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>0.001389563</td>
<td>0.000904024</td>
<td>0.543807447</td>
</tr>
<tr>
<td>Slow</td>
<td>0.001389563</td>
<td>0.000904024</td>
<td>0.543807447</td>
</tr>
<tr>
<td>Control</td>
<td>0.000904024</td>
<td>0.543807447</td>
<td></td>
</tr>
</tbody>
</table>

Table A.6: P-values for the Newman-Keuls post hoc analysis in the ventral column.

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>0.035441101</td>
<td>0.040415227</td>
<td>0.621406317</td>
</tr>
<tr>
<td>Slow</td>
<td>0.035441101</td>
<td>0.040415227</td>
<td>0.621406317</td>
</tr>
<tr>
<td>Control</td>
<td>0.040415227</td>
<td>0.621406317</td>
<td></td>
</tr>
</tbody>
</table>
The effect of impact velocity on acute spinal cord injury

Appendix A

Table A. 7: P-values for the Newman Keuls post hoc analysis in the medial-ventral columns.

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>0.04983151</td>
<td>0.055193365</td>
<td>0.04983151</td>
</tr>
<tr>
<td>Slow</td>
<td>0.055193365</td>
<td>0.04983151</td>
<td>0.628098488</td>
</tr>
<tr>
<td>Control</td>
<td>0.04983151</td>
<td>0.628098488</td>
<td></td>
</tr>
</tbody>
</table>

Table A. 8: The results of the Shapiro-Wilk statistic to analyse the normality of distribution in each group. A significant value indicates a non-normal distribution.

<table>
<thead>
<tr>
<th>Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast1</td>
<td>0.7106</td>
</tr>
<tr>
<td>Fast2</td>
<td>0.0805</td>
</tr>
<tr>
<td>Fast3</td>
<td>0.1107</td>
</tr>
<tr>
<td>Fast4</td>
<td>0.9927</td>
</tr>
<tr>
<td>Slow1</td>
<td>0.0507</td>
</tr>
<tr>
<td>Slow2</td>
<td>0.4275</td>
</tr>
<tr>
<td>Slow3</td>
<td>0.4908</td>
</tr>
<tr>
<td>Slow4</td>
<td>0.3125</td>
</tr>
<tr>
<td>Control1</td>
<td>0.2821</td>
</tr>
<tr>
<td>Control2</td>
<td>0.1348</td>
</tr>
<tr>
<td>Control3</td>
<td>0.1406</td>
</tr>
<tr>
<td>Control4</td>
<td>0.5816</td>
</tr>
</tbody>
</table>

Levene's test for equal variance showed comparable variances in the groups (p=0.16).

**SMI-32 Antibody Reactivity in the Grey Matter**

Table A. 9: Summary of all Effects: MANOVA

<table>
<thead>
<tr>
<th>Wilks’ Lambda</th>
<th>Rao’s R</th>
<th>df 1</th>
<th>df 2</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.347365</td>
<td>2.368801</td>
<td>10</td>
<td>34</td>
<td>0.029811</td>
</tr>
</tbody>
</table>

Table A. 10: Means of each data group

<table>
<thead>
<tr>
<th></th>
<th>Lateral</th>
<th>Lateral-ventral column</th>
<th>Ventral column</th>
<th>Medial-ventral column</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAST</td>
<td>1.231928</td>
<td>2.447996</td>
<td>1.319747</td>
<td>2.808093</td>
</tr>
<tr>
<td>SLOW</td>
<td>1.005096</td>
<td>1.637273</td>
<td>1.047982</td>
<td>2.029213</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1</td>
<td>0.981227</td>
<td>1.027445</td>
<td>1.018773</td>
</tr>
</tbody>
</table>

Table A. 11: Main effect of each group

<table>
<thead>
<tr>
<th></th>
<th>Mean sqr Effect</th>
<th>Mean sqr Error</th>
<th>F(df1,2)</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most rostral</td>
<td>0.140359</td>
<td>0.209853</td>
<td>0.66842</td>
<td>0.52288</td>
</tr>
<tr>
<td>Rostral</td>
<td>4.318772</td>
<td>0.69943</td>
<td>6.17470</td>
<td>0.007778</td>
</tr>
<tr>
<td>Epicenter</td>
<td>0.212958</td>
<td>1.145736</td>
<td>0.18587</td>
<td>0.831733</td>
</tr>
<tr>
<td>Caudal</td>
<td>6.439078</td>
<td>0.517702</td>
<td>12.43781</td>
<td>0.000273</td>
</tr>
<tr>
<td>Most caudal</td>
<td>1.19477</td>
<td>0.275147</td>
<td>4.342295</td>
<td>0.026407</td>
</tr>
</tbody>
</table>

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Appendix A

Table A. 12: P-values for the Newman-Keuls post hoc analysis in the grey matter rostral to the injury epicenter.

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>0.066219</td>
<td>0.005794</td>
<td></td>
</tr>
<tr>
<td>Slow</td>
<td>0.066219</td>
<td>0.131763</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.005794</td>
<td>0.131763</td>
<td></td>
</tr>
</tbody>
</table>

Table A. 13: P-values for the Newman-Keuls post hoc analysis in the grey matter caudal to the injury epicenter.

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>0.042174</td>
<td>0.000301</td>
<td></td>
</tr>
<tr>
<td>Slow</td>
<td>0.042174</td>
<td>0.010672</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.000301</td>
<td>0.010672</td>
<td></td>
</tr>
</tbody>
</table>

Table A. 14: P-values for the Newman Keuls post hoc analysis in the grey matter most caudal to the injury epicenter.

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>0.050189</td>
<td>0.025131</td>
<td></td>
</tr>
<tr>
<td>Slow</td>
<td>0.050189</td>
<td>0.450497</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.025131</td>
<td>0.450497</td>
<td></td>
</tr>
</tbody>
</table>

Table A. 15: The results of the Shapiro-Wilk statistic to analyse the normality of distribution in each group. A significant value indicates a non-normal distribution.

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast1</td>
<td>0.0971</td>
</tr>
<tr>
<td>Fast2</td>
<td>0.014</td>
</tr>
<tr>
<td>Fast3</td>
<td>0.3053</td>
</tr>
<tr>
<td>Fast4</td>
<td>0.1723</td>
</tr>
<tr>
<td>Fast5</td>
<td>0.0023</td>
</tr>
<tr>
<td>Slow1</td>
<td>0.4869</td>
</tr>
<tr>
<td>Slow2</td>
<td>0.327</td>
</tr>
<tr>
<td>Slow3</td>
<td>0.0155</td>
</tr>
<tr>
<td>Slow4</td>
<td>0.0829</td>
</tr>
<tr>
<td>Slow5</td>
<td>0</td>
</tr>
<tr>
<td>Control1</td>
<td>1</td>
</tr>
<tr>
<td>Control2</td>
<td>0.7054</td>
</tr>
<tr>
<td>Control3</td>
<td>0.2385</td>
</tr>
<tr>
<td>Control4</td>
<td>0.7054</td>
</tr>
<tr>
<td>Control5</td>
<td>1</td>
</tr>
</tbody>
</table>

Levene's test for equal variance showed that the variance was not equal in all groups (p=0.000001).
Appendix B – Parameters of injury for each specimen

Figure B. 1: Injury parameters for specimen CS15, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 2: Injury parameters for specimen CS16, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 3: Injury parameters for specimen CS17, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 4: Injury parameters for specimen CS29, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 5: Injury parameters for specimen CS30, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 6: Injury parameters for specimen CS31, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 7: Injury parameters for specimen CS32, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 8: Injury parameters for specimen CS34, in the fast impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 9: Injury parameters for specimen CS20, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 10: Injury parameters for specimen CS21, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B.11: Injury parameters for specimen CS22, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 12: Injury parameters for specimen CS23, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 13: Injury parameters for specimen CS37, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 14: Injury parameters for specimen CS39, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 15: Injury parameters for specimen CS42, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 16: Injury parameters for specimen CS43, in the slow impact group. Displacement and force were directly observed, velocity, impulse power and energy were derived from the observed data.
Figure B. 17: Injury parameters for specimen CS11. This specimen was not included in the study due to inconsistencies in tissue preparation following harvest.
Figure B. 18: Injury parameters for specimen CS13. This specimen was not included in the study. The force curve indicated the animal slipped in the device clamps. The plateau seen in the loading portion of the force curve, circled in red, was considered indicative of slipping.
Figure B. 19: Injury parameters for specimen CS18. This specimen was not included in the study. The force curve indicated the animal slipped in the device clamps. The plateau seen in the loading portion of the force curve, circled in red, was considered indicative of slipping.
Figure B. 20: Injury parameters for specimen CS24. This specimen was not included in the study. The force curve indicated the animal slipped in the device clamps. The plateau seen in the loading portion of the force curve, circled in red, was considered indicative of slipping.
Figure B. 21: Injury parameters for specimen CS25. This specimen was not included in the study due to inconsistencies in tissue preparation following harvest.
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Appendix B

Figure B. 22: Injury parameters for specimen CS38. The force curve did not show the same linear loading response as all of the other animals. While there was no sudden plateau in the curve indicating slippage in the clamps, the force curve suggested a lack of rigidity in the clamping system. This specimen was not included in the study.
Appendix C – SMI-32 Reactivity in the Grey Matter

Figure C.1 Grey matter reactivity in fast impact specimens CS15 (A) and CS16 (B)
Figure C. 2 Grey matter reactivity in fast impact specimens CS17 (A) and CS29 (B)
Figure C. 3 Grey matter reactivity in fast impact specimens CS30 (A) and CS31 (B)
Figure C.4 Grey matter reactivity in fast impact specimens CS32 (A) and CS34 (B)
Figure C. 5 Grey matter reactivity in slow impact specimens CS20 (A) and CS21 (B).
Figure C. 6 Grey matter reactivity in slow impact specimens CS22 (A) and CS23 (B).
Figure C. 7 Grey matter reactivity in slow impact specimens CS37 (A) and CS39 (B).
Figure C. 8 Grey matter reactivity in slow impact specimens CS42 (A) and CS43 (B).
Figure C. 9 Grey matter reactivity in control specimens CS25 (A), CS26 (B), CS27 (C) and CS33 (D).
Figure C. 10 Grey matter reactivity in control specimens CS35 (A), CS36 (B), CS40 (C) and CS41 (D).