

CHARACTERISING THE ROLE OF SUBSTANCE P FOLLOWING TRAUMATIC SPINAL CORD INJURY

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degree of Doctor of Philosophy

DECLARATION

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PUBLICATIONS AND PRESENTATIONS

The following articles have been published or accepted for publication or presentation during the period of my PhD candidature. Sections thereof have been included in the present thesis with the permission of the copyright owner.

Publications

Leonard AV, Blumbergs P, Vink R (2012). The effect of an NK1 receptor antagonist on blood spinal cord barrier permeability following a balloon compression model of spinal cord injury. *acta neurochir (suppl)*. In press 2012.

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Abstracts

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ABBREVIATIONS

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
ANOVA	analysis of variance
APP	amyloid precursor protein
AQP	aquaporin
ASIA	American spinal cord injury association
ATP	adenosine triphosphate
BBB	blood brain barrier
BBB	Basso Beattie Bresnahan motor score
BF	burst fracture
BSCB	blood spinal cord barrier
C	cervical
Ca ²⁺	calcium
CBF	cerebral blood flow
CGRP	calcitonin gene-related peptide
Cl ⁻	chloride
CNS	central nervous system
COX	cyclooxygenase
CPP	cerebral perfusion pressure
CSF	cerebrospinal fluid
CT	X-ray computed tomography
DAB	diaminobenzidene
DNA	deoxyribose nucleic acid
EB	Evan's blue
F	female
FD	fracture dislocation
GFAP	glial fibrillary associated protein

H&E	hematoxylin and eosin
H ₂ O ₂	hydrogen peroxide
HRP	horseradish peroxidase
HV	high velocity
ICP	intracranial pressure
IMVS	institute of medical and veterinary sciences
ITP	intrathecal pressure
i.v.	intravenous
K ⁺	potassium
L	lumbar
M	male
MABP	mean arterial blood pressure
MP	methylprednisolone
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MVA	motor vehicle accident
Na ⁺	sodium
NaCl	sodium chloride
NaOH	sodium hydroxide
NASCIS	National Acute Spinal Cord Injury Studies
NAT	N-acetyl L-tryptophan
NeuN	neuronal nuclei
NHS	normal horse serum
NKA	neurokinin A
NKB	neurokinin B
NMDA	N-methyl-D-aspartate
NO	nitric oxide

NOS	nitric oxide synthase
O ₂	oxygen
PBS	phosphate buffered solution
PNS	peripheral nervous system
PPTA	preprotachykinin A
PPTB	preprotachykinin B
ROS	reactive nitrogen species
S	sacral
SA	South Australian
SCBF	spinal cord blood flow
SCI	spinal cord injury
SCPP	spinal cord perfusion pressure
SEM	standard error of the mean
SP	substance P
SPC	streptavidin peroxidase conjugate
T	thoracic
TAA	thoracoabdominal aortic aneurysm
TBI	traumatic brain injury
TNF- α	tumor necrosis factor- α
VO	vehicle occupant

TABLE OF CONTENTS

DECLARATION	ii
PUBLICATIONS AND PRESENTATIONS	iii
ACKNOWLEDGEMENTS	iv
ABBREVIATIONS	vi
TABLE OF CONTENTS	ix
LIST OF FIGURES AND TABLES	xviii
ABSTRACT	xxiii

CHAPTER 1: INTRODUCTION

1.1 Overview	2
1.2 Epidemiology of Spinal Cord Injury	3
1.3 Classification of Spinal Cord Injury	3
1.4 Mechanism of Injury	5
1.4.1 Primary Injury	5
1.4.2 Secondary Injury	6
<i>Vascular Abnormalities</i>	<i>6</i>
<i>Free Radical Generation and Lipid Peroxidation</i>	<i>7</i>
<i>Excitotoxicity and Electrolyte Imbalances</i>	<i>7</i>
<i>Necrotic and apoptotic cell death</i>	<i>8</i>
<i>Inflammatory Response</i>	<i>8</i>
1.5 Blood Spinal Cord Barrier Permeability	9
1.5.1 Temporal and Spatial Profile of BSCB Permeability	10
1.6 Edema	11
1.6.1 Vasogenic edema	11
1.6.2 Cytotoxic Edema	11
1.6.3 Edema following SCI	12
1.6.4 Aquaporins and Edema	12
<i>Aquaporin4 in spinal cord injury</i>	<i>12</i>
1.6.5 Temporal profile and distribution of Edema	13

