

The Role of Substance P

In

Experimental Intracerebral Haemorrhage

Timothy J. Kleinig, M.B.B.S. (Hons.), B.A., F.R.A.C.P.

Discipline of Pathology,
School of Medical Sciences,
University of Adelaide

May 2010

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

DEDICATION

This thesis is dedicated to my wife Pagan, for her love, support, hard work and encouragement through busy and challenging times, not only through the PhD years, but every year since we've been married – more so since the children started arriving.

First you guess. Don't laugh, this is the most important step. Then you compute the consequences. Compare the consequences to experience. If it disagrees with experience, the guess is wrong. In that simple statement is the key to science. It doesn't matter how beautiful your guess is or how smart you are or what your name is. If it disagrees with experience, it's wrong. That's all there is to it.

Richard P. Feynman (1918-1988)

Table of Contents

1. Spontaneous intracerebral haemorrhage: topic review and a putative pathophysiological role for substance P	1
1.1 Definition.....	1
1.2 Epidemiology.....	1
1.3 Aetiology and prognostic factors	2
1.4 Histological and imaging appearances and their changes over time	5
1.5 Secondary injury in intracerebral haemorrhage.....	6
1.5.1 Secondary pathological events in ICH	7
1.5.2 Mediators of perihæmatomal secondary injury.....	14
1.6 Approaches to limit secondary injury after ICH.....	23
1.6.1 Medical approaches.....	23
1.6.2 Surgical approaches	24
1.7 SP as a potential mediator of secondary injury in ICH.....	25
1.7.1 Background, structure and receptors.....	25
1.7.2 Peripheral actions	27
1.7.3 Central nervous system SP	30
1.7.4 A putative role for substance P in intracerebral haemorrhage.....	33
1.8 Animal models of intracerebral haemorrhage.....	34
1.9 Hypotheses.....	35
2 Materials and methods.....	37
2.1 Animal Care	37
2.1.1 Animal ethics.....	37
2.1.2 General.....	37
2.2 Experimental procedures	37
2.2.1 Anaesthesia.....	37
2.2.2 Stereotactic injection of substance P	38
2.2.3 Autologous blood infusion intracerebral haemorrhage	39
2.2.4 Collagenase infusion intracerebral haemorrhage	39
2.2.5 Stereotactic thrombin injection.....	40
2.2.6 Splenectomy	40
2.2.7 Post-surgery recovery.....	40
2.2.8 Perfuse fixation	41
2.3 Drug treatments.....	41

2.3.1	N-acetyl-L-tryptophan.....	41
2.3.2	L-733,060	41
2.4	Neurological assessment.....	42
2.4.1	Rotarod	42
2.4.2	Sticky label test	43
2.4.3	Tapered ledged beam test.....	44
2.4.4	Vibrissae-elicited stimulation test	45
2.4.5	Elevated drag test	46
2.4.6	Other pilot behavioural tests.....	47
2.5	Histological analysis	47
2.5.1	Brain sectioning	47
2.5.2	Haemorrhage quantification	48
2.5.3	Brain processing for histological assessment	48
2.5.4	Haematoxylin and eosin (H&E) staining	49
2.5.5	Immunohistochemistry.....	49
2.5.6	Immunofluorescence double labelling	50
2.5.7	Fluoro-Jade staining.....	50
2.6	Oedema measurement	51
2.7	Assessment of blood-brain barrier permeability	51
2.8	ELISA for substance P	52
2.9	Real-time RT-PCR for SP and NK1 receptor mRNA	53
2.10	Quantitation of immunohistochemical staining	55
2.11	Automated cell counting	60
2.12	Power calculations.....	62
2.13	Statistical analysis.....	62
3	Characterisation of substance P immunostaining and expression following collagenase and autologous blood experimental ICH	63
3.1	Introduction	63
3.2	Experimental design.....	64
3.2.1	Haematoma quantification.....	64
3.2.2	Histology and immunohistochemistry.....	65
3.2.3	Semi-quantitation of SP immunostaining.....	65
3.2.4	Cell counting	66
3.2.5	ELISA.....	66

