

**CHARACTERISING THE ROLE OF SUBSTANCE P
IN ACUTE ISCHAEMIC STROKE**

RENÉE JADE TURNER

B.Sc (Hons)

Discipline of Pathology, School of Medical Sciences

The University of Adelaide

November 2007

A thesis submitted to the University of Adelaide in 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 that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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

Renée Turner

Date:

DEDICATION

This thesis is dedicated in memory of Jack Byrne.

PUBLICATIONS AND PRESENTATIONS

The following articles have been published or accepted for publication or presentation during the period of my PhD candidature, and sections of these articles are included in the present thesis.

Publications

Turner RJ, Blumbergs, PC, Sims, NR., Helps, SC, Rodgers, KM, Vink, R. (2006) Increased substance P immunoreactivity and edema formation following reversible ischemic stroke. *Acta Neurochir (Suppl)*, 96:263-266.

Turner RJ and Vink R. (2007) Neurogenic inflammation as a novel treatment for ischaemic stroke. *Drug News and Perspectives*, 20:221-226 .

Donkin J, **Turner RJ**, Hassan I, Vink R. (2007) Substance P in traumatic brain injury. *Prog Brain Res*, 161:97-109.

Turner RJ and Vink R. (2006). Magnesium in the central nervous system, in *New Perspectives in Magnesium Research*. (Eds. Nishizawa Y, Morii H, Durlach J). Springer, Tokyo, p 338-353.

Turner RJ. (2007) New hope for stroke. *Australasian Science*. 28(1):38-40.

Abstracts

Turner, R.J., Blumbergs, P.C., Vink, R. (2005) A substance P antagonist improves outcome following reversible middle cerebral artery occlusion in rats. *J Cereb Blood Flow Metab*, 25(Suppl):32.

Turner, R.J., Blumbergs, P.C., Sims, N.R., Helps, S.C., Rodgers, K.M., Vink, R. (2005) Increased substance P immunoreactivity and edema formation following reversible ischaemic stroke. Abstracts of the *XIIIth International Symposium on Brain Edema and Tissue Injury*. Ann Arbor, MI, USA, May 31-June 3, p70.

Turner, R.J., and Vink, R. (2006) Novel approaches to the management of oedema following stroke. *Stroke Society of Australasia – Annual Scientific Meeting*. Adelaide, Australia, October 11-13, p27.

Turner, R.J., Vink, R. (2007) Capsaicin pre-treatment in cerebral ischaemia. *XXIIrd International Symposium on Cerebral Blood Flow, Metabolism and Function*. Osaka, Japan, May 20-25, In press.

Patents:

Vink R, Nimmo A, Whitfield K, **Turner R.J.** (2007) *Methods and composition for reducing reperfusion injury*. Australian Provisional Patent Number 2006906859.

ACKNOWLEDGEMENTS

My gratitude cannot be expressed adequately in words, however I wish to convey my appreciation to the following people.

Professor Robert Vink, my primary supervisor, thankyou for providing me with the opportunity to undertake my PhD and to introduce stroke research into the lab. Bob, thankyou for sharing your wealth of research knowledge and experience. Your support, guidance, feedback and belief in my ability, has provided me with the tools and confidence to pursue a research career. Finally, for the many wine centre chats and for your unwavering, assurance that everything will “ be fine”.

Professor Peter Blumbergs, my co-supervisor, for your amazing and seemingly never-ending neuropathology knowledge.

Dr Stephen Helps (aka: HeadRat) for teaching me the MCAO surgery and helping to trouble-shoot the initial teething problems. Your expertise and assistance in the analysis of the TTC data was invaluable, I appreciate the pain-staking hours spent in Photoshop.

Emma Thornton, first and foremost for your friendship and support. You were there for many of the laughs and tears, frustrations and celebrations. I wish you all the best for the remainder of your PhD and research career.

Jim Manavis, for your immunohistochemistry expertise. Your help with trouble-shooting the immuno's was greatly appreciated.

Kathryn Baht, for your assistance with sectioning of the blocks, without your help I would still be on the Microtome cutting to this day!

Dr Corinna van den Heuvel, for your friendship and support throughout, and for listening when things were or weren't going so well, it was greatly appreciated.

Dr Barbara Koszyca, for your running commentary of events that never failed to make me laugh – thankyou for laughing with me.

Tuyet Tran, for your friendship and ability to put a smile on my face regardless. I wish you all the best for completing your PhD and the future.

Dr James Donkin, for your friendship and for sharing the PhD experience with me. I wish you all the best for you adventure overseas.

Rena Hirani, for your insight and help with trouble-shooting the ELISA.

Felicity Johnson, for your friendship, and for attempting to teach me the finer points of molecular biology!

The IMVS animal house staff, for taking such good care of my animals.

Dale Caville, for the wonderful H&E shots.

To all of Team Neuro, for sharing many thoughts, ideas, laughs, (and wines!), throughout the year, thankyou for making the Vink lab a great place to study and work.

My friends, for your support, encouragement and understanding, but also for being there when I needed a shoulder or a wine.

My family, in particular, Mum, Dad and Nicole, for your love, support, and encouragement, as well as your confidence and belief in me.