1.6.6 Consequences of Edema	14
1.7 Intrathecal Pressure	14
1.7.1 Spinal Cord Oxygenation	16
1.8 Treatment of SCI	17
1.8.1 Pharmacological Intervention	18
<i>Methylprednisolone (MP)</i>	18
<i>Opioid Antagonism - Naloxone</i>	19
1.8.2 Surgical/Nonpharmacological Intervention	20
<i>Decompression</i>	20
<i>Hypothermia</i>	21
<i>Cerebrospinal Fluid Drainage</i>	21
<i>Omental Transposition</i>	22
1.8.3 Alternative treatment	23
1.9 Substance P	23
1.9.1 Synthesis and Degradation.....	23
1.9.2 Location	24
1.9.3 SP Receptor – the NK1 receptor.....	24
1.9.4 Role of Substance P in Neurogenic Inflammation.....	25
1.10 Experimental models of spinal cord injury.....	26
1.10.1 Weight Drop	27
1.10.2 Transection and Hemisection	28
1.10.3 Clip Compression.....	28
1.10.4 Balloon compression model.....	29
1.11 Synopsis	31
<u>CHAPTER 2: MATERIALS AND METHODS</u>	
2.1 Animal Care	33
2.1.1 Ethics.....	33
2.1.2 General.....	33
2.2 Experimental Procedures	33
2.2.1 Clip compression model of spinal cord injury	33

<i>Anaesthesia</i>	34
Isoflurane	34
<i>Analgesic</i>	34
Lignocaine	34
Buprenorphine (Temgesic).....	34
<i>Surgery</i>	34
<i>Post-surgery recovery</i>	36
<i>Assessment</i>	36
2.2.2 Balloon compression model of spinal cord injury	36
<i>Anaesthesia</i>	36
Ketamine/Domitor	36
Isoflurane	37
<i>Analgesic</i>	37
Lignocaine	37
Buprenorphine (Temgesic).....	37
<i>Euthanasia</i>	37
Pentobarbital (Lethobarb)	37
<i>Surgery</i>	38
<i>Post-surgery recovery</i>	40
<i>Assessments</i>	40
2.2.3 Perfusion	40
2.3 Drug Treatment	41
2.3.1 Saline.....	41
2.3.2 N-Acetyl- L-Tryptophan (NK1 receptor antagonist)	41
2.4 BSCB Measurements	41
2.4.1 Evan’s Blue Extravasation.....	41
2.5 Edema Measurements	42
2.5.1 Wet Weight - Dry Weight	42
2.6 Intrathecal Pressure monitoring	43
2.6.1 Tracheotomy	43
2.6.2 Femoral arterial dissections	43
2.6.3 ITP Probe placement	44
2.6.4 Powerlab ITP and BP monitoring.....	44

2.7 Functional assessment	46
2.7.1 Sensory Function	46
<i>Prick Test</i>	46
2.7.2 Motor Function.....	47
<i>Modified Tarlov Score</i>	47
<i>Forelimb to Hindlimb step ratio</i>	47
2.8 Histological analysis	47
2.8.1 Tissue Assessed	47
<i>Archived tissue</i>	47
Human Tissue.....	47
Rat – Weight drop model of SCI.....	47
Sheep – Dorsal Hemi-section model of SCI.....	48
<i>Generated tissue</i>	48
2.8.2 Perfusion Fixation and Tissue Sampling	48
2.8.3 Hematoxylin and Eosin Staining	49
2.8.4 Immunohistochemistry & Lectin-histochemistry.....	49
<i>Antibodies Used</i>	50
Substance P	50
NK1 Receptor	50
Albumin	50
Claudin – 5.....	51
Amyloid Precursor Protein (APP)	51
Microglia	51
Glial fibrillary acidic protein (GFAP)	51
NeuN	51
Aquaporin 4 (AQP4)	51
2.8.5 Assessment of Immunohistochemistry	52
<i>Hamamatsu Nanozoomer</i>	52
<i>Colour Deconvolution</i>	52
<i>Axonal injury</i>	52
2.9 Statistical Analysis	52

CHAPTER 3: CHARACTERISING SUBSTANCE P IMMUNOREACTIVITY FOLLOWING ACUTE TRAUMATIC SPINAL CORD INJURY IN HUMANS

3.1 Introduction	54
3.2.1 Immunohistochemistry	55
3.2.2 Statistical Analysis	55
3.3 Results	55
3.3.1 SCI Case Data	55
3.3.2 Morphological features of SCI	57
3.3.3 Substance P response following human SCI	59
3.3.4 NK1 receptor immunoreactivity following human SCI	62
3.3.5 APP expression following human SCI	65
3.3.6 Claudin-5 expression following human SCI	68
3.3.7 AQP4 expression following human SCI	71
<i>Perivascular region</i>	71
<i>Central canal region</i>	71
<i>Subpial region</i>	72
<i>Figure 3.14: AQP4 immunoreactivity surrounding the central canal following SCI.</i>	76
3.4 Discussion	79
3.5 Conclusions	83

CHAPTER 4: CHARACTERISING THE ROLE OF SP FOLLOWING OPEN MODELS OF EXPERIMENTAL SPINAL CORD INJURY

4.1 Introduction	85
4.2 Study Design	86
4.2.1 Experimental Injury Models	86
<i>Weight Drop</i>	86
<i>Hemisection</i>	86
<i>Clip compression</i>	86
4.2.2 Immunohistochemistry	87
4.2.3 Statistical Analysis	87
4.3 Results	87

4.3.1 Weight drop model of SCI histological outcome.....	87
<i>Weight drop model of SCI – General morphology (H&E)</i>	87
<i>Weight drop model of SCI – Substance P Immunoreactivity</i>	89
<i>Weight drop model of SCI – NK1 receptor Immunoreactivity</i>	91
<i>Weight drop model of SCI – Albumin Immunoreactivity</i>	92
<i>Weight drop model of SCI – APP Immunoreactivity</i>	94
4.3.2 Hemisection model of SCI histological outcome.....	96
<i>Hemisection model of SCI – General morphology (H&E)</i>	96
<i>Hemisection model of SCI – Substance P Immunoreactivity</i>	97
<i>Hemisection model of SCI – NK1 receptor Immunoreactivity</i>	98
<i>Hemisection model of SCI – Albumin Immunoreactivity</i>	100
<i>Hemisection model of SCI – APP Immunoreactivity</i>	101
4.3.3 Clip compression model of SCI histological outcome	101
<i>Clip compression model of SCI – General morphology (H&E)</i>	101
<i>Clip compression model of SCI – SP Immunoreactivity</i>	103
<i>Clip compression model of SCI – NK1 receptor Immunoreactivity</i>	105
<i>Clip compression model of SCI – Albumin Immunoreactivity</i>	107
<i>Clip compression model of SCI – APP Immunoreactivity</i>	109
4.4 Discussion	111
4.5 Conclusion	118

CHAPTER 5: CHARACTERISATION OF THE BALLOON COMPRESSION MODEL OF SPINAL CORD INJURY

5.1 Introduction	120
5.2 Study Design.....	121
5.2.1 Experimental Injury Model.....	121
<i>Balloon compression model of SCI</i>	121
5.2.2 BSCB permeability	121
5.2.3 Edema measurement	121
5.2.4 Intrathecal pressure measurement.....	121
5.2.5 Functional outcome.....	122
5.2.6 Histological outcome.....	122
5.3 Results.....	123

5.3.1 BSCB Permeability – Evan’s Blue extravasation	123
5.3.2 Edema measurement	124
5.3.3 Intrathecal pressure measurement.....	125
5.3.4 Functional outcome.....	126
<i>Sensory function – Plantar Prick Test</i>	126
<i>Motor function</i>	127
Modified Tarlov scale	127
Hindlimb to forelimb ratio	127
5.3.5 Histological outcome	128
<i>Morphological features – H&E staining</i>	128
<i>Substance P immunoreactivity</i>	130
Grey Matter - Dorsal Horn	130
Perivascular	133
<i>NK1 immunoreactivity</i>	135
Grey Matter.....	135
Perivascular	138
<i>Albumin immunoreactivity</i>	Error! Bookmark not defined.
<i>Albumin immunoreactivity</i>	140
<i>APP immunoreactivity</i>	143
<i>NeuN immunoreactivity</i>	146
<i>GFAP immunoreactivity</i>	149
<i>Microglial immunoreactivity (ISOB4)</i>	150
White Matter	150
Grey Matter.....	150
<i>AQP4 immunoreactivity</i>	153
Perivascular	153
Central canal	156
5.4 Discussion	158
5.5 Conclusion	166
<u>CHAPTER 6: THE EFFECT OF AN NK1 RECEPTOR ANTAGONIST IN THE BALLOON COMPRESSION MODEL OF SPINAL CORD INJURY</u>	
6.1 Introduction	168
6.2 Study Design.....	168

6.2.1 Experimental Injury Model	168
<i>Balloon compression model of SCI</i>	168
6.2.2 n-acetyl L-tryptophan (NAT) dose-response	169
6.2.3 BSCB permeability	169
6.2.4 Edema measurement	169
6.2.5 Intrathecal pressure measurement.....	169
6.2.6 Functional outcome.....	170
6.2.7 Histological outcome	170
6.3 Results.....	171
6.3.1 NAT dose response.....	171
6.3.2 BSCB Permeability– Evan’s Blue extravasation	172
6.3.3 Edema measurement	173
6.3.4 Intrathecal pressure measurement.....	174
6.3.5 Functional outcome.....	175
<i>Sensory function – prick test</i>	175
<i>Motor function - Modified Tarlov Scale</i>	176
<i>Motor function - hindlimb to forelimb ratio</i>	176
6.3.6 Histological outcome	177
<i>Morphological features – H&E staining</i>	177
<i>Substance P immunoreactivity</i>	179
Grey Matter – Dorsal Horn.....	179
Perivascular	182
<i>NK1 immunoreactivity</i>	185
Grey matter	185
Perivascular	188
<i>Albumin immunoreactivity</i>	191
<i>APP immunoreactivity</i>	194
<i>NeuN immunoreactivity</i>	197
<i>GFAP immunoreactivity</i>	200
<i>Microglial immunoreactivity (ISOB4)</i>	202
White Matter	202
Grey Matter.....	202
<i>AQP4 immunoreactivity</i>	205

	Perivascular region.....	205
	Central Canal region.....	208
6.4	Discussion	211
6.5	Conclusion.....	213

CHAPTER 7: GENERAL DISCUSSION

7.1	Summary of Principle Findings	215
7.2	Conclusions.....	223
	Reference List.....	224

LIST OF FIGURES AND TABLES

Figure 1.1: Weight drop models of SCI	27
Figure 1.2: Clip compression model of spinal cord injury:.....	29
Figure 1.3: The balloon compression model of SCI.....	30
Figure 2.1: Clip compression model of spinal cord injury:.....	35
Figure 2.2: Laminectomy:.....	39
Figure 2.3: Balloon compression model of spinal cord injury.....	39
Figure 2.4: Spinal cord segmentation for Evan’s Blue extravasation and edema measurements.	42
Figure 2.5: Tracheotomy:	43
Figure 2.6: Femoral arterial dissection:	44
Figure 2.7: PowerLab system:	45
Figure 2.8: LabChart software screenshot:	45
Figure 2.9: Sensory prick test:.....	46
Figure 2.10: Spinal cord segmentation for histological processing:	48
Figure 3.1: Axonal Swelling	57
Figure 3.2: H&E stain – general morphological features.	58
Figure 3.3: Assessment of human SP Immunoreactivity within the dorsal horn region post-SCI.	60
Figure 3.4: Human SP immunoreactivity following SCI within the dorsal horn region.....	61
Figure 3.5: Assessment of NK1 receptor immunoreactivity following human SCI.	63
Figure 3.6: NK1 receptor immunoreactivity following human SCI – perivascular.....	64
Figure 3.7: Assessment of APP immunoreactivity following human SCI.	66
Figure 3.8: APP immunoreactivity following human SCI.....	67
Figure 3.9: Assessment of Claudin-5 immunoreactivity following human SCI.	69
Figure 3.10: Claudin-5 immunoreactivity following human SCI.....	70
Figure 3.11: Assessment of AQP4 immunoreactivity within the perivascular region following SCI.....	73
Figure 3.12: AQP4 immunoreactivity in the perivascular region following SCI	74
Figure 3.13: Assessment of AQP4 immunoreactivity surrounding the central canal following SCI.	75
Figure 3.15: Assessment of AQP4 immunoreactivity within the subpial region following SCI.....	77
Figure 3.16: AQP4 immunoreactivity within the subpial region following SCI.	78
Figure 4.1: H&E staining – general morphological features following the weight drop model of SCI. .	88
Figure 4.2: Assessment of SP immunoreactivity following the weight drop model of SCI.....	89
Figure 4.3: SP immunoreactivity following the weight drop model of SCI	90
Figure 4.4: NK1 receptor immunoreactivity following the weight drop model of SCI.....	91
Figure 4.5 Assessment of albumin immunoreactivity following the weight drop model of SCI.....	92
Figure 4.6 Albumin immunoreactivity following the weight drop model of SCI	93

Figure 4.7: Assessment of APP immunoreactivity within the injury epicentre following the weight drop model of SCI.....	94
Figure 4.8: APP immunoreactivity following the weight drop model of SCI.....	95
Figure 4.9: H&E staining – general morphological features following the hemisection model of SCI. .	96
Figure 4.10: Assessment of SP immunoreactivity following a hemisection model of SCI	97
Figure 4.11: SP immunoreactivity following a hemisection model of SCI	98
Figure 4.12: Assessment of NK1 receptor immunoreactivity following the hemisection model of SCI.	99
Figure 4.13: NK1 receptor immunoreactivity following the hemisection model of SCI.	99
4.14: Semi-quantification of albumin immunoreactivity following a hemisection model of SCI.	100
Figure 4.15: Albumin immunoreactivity following a hemisection model of SCI.....	100
Figure 4.16: APP immunoreactivity following a hemisection model of SCI.....	101
Figure 4.17: H&E staining – General morphological features following the clip compression model of SCI.....	102
Figure 4.18: Assessment of SP immunoreactivity following the clip compression model of SCI.	103
Figure 4.19: SP immunoreactivity following the clip compression model of SCI.....	104
Figure 4.20: Assessment of NK1 receptor immunoreactivity following the clip compression model of SCI.....	105
Figure 4.21: NK1 receptor immunoreactivity following the clip compression model of SCI.	106
Figure 4.22: Assessment of albumin immunoreactivity following the clip compression model of SCI.	107
Figure 4.23: Albumin immunoreactivity following the clip compression model of SCI.....	108
Figure 4.24: Assessment of APP immunoreactivity following the clip compression model of SCI.....	109
Figure 4.25: APP immunoreactivity following the clip compression model of SCI.	110
Figure 5.1: Evan’s Blue extravasation following the balloon compression model of SCI.	123
Figure 5.2: Spinal cord edema following the balloon compression model of SCI.	124
Figure 5.3: Intrathecal pressure following the balloon compression model of SCI.....	125
Figure 5.4: Sensory function (plantar prick test) following the balloon compression model of SCI. ...	126
Figure 5.5: Motor function (Modified Tarlov Scale) following the balloon compression model of SCI.	127
Figure 5.6: H&E staining – general morphological features following the balloon compression model of SCI.	129
Figure 5.7: Assessment of SP immunoreactivity within the dorsal horn following the balloon compression model of SCI.....	131
Figure 5.8: SP immunoreactivity within the dorsal horn following the balloon compression model of SCI.....	132
Figure 5.9: Assessment of SP immunoreactivity within the perivascular region following the balloon compression model of SCI.....	133

Figure 5.10: SP immunoreactivity within the perivascular region following the balloon compression model of SCI.	134
Figure 5.11: Assessment of NK1 receptor immunoreactivity following the balloon compression model of SCI.	136
Figure 5.12: NK1 receptor immunoreactivity following the balloon compression model of SCI	137
Figure 5.13: Assessment of NK1 receptor immunoreactivity within the perivascular region following the balloon compression model of SCI.	138
Figure 5.14: NK1 receptor immunoreactivity within the perivascular region following the balloon compression model of SCI.....	139
Figure 5.15: Semi-quantitation of albumin immunoreactivity following the balloon compression model of SCI.	141
Figure 5.16: Albumin immunoreactivity following the balloon compression model of SCI	142
Figure 5.17: Assessment of APP immunoreactivity following the balloon compression model of SCI.	144
Figure 5.18: APP immunoreactivity following the balloon compression model.....	145
Figure 5.19: Assessment of NeuN immunoreactivity following the balloon compression model of SCI.	147
Figure 5.20: NeuN immunoreactivity following the balloon compression model of SCI.....	148
Figure 5.21: GFAP immunoreactivity following the balloon compression model of SCI.	149
Figure 5.22: Representative images of microglia activation.....	150
Figure 5.23: Microglia immunoreactivity within the white matter following the balloon compression model of SCI.	151
Figure 5.24: Microglia immunoreactivity within the grey matter following the balloon compression model of SCI.	152
Figure 5.25: Assessment of AQP4 immunoreactivity in the perivascular region following the balloon compression model of SCI.....	154
Figure 5.26: AQP4 immunoreactivity in the perivascular region following the balloon compression model of SCI.	155
Figure 5.27: Assessment of AQP4 immunoreactivity in the central canal region following the balloon compression model of SCI.....	156
Figure 5.28: AQP4 immunoreactivity in the central canal region following the balloon compression model of SCI.	157
Figure 6.2: NAT dose-response – EB extravasation at 5 hours post-SCI.....	171
Figure 6.1: The effect of NAT administration on Evan’s Blue extravasation following SCI.....	172
Figure 6.3: The effect of NAT administration on edema development following SCI	173
Figure 6.4: The effect of NAT administration on ITP following SCI.....	174
Figure 6.5: The effect of NAT administration on sensory function following SCI.	175
Figure 6.6: The effect of NAT administration of motor function following SCI.	177

Figure 6.7: H&E Staining – The effect of NAT administration of the general morphological features following SCI:.....	178
Figure 6.8: Assessment of the effect of NAT treatment on SP immunoreactivity within the dorsal horn region following SCI.	180
Figure 6.9: The effect of NAT administration on SP immunoreactivity within the dorsal horn region following SCI.....	181
Figure 6.10: Assessment of the effect of NAT treatment on SP immunoreactivity within the perivascular region following SCI.....	183
Figure 6.11: The effect of NAT treatment of SP immunoreactivity within the perivascular region following SCI.....	184
Figure 6.12: Assessment of the effect of NAT administration on NK1 receptor immunoreactivity within the grey matter following SCI.	186
Figure 6.13: The effect of NAT administration on NK1 receptor immunoreactivity within the grey matter following SCI.....	187
Figure 6.14: Assessment of the effect of NAT administration on NK1 receptor immunoreactivity within the perivascular region following SCI.	189
Figure 6.15: The effect of NAT administration on NK1 receptor immunoreactivity within the perivascular region following SCI.	190
Figure 6.16: Assessment of the effect of NAT treatment on albumin immunoreactivity following SCI.	192
Figure 6.17: The effect of NAT treatment on albumin immunoreactivity following SCI.	193
Figure 6.18: Assessment of the effect of NAT on APP immunoreactivity following SCI.	195
Figure 6.19: The effect of NAT administration on APP immunoreactivity following SCI.	196
Figure 6.20: Assessment of the effect of NAT administration on NeuN immunoreactivity following SCI.	198
Figure 6.21: The effect of NAT administration on NeuN immunoreactivity following SCI.	199
Figure 6.22: The effect of NAT administration on GFAP immunoreactivity following SCI.....	201
Figure 6.23: The effect of NAT administration on microglial immunoreactivity following SCI within the white matter.	203
Figure 6.24: The effect of NAT administration on microglial immunoreactivity following SCI within the grey matter.	204
Figure 6.25: Assessment of the effect of NAT treatment on AQP4 immunoreactivity following SCI.	206
Figure 6.26: The effect of NAT administration of AQP4 immunoreactivity following SCI.	207
Figure 6.27: Assessment of the effects of NAT administration on AQP4 immunoreactivity surrounding the central canal following SCI.....	209
Figure 6.28: The effect of NAT administration on AQP4 immunoreactivity surrounding the central canal following SCI in adjacent segments.	210
Table 1.1: ASIA classification of complete versus incomplete spinal cord injury	4

