Cellular Pathology and Apoptosis in Experimental and Human Acute and Chronic Compressive Myelopathy

ROWENA ELIZABETH ANNE NEWCOMBE
M.B.B.S.  B.Med Sci. (Hons.)

Discipline of Pathology, School of Medical Sciences
University of Adelaide

June 2010

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I give consent to this copy of my thesis, when deposited in the University library, being made available for loan and copying.

Signed:      Date

Rowena Elizabeth Anne Newcombe
I acknowledge the invaluable and expert teaching, encouragement, patience and wisdom of my supervisors, Professor Peter Blumbergs, Professor Robert Vink, Dr John Finnie, Professor Peter Reilly, and Professor Nigel Jones. In particular I thank Professor Blumbergs who first enabled me this opportunity, providing unwavering guidance until its completion.

I sincerely thank Mr Jim Manavis for his technical expertise, enthusiasm and assistance over many years.

I am ever grateful for the encouragement and insights provided by my dear husband Matthew, my wise mother Carolyn, and my family and friends throughout this process of learning. I could not have achieved this without you.

I make special mention of my father, Doctor Raymond Newcombe, to whom I dedicate this thesis, for his careful, inspiring and excellent mentoring. He is exemplary of those qualities desired in any doctor of medicine; integrity, perseverance, compassion, precise clinical acumen, curiosity, and an unyielding belief in the human spirit.

„He who has health has hope, and he who has hope has everything.”

- Arabian proverb
Publications and Presentations


Prizes and Scholarships

2009  International Neurotrauma Society Student Travel Grant
2007  National Health and Medical Research Council Training Postgraduate Fellowship
2004  Spine Society of Australia Award for Spinal Research
2003  The Douglas Hardy Research Project Prize – The University of Adelaide
2003  Australian Medical Students’ Association Research Scholarship
2003  John Curtin School of Medical Research Summer Scholarship
2003  Spine Society of Australia Award for Spinal Research
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>AHC</td>
<td>Anterior Horn Cell</td>
</tr>
<tr>
<td>ALA</td>
<td>Anterolateral White Matter Area</td>
</tr>
<tr>
<td>AIF</td>
<td>Apoptosis Inducing Factor</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid Precursor Protein</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>aC3</td>
<td>Active Caspase-3</td>
</tr>
<tr>
<td>BACE</td>
<td>β-site APP-cleaving Enzyme</td>
</tr>
<tr>
<td>Bak</td>
<td>Bcl-2 Antagoinist Killer</td>
</tr>
<tr>
<td>Bax</td>
<td>B-Cell Lymphoma-Associated X</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>BBB Score</td>
<td>Beattie Basso Bresnahan Score</td>
</tr>
<tr>
<td>Bcl-2/x</td>
<td>B-cell Lymphoma 2/x</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-derived Neurotrophic Factor</td>
</tr>
<tr>
<td>C3</td>
<td>Caspase-3</td>
</tr>
<tr>
<td>C9</td>
<td>Caspase-9</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>CCA</td>
<td>Caspase-3 and Caspase-mediated Cleavage of APP</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre(s)</td>
</tr>
<tr>
<td>CMAP</td>
<td>Caspase-3-mediated APP Proteolytic Peptide Antibody</td>
</tr>
<tr>
<td>CNPase</td>
<td>Cyclic Nucleotide Phosphodiesterase</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CSM</td>
<td>Cervical Spondylotic Myelopathy</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variance</td>
</tr>
<tr>
<td>DAB</td>
<td>Diaminobenzidine</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DNA-PK</td>
<td>DNA-dependent Protein Kinase</td>
</tr>
<tr>
<td>DNA-PKcs</td>
<td>DNA-dependent Protein Kinase Catalytic Subunit</td>
</tr>
<tr>
<td>DR3/6</td>
<td>Death Receptor 3/6</td>
</tr>
<tr>
<td>e.g.</td>
<td>Exempli Gratia (for example)</td>
</tr>
<tr>
<td>EDAR</td>
<td>Ectodermal Dysplasia Receptor</td>
</tr>
</tbody>
</table>
NMDA  N-methyl-D-aspartate
nNOS  Neuronal Nitric Oxide Synthase
NO  Nitric Oxide
NOS  Nitric Oxide Synthase
NT-3  Neurotrophin-3
Olig2  Oligodendrocyte Transcription Factor 2
p  Probability
PAR  Poly (ADP-ribose)
PARG  Poly (ADP-ribose) Glycohydrolase
PARP  Poly (ADP-ribose) Polymerase
PBS  Phosphate Buffered Saline
PCA  Posterior Column Area
PCD  Programmed Cell Death
PMT  Photomultiplier Tube(s)
PNS  Peripheral Nervous System
PS-1  Presenelin-1
RPM  Revolutions Per Minute
ROS  Reactive Oxygen Species
SCI  Spinal Cord Injury
SEM  Standard Error of the Mean
Smac/ DIABLO Second Mitochondria-derived Activator of Caspases/Direct IAP Binding Protein
with Low PI
TBI  Traumatic Brain Injury
TBS  Tris-buffered saline
TMRM  Tetramethyl Rhodamine Methyl Ester
TNF  Tumour Necrosis Factor
TNF-α  Tumour Necrosis Factor-α
TPA  Tissue Polypeptide Antigen
TRAIL-R1/2  TNF-related Apoptosis Inducing Ligand Receptor 1/2
TUNEL  Terminal in situ Nick-end Labelling
μl  Microlitre(s)
UPLAPO  Universal Plan Apochromatic Objectives
WM  White Matter
XRCC1  X-ray Repair Cross-complimenting Group 1
°C  Degrees Celsius
3-AB  3-aminobenzamide
5-AIQ  5-aminoisoquinolinone
Table of Contents

ACKNOWLEDGEMENTS........................................................................................ ii
PUBLICATIONS AND PRESENTATIONS............................................................... iii
ABBREVIATIONS.................................................................................................... iv
FIGURES............................................................................................................... x
TABLES.................................................................................................................... xii
ABSTRACT.............................................................................................................. xviii

CHAPTER 1. INTRODUCTION............................................................................... 1
  1.1 Human Chronic Compressive Myelopathy................................................ 4
  1.1.1 History...................................................................................................... 4
  1.1.2 Definitions.............................................................................................. 4
  1.1.3 Aetiology and Classification.................................................................. 5
  1.1.4 Clinical History and Features.............................................................. 10
  1.1.5 Diagnostic Investigations.................................................................... 11
  1.1.6 Management and Outcome.................................................................. 13
  1.1.7 Spinal Cord Repair................................................................................ 15
  1.2 The Apoptosis-Necrosis Continuum......................................................... 17
  1.3 Human Chronic Compressive Myelopathy............................................. 23
    1.3.1 Apoptosis in Chronic Compressive Myelopathy.............................. 26
    1.3.2 Experimental Models of Chronic Spinal Cord Compression.......... 27
    1.3.3 The Role of Surgical Decompression.......................................... 30
  1.4 Axonal Injury.............................................................................................. 32
  1.5 Human Acute Compressive Myelopathy.................................................. 40
    1.5.1 History.................................................................................................. 40
    1.5.2 Definitions.......................................................................................... 40
    1.5.3 Aetiology and Classification................................................................ 40
    1.5.4 Clinical Manifestations...................................................................... 41
    1.5.5 Diagnostic Investigations................................................................... 42
    1.5.6 Management and Outcome.............................................................. 42
  1.6 Aims and Hypotheses.................................................................................. 53

CHAPTER 2. METHODS...................................................................................... 54
  2.1 Experimental Chronic Compressive Myelopathy..................................... 55
    2.1.1 Animals............................................................................................... 55
    2.1.2 Experimental Groups of Chronic Spinal Cord Compression .......... 55
    2.1.3 Control Groups.................................................................................... 56
  2.2 Experimental Acute Compressive Myelopathy........................................ 63
    2.2.1 Functional testing................................................................................ 64
  2.3 Human Compressive Myelopathy.............................................................. 68
  2.4 Processing of Experimental Tissue............................................................ 71
# CHAPTER 3. RESULTS - CHRONIC SPINAL CORD COMPRESSION