I wish to respectfully acknowledge that the sacrifice of rats was central to this body of research.

ABBREVIATIONS

Ab	antibody
ACE	angiotensin converting enzyme
AF	atrial fibrillation
ANOVA	analysis of variance
APP	amyloid precursor protein
APP- α	amyloid precursor protein α
ATP	adenosine triphosphate
BBB	blood brain barrier
BGL	blood glucose levels
bFGF	basic fibroblast growth factor
BI	barthel index
BP	blood pressure
CGRP	calcitonin gene-related peptide
cAMP	cyclic adenosine monophosphate
CA neurons	cornu ammonis neurons
CAST	Chinese Acute Stroke Trial collaborative group
CBF	cerebral blood flow
CCA	common carotid artery
CSF	cerebrospinal fluid
cm	centimetres
CNS	central nervous system
COX	cyclooxygenase

CPP	cerebral perfusion pressure
d	day
DAB	diaminobenzidene
DCC	dark cell change
DISC	death inducing signalling complex
DPX	mounting medium
EB	Evan's Blue
ECA	external carotid artery
ECASS	European Cooperative Acute Stroke Study
ECM	extracellular matrix
ED-1	anti- CD-68 antibody
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
FJC	Fluoro Jade C
FR	free radicals
G	gauge
GOS	Glasgow outcome scale
GFAP	glial fibrillary associated protein
h	hour
HDL	high-density lipoproteins
H&E	haematoxylin and eosin
HRP	horseradish peroxidase
i.c.v.	intracerebroventricular
ICA	internal carotid artery

ICH	intracerebral haemorrhage
ICP	intracranial pressure
IFN- γ	interferon- γ
iNOS	inducible nitric oxide synthase
1L-1 β	interleukin-1 β
Il-6	interleukin-6
IMVS	Institute of Medical and Veterinary Science
IP ₃	inositol 1,4,5-triphosphate
IST	international stroke trial collaborative group
I.U/ml	international units per millilitre
kDA	kilodaltons
L 760 735	NK ₁ receptor antagonist
LDL	low-density lipoprotein
L/min	litres per minute
LRP	low-density lipoprotein-related receptor protein
M	molar
m	metre
MCA	middle cerebral artery
MCAO	middle cerebral artery occlusion
mins	minutes
mg/ml	milligrams per millilitre
MK-801	NMDA receptor antagonist
ml	millilitre
mm	millimetre
mmHg	millimetres of mercury

ml/100g/min	millilitres per 100g of tissue per minute
mmol/L	millimoles per litre
MMP	matrixmetalloproteinase
mNSS	modified neuroseverity score
mRNA	messenger riboneucleic acid
MRS	modified rankin scale
mw	molecular weight
n	number
Na ⁺ /K ⁺ ATPase	sodium/potassium ATP-dependent ion pump
NAT	n-acetyl-tryptophan
NEP	neutral endopeptidase
NF-κB	nuclear factor κB
NGF	nerve growth factor
NH ₂	amino terminus
NH&MRC	National Health and Medical Research Council
NIHSS	National Institute of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NK ₁	neurokinin 1
NK ₂	neurokinin 2
NK ₃	neurokinin 3
NKA	neurokinin A
NKB	neurokinin B
NK _γ	neurokinin γ
NMDA	N-methyl-D-aspartate
Nm	nanometres

nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
O ₂	oxygen
PAI-1	plasminogen activator inhibitor 1
PBS	phosphate buffered saline
PET	Positron Emission Tomography
PKC	protein kinase C
PLA ₂	phospholipase A2
PLC	protein lipase C
pMCAO	permanent middle cerebral artery occlusion
PNS	peripheral nervous system
PPT	preprotackytinin
RCC	red cell change
RP 67580	NK ₁ receptor antagonist
rpm	revolutions per minute
s	seconds
SAH	subarachnoid haemorrhage
SEM	standard error of the mean
s/min	strokes per minute
SP	substance P
SPC	streptavidin peroxidase conjugate
SR 140333	NK ₁ receptor antagonist
TBI	traumatic brain injury
TGF-β	transforming growth factor-β

TIA	transient ischaemic attack
tMCAO	transient middle cerebral artery occlusion
TMB	3,3',5,5'-tetramethylbenzidine
TNF- α	tumour necrosis factor- α
tPA	tissue plasminogen activator
TRPV1	transient receptor potential V1
TTC	2,3,5-triphenyltetrazolium chloride
UV	ultraviolet
VEGF	vascular endothelial growth factor
WHO	World Health Organization
WIN 51 708	NK ₁ receptor antagonist
ww dw	wet weight dry weight
μ l	microlitres
μ mol/kg	micromoles per kilogram
$^{\circ}$ C	degrees celcius

TABLE OF CONTENTS

<i>CHAPTER 1:</i>	1
<i>INTRODUCTION AND LITERATURE REVIEW</i>	1
1.0 Introduction	2
1.1 Epidemiology	3
1.2 The Cerebral Vasculature	3
1.3 Types of Stroke	5
1.3.1 Ischaemic Stroke.....	5
1.3.2 Haemorrhagic Stroke.....	6
1.3.3 Lacunar Stroke.....	7
1.3 Outcome of Stroke	7
1.3.1 Mortality.....	8
1.4.1 Factors Influencing Outcome Following Stroke.....	9
1.3.2 Rehabilitation.....	10
1.3.3 Depression.....	11
1.3.4 Physiological Predictors of Post-Stroke Outcome.....	12
1.3.5 Measures of Post-Stroke Outcome.....	15
1.4 Stroke Risk Factors and Prevention	17
1.4.1 Risk Factors.....	17
1.4.2 Prevention.....	21
1.4.3 Recurrent Stroke.....	22
1.5 Neuropathology and Pathophysiology of Stroke	23
1.5.1 Cerebral Blood Flow and Perfusion Reserve.....	23
1.5.2 Cerebral Infarction.....	24
1.5.3 The Ischaemic Injury Cascade.....	27
1.5.4 Neuronal Death: Necrosis, Apoptosis and Non-Apoptotic Programmed Cell Death following stroke.....	30
1.5.5 Reperfusion Injury.....	32
1.6 Ischaemic Brain Oedema	33
1.6.1 Classification.....	33
1.6.3 Blood-Brain-Barrier Permeability.....	35
1.6.4 Consequences of Cerebral Oedema.....	39
1.6.5 Treatment.....	40
1.7 Substance P	41
1.7.1 Neuropeptide Synthesis.....	42
1.7.2 Neuropeptide Release.....	43
1.7.3 Neuropeptide Localisation.....	43
1.7.4 Metabolism.....	44
1.7.5 Receptors.....	46
1.7.6 NK ₁ Receptor Agonists and Antagonists.....	48
1.7.7 Functions of Substance P.....	48
1.7.8 Neurogenic Inflammation.....	52
1.7.9 Substance P in Hypoxia and Ischaemia.....	56
1.8 Treatment of Ischaemic Stroke	57
1.8.1 Thrombolytic Therapy.....	57
1.8.2 Neuroprotection.....	60
1.9 Experimental Modelling of Stroke	61
1.9.1 Model of ischaemic stroke: middle cerebral artery occlusion.....	63
1.10 Synopsis	64

CHAPTER 2:	65
MATERIALS AND METHODS	65
2.1 Animal Care	66
2.1.1 Ethics.....	66
2.1.2 General.....	66
2.2 Experimental Procedures	66
2.2.1 Anaesthesia.....	66
2.2.2 Rodent Model of Reversible Middle Cerebral Artery Occlusion	68
2.2.3 Perfusion	72
2.3 Drug Treatments	73
2.3.1 Saline.....	73
2.3.2 N-acetyl-L-Tryptophan (SP Antagonist)	73
2.3.3 Actilyse (tPA).....	74
2.3.4 Capsaicin.....	74
2.4 Neurological Assessment	74
2.4.1 Rotarod.....	75
2.4.3 Open Field	77
2.4.4 Modified Neuroseverity Score (mNSS).....	80
2.4.5 Angleboard	80
2.5 Histological Analysis	84
2.5.1 Perfusion Fixation and Brain Sampling.....	84
2.5.2 Haematoxylin & Eosin Staining (H&E).....	84
2.5.3 Immunohistochemistry for Substance P (SP).....	85
2.5.4 Immunohistochemistry for Amyloid Precursor Protein (APP).....	85
2.5.5 Immunohistochemistry for Glial Fibrillary Associated Protein (GFAP).....	86
2.5.6 Immunohistochemistry for ED-1	86
2.5.7 Fluoro Jade C (FJC)	87
2.6 Oedema Study	87
2.5.1 Wet Weight – Dry Weight.....	87
2.7 Assessment of Blood Brain Barrier Permeability	88
2.6.1 Evan’s Blue Extravasation.....	88
2.7 Assessment of Infarct Volume	89
2.7.1 TTC	89
2.8 ELISA for SP	91
2.9 Statistical Analysis	91
CHAPTER 3:	93
CHARACTERISATION OF THE SUBSTANCE P RESPONSE FOLLOWING PERMANENT VERSUS TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION	93
3.1 Introduction	94
3.2 Study Design	95
3.2.1 Immunohistochemistry	95
3.2.2 Enzyme Linked Immunosorbent Assay (ELISA)	96
3.2.3 Statistical Analysis	96
3.3 Results	96
3.3.1 pMCAO	99
3.3.2 tMCAO	108

3.3.3 pMCAO versus tMCAO	127
3.3.4 ELISA for Substance P	129
3.4 Discussion.....	131
3.5 Conclusions.....	135
CHAPTER 4:.....	137
<i>CHARACTERISATION OF N-Acetyl-L-Tryptophan TREATMENT IN ACUTE ISCHAEMIC STROKE.....</i>	<i>137</i>
4.1 Introduction.....	138
4.2 Study Design.....	139
4.2.1 ELISA	139
4.2.2 Blood Brain Barrier Permeability	139
4.2.3 Oedema	140
4.2.4 Infarct Volume.....	140
4.2.5 Functional Outcome	140
4.2.6 Histological Outcome	140
4.2.7 Statistical analysis	141
4.3 Results	141
4.3.1 Mortality	141
4.3.2 ELISA (24 h post-reperfusion) - Effect of NAT treatment on SP protein levels.....	141
4.3.3 Blood Brain Barrier (24 h post-reperfusion) - Effect of NAT treatment on blood brain barrier permeability	144
4.3.4 Cerebral Oedema (24 h post-reperfusion) - Effect of NAT treatment on brain water content	144
4.3.5 Infarct Volume (24 h post-reperfusion) – Effect of NAT treatment on infarct volume ...	147
4.3.6 Functional Outcome.....	147
4.3.7 Histological Outcome (24 h, 7 d post-reperfusion) - Effect of NAT treatment on histological outcome.....	157
4.4 Discussion.....	174
4.5 Conclusions.....	182
CHAPTER 5:.....	183
<i>COMBINATION THERAPY OF AN NK₁ RECEPTOR ANTAGONIST AND TISSUE PLASMINOGEN ACTIVATOR IN ISCHAEMIC STROKE.....</i>	<i>183</i>
5.1 Introduction.....	184
5.2 Study Design.....	187
5.2.1 Histology Study	188
5.2.2 Blood Brain Barrier Study	188
5.2.3 Functional Outcome	188
5.2.4 Statistical Analysis	188
5.3 Results	189
5.3.1 Mortality	189
5.3.2 Effect of tPA on mortality and incidence of intracerebral haemorrhage.....	189
5.3.3 The effects of tPA on the blood brain barrier in naïve animals	192
5.3.4 The effect of tPA and NAT on blood brain barrier breakdown following stroke.....	192
5.3.5 Effect of tPA on Histological Outcome	195
5.3.4 Functional Outcome	221
5.3.6 Effect of tPA on infarct volume	230

5.4 Discussion.....	232
5.5 Conclusions.....	241
<u>CHAPTER 6:</u>	242
<i>THERAPEUTIC WINDOW FOR ADMINISTRATION OF AN NK₁ RECEPTOR ANTAGONIST FOLLOWING ISCHAEMIC STROKE</i>	242
6.1 Introduction.....	243
6.2 Study Design.....	243
6.2.1 Functional Outcome.....	244
6.2.2 Histological Outcome	244
6.2.3 Statistical Analysis.....	244
6.3 Results	244
6.3.1 Functional Outcome.....	244
6.3.2 Histological Outcome	253
6.4 Discussion.....	269
6.5 Conclusions.....	273
<u>CHAPTER 7:</u>	274
<i>NK₁ RECEPTOR ANTAGONIST FOLLOWING MILD, MODERATE AND SEVERE ISCHAEMIC STROKE</i>	274
7.1 Introduction.....	275
7.2 Study Design.....	276
7.2.1 Functional Outcome.....	276
7.2.2 Histological Outcome	276
7.2.3 Statistical Analysis.....	276
7.3 Results	277
7.3.1 Functional Outcome.....	277
7.3.2 Histological Outcome	291
7.4 Discussion.....	321
7.5 Conclusions.....	323
<u>CHAPTER 8:</u>	325
<i>CHARACTERISATION OF THE EFFECTS OF NEUROPEPTIDE DEPLETION WITH CAPSAICIN FOLLOWING ACUTE ISCHAEMIC STROKE</i>	325
8.1 Introduction.....	326
8.2 Study Design.....	327
8.2.1 Functional Outcome.....	327
8.2.2 Histological Outcome	328
8.2.3 Statistical Analysis.....	328
8.3 Results	328
8.3.1 Functional Outcome.....	328
8.3.2 Histological outcome	337

8.4 Discussion.....	353
8.5 Conclusions.....	357
<i>CHAPTER 9:</i>	359
<i>GENERAL DISCUSSION</i>	359
9.1 Conclusions.....	375
<i>REFERENCES</i>	376

LIST OF FIGURES AND TABLES

<i>Figure 2.1 Middle cerebral artery territory anatomy. Error! Bookmark not defined.</i>	
<i>Figure 2.2 Motor function- Rotarod.Error! Bookmark not defined.</i>	
<i>Figure 2.3 Sensory function – bilateral asymmetry test..... Error! Bookmark not defined.</i>	
<i>Figure 2.4 Spontaneous Exploratory Behaviour – Open Field.Error! Bookmark not defined.</i>	
<i>Figure 2.5 Neurological function – modified neuroseverity score. Error! Bookmark not defined.</i>	
<i>Figure 2.6 Hemiparesis – Angleboard.....Error! Bookmark not defined.</i>	
<i>Figure 2.7 Degree of Infarction – TTC staining.....Error! Bookmark not defined.</i>	
<i>Figure 3.1 Permanent versus transient stroke. Haemorrhagic Transformation – H&E stained sections (Bar = 100 μm).....</i>	97
<i>Figure 3.2 Permanent versus transient stroke. Infarction – H&E stained sections (10x).....</i>	98
<i>Figure 3.3 Permanent versus transient stroke. pMCAO Cortex – H&E stained sections (Bar = 100 μm).....</i>	100
<i>Figure 3.4 Permanent versus transient stroke. pMCAO White Matter – H&E stained sections (Bar = 100 μm).....</i>	101
<i>Figure 3.5 Permanent versus transient stroke. pMCAO – H&E stained sections (Bar = 100 μm).....</i>	102
<i>Figure 3.6 Permanent versus transient stroke. pMCAO Perivascular Tissue – SP stained sections (Bar = 100 μm).....</i>	104
<i>Figure 3.7 Permanent versus transient stroke. pMCAO Cortex – SP stained sections (Bar = 100 μm).....</i>	105
<i>Figure 3.8 Permanent versus transient stroke. pMCAO White Matter – APP stained sections (Bar = 100 μm).....</i>	106
<i>Figure 3.9 Permanent versus transient stroke. pMCAO Cortex – APP stained sections (Bar = 100 μm).....</i>	107
<i>Figure 3.10 Permanent versus transient stroke. pMCAO Cortex-Degenerating Neurons – FJC stained sections (Bar = 100 μm).</i>	109
<i>Figure 3.11 Permanent versus transient stroke. pMCAO White Matter-Degenerating Neurons – FJC stained sections (Bar = 100 μm).....</i>	110

<i>Figure 3.12 Permanent versus transient stroke. tMCAO Cortex – H&E stained sections (Bar = 100 μm)</i>	111
<i>Figure 3.13 Permanent versus transient stroke. tMCAO White Matter – H&E stained sections (Bar = 100 μm)</i>	113
<i>Figure 3.14 Permanent versus transient stroke - tMCAO</i>	114
<i>Figure 3.15 Permanent versus transient stroke. tMCAO Perivascular Tissue – SP stained sections (Bar = 100 μm)</i>	115
<i>Figure 3.16 Permanent versus transient stroke. tMCAO Cortex – SP stained sections (Bar = 100 μm)</i>	116
<i>Figure 3.17 Permanent versus transient stroke. tMCAO White Matter – APP stained sections (Bar = 100 μm)</i>	118
<i>Figure 3.18 Permanent versus transient stroke. tMCAO Cortex – APP stained sections (Bar = 100 μm)</i>	119
<i>Figure 3.19 Permanent versus transient stroke. tMCAO Cortex-Degenerating Neurons – FJC stained neurons (Bar = 100 μm)</i>	121
<i>Figure 3.20 Permanent versus transient stroke. tMCAO White Matter-Degenerating Neurons – FJC stained neurons (Bar = 100 μm)</i>	122
<i>Figure 3.21 Permanent versus transient stroke. tMCAO - GFAP stained sections (4x)</i>	123
<i>Figure 3.22 Permanent versus transient stroke. tMCAO GFAP stained sections (Bar = 100 μm)</i>	124
<i>Figure 3.23 Permanent versus transient stroke. tMCAO – Macrophage/Activated Microglia response - ED-1 Stained sections (Bar = 100 μm)</i>	126
<i>Figure 3.24 Permanent versus transient stroke. pMCAO versus tMCAO – SP stained sections (Bar = 100 μm)</i>	128
<i>Figure 3.25 Permanent versus transient stroke. Level of SP within the infarcted hemisphere measured at 24 h post-reperfusion, as assessed by ELISA</i>	130
<i>Figure 4. 1 NK₁ receptor antagonist treatment. Survival at 7 d post-stroke</i>	142
<i>Figure 4.2 NK₁ receptor antagonist treatment. Level of SP within the infarcted hemisphere measured at 24 h post-reperfusion, as assessed by ELISA</i>	143
<i>Figure 4.3 NK₁ receptor antagonist treatment. Blood brain barrier permeability of the infarcted hemisphere measured at 24 h post-reperfusion, as assessed by Evan's Blue extravasation</i>	145

<i>Figure 4. 4 NK₁ receptor antagonist treatment. Oedema within the infarcted hemisphere measured at 24 h post-reperfusion, as assessed by wet weight dry weight.</i>	146
<i>Figure 4.5 NK₁ receptor antagonist treatment. Percentage of infarction within the cortex and striatum as assessed by TTC staining.</i>	148
<i>Figure 4.6 NK₁ receptor antagonist treatment. Motor performance as assessed by the rotarod.</i>	149
<i>Figure 4.7 NK₁ receptor antagonist treatment. Sensory function as assessed by the bilateral asymmetry test.</i>	151
<i>Figure 4. 8 NK₁ receptor antagonist treatment. Stress and anxiety as assessed by the open field.</i>	152
<i>Figure 4.9 NK₁ receptor antagonist treatment. Neurological function as assessed by the modified neuroseverity score.</i>	154
<i>Figure 4. 10 NK₁ receptor antagonist treatment. Hemiparesis as assessed by the angleboard.</i>	156
<i>Figure 4.11 NK₁ receptor antagonist treatment. Cortex – H&E stained sections.</i>	158
<i>Figure 4.12 NK₁ receptor antagonist treatment. White Matter – H&E stained sections.</i>	159
<i>Figure 4.13 NK₁ receptor antagonist treatment. Perivascular SP response – SP stained sections.</i>	161
<i>Figure 4. 14 NK₁ receptor antagonist treatment. Cortical SP response – SP stained sections.</i>	162
<i>Figure 4.15 NK₁ receptor antagonist treatment. Axonal Injury – APP stained sections.</i>	164
<i>Figure 4.16 NK₁ receptor antagonist treatment. Cortex – APP stained sections.</i>	165
<i>Figure 4.17 NK₁ receptor antagonist treatment. Degenerating Neurons Cortex – Fluoro Jade C stained sections.</i>	167
<i>Figure 4. 18 NK₁ receptor antagonist treatment. Degenerating Neurons White Matter – Fluoro Jade C stained sections.</i>	168
<i>Figure 4.19 NK₁ receptor antagonist treatment. Cortical Macrophage Response – ED-1 stained sections.</i>	170
<i>Figure 4.20 NK₁ receptor antagonist treatment. Perivascular Macrophage response ED-1 stained sections.</i>	171
<i>Figure 4. 21 NK₁ receptor antagonist treatment. GFAP Perivascular – GFAP stained sections.</i>	172
<i>Figure 4.22 NK₁ receptor antagonist treatment. GFAP Penumbra – GFAP stained sections.</i>	173