3.2.6	RT-PCR.....	66
3.2.7	Pilot behavioural studies	66
3.2.8	Statistical analysis	67
3.3	Results	67
3.3.1	Baseline and surgical parameters.....	67
3.3.2	Haematoma volumes.....	67
3.3.3	Collagenase ICH Histology	68
3.3.4	Autologous ICH Histology	80
3.4	Discussion.....	92
3.5	Conclusion	94
4	Inhibition of substance P via NK1 receptor antagonists: histological, behavioural, oedema and blood-brain-barrier experiments.....	95
4.1	Introduction	95
4.2	Experimental design.....	96
4.2.1	Haematoma quantification.....	96
4.2.2	Brain lesion volume assessment.....	97
4.2.3	Histology and immunohistochemistry.....	97
4.2.4	Semi-quantitation of SP immunostaining.....	97
4.2.5	Cell counting and evaluation of subventricular zone cellular proliferation	97
4.2.6	Brain oedema.....	98
4.2.7	Blood-brain barrier permeability.....	98
4.2.8	Behavioural studies.....	98
4.2.9	Statistical analysis	98
4.3	Results	98
4.3.1	Operative and physiological parameters.....	98
4.3.2	Haematoma volumes.....	99
4.3.3	Lesion volumes 28 days after collagenase ICH	99
4.3.4	Histology and immunohistochemistry.....	100
4.3.5	Cellular proliferation in the SVZ.....	104
4.3.6	Brain oedema.....	104
4.3.7	Blood-brain barrier dysfunction	105
4.3.8	The effect of NK1R antagonists on functional outcome	106
4.4	Discussion.....	109
4.5	Conclusion	111

5	Investigation of the effect of thrombin on substance P: is thrombin the factor which triggers SP-mediated oedema?	112
5.1	Introduction	112
5.2	Experimental design.....	113
5.2.1	24 hour lesion comparison with collagenase ICH.....	114
5.2.2	Histology and immunohistochemistry.....	114
5.2.3	Semi-quantitation of SP immunostaining.....	114
5.2.4	Brain oedema.....	114
5.2.5	Blood-brain barrier permeability.....	115
5.2.6	Statistical analysis	115
5.3	Results	115
5.3.1	Baseline and surgical parameters.....	115
5.3.2	Dose titration - histology	116
5.3.3	Thrombin dose titration – oedema.....	117
5.3.4	Histology and immunohistochemistry.....	118
5.3.5	Double labelling	126
5.3.6	Brain oedema.....	127
5.3.7	Blood-barrier dysfunction.....	128
5.4	Discussion.....	129
5.5	Conclusion	132
6	Effects of intracerebral substance P injections.	134
6.1	Introduction	134
6.2	Experimental design.....	135
6.2.1	Histology and immunohistochemistry.....	135
6.2.2	Brain oedema.....	135
6.2.3	Blood-brain barrier permeability.....	136
6.2.4	Statistical analysis	136
6.3	Results	136
6.3.1	Baseline and experimental parameters.....	136
6.3.2	Dose finding experiments	136
6.3.3	Histology and immunohistochemistry.....	137
6.3.4	Brain oedema.....	143
6.3.5	Blood-brain barrier dysfunction	144
6.4	Discussion.....	144

6.5	Conclusion	147
7	Exploration of the potential mechanisms of NK1RA-mediated oedema reduction following collagenase ICH.	148
7.1	Introduction	148
7.2	Experimental design.....	149
7.3	Results	149
7.3.1	Baseline and experimental parameters.....	149
7.3.2	Brain oedema following autologous ICH	150
7.3.3	Brain oedema in splenectomised rats	150
7.4	Discussion.....	151
7.5	Conclusion	153
8	Summary and future directions.....	154
	Conclusion.....	158
	References:	159

ABSTRACT

Background:

Elevated levels of substance P (SP) have previously been found following ischaemic stroke and traumatic brain injury. Inhibiting the main SP receptor (neurokinin-1 (NK1)) reduces oedema and improves functional outcome in both settings. As this thesis details, we hypothesised that SP plays a similarly deleterious role following intracerebral haemorrhage (ICH).

We further hypothesised that the post-ICH effects of intracerebral thrombin (which is known to play a major role in post-ICH secondary injury) are at least partly SP-mediated. Thrombin, similarly to SP, is known to play a deleterious role following both ischaemic stroke and traumatic brain injury. Previous research has also demonstrated that thrombin causes cutaneous oedema by an SP-dependent mechanism.

Methods:

Three hundred and forty three male Sprague-Dawley rats were used, and variously subjected to collagenase ICH, autologous ICH, intracerebral thrombin injection and intracerebral injection of SP. The sequelae of these various injuries was assessed, as well as the effect of antagonists to the main substance P receptor (NK1R), using functional testing, histological analysis, ELISA, real-time RT-PCR, wet-weight dry weight (for assessment of oedema) and Evans blue (for assessment of blood-brain barrier integrity). The effect of prior splenectomy on oedema following ICH was also assessed.

Results:

Elevated levels of SP were demonstrated post-ICH in the two different ICH models, and localised to astrocytes. Following collagenase ICH, two structurally unrelated NK1R antagonists reduced oedema and blood-brain barrier (BBB) dysfunction, but failed to reduce cellular inflammation, brain lesion volume and functional deficits.

Stereotactic thrombin injections caused both oedema and elevated intracerebral SP, however, NK1R antagonism post-intracerebral thrombin failed to reduce brain oedema, largely disproving the hypothesis that thrombin causes intracerebral oedema post-ICH by a SP-dependent mechanism.

Supraphysiological levels of SP injected stereotactically caused surprisingly little oedema and BBB dysfunction. Additional exploratory experiments demonstrated that NK1R antagonism did not reduce oedema caused by autologous ICH and also that the oedema-reducing effects of NK1R antagonism following collagenase ICH were abrogated by prior splenectomy.

Conclusion:

These results demonstrate that the oedematogenic actions of substance P following ICH are complex, and may predominantly be peripherally-mediated. Future experiments are planned to characterise further the role of SP in neuroinflammatory conditions.

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 to Timothy Kleinig and, to the best of my knowledge and belief, 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 loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Tim Kleinig

Date

Papers and presentations during period of candidature

Portions of the work contained in this thesis have been presented at conferences and/or published in the literature (or submitted for publication).

Published

- Kleinig TJ, Helps SC, Ghabriel MN, Manavis J, Leigh C, Blumbergs PC, Vink R. Hemoglobin crystals: a pro-inflammatory potential confounder of rat experimental intracerebral hemorrhage. *Brain Res.* 2009; 1287: 164-72.

- Kleinig TJ, Vink R. Inflammation in ischaemic and hemorrhagic stroke: therapeutic options. *Curr Opin Neurol* 2009; 22: 294-301.

- Cook NL, Kleinig TJ, Vink R, van den Heuvel C. Selection of reference genes for normalising gene expression data in the collagenase model of intracerebral haemorrhage in rats. *BMC Molecular Biology.* 2010; 11: 7.

Submitted

- Helps SC, Kleinig TJ. Automated semiquantitation of immunohistochemistry using color deconvolution. *J Neurosci Methods.*

Abstracts/presentations

- Kleinig TJ, Helps SC, Manavis J, Blumbergs PC, Vink R. Substance P: a potential role in the pathophysiology of intracerebral haemorrhage. (Presented at the Australasian Stroke Society Annual Meeting, Sydney 2009) *Int Med Journal* 2008; 38 (Suppl 4): S101.

- Kleinig TJ, Helps SC, Cook N, Blumbergs P, Vink R. Inhibition of Substance P reduces Edema and Blood-Brain Barrier Dysfunction following Rat Collagenase Intracerebral Hemorrhage, but does not improve Functional Outcome. (Accepted for Platform presentation at the International Stroke Conference, Feb 23-26, 2010) To be published in *Stroke*.

Other papers published during period of candidature

- Broadley SA, Ghali JR, Kleinig TJ, Ooi SYJ, Allison DL, Taylor J, Allison DL, Thompson PD. Serum elevations of Troponin T in acute stroke: what do they mean? *J Clin Neuroscience.* doi:10.1016/j.jocn.2009.03.017.

- Kleinig TJ, Kimber TE, Thompson PD. Prevention of stroke and stroke disability: quantifying potential benefits of best practice therapies. *Med J Aust* 2009; 190:678-82.

- Kleinig TJ, Kimber TE, Thompson PD. Convexity subarachnoid haemorrhage as initial manifestation of bilateral internal carotid artery stenoses. *J Neurol* 2009; 256: 669-71.

- Kleinig T, Koszyca B, Thompson P, Blumbergs P. Acute fatal leucocytoclastic cerebral vasculitis in a patient with otherwise indolent systemic lupus erythematosus. *Lupus* 2009; 18: 486-90.

- Kleinig TJ, Thompson PD, Kneebone CS. Chorea, transverse myelitis, neuropathy and a distinctive MRI: paraneoplastic manifestations of probable small-cell lung cancer. *J Clin Neuro Sci* 2009; 16: 966-968.
- Kumar K, Kleinig T. 'Malignant' Ischemic Stroke of an Entire Cerebral Hemisphere. *J Neurol Neurosurg Psych.* 2009; 80: 87.
- Kong A, Kleinig T, van der Vliet A, Bergin P, Coscia C, Ring S, Brooder R. MRI of sporadic Creutzfeldt-Jacob disease. *J Med Imaging Radiat Oncol* 2008; 52:318-24.
- Leyden J, Kleinig T. The role of the basal ganglia in data processing. *Med Hypotheses* 2008;71:61-64.
- Kleinig TJ, Thompson PD, Matar W, Duggins A, Kimber TE, Morris JG, Kneebone CS, Blumbergs PC. The distinctive movement disorder of ovarian teratoma-associated encephalitis. *Mov Disord* 2008; 23: 1256-61.
- Kleinig TJ, Kiley M, Thompson PD. Acute convexity subarachnoid haemorrhage: a cause of aura-like symptoms in the elderly. *Cephalalgia.* 2008;28:658-63.
- Kleinig TJ, Harley H, Thompson PD. Neurological deterioration during treatment in Wilson's disease. *J Clin Neurosci* 2008; 575, 607.
- Fleury V, Kleinig TJ, Thompson PD, Ravindran J. Cardio-embolic cerebellar stroke secondary to mitral valve chordae rupture as a delayed complication of a high-voltage electrical injury. *J Clin Neurosci.* 2008 ;15:210-2.
- Kleinig T, Thompson PD (2008). A woman with recurrent ataxia and facial myoclonus. In: *Movement Disorders: 100 Instructive cases.* Edited by S Reich. Informa Healthcare, London, 171-173.
- Kleinig TJ. Reply to: Health effects of war are devastating: pre-emptive war demands pre-emptive criticism by the medical profession. *Intern Med J* 2007; 37: 585.
- Kleinig TJ, Kimber T, Thompson PD. Acute encephalopathy as the initial symptom of CADASIL. *Intern Med J.* 2007 ;37:786-7.

Accepted, publication pending

- Field DK, Kleinig TJ, Thompson PD, Kimber TE, Kleinig TJ. Reversible cerebral vasoconstriction, internal carotid artery dissection and renal artery stenosis. *Cephalalgia.*

Acknowledgements

Although, by definition, a PhD thesis must be primarily the work of the candidate, it goes almost without saying the research detailed subsequently could never have occurred, had it not been for the innumerable contributions, past and present, of many colleagues, friends and family members.

I owe an immense debt of gratitude to Professor Bob Vink, primary supervisor, head of the research group and of the School of Medical Sciences, who took a great risk in accepting into his laboratory a head-strong and overly-opinionated Medico with no experience in laboratory research. Thankyou for supporting me through the early (and not so early!) stages of project choice, and for allowing me a great deal of independence, while always being ready to chat when needed and intervene when warranted.

My second supervisor, Professor Peter Blumbergs, I have known and respected since starting my neurology training, and having worked with him for the last few years has only served to elevate further my stratospherically high regard for him. You are a walking textbook, a sage philosopher, an independent and iconoclastic thinker and a man of great integrity and personal kindness.

Many of the research techniques in this thesis could not have been implemented or developed without the assistance of Dr Stephen Helps, whose surname could not be more apt. Always ready to jump to the aid of anyone in the Lab, his influence on the research quality and also the generally supportive and encouraging atmosphere of the Lab is immense. I value greatly your quirky intellect, fresh perspective and stimulating conversation (and your wife's apple pies).

Jim Manavis, head of the Laboratory and human compendium of all things histopathological, has likewise been of incalculable assistance. Similarly encouraging and supportive, his can-do attitude contributes significantly to the success of the research group. My gratitude also extends to Sven, Sophie, the Kathies, Yvonne, Mark, Glynn and John for their numerous little and not-so-little acts of assistance and kindness along the way. The help of Alan, Sandy, Ghafar and Sook-Chin with confocal microscopy and the Nanozoomer is likewise greatly appreciated.

My fellow researchers in Team Neuro have contributed in many ways to this thesis, to the theoretical underpinning (especially Renee Turner, James Donkin and Emma Thornton), the practical techniques and also to the provision of personal and emotional support. Special thanks go to Naomi Cook for the PCR work contained in this thesis and to Christine Barry for innumerable theoretical discussions. I am grateful likewise to the other current PhD students, Frances, Kate, Liz, Anna, Levon, and the many honours/masters degree students for their help, both practical and theoretical, and for working around the inflexibility impressed by my other professional and personal commitments.

Thanks are also due to the RAH Dawes' Foundation, the NHMRC and National Heart foundation, Pfizer Neuroscience Research Grants and the Neurosurgical Research Foundation, all of whom have provided funding for living expenses, conference travel and research costs.

In general terms I owe a great deal to my father, John, and my recently-deceased and very much missed Papa William Bentley for embodying the virtues of scholarship. Dad and my mother, Claire, were exemplary parents and any of their children's strengths are in large measure inherited.

My greatest debt of appreciation is owed to my immediate family, my sons Oliver and Oscar, my daughter Allegra and, most especially, my wife Pagan. Looking after a family of five is a challenge in itself and doing this while married to a doctor doing research doubly so. However, doing all this while working yourself, subspecialising in Radiology and renovating a house is an astonishing feat, little appreciated (but not by me). I cannot begin to imagine how impoverished my existence would be without your love, hard work and thoughtfulness, and hope that in this new phase of our life we will have more time to stop and sniff the gardenias, my hayfever notwithstanding.

Abbreviations

ACE	Angiotensin converting enzyme
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
Bp	Base pairs
BSA	Bovine serum albumin
CGRP	Calcitonin gene related peptide
cICH	collagenase ICH
CNS	Central nervous system
COX-2	Cyclooxygenase-2
CSF	Cerebrospinal fluid
Ct	Cycle threshold
DAB	3,3' Diaminobenzidine
DAMP	Damage-associated molecular patterns
DMFA	Dimethylformamide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EB	Evans blue
EDTA	Ethylenediaminetetraacetic acid
FJC	Fluoro-jade C
GFAP	Glial fibrillary Acidic Protein
H&E	Haematoxylin and eosin
HMGB-1	High mobility group box-1
HO	Haem oxygenase
IBA-1	Ionized calcium binding adaptor molecule-1
ICAM-1	Intercellular adhesion molecule-1
ICH	Intracerebral haemorrhage
IR (-IR)	Immunoreactivity
IVH	Intraventricular haemorrhage
LFA-1	Leucocyte function antigen-1
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MPOX	Myeloperoxidase
mRNA	messenger ribonucleic acid
MRI	Magnetic resonance imaging
NADA	N-arachidonoyl-dopamine
NAT	N-acetyl-L-tryptophan
NBF	Neutral buffered formalin
NEP	Neutral endopeptidase

NF- κ B	Nuclear factor-kappa B
NK	Neurokinin
NK1R	Neurokinin-1 receptor
NK1RA	Neurokinin-1 receptor antagonist
NO	Nitric oxide
NPC	Neural progenitor cells
NS	Not significant
NSC	Neural stem cells
PAR	Proteinase-activated receptor
PBS	Phosphate buffered saline
PET	Positron emission tomography
pMCAO	permanent middle cerebral artery occlusion
PPAR- γ	Peroxisome proliferator-activated receptor-gamma
PPTA	Pre-protachykinin A
RANTES	Regulated on activation, normal T-cell expressed and secreted
RAGE	Receptor for advanced glycosylation end-products
RGB	Red green blue
ROS	Reactive oxygen species
RNA	Ribonucleic acid
SD	Standard deviation
SEM	Standard error of the mean
siRNA	Small interfering ribonucleic acid
SP	Substance P
STAIR	Stroke academic industry roundtable
SVZ	Subventricular zone
TAC-1 (-2, -3, -4)	Tachykinin-1 (-2, -3, -4)
TBI	Traumatic brain injury
TBS	TRIS-buffered saline
TGF- β	Transforming growth factor- β
TLR	Toll-like receptor
TMB	3,3,5,5-Tetramethylbenzidine
tMCAO	transient middle cerebral artery occlusion
TNF- α	Tumour necrosis factor-alpha
tPA	tissue plasminogen activator
TPRV-1	Transient potential receptor vanilloid 1
TUNEL	Terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling

Table of Figures

Figure	Title	Page
Figure 1	Typical ICH locations	3
Figure 2	Atrophy following ICH	6
Figure 3	Progressive oedema following ICH	7
Figure 4	Secondary injury mechanisms following ICH	9
Figure 5	Main constituents of the blood-brain barrier	11
Figure 6	Blocking thrombin reduces oedema following ICH (Xi <i>et al</i> 1998)	16
Figure 7	Probable and proven causes and consequences of post-ICH inflammation	19
Figure 8	Transcription and translation of tachykinins	26
Figure 9	Peripheral inflammatory actions and triggers of SP release	28
Figure 10	Rotarod	42
Figure 11	Sticky label test	43
Figure 12	Tapered ledged beam	44
Figure 13	Vibrissae elicited stimulation test	45
Figure 14	Elevated drag test	46
Figure 15	Brain sectioning	48
Figure 16	Evans Blue	52
Figure 17	Correlation of DABwt% with antibody dilution and incubation time	57
Figure 18	Intrastratial thrombin causes a progressive increase in albumin immunoreactivity	57
Figure 19	Semi-quantitation by colour deconvolution: albumin immunoreactivity post-thrombin injection	58
Figure 20	Variations in processing technique alter levels of SP-IR	59
Figure 21	Adjusting semi-quantitated deconvolved images by reference to a control area reduces errors introduced by staining variability	60
Figure 22	Automated cell counting of Fluoro-Jade C-positive neurons	61
Figure 23	Microdissection of areas for SP image analysis	65
Figure 24	Haematoma volumes following autologous and collagenase ICH	68
Figure 25	H&E staining of collagenase ICH	69
Figure 26	SP immunostaining 5 hours after collagenase ICH	70
Figure 27	SP immunostaining, collagenase ICH at later timepoints	71
Figure 28	SP immunostaining following collagenase ICH, semi-quantitated	72
Figure 29	NK1R following collagenase ICH	73
Figure 30	Fluoro-Jade C staining following collagenase ICH	73
Figure 31	Myeloperoxidase staining post-collagenase ICH	74
Figure 32	Macrophage/activated microglia response following collagenase	75

	ICH	
Figure 33	Astrocytic response following collagenase ICH	76
Figure 34	Albumin immunostaining following collagenase ICH	77
Figure 35	Double labelling post-collagenase ICH localises increased SP immunostaining to astrocytes	78
Figure 36	Double labelling post-collagenase ICH localises increased NK1R immunostaining to astrocytes	79
Figure 37	H&E staining following autologous ICH	81
Figure 38	SP immunostaining following autologous ICH	82
Figure 39	SP immunostaining following autologous ICH, semi-quantitated	83
Figure 40	NK1R immunostaining 24 hours after autologous ICH	84
Figure 41	Fluoro-Jade C staining following autologous ICH	84
Figure 42	Myeloperoxidase immunostaining following autologous ICH	85
Figure 43	Monocyte/microglial responses following autologous ICH and leucocyte quantification (both models)	86
Figure 44	Astrocytic response following autologous ICH	87
Figure 45	Albumin immunostaining following autologous ICH	88
Figure 46	SP ELISA post-collagenase ICH	89
Figure 47	SP and NK1R mRNA following collagenase ICH	90
Figure 48	Pilot behavioural tests post-collagenase ICH	91
Figure 49	The effect of NK1R antagonisms on 24 hour haematoma volumes post-collagenase ICH	99
Figure 50	Effect of NK1 receptor antagonists on lesion volumes	100
Figure 51	The effect of NK1R antagonism on H&E staining after collagenase ICH	101
Figure 52	Fluoro-Jade C staining, astrocyte response and leucocyte response following NK1R antagonism	102
Figure 53	The effect of NK1R antagonism on SP immunostaining and cell counts (quantified).	103
Figure 54	The effect of NK1R antagonism on cellular proliferation in the subventricular zone	104
Figure 55	Brain oedema following treatment with NK1R antagonists	105
Figure 56	The effect of NK1R antagonism on BBB disruption	106
Figure 57	Functional changes post-ICH in animals treated with NAT or vehicle	107
Figure 58	Functional changes post-ICH in animals treated with L733,060 or vehicle	108
Figure 59	EB extravasation - pilot	115
Figure 60	Lesion size following high- and low-dose intracerebral thrombin	117
Figure 61	Thrombin oedema dose-response	118
Figure 62	H&E staining following intracerebral thrombin injections	119

Figure 63	SP immunostaining following intracerebral thrombin injections	120
Figure 64	SP immunostaining following intracerebral thrombin injections, semi-quantitated	121
Figure 65	Semiquantitative comparison of ipsilateral SP immunostaining following thrombin injection and collagenase ICH	122
Figure 66	Fluoro-Jade C staining following thrombin injections	122
Figure 67	Neutrophilic infiltrate (myeloperoxidase immunostaining) following thrombin injections	123
Figure 68	Macrophage/activated microglia response following intracerebral thrombin injections	124
Figure 69	Astrogliosis following intracerebral thrombin injections	125
Figure 70	Albumin staining following intracerebral thrombin injections	126
Figure 71	Double labelling post-thrombin injection localises increased SP immunostaining to astrocytes	126
Figure 72	NAT fails to reduce oedema after injecting 5U intracerebral thrombin	127
Figure 73	L733,060 fails to reduce oedema after injecting 1 and 3U intracerebral thrombin	128
Figure 74	Evans Blue extravasation 4-8 hours after thrombin 1U and L733,060 or vehicle	129
Figure 75	Dose finding studies for intracerebral substance P injections	136
Figure 76	H&E staining following intracerebral substance P injections	137
Figure 77	Fluoro-Jade C staining following intracerebral substance P injections	138
Figure 78	Substance P immunostaining following intracerebral substance P injections	139
Figure 79	Leucocyte infiltration/activation following intracerebral substance P injections	140
Figure 80	Astrocyte response following intracerebral substance P injections	141
Figure 81	Albumin immunostaining following intracerebral substance P injections	142
Figure 82	Brain oedema following intracerebral substance P injections	142
Figure 83	Blood-brain barrier dysfunction following intracerebral substance P injections	143
Figure 84	Brain oedema after autologous ICH and the effect of an NK1R antagonist	149
Figure 85	Brain oedema in splenectomised collagenase ICH rats and the effect of an NK1R antagonist	150