Table 1.2: Classification of Incomplete SCI Syndromes	4
Table 1.3: Mechanisms of Acceleration-Deceleration Injuries	6
Table 3.1: Case data for human spinal cord injury	56

ABSTRACT

Spinal cord injury (SCI) is a devastating injury that commonly results in permanent physical disability. The highest incidence of SCI occurs in younger populations, causing an enormous financial burden to both individuals and society amounting to almost \$1 billion annually within Australia spent on hospitalisation, treatment and rehabilitation of spinal cord injured individuals. To date, an effective clinical treatment for SCI remains elusive, highlighting the need for research aimed at developing therapeutic interventions that improve functional outcome. Spinal cord edema is recognised as a common complication of SCI which continues to develop, spreading in a rostrocaudal direction days after injury, resulting in greater tissue damage and functional deficits. Reducing edema following SCI is therefore of utmost importance and represents an attractive target for therapeutic intervention.

Substance P (SP) is a neuropeptide known to facilitate the process of neurogenic inflammation, which has previously been shown to result in blood brain barrier (BBB) disruption and subsequent edema development following both traumatic brain injury (TBI) and stroke. Furthermore, inhibition of the high-affinity SP receptor, the tachykinin NK1 receptor, resulted in reduced BBB permeability, edema and improved functional outcome in both of these conditions. Accordingly, the current thesis sought to determine whether SP played a similar role as a mediator of neurogenic inflammation following traumatic SCI.

Immunohistochemical assessment of human SCI demonstrated a loss of SP from the dorsal horn region, suggesting that SP release increased following injury. NK1 receptor immunoreactivity was also initially increased post-injury before declining, indicating that receptor activation and subsequent internalisation occurred. Assessment of various open experimental injury models, including the weight drop, hemisection and clip compression models, demonstrated similar SP immunoreactivity as that observed in human tissue, although NK1 receptor immunoreactivity varied in localisation and response to injury. These results highlighted the need for experimental models to accurately replicate the primary injury mechanisms observed clinically, especially the closed environment rather than the open nature of most experimental models. The balloon compression model was subsequently employed for the remainder of the study, given its closed nature and its ability to mimic primary injury mechanisms such as an initial impact followed by persisting compression. This model also proved to replicate many other facets of human injury such as severe hemorrhage, axonal injury, neuronal loss, microglial activation, as well as increased BSCB disruption, edema, intrathecal pressure (ITP) and reduced functional outcome.

Balloon compression induced SCI was also associated with reduced SP immunoreactivity, suggesting increased SP release, and increased NK1 receptor immunoreactivity. Such observations implicate a potential role for SP in mediating neurogenic inflammation following SCI. However, administration of an NK1 receptor antagonist, n-acetyl tryptophan (NAT), resulted in no improvement in any assessed outcomes, suggesting that SP mediated neurogenic inflammation might not play a major role in the development of BSCB disruption following SCI. Indeed, the physiological effects of SP on the barrier may be outweighed by the substantial mechanical disruption observed. Interestingly, changes in the immunoreactivity of the water channel protein, aquaporin 4 (AQP4), were observed following both human SCI and the balloon compression model. These alterations implicate the involvement of AQP4 in facilitating the removal of excess water from the spinal cord. As such, modulation of AQP4 may represent a novel therapeutic intervention following SCI.

We conclude that SP mediated neurogenic inflammation is a minor player in the injury cascade after SCI. At times, NAT administration resulted in worsened outcomes and as such raises the question as to whether SP might be beneficial following SCI rather than detrimental. Further investigation of the role of SP following SCI, especially at later time points, is required to better elucidate its potential role. Nonetheless, substantial edema development remained a serious consequence following SCI and given the observed changes in AQP4 immunoreactivity, investigation of AQP4 modulation following SCI is warranted.