<i>Figure 5.1 NAT/tPA combination therapy. Percentage survival at 7 d post-stroke.</i>	190
<i>Figure 5.2 NAT/tPA combination therapy. Deaths attributable to intracerebral haemorrhage (ICH).</i>	191
<i>Figure 5.3 NAT/tPA combination therapy. Effect of tPA administration on EB extravasation in naïve animals.</i>	193
<i>Figure 5.4 NAT/tPA combination therapy. EB extravasation at 24 h post-reperfusion.</i>	194
<i>Figure 5.5 NAT/tPA combination therapy. Cortex at 24 h post-reperfusion. H&E stained sections.</i>	196
<i>Figure 5.6 NAT/tPA combination therapy. Cortex at 7 d post-reperfusion. H&E stained sections.</i>	197
<i>Figure 5.7 NAT/tPA combination therapy. White matter at 24 h post-reperfusion. H&E stained sections.</i>	198
<i>Figure 5.8 NAT/tPA combination therapy. White matter at 7 d post-reperfusion. H&E stained sections.</i>	199
<i>Figure 5.9 NAT/tPA combination therapy. SP Perivascular tissue at 24 h post-reperfusion. SP stained sections.</i>	201
<i>Figure 5.10 NAT/tPA combination therapy. Perivascular SP immunoreactivity at 7 d post-reperfusion. SP stained sections.</i>	202
<i>Figure 5.11 NAT/tPA combination therapy. Cortical SP Immunoreactivity at 24 h post-reperfusion. SP stained sections.</i>	203
<i>Figure 5.12 NAT/tPA combination therapy. Cortical SP immunoreactivity at 7 d post-reperfusion. SP stained sections.</i>	204
<i>Figure 5.13 NAT/tPA combination therapy. Axonal Injury at 24 h post-reperfusion. APP stained sections.</i>	206
<i>Figure 5.14 NAT/tPA combination therapy. Axonal Injury at 7 d post-reperfusion. APP stained sections.</i>	207
<i>Figure 5.15 NAT/tPA combination therapy. Cortical APP Immunoreactivity at 24 h post-stroke. APP stained sections.</i>	208
<i>Figure 5.16 NAT/tPA combination therapy. Cortical APP immunoreactivity at 7 d post-reperfusion. APP stained sections.</i>	209
<i>Figure 5.17 NAT/tPA combination therapy. Degenerating neurons in the cortex at 24 h post-reperfusion. FJC stained sections.</i>	212
<i>Figure 5.18 NAT/tPA combination therapy. Degenerating neurons in the cortex at 7 d post-reperfusion. FJC stained sections.</i>	213
<i>Figure 5.19 NAT/tPA combination therapy. Degenerating neurons in the white matter at 24 h post-reperfusion. FJC stained sections.</i>	214

<i>Figure 5.20 NAT/tPA combination therapy. Degenerating neurons within the white matter at 7 d post-reperfusion. FJC stained sections.</i>	215
<i>Figure 5.21 NAT/tPA combination therapy. Perivascular astrocytic response at 7 d post-reperfusion. GFAP stained sections.</i>	217
<i>Figure 5.22 NAT/tPA combination therapy. Astrocytic response within the infarct border zone at 7 d post-reperfusion. GFAP stained sections.</i>	218
<i>Figure 5.23 NAT/tPA combination therapy. Perivascular macrophage response at 7 d post-reperfusion. ED-1 stained sections.</i>	219
<i>Figure 5.24 NAT/tPA combination therapy. Cortical Macrophage response within the infarct at 7 d post-reperfusion. ED-1 stained sections.</i>	220
<i>Figure 5.25 NAT/tPA combination therapy. Motor performance as assessed by the rotarod.</i>	222
<i>Figure 5.26 Figure 5.26 NAT/tPA combination therapy. Sensory neglect as assessed by the bilateral asymmetry test.</i>	223
<i>Figure 5.27 NAT/tPA combination therapy. Post-stroke stress and anxiety as assessed by the open field.</i>	227
<i>Figure 5.28 NAT/tPA combination therapy. Post-stroke neurological function as assessed by the mNSS.</i>	228
<i>Figure 5.29 NAT/tPA combination therapy. Post-stroke hemiparesis as assessed by the angleboard.</i>	229
<i>Figure 5.30 NAT/tPA combination therapy. Infarct volume at 24 h post-reperfusion, as assessed by TTC staining.</i>	231
<i>Figure 6.1 Therapeutic window - Motor performance as assessed by the rotarod.</i>	246
<i>Figure 6.2 Therapeutic Window – Sensory function as assessed by the bilateral asymmetry test.</i>	247
<i>Figure 6.3 Therapeutic Window – Stress and anxiety as assessed by the open field.</i>	249
<i>Figure 6.4 Therapeutic Window – Neurological function as assessed by the modified neuroseverity score.</i>	251
<i>Figure 6.5 Therapeutic Window – Hemiparesis as assessed by the angleboard.</i>	252
<i>Figure 6.6 Therapeutic Window – Cortex at 7 d post-reperfusion. H&E stained sections.</i>	254
<i>Figure 6.7 Therapeutic Window – White matter at 7 d post-reperfusion. H&E stained sections.</i>	255
<i>Figure 6.8 Therapeutic Window – Perivascular tissue at 7 d post-reperfusion. SP stained sections.</i>	257

<i>Figure 6.9 Therapeutic Window – Cortex at 7 d post-reperfusion. SP stained sections.</i>	258
<i>Figure 6.10 Therapeutic Window – Axonal injury within the white matter at 7 d post-reperfusion. APP stained sections.</i>	259
<i>Figure 6.11 Therapeutic Window – Cortex at 7 d post-reperfusion. APP stained sections.</i>	260
<i>Figure 6.12 Therapeutic Window – Degenerating neurons within the cortex at 7 d post-reperfusion. FJC stained sections.</i>	262
<i>Figure 6.13 Therapeutic Window – Degenerating neurons within the white matter at 7 d post-reperfusion. FJC stained sections.</i>	263
<i>Figure 6.14 Therapeutic Window – Perivascular astrocytic response at 7 d post-reperfusion. GFAP stained sections.</i>	265
<i>Figure 6.15 Therapeutic Window – Astrocytic response within the infarct border zone at 7 d post-reperfusion. GFAP stained sections.</i>	266
<i>Figure 6.16 Therapeutic Window – Perivascular macrophage response at 7 d post-reperfusion. ED-1 stained sections.</i>	267
<i>Figure 6.17 Therapeutic Window – Macrophage response within the infarct at 7 d post-reperfusion. ED-1 stained sections.</i>	268
<i>Figure 7.1 Mild, moderate and severe ischaemia – NAT at 8 h. Motor function as assessed by the rotarod.</i>	278
<i>Figure 7.2 Mild, moderate and severe ischaemia – NAT at 8 h. Sensory function, as assessed by the bilateral asymmetry test.</i>	281
<i>Figure 7.3 Mild, moderate and severe ischaemia – NAT at 8 h. Spontaneous exploratory behaviour, as assessed by the open field.</i>	285
<i>Figure 7.4 Mild, moderate and severe ischaemia – NAT at 8 h. Sensory function, as assessed by the bilateral asymmetry test.</i>	287
<i>Figure 7.5 Mild, moderate and severe ischaemia – NAT at 8 h. Hemiparesis as assessed by the angleboard.</i>	289
<i>Figure 7.6 Mild, moderate and severe ischaemia – NAT at 8 h. Cortex at 7 d following stroke. H&E stained sections.</i>	292
<i>Figure 7.7 Mild, moderate and severe ischaemia – NAT at 8 h. White matter at 7 d following stroke. H&E stained sections.</i>	295
<i>Figure 7.8 Mild, moderate and severe ischaemia – NAT at 8 h. Perivascular SP response at 7 d following stroke. SP stained sections.</i>	298
<i>Figure 7.9 Mild, moderate and severe ischaemia – NAT at 8 h. Cortical SP response at 7 d following stroke. H&E stained sections.</i>	300
<i>Figure 7.10 Mild, moderate and severe ischaemia – NAT at 8 h. Axonal injury within the white matter following stroke. APP stained sections.</i>	303

<i>Figure 7.11 Mild, moderate and severe ischaemia – NAT at 8 h. Cortical APP response at 7 d following stroke. APP stained sections.</i>	305
<i>Figure 7.12 Mild, moderate and severe ischaemia – NAT at 8 h. Degenerating neurons within the cortex at 7 d following stroke. FJC stained sections.</i>	308
<i>Figure 7.13 Mild, moderate and severe ischaemia – NAT at 8 h. Degenerating neurons within the white matter at 7 d following stroke. FJC stained sections.</i>	310
<i>Figure 7.14 Mild, moderate and severe ischaemia – NAT at 8 h. Astrocytic response within the infarct boundary zone at 7 d following stroke. GFAP stained sections.</i>	313
<i>Figure 7.15 Mild, moderate and severe ischaemia – NAT at 8 h. Perivascular astrocytic response within the infarct boundary zone at 7 d following stroke. GFAP stained sections.</i>	315
<i>Figure 7.16 Mild, moderate and severe ischaemia – NAT at 8 h. Macrophage response within the infarct at 7 d following stroke. ED-1 stained sections.</i>	317
<i>Figure 7.17 Mild, moderate and severe ischaemia – NAT at 8 h. Perivascular macrophage response at 7 d following stroke. ED-1 stained sections.</i>	319
<i>Figure 8.1 Capsaicin pre-treatment – motor function as assessed by the rotarod.</i>	330
<i>Figure 8.2 Capsaicin pre-treatment –sensory function as assessed by the bilateral asymmetry test.</i>	331
<i>Figure 8.3 Capsaicin pre-treatment –Spontaneous exploratory behaviour, as assessed by the open field test.</i>	334
<i>Figure 8.4 Capsaicin pre-treatment –Neurological function, as assessed by the modified neuroseverity score.</i>	335
<i>Figure 8.5 Capsaicin pre-treatment –Hemiparesis, as assessed by the angleboard.</i>	336
<i>Figure 8.6 Capsaicin pre-treatment –Cortex at 7 d following stroke. H&E stained sections.</i>	338
<i>Figure 8.7 Capsaicin pre-treatment –White matter at 7 d following stroke. H&E stained sections.</i>	339
<i>Figure 8.8 Capsaicin pre-treatment –Perivascular SP immunoreactivity at 7 d post-stroke. SP stained sections.</i>	341
<i>Figure 8.9 Capsaicin pre-treatment –Cortical SP immunoreactivity at 7 d following stroke. SP stained sections.</i>	342
<i>Figure 8.10 Capsaicin pre-treatment –Axonal injury within the white matter at 7 d following stroke. APP stained sections.</i>	343
<i>Figure 8.11 Capsaicin pre-treatment –Cortical APP immunoreactivity at 7 d following stroke. APP stained sections.</i>	344

<i>Figure 8.12 Capsaicin pre-treatment –Degenerating neurons within the cortex at 7 d following stroke. FJC stained sections.</i>	346
<i>Figure 8.13 Capsaicin pre-treatment –Degenerating neurons within the white matter at 7 d following stroke. FJC stained sections.</i>	347
<i>Figure 8.14 Capsaicin pre-treatment –GFAP immunoreactivity within the infarct border zone at 7 d following stroke. GFAP stained sections.</i>	348
<i>Figure 8.15 Capsaicin pre-treatment –Perivascular GFAP immunoreactivity at 7 d following stroke. GFAP stained sections.</i>	349
<i>Figure 8.16 Capsaicin pre-treatment –Macrophage response within the infarct at 7 d post-reperfusion. ED-1 stained sections.</i>	351
<i>Figure 8.17 Capsaicin pre-treatment – perivascular ED-1 immunoreactivity at 7 d following stroke. ED-1 stained sections.</i>	352
<i>Figure 9. 1 The involvement of SP and neurogenic inflammation in CNS injury</i>	373
<i>Table 2. 1 Modified neuroseverity score.</i>	81

ABSTRACT

More than 15 million people worldwide will suffer a stroke each year two thirds will die or be left permanently disabled. Accordingly, stroke represents an enormous financial burden on the community, due to the cost of hospitalisation, treatment and rehabilitation of stroke patients. Despite the significance of this public health problem, a safe and widely applicable stroke therapeutic remains elusive. Cerebral oedema is widely recognised as a common and often fatal complication of stroke that is associated with worsened outcome. However, the exact mechanisms of oedema formation remain unclear, with current therapies largely ineffective in addressing the mechanisms of cerebral swelling, and also being associated with their own negative side-effect profile.

This thesis characterises the role of neurogenic inflammation and the neuropeptide, substance P (SP), in mediating the development of blood brain barrier breakdown, cerebral oedema and resultant functional deficits following stroke, using a rodent model of reversible cerebral ischaemia. The findings of this thesis demonstrate that increased SP immunoreactivity, particularly of the penumbral tissue vasculature, is a feature of tissue perfusion following stroke, but not in non-reperfused infarcts. The central role for SP in the breakdown of the BBB following stroke and the associated deleterious effects of such breakdown was confirmed by studies using an NK₁ receptor antagonist. These antagonists conferred a profound attenuation of BBB breakdown, cerebral oedema formation, neuronal death and injury, and the associated development of functional deficits following reversible stroke. Similarly, depletion of all neuropeptides by capsaicin pre-treatment also reduced

both histological abnormalities and functional deficits following stroke, confirming the central role of neuropeptides in the secondary injury process after stroke.

The NK₁ receptor antagonist was able to be safely combined with the currently approved treatment for stroke, tPA, producing a synergistic effect of greater protection from the ischaemic insult. In particular, histological and functional outcome were markedly improved, as well as a reduction in the risk of intracerebral haemorrhage and death. Furthermore, the NK₁ receptor antagonist was effective even when administered up to 8 h following the onset of ischaemia, and in a variety of stroke severities.

We conclude that SP plays a central role in the secondary injury that occurs following stroke, in particular, the genesis of BBB breakdown and cerebral oedema. Accordingly, combination therapy of tPA and an NK₁ receptor antagonist may offer a novel therapeutic strategy for the clinical management of ischaemic stroke of varying severity.