<table>
<thead>
<tr>
<th>3.1 Experimental Chronic Spinal Cord Compression</th>
<th>98</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.1 Histopathology</td>
<td>98</td>
</tr>
<tr>
<td>3.1.2 Apoptosis</td>
<td>103</td>
</tr>
<tr>
<td>3.1.3 Axonal Injury (APP)</td>
<td>112</td>
</tr>
<tr>
<td>3.2 Quantitative Studies – Cross-Sectional Area</td>
<td>113</td>
</tr>
<tr>
<td>3.2.1 Area – Posterior white matter</td>
<td>113</td>
</tr>
<tr>
<td>3.2.2 Area – Total cross-section of spinal cord (mm²)</td>
<td>116</td>
</tr>
<tr>
<td>3.3 Functional Assessment</td>
<td>118</td>
</tr>
<tr>
<td>3.3.1 BBB Score</td>
<td>118</td>
</tr>
<tr>
<td>3.3.2 Rotarod</td>
<td>122</td>
</tr>
<tr>
<td>3.3.3 Tail Flick</td>
<td>127</td>
</tr>
<tr>
<td>3.3.4 Ledge Beam</td>
<td>131</td>
</tr>
<tr>
<td>3.4 Chronic Human Spinal Cord Compression</td>
<td>132</td>
</tr>
<tr>
<td>3.4.1 Histopathology</td>
<td>132</td>
</tr>
<tr>
<td>3.4.2 Apoptosis</td>
<td>138</td>
</tr>
<tr>
<td>3.4.3 Axonal Injury</td>
<td>149</td>
</tr>
<tr>
<td>3.5 Discussion</td>
<td>162</td>
</tr>
<tr>
<td>3.6 Conclusions</td>
<td>182</td>
</tr>
<tr>
<td>3.6.1 Apoptosis</td>
<td>182</td>
</tr>
<tr>
<td>3.6.2 Axonal Injury</td>
<td>183</td>
</tr>
</tbody>
</table>

# CHAPTER 4. RESULTS - ACUTE COMPRESSIVE MYELOPATHY

<table>
<thead>
<tr>
<th>4.1 Experimental Acute Compressive Myelopathy</th>
<th>187</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.1 Histopathology</td>
<td>187</td>
</tr>
<tr>
<td>4.1.2 Apoptosis</td>
<td>190</td>
</tr>
<tr>
<td>4.2 Functional Assessment</td>
<td>193</td>
</tr>
<tr>
<td>4.2.1 BBB Score</td>
<td>193</td>
</tr>
<tr>
<td>4.2.2 Tail Flick</td>
<td>196</td>
</tr>
<tr>
<td>4.2.3 Rotarod</td>
<td>198</td>
</tr>
<tr>
<td>4.3 Axonal Injury</td>
<td>199</td>
</tr>
</tbody>
</table>
4.4 Human Acute Compressive Myelopathy.................................................... 201
  4.4.1 Histopathology.................................................................................. 201
  4.4.2 Apoptosis.......................................................................................... 203
  4.4.3 Axonal Injury.................................................................................... 209
4.5 Discussion – Experimental and Human Acute Compressive Myelopathy. 215
  4.5.1 Apoptosis.......................................................................................... 215
  4.5.2 Axonal Injury.................................................................................... 223
4.6 Conclusions – Histopathological Changes in Experimental and Human
  Acute Compressive Myelopathy............................................................... 226
  4.6.1 Apoptosis.......................................................................................... 226
  4.6.2 Axonal Injury.................................................................................... 226

CHAPTER 5. GENERAL DISCUSSION –
  CHRONIC AND ACUTE COMPRESSIVE MYELOPATHY....................... 227
5.1 Conclusions............................................................................................. 235

REFERENCES............................................................................................... 236

APPENDIX – INDIVIDUAL HUMAN CASES.............................................. 263
Re: Thesis emendations for PhD thesis by Dr Rowena Newcombe

During printing of the thesis a variation occurred in page numbering between the electronic and the printed copies. Subsequently, Examiner 1’s emendations are 19-20 pages in advance of the correct numbering. In addition, Examiner 1 identified that the list of corrections given was incomplete, and that further review of spelling and grammatical errors be made, further altering the page structure. Thus, the actual page at which the correction is located in the thesis correlates to the page number and line set in bold at the end of the noted correction.

The following emendations have been made to the thesis as recommended by the examiners.

Examiner 1

**Minor changes that would enhance the thesis**

P48 Description of the model used in the studies in comparison to previous models might be better in the discussion than in the introduction, unless the model was previously used and published.

**Response:** A model of chronic compressive myelopathy as used by Kim et al. 2004 is described within the introduction. This is a partial reference which is expanded in more detail in the discussion, in agreement with the referee’s comments **Page 29 Line 23**

P48 Syringomyelia is not really a form of cord compression in the usual sense. Although it may result in some compression of cord tissue, the pathology is likely to be quite different to forms of external compression. Although it is valid to include the studies of syringomyelia in this work, it would be preferable if this distinction were clarified in the text.

**Response:** The probable differing mechanism of syringomyelia and spondylotic myelopathy is noted **Page 24 Line 2**

P89: what pressure was used for perfusion fixation?

**Response:** A pressure of 80-120mmHg was used for perfusion fixation **Page 71 Line 11**

P125 ‘...a subtype of oligodendrocytes was identified,’ is not clear. What was the subtype?

**Response:** The use of the term ‘subtype’ was removed from the text to simply describe the cell ‘oligodendrocyte’ throughout the thesis. **Page 103**

It would be preferable to reduce the number of significant figures used for the cord cross-sectional area results, BBB score results, and rotarod times. For example, reporting mean BBB scores to 2 decimal places has no meaning.
Response: The significant figures were rounded to a consistent 2 decimal places.

Table 112 ‘Communicating syrinx’ is usually used to refer to cysts that communicate with the fourth ventricle rather than the central canal. Did these syrinxes really communicate with the fourth ventricle? (It would be highly unusual if associated with Chiari I malformations).

Response: There is no table 112, and it is suspected that the Examiner may be referring to Table 28, in which communicating syrinx was correctly defined as a communication with the central canal.

Grammatical and spelling errors that require correction

P23: “during the 1952”
As recommended this was changed to, ‘during 1952’. Page 4 Line 5

P23: ‘canalicular’
As recommended this was changed to, ‘cannalicular’. Page 5 Line 18

P28: ‘An association between osteophytes and concave, load baring areas within the spine were recognised early’ should be ‘An association between osteophytes and concave, load bearing areas within the spine was recognised early...’.
A correction was made as recommended. Page 9 Line 4

P35:
‘Multiple forms of programmed cell death (PCD) and classified as types I, II, and III PCD’ Should this be, ‘Multiple forms of programmed cell death (PCD) are classified as types I, II, and III PCD.’
A correction was made as recommended. Page 17 Line 6

P36: The ontology of apoptosis was given twice.
This was corrected as appropriate. Page 17 Line 21

P47: The words ‘central canal’ should be ’spinal canal’
This was corrected Page 28 Line 14
This current study aims to assess the effects of decompression in an experimental model of mild chronic cord compression.

A correction was made as recommended. Page 31 Line 6

…a minimum of approximately 10% of axons’. Should this be ‘…a maximum of approximately 10% of axons’?

No change was necessary as the sentence reads correctly Page 32 Line 15

‘transaction’ was corrected to ‘transection’.

This was corrected Page 48 Line 1

Tables 86-92 are out of order and not referenced in the text or the table of tables.

This was corrected Page 69 Line 1

‘Processotomy’ is an unusual term. Would ‘removal of the spinous process and laminectomy’ be better?

The term, ‘processotomy’ is consistent with surgical terminology to describe full resection of the spinous process to expose the dura, and thus the term was retained. Page 57 Line 7

The Gracile fasciculus was labelled. Page 73

‘tetromethylbenzidine’ was changed to ‘tetramethylbenzidine’. Page 92 Line 15

‘data is’ was changed to ‘data are’ as recommended. Page 93 Line 8

‘emersion’ was corrected to ‘immersion’. Page 95 Line 15

‘A complete representation of pathological and apoptotic changes in human cases are documented...’ should be ‘A complete representation of pathological and apoptotic changes in human cases is documented...’

This was corrected as suggested. Page 95, Line 12

‘A similar panel of apoptotic markers were used...’ should be ‘A similar panel of apoptotic markers was used...’
A correction was made as suggested Page 95 Line 21
P114: ‘A subset of enlarged axons were immunopositive...’ should be ‘A subset of enlarged axons was immunopositive...’.

A correction was made as suggested. Page 97 Line 4
P123: ‘Rare of occasional immunopositive glia was seen...’ should be ‘Rare or occasional immunopositive glia were seen...’.

A correction was made as suggested. Page 105 Line 19
P124: ‘At 9 week,...’ was changed to, ‘At 9 weeks,...’ as suggested. Page 106 Line 12
P125: ‘...frequent glial staining was seen in the majority cases...’ was changed to, ‘...frequent glial staining was seen in the majority of cases...’ as recommended. Page 107 Line 12
P126: ‘...TUNEL was either absent of rarely present,’ was changed to, ‘...TUNEL was either absent or rarely present,’ as recommended. Page 108 Line 17
P130: ‘APP axonal immunopositivity was rare or occasionally present in compression groups but were frequently present...’ should be ‘APP axonal immunopositivity was rare or occasionally present in compression groups but was frequently present...’

A correction was made as recommended. Page 112 Line 11
P131: ‘...the ratio or posterior to anterolateral white matter at the site was comparable to controls...’ should be ‘...the ratio of posterior to anterolateral white matter at the site was comparable to controls...’.

A correction was made as recommended. Page 113 Line 14
P140: ‘Statistically, the rotarod results were compared between the seven groups using a Cox proportional hazards model. A value of 120 second was considered to be right censored’ was repeated.

A correction was made as recommended. Page 122 Line 7
P184: It was recommended that, ‘In experimental chronic compressive myelopathy, caspase-9, PARP and aC3 staining was found...’ be changed to, ‘In experimental chronic compressive myelopathy, caspase-9, PARP and aC3 staining were found...’

A correction was made as recommended. Page 166 Line 13
P184: As recommended, ‘Although our data...’ was changed to, ‘Although our results...’. Page 169 Line 1
P202: As recommended, ‘Tissue from human cases was also studies for apoptosis...’ was changed to, ‘Tissue from human cases was also studied for apoptosis...’. Page 185 Line 3

As recommended, ‘Glial positivity to TUNEL, the gold standard biochemical marker of apoptosis, TUNEL, was seen at 24 hours and at 1 week post-injury,’ was changed to, ‘Glial positivity to TUNEL, the “gold standard” biochemical marker of apoptosis, was seen at 24 hours and at 1 week post-injury. Page 185 Line 10

Examiner 2

Minor grammatical changes and errors:

Page 2

2nd and 3rd paragraph – suggest moving first 2 sentences of the third paragraph to before the 2nd sentence of the 2nd paragraph.

A correction was made as recommended. Page 2 Line 18

Page 9

Paragraph starting ‘…Theories vary…’ suggest putting a ‘comma’ after ‘syringomyelia’ and delete the word ‘where’ before ‘the Venturi.

A correction was made as recommended. Page 10 Line 10 and 11

Page 15

Paragraph starting ‘Principal modes of cell death…’, Line 4 - change ‘apoptotic forms’ to ‘apoptotic process’.

A correction was made as recommended. Page 17 Line 5

Page 49

2nd paragraph, Line 1 ‘suggest’ should be ‘suggests’.

A correction was made as recommended. Page 51 Line 10

Page 120 In the 1st paragraph Line 1 should read ‘The rotarod score was used in the assessment of...’

A correction was made as recommended. Page 122 Line 2

Page 163

In the 2nd paragraph it was recommended to remove ‘comma’ after ‘a’ and the inverted commas around ‘percentage of apoptotic cells.'
The correction was made as recommended. **Page 165 Line 12**

**Page 217**

Paragraph starting ‘In similarity’ suggest change to more common word usage, Eg ‘Similar to chronic compressive…’

A correction to, ‘Similar to chronic compressive…’ was made. **Page 221 Line 1**

**Page 227**

Paragraph starting with ‘The principal aims…’ this 1st sentence is too long and should be changed to 2 sentences at least.

The change was made as recommended. **Page 229 Line 10**

Paragraph starting, ‘The experimental model…’ Line 1 the word ‘newly’is inappropriate and should be changed. Eg. delete and leave sentence as is or use ‘recently’ instead.

The word ‘newly’ was deleted. **Page 229 Line 22**

**Page 228**

Paragraph starting with, ‘Our studies…’ again the word ‘newly’ is inappropriate and should be deleted and rework the sentence or change the word to a more appropriate word.

The word ‘newly’ was deleted. **Page 230 Line 18**

**Page 233**

Point 1: Line 3 - the word ‘in’ should read ‘at.

The correction was made as recommended. **Page 235 Line 3**

**References**

The recommendation was to be consistent with use of a ‘stop’ and a ‘space’ after the Journal listed.

This was amended for each reference to consistent use of the ‘stop’ and ‘space’.

Page 241, 3rd reference – no ‘year’ is noted in this reference.

This was corrected. **Page 243 Line 9**
Appendix

Page 261 Point ‘1’ Fullstop be used after the word ‘right’.

Page 271 Line 5 sentence should read, ‘There was a past…right arm and right leg weakness…’

The corrections were made as recommended Page 263 Line 15, 273 Line 6.
## Figures

| Figure 1.1 | Pathological changes in human chronic compressive myelopathy | 8 |
| Figure 1.2 | Intrinsic and extrinsic molecular pathways in apoptosis | 20 |
| Figure 1.3 | APP cleavage sites for the production of soluble Amyloid Precursor Protein beta and Amyloid-beta 42 protein fragment via beta- and gamma-secretases | 39 |
| Figure 2.1 | Anatomy of the rodent spine and spinal cord | 58 |
| Figure 2.2 | Polymer preparation | 59 |
| Figure 2.3 | Surgical technique for a rodent model of chronic compressive myelopathy | 60 |
| Figure 2.4 | The rodent spinal cord in a model of chronic compressive myelopathy | 62 |
| Figure 2.5 | Tests of motor and mixed motor-sensory function in a rodent model of acute and chronic compressive myelopathy | 66 |
| Figure 2.6 | Major tracts of the human spinal cord at the mid-cervical level | 73 |
| Figure 2.7 | Long tracts of the rodent spinal cord | 73 |
| Figure 2.8 | Semi-quantitative assessment – Spatial distribution of staining | 88 |
| Figure 2.9 | Key – Spatial distribution of staining | 89 |
| Figure 3.1 | Histopathological changes on haematoxylin and eosin staining in rodent chronic compressive myelopathy | 101 |
| Figure 3.2 | White matter (WM) changes in a rodent model of chronic compressive myelopathy | 102 |
| Figure 3.3 | Immunohistochemical staining for apoptosis in chronic compressive myelopathy | 110 |
| Figure 3.4 | PARP immunohistochemical staining following early decompression in a rodent model of chronic compressive myelopathy | 111 |
| Figure 3.5 | Histopathological changes in experimental chronic compressive myelopathy | 117 |
| Figure 3.6 | Weil staining in human chronic compressive myelopathy | 137 |
| Figure 3.7 | Immunoreactivity within cortical neurons of an Alzheimer’s disease control case | 150 |
| Figure 3.8 | APP and Amyloid-beta antibody staining in positive control Alzheimer’s disease neuritic plaque | 151 |
Figure 3.9  APP, AIF and Amyloid-beta antibody staining in human chronic compressive myelopathy ................................................................. 152

Figure 3.10  APP and Amyloid-beta immunopositivity in human chronic compressive myelopathy ................................................................. 153

Figure 3.11  Potential role of apoptosis in the pathophysiology of chronic compressive myelopathy ................................................................. 164

Figure 4.1  Central haemorrhagic necrosis following acute weight drop spinal cord injury in the rodent ........................................................................ 189

Figure 4.2  Immunohistochemical staining in experimental acute compressive myelopathy using PARP and Caspase-3 ........................................ 192

Figure 4.3  APP immunopositivity in axonal swellings in a rodent model of acute spinal cord injury ......................................................................... 200
## Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Medications administered perioperatively for acute and chronic spinal cord compression in rats</td>
<td>57</td>
</tr>
<tr>
<td>Table 2</td>
<td>BBB score categories</td>
<td>67</td>
</tr>
<tr>
<td>Table 3</td>
<td>Case Summary – Human Cervical Spondylotic Myelopathy</td>
<td>69</td>
</tr>
<tr>
<td>Table 4</td>
<td>Case Summary – Human Neoplastic Compressive Myelopathy</td>
<td>69</td>
</tr>
<tr>
<td>Table 5</td>
<td>Case Summary – Human Syringomyelia</td>
<td>70</td>
</tr>
<tr>
<td>Table 6</td>
<td>Case Summary – Human Traumatic Compressive Myelopathy</td>
<td>70</td>
</tr>
<tr>
<td>Table 7</td>
<td>Antibody panel</td>
<td>75</td>
</tr>
<tr>
<td>Table 8</td>
<td>Haematoxylin and eosin findings in a rodent model of chronic compressive myelopathy</td>
<td>98</td>
</tr>
<tr>
<td>Table 9</td>
<td>Immunopositivity in a rodent model of chronic compressive myelopathy</td>
<td>103</td>
</tr>
<tr>
<td>Table 10</td>
<td>Mean posterior column area (a) for injury groups at 1, 3, 9 and 20 weeks compression and (b) in 9 week compression, 24 hour decompression and 3 week decompression groups</td>
<td>115</td>
</tr>
<tr>
<td>Table 11</td>
<td>(a) Mean and Median BBB scores at sacrifice in Sprague-Dawley rats following spinous processotomy at T12 and insertion of an aquaprene polymer</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>(b) Median and Mean BBB scores at sacrifice in Sprague-Dawley rats following decompression laminoplasty for removal of expandable polymer at 24 hours and 3 weeks</td>
<td>119</td>
</tr>
<tr>
<td>Table 12</td>
<td>Mean and (b) median BBB score in rat chronic compressive myelopathy up to 20 weeks duration</td>
<td>120</td>
</tr>
<tr>
<td>Table 13</td>
<td>Mean and (b) median BBB scores in the 9 week continuous compression group versus decompression groups</td>
<td>121</td>
</tr>
<tr>
<td>Table 14</td>
<td>Mean and (b) median rotarod scores in rat chronic compressive myelopathy up to 20 weeks duration</td>
<td>125</td>
</tr>
</tbody>
</table>
Table 16. (a) Mean and (b) median rotarod scores in a rodent model of chronic compressive myelopathy................................................................. 126
Table 17. Tail flick scores (seconds) at sacrifice in Sprague-Dawley rats following spinous processotomy at T12 and insertion of an aquaprene polymer...... 128
Table 18. Tail flick scores (seconds) at sacrifice in Sprague-Dawley rats following decompression laminoplasty and removal of expandable polymer at 24 hours and 3 weeks post initial laminoplasty.............................................. 128
Table 19. (a) Mean and (b) median tail flick score in rat chronic compressive myelopathy.................................................................................................. 129
Table 20. (a) Mean and (b) median tail flick score in persisting compression versus decompression and control groups........................................ 130
Table 21. List of cervical spondylotic myelopathy cases...................................................... 134
Table 22. Summary of histopathological changes in cervical spondylotic myelopathy cases.......................................................................................... 134
Table 23. List of neoplastic compressive myelopathy cases................................................. 135
Table 24. Summary of histopathological changes in neoplastic compressive myelopathy.......................................................................................... 135
Table 25. List of syringomyelia cases.................................................................................. 136
Table 26. Histopathological changes in human syringomyelia – Morphological findings............................................................................................ 136
Table 27. Summary of immunohistochemical results in human cervical spondylotic myelopathy – Immunohistochemical markers of apoptosis and TUNEL........................................................................................................ 139
Table 28. CASE 1 – Compression C3/4 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 139
Table 29. CASE 2 – Compression C4/5 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles.............................................................................. 140
Table 30. CASE 3 – Compression C4/5 and C5/6 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles........................................ 140
Table 31. Summary of immunohistochemical results in human neoplastic compressive myelopathy – Markers of apoptosis........................................... 140
Table 32. CASE 4 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles.................................................................................. 143
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 33.</td>
<td>CASE 5 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 34.</td>
<td>CASE 6 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 35.</td>
<td>CASE 7 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 36.</td>
<td>CASE 8 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 37.</td>
<td>CASE 9 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 38.</td>
<td>CASE 10 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 39.</td>
<td>Immunohistochemical staining using a panel of markers of apoptosis in human syringomyelia – Morphological findings</td>
</tr>
<tr>
<td>Table 40.</td>
<td>CASE 19 – Syringomyelia series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 41.</td>
<td>CASE 20 – Syringomyelia series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 42.</td>
<td>CASE 21 – Syringomyelia series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 43.</td>
<td>Immunohistochemical and histopathological findings in normal human spinal cord cases</td>
</tr>
<tr>
<td>Table 44.</td>
<td>Summary of immunohistochemical results in human cervical spondylotic myelopathy – APP, Caspase-3, CMAP and Amyloid-beta antibodies</td>
</tr>
<tr>
<td>Table 45.</td>
<td>CASE 1 – Compression C3/4 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 46.</td>
<td>CASE 2 – Compression C4/5 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
<tr>
<td>Table 47.</td>
<td>CASE 3 – Compression C4/5 and C5/6 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles</td>
</tr>
</tbody>
</table>
Table 48. Summary of immunohistochemical results in human neoplastic compressive myelopathy – APP, caspase-3, CMAP and Amyloid-beta antibodies

Table 49. CASE 4 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 50. CASE 5 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 51. CASE 6 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 52. CASE 7 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 53. CASE 8 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 54. CASE 9 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 55. CASE 10 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 56. Immunohistochemical staining in syringomyelia cases – Morphological findings

Table 57. CASE 19 – Syringomyelia series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 58. CASE 20 – Syringomyelia series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 59. CASE 21 – Syringomyelia series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles

Table 60. Immunopositivity in a rodent model of acute compressive myelopathy...

Table 61. Mean and median rotarod scores in a weight drop model of acute spinal cord injury in the rat

Table 62. Mean and median BBB scores in a weight drop model of acute spinal cord injury in the rat
Table 63. Mean tail flick scores (a) by time and (b) versus control in a weight drop model of acute spinal cord injury in the rat................................................. 196
Median tail flick scores (c) by time and (d) versus control in a weight drop model of acute spinal cord injury in the rat................................................. 197
Table 64. List of traumatic spinal cord injury cases................................................. 202
Table 65. Summary of histopathological changes in human traumatic spinal cord injury cases....................................................... 202
Table 66. CASE 11 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 204
Table 67. CASE 12 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 204
Table 68. CASE 13 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 205
Table 69. CASE 14 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 205
Table 70. CASE 15 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 206
Table 71. CASE 16 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 206
Table 72. CASE 17 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 207
Table 73. CASE 18 – Trauma series – Compression C7 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles........................................ 207
Table 74. Immunohistochemical staining in human traumatic spinal cord injury cases – Morphological findings................................................................. 208
Table 75. CASE 11 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 210
Table 76. CASE 12 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 210
Table 77. CASE 13 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 211
Table 78. CASE 14 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles................................................................. 211
Table 79. CASE 15 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles.................................................................................................................. 212

Table 80. CASE 16 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles.................................................................................................................. 212

Table 81. CASE 17 – Trauma series – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles.................................................................................................................. 213

Table 82. CASE 18 – Trauma series – Compression C7 – Immunohistochemical positivity (+) in glial, axonal or neuronal profiles.......................................................... 213

Table 83. Immunohistochemistry human traumatic spinal cord injury – Morphological findings.................................................................................................................. 214
ABSTRACT

Evidence suggests that apoptosis of neurons and glia may play an important role in the pathophysiology and functional outcome of spinal cord compression. In the current thesis, chronic and acute rodent experimental models analysed the functional, cellular and apoptotic marker changes produced by compression and subsequent surgical decompression.

In experimental mild chronic compression there was a loss of posterior white matter maximal at the compression site. Total cross-sectional area decreased with a longer duration of compression (3 weeks) but resolved with decompression (e.g. 3 week group mean 3.05 mm$^2$ increasing following decompression at 3 weeks to 5.75 mm$^2$). A significant increase in posterior white matter area was found above and below the site at 3 weeks. Caspase-9, PARP, AIF and active caspase-3 staining was found in glia at, above and below the site in all groups. Caspase-3 was greater expressed in the 24 hour (mean 0.32, p = 0.01) and 3 week (mean 0.31, p = 0.02) decompression groups when compared with the 9 week compression group (mean 0.19). APP axonal immunopositivity was frequently seen after decompression.

Following experimental acute compression, central necrosis was seen, surrounded by axonal swellings and inflammatory infiltrate. Glial positivity using TUNEL occurred at 24 hours and 1 week post-injury. PARP, DNA-PKcs and AIF immunopositivity occurred in glia at, above and below the site. APP immunopositivity was present in axonal swellings.

In human chronic compression, axonal swellings, loss of anterior horn cells, and cystic change were seen in severe cases. TUNEL, DNA-PKcs, PARP and AIF immunopositivity in glia were seen at, above and below the compression. APP immunopositivity was seen in axonal swellings.

In human acute compression, the central cord showed haemorrhagic necrosis and inflammatory cells. TUNEL, DNA-PKcs and PARP immunopositive glia were found at, above and below the site. Axonal swellings, a subset of which were APP immunopositive,
occurred in the penumbra. APP immunopositive axonal swellings were found above and below the site of compression, indicating widespread changes in fast axoplasmic transport.

We conclude that mild, chronic, fixed posterior compression results in a potentially reversible reduction of white matter at the site and increased white matter above and below the site of compression. This, combined with evidence of axonal injury, may indicate altered axoplasmic transport. Decompressive surgery results in increased immunostaining for apoptotic markers and increased axonal injury despite restoration of spinal cord anatomy. These studies provide novel evidence that neuronal and glial apoptosis occurs in acute and chronic compressive myelopathy at various time points of compression, maximal at the site of injury.