

**THE ROLE OF SUBSTANCE P IN EARLY EXPERIMENTAL
PARKINSON'S DISEASE**

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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, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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PUBLICATIONS AND PRESENTATIONS

The following articles plus lodged patent 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.

Patent

Thornton E, Vink R. (Applicant: University of Adelaide) Method for Treating Dopaminergic Cell Death. Australian Provisional Patent Application (Filed February, 2008). PCT to be lodged February, 2009.

Publications

Thornton E, Vink, R, Blumbergs PC and Van Den Heuvel C. (2006) Soluble amyloid precursor protein α reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Research*, 1094, p38-46.

Van Den Heuvel C, **Thornton E** and Vink R. (2007) Traumatic brain injury and Alzheimer's disease: A review. *Progress in Brain Research*, 161, p303-16.

Abstracts

Thornton E and Vink R. (2007) Chronic Captopril treatment accelerates injury in an early stage rat model of Parkinson's disease. *Movement Disorders (Suppl)*, 22(16) pS230.

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Finally, I wish to respectfully acknowledge the sacrifice of animal life, as without them we could not have acquired the greater knowledge into Parkinson's disease.

ABBREVIATIONS

3-MT	3-methoxytyramine
5-HT	serotonin
6-OHDA	6-hydroxydopamine
AA	arachidonic acid
ACE	angiotensin converting enzyme
ACh	acetylcholine
AChE	acetylcholine esterase
AD	Alzheimer's disease
ADH	alcohol dehydrogenase
AEA	anandemide
AIF	apoptosis-inducing factor
Ang II	angiotensin II
ANOVA	analysis of variance
AP	anterior-posterior
AT1	angiotensin II type 1
ATP	adenosine triphosphate
BBB	blood brain barrier
BDNF	brain derived growth factor
BG	basal ganglia
BSA	bovine serum albumin
Ca ²⁺	calcium
CaCl	calcium chloride
cAMP	cyclic adenosine monophosphate
CGRP	calcitonin gene-related peptide

CNS	central nervous system
COMT	catechol- σ -methyltransferase
CREB	cAMP binding protein
CSF	cerebrospinal fluid
DA	dopamine
DAB	3,3'-diaminobenzidine
DAT	dopamine transporter
DNA	deoxyribose nucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
Dyn	dynorphin
ED-1	anti-CD-68 antibody
EDTA	ethylenediametetraacetic acid
ELISA	enzyme-linked immunosorbent assay
Enk	enkephalin
Fe ²⁺	iron
Fe ³⁺	ferritin
FGF	fibroblast growth factor
FR	free radical
G	gauge
GABA	γ -aminobutyric acid
GBSS	Gey's balanced salt solution
GDNF	glial derived growth factor
GFAP	glial fibrillary associated protein
GP	globus pallidus
GPe	globus pallidus external

GPI	globus pallidus internal
GSH	glutathione
HD	Huntingdon's disease
H&E	haematoxylin and eosin
H ₂ O ₂	hydrogen peroxide
hr	hour
HRP	horseradish peroxidase
H ₂ SO ₄	sulphuric acid
HVA	homovanillic acid
i.c.v	intracerebroventricular
IL-1 β	interleukin-1 β
IL-2	interleukin-2
IL-6	interleukin-6
iNOS	inducible NOS
IP ₃	inositol 1,4,5-triphosphate
K ⁺	potassium
K ₂ HPO ₄	potassium phosphate
LB	Lewy body
LDH	lactate dehydrogenase
L-DOPA	levadopa
MAO	monoamine oxidase
MFB	medial forebrain bundle
Mg ²⁺	magnesium
mGluR	metabotropic glutamate receptors
MgSO ₄	magnesium sulphate

ML	medial-lateral
mNSS	modified Neurological Severity Score
MPDP+	1-methyl-1-4-phenyl-1,2,3-dihydropyridinium
MPP+	1-methyl-1-4-phenylpyridinium
MPTP	1-methyl-1-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
MSN	medium spiny neuron
Na ⁺	sodium
NaCl	sodium chloride
NADA	N-archidonoyldopamine
NAT	N-acetyl-L-tryptophan
NEP	neutral endopeptidase
NGF	nerve growth factor
NHS	normal horse serum
NK ₁	neurokinin 1
NK ₂	neurokinin 2
NK ₃	neurokinin 3
NKA	neurokinin A
NKB	neurokinin B
NM	neuromelanin
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthetase
nNOS	neuronal nitric oxide
NPK	neuropeptide K

NP γ	neuropeptide gamma
NSAID	non-steroidal anti-inflammatory drug
O ₂	oxygen
OH	hydroxyl radical
ONOO ⁻	peroxynitrite
PBS	phosphate buffered solution
PD	Parkinson's disease
PLA ₂	phospholipase A ₂
PLC _B	phospholipase C _B
PNS	peripheral nervous system
PMSF	phenylmethanesulfonylfluoride
PPT	preprotachykinin
PPTg	pedunculopontine tegmental nucleus
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
rpm	revolutions per minute
SEM	standard error of the mean
SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticula
SOD	superoxide dismutase
SP	substance P
SPC	streptavidin peroxidase conjugate
STN	subthalamic nucleus

TBI	traumatic brain injury
TBS	tris-buffered saline
TH	tyrosine hydroxylase
TMB	3,3'-5,5'-tertamethylbenzadine
TNF- α	tumour necrosis factor- α
TNF- α R1	tumour necrosis factor- α receptor 1
TRPV1	transient receptor potential vanilloid 1
VIP	vasoactive intestinal peptide
VM	ventral mesencephalon
VMAT	vesicular monoamine transporters
VTA	ventral tegmental area

TABLE OF CONTENTS

DECLARATION.....	II
PUBLICATIONS AND PRESENTATIONS.....	III
ACKNOWLEDGEMENTS.....	IV
ABBREVIATIONS.....	VII
TABLE OF CONTENTS.....	XIII
LIST OF FIGURES AND TABLES	XXII
ABSTRACT	XXX

CHAPTER 1: INTRODUCTION

1.1 EPIDEMIOLOGY OF PARKINSON'S DISEASE	3
1.2 THE BASAL GANGLIA	3
1.2.1 STRIATUM.....	4
1.2.2 SUBSTANTIA NIGRA	6
1.2.3 THE GLOBUS PALLIDUS	7
1.2.4 THE SUBTHALAMIC NUCLEUS	7
1.2.5 NEUROTRANSMITTERS IN THE BASAL GANGLIA.....	7
<i>Dopamine</i>	8
<i>Glutamate</i>	10
<i>GABA</i>	10
<i>Substance P</i>	11
<i>Neurokinin A</i>	12
<i>Acetylcholine</i>	13
<i>Enkephalin and Dynorphin</i>	13
1.2.6 THE DIRECT AND INDIRECT SIGNALLING PATHWAYS OF THE BASAL GANGLIA.....	14

1.4 MECHANISMS OF DOPAMINERGIC CELL LOSS IN PD	19
1.4.1 OXIDATIVE STRESS	19
1.4.2 MITOCHONDRIAL DYSFUNCTION	21
1.4.3 GLUTAMATE EXCITOTOXICITY	22
1.4.4 INFLAMMATORY PROCESSES	23
1.4.5 APOPTOSIS	24
1.4.6 LEWY BODIES	25
1.4 SYMPTOMS OF PARKINSON'S DISEASE.....	26
1.4.1 MEASURE OF OUTCOME IN PD	27
1.5 PARKINSON'S DISEASE RISK FACTORS AND PREVENTION.....	27
1.6 L-DOPA THERAPY IN PARKINSON'S DISEASE.....	28
1.7 EXPERIMENTAL MODELS OF PARKINSON'S DISEASE.....	29
1.7.1 CULTURE MODELS OF PD	30
1.7.2 6-OHDA MODELS	30
1.7.3 MPTP MODELS	32
1.7.4 ROTENONE MODEL	33
1.7.5 TRANSGENIC AND KNOCKOUT MICE MODELS OF PD	34
1.8 SUBSTANCE P.....	34
1.8.1 TACHYKININ RECEPTORS.....	35
1.8.2 SYNTHESIS AND METABOLISM OF SP.....	36
1.8.3 DISTRIBUTION OF SP.....	38
1.8.4 FUNCTIONS OF SP.....	39
<i>Contractile Properties of SP</i>	40
<i>Nociception</i>	40
<i>Neuronal Growth and Protection</i>	40
<i>Neurogenic Inflammation</i>	41
<i>Behavioural Effects of SP</i>	42
<i>Programmed Cell Death</i>	42

<i>NK₁</i> antagonists	43
1.9 PREVIOUS STUDIES OF SUBSTANCE P IN PARKINSON'S DISEASE	43
1.9.1 HUMAN STUDIES	43
1.9.2 ANIMAL STUDIES	44
1.10 SYNOPSIS	45
<u>CHAPTER 2: MATERIALS AND METHODS</u>	
2.1 ANIMALS	48
2.1.1 ETHICS	48
2.1.2 ANIMAL PREPARATION	48
2.2 EXPERIMENTAL PROCEDURES.....	48
2.2.1 ORGANOTYPIC SLICE CULTURE.....	48
<i>Dissection of the Ventral Mesencephalon and Striatum</i>	49
<i>Mounting the Slices</i>	50
<i>Maintenance of Cultures</i>	51
<i>Treatment of Cultures</i>	51
<i>Fixation of Cultures and Immunocytochemistry for Tyrosine Hydroxylase</i>	52
2.2.2 6-OHDA RODENT MODEL OF THE EARLY STAGES PARKINSON'S DISEASE.....	52
<i>Anaesthesia</i>	53
<i>Induction of Experimental Parkinson's Disease</i>	54
<i>Post-Surgery Recovery</i>	55
2.2.4 PERFUSION FIXATION.....	55
2.3 DRUG PREPARATION.....	56
2.4 FUNCTIONAL ASSESSMENT.....	58
2.4.1 ROTAROD.....	59
2.4.2 BILATERAL ASYMMETRY TEST	61
2.4.3 STEPPING TESTS	61

2.4.5 MODIFIED NEUROLOGICAL SEVERITY SCORE	66
2.4.6 OPEN FIELD	68
2.4.7 ROTOMETER	70
2.5 HISTOLOGICAL ANALYSIS.....	72
2.5.1 PERFUSION FIXATION AND BRAIN SAMPLING.....	72
2.5.2 HAEMATOXYLIN & EOSIN STAINING	72
2.5.3 IMMUNOHISTOCHEMISTRY FOR TYROSINE HYDROXYLASE	73
2.5.4 IMMUNOHISTOCHEMISTRY FOR SUBSTANCE P.....	74
2.5.5 IMMUNOCYTOCHEMISTRY FOR GLIAL FIBRILLARY ASSOCIATED PROTEIN	74
2.5.6 IMMUNOCYTOCHEMISTRY FOR ED-1	75
2.5.7 IMMUNOCYTOCHEMISTRY FOR ALBUMIN	75
2.6 ELISA	76
2.6.1 PREPARATION OF BRAIN HOMOGENATE	76
2.6.2 PROTEIN ESTIMATION ASSAY	77
2.6.3 ELISA FOR SP.....	78
2.6.4 ELISA FOR LACTATE DEHYDROGENASE.....	78
2.7 STATISTICAL ANALYSIS	79
<u>CHAPTER 3: THE EFFECTS OF SP AND THE NK₁ RECEPTOR ANTAGONIST, N-ACETYL-L-TRYPTOPHAN, IN AN <i>IN VITRO</i> MODEL OF PARKINSON'S DISEASE</u>	
3.1 INTRODUCTION	81
3.2 STUDY DESIGN	82
3.2.1 ELISA.....	83
3.2.2 IMMUNOHISTOCHEMISTRY FOR TH	83
3.2.3 STATISTICAL ANALYSIS.....	83
3.3 RESULTS.....	84
3.3.1 PRESENCE OF DOPAMINERGIC NEURONS IN CULTURE	84
3.3.2 SP CONTENT FOLLOWING DIFFERENT 6-OHDA TREATMENT TIMES.....	84

3.3.3 SP CONTENT FOLLOWING 6-OHDA EXPOSURE COMBINED WITH SP OR NAT	85
3.3.4 LDH CONTENT FOLLOWING 6-OHDA TREATMENT COMBINED WITH SP OR NAT	89
3.3.5 COMPARISON OF SP AND LDH EXPRESSION.....	92
3.4 DISCUSSION	94
3.5 CONCLUSIONS	98

CHAPTER 4: CHARACTERISATION OF THE INTRASTRIATAL 6-OHDA MODEL OF EARLY PARKINSON’S DISEASE

4.1 INTRODUCTION	101
4.2 STUDY DESIGN	102
4.2.1 FUNCTIONAL OUTCOME.....	102
4.2.2 HISTOLOGICAL OUTCOME	103
4.2.3 ELISA ASSAY	103
4.2.4 STATISTICAL ANALYSIS.....	103
4.3 RESULTS.....	103
4.3.1 FUNCTIONAL OUTCOME.....	103
<i>Motor Outcome – Rotarod</i>	<i>103</i>
<i>Motor Outcome – Stepping Tests.....</i>	<i>104</i>
<i>Motor Outcome - Bilateral Asymmetry Test</i>	<i>115</i>
<i>Neurological Outcome – mNSS.....</i>	<i>115</i>
<i>Behavioural Outcome – Open Field.....</i>	<i>119</i>
<i>Estimation of Lesion Size – Rotometer.....</i>	<i>119</i>
4.3.2 HISTOLOGICAL OUTCOME	121
<i>Dopaminergic Response: TH Immunocytochemistry</i>	<i>121</i>
<i>General Pathology - H&E.....</i>	<i>135</i>
<i>Substance P Response – SP Immunohistochemistry / SP ELISA.....</i>	<i>140</i>
<i>Astrocytic Response – GFAP Immunohistochemistry</i>	<i>146</i>
<i>Activated Microglial Response – ED-1 Immunohistochemistry.....</i>	<i>153</i>

<i>Inflammatory Response in the Striatum Surrounding the Needle Tract</i>	158
<i>Blood Brain Barrier Dysfunction – Albumin Immunohistochemistry</i>	158
4.4 DISCUSSION	161
4.5 CONCLUSIONS	171
<u>CHAPTER 5: THE EFFECTS OF CAPTOPRIL IN THE INTRASTRIATAL 6-OHDA</u>	
<u>MODEL OF EARLY PARKINSON’S DISEASE</u>	
5.1 INTRODUCTION	173
5.2 STUDY DESIGN	174
5.2.1 FUNCTIONAL OUTCOME.....	175
5.2.2 HISTOLOGICAL ANALYSIS	175
5.2.3 STATISTICAL ANALYSIS.....	176
5.3 RESULTS	176
5.3.1 FUNCTIONAL OUTCOME.....	176
<i>Motor Function - Rotarod</i>	176
<i>Motor Function - Stepping Tests</i>	178
<i>Motor Outcome – Bilateral Asymmetry Test</i>	186
<i>Neurological Outcome - mNSS</i>	189
<i>Behavioural Outcome – Open Field</i>	189
<i>Estimation of Lesion Size - Rotometer</i>	192
5.3.2 HISTOLOGICAL OUTCOME	194
<i>Dopaminergic Response: TH Immunohistochemistry</i>	194
<i>General Pathology – H&E</i>	199
<i>Substance P Response – SP Immunohistochemistry</i>	205
<i>Astrocytic Response – GFAP Immunohistochemistry</i>	210
<i>Activated Microglial Response – ED-1 Immunohistochemistry</i>	215
<i>Blood Brain Barrier Dysfunction – Albumin Immunohistochemistry</i>	220
5.4 DISCUSSION	222

5.5 CONCLUSIONS	229
------------------------------	------------

**CHAPTER 6: EFFECTS OF NEUROPEPTIDE DEPLETION WITH CAPSAICIN IN THE
INTRASTRIATAL 6-OHDA MODEL OF EARLY PARKINSON'S DISEASE**

6.1 INTRODUCTION	231
-------------------------------	------------

6.2 STUDY DESIGN	232
-------------------------------	------------

6.2.1 FUNCTIONAL OUTCOME.....	233
-------------------------------	-----

6.2.2 HISTOLOGICAL ANALYSIS	233
-----------------------------------	-----

6.2.3 STATISTICAL ANALYSIS.....	233
---------------------------------	-----

6.3 RESULTS.....	234
-------------------------	------------

6.3.1 FUNCTIONAL OUTCOME.....	234
-------------------------------	-----

<i>Motor Function – Rotarod</i>	<i>234</i>
---------------------------------------	------------

<i>Motor Function – Stepping Tests</i>	<i>234</i>
--	------------

<i>Motor Outcome – Bilateral Asymmetry Test.....</i>	<i>241</i>
--	------------

<i>Neurological Outcome – mNSS.....</i>	<i>242</i>
---	------------

<i>Behavioural Outcome – Open Field</i>	<i>248</i>
---	------------

<i>Estimation of Lesion Size – Rotometer</i>	<i>248</i>
--	------------

6.3.2 HISTOLOGICAL OUTCOME	251
----------------------------------	-----

<i>Dopaminergic Response: TH Immunocytochemistry</i>	<i>251</i>
--	------------

<i>General Pathology – H&E.....</i>	<i>254</i>
---	------------

<i>Substance P Response – SP Immunohistochemistry.....</i>	<i>258</i>
--	------------

<i>Astrocytic Response – GFAP Immunohistochemistry</i>	<i>258</i>
--	------------

<i>Activated Microglial Response – ED-1 Immunohistochemistry</i>	<i>261</i>
--	------------

6.4 DISCUSSION	266
-----------------------------	------------

6.5 CONCLUSIONS	273
------------------------------	------------

CHAPTER 7: THE EFFECTS OF SP AND AN NK₁ ANTAGONIST, N-ACETYL-L-TRYPTOPHAN, IN THE INTRASTRIATAL 6-OHDA MODEL OF EARLY PARKINSON'S DISEASE

7.1 INTRODUCTION	275
7.2 STUDY DESIGN	276
7.2.1 FUNCTIONAL OUTCOME.....	276
7.2.2 HISTOLOGICAL OUTCOME	277
7.2.3 ELISA.....	277
7.2.4 STATISTICAL ANALYSIS.....	277
7.3 RESULTS.....	278
7.3.1 FUNCTIONAL OUTCOME.....	278
<i>Motor Function - Rotarod</i>	278
<i>Motor Function – Stepping Tests</i>	278
<i>Motor Outcome – Bilateral Asymmetry Test</i>	286
<i>Neurological Outcome – mNSS</i>	289
<i>Behavioural Outcome – Open Field</i>	293
<i>Estimation of Lesion Size – Rotometer</i>	295
7.3.2 HISTOLOGICAL OUTCOME	297
<i>Dopaminergic Response: TH Immunocytochemistry</i>	297
<i>General Pathology – H&E</i>	305
<i>Substance P Response – SP Immunohistochemistry and ELISA</i>	308
<i>Astrocytic Response – GFAP Immunohistochemistry</i>	314
<i>Activated Microglial Response – ED-1 Immunohistochemistry</i>	319
7.4 DISCUSSION	324
7.5 CONCLUSIONS	332

CHAPTER 8: COMPARISON OF THE EFFECTS OF NK₁ ANTAGONISTS, L-333,060 AND N-ACETYL-L-TRYPTOPHAN, IN THE INTRASTRIATAL 6-OHDA MODEL OF EARLY PARKINSON'S DISEASE

8.1 INTRODUCTION	334
8.2 STUDY DESIGN	335
8.2.1 FUNCTIONAL OUTCOME.....	335
8.2.2 HISTOLOGICAL OUTCOME	336
8.2.3 STATISTICAL ANALYSIS.....	336
8.3 RESULTS.....	336
8.3.1 FUNCTIONAL OUTCOME.....	336
<i>Motor Function – Rotarod</i>	<i>336</i>
<i>Motor Function – Stepping Tests</i>	<i>337</i>
<i>Motor Outcome – Bilateral Asymmetry Test.....</i>	<i>347</i>
<i>Neurological Outcome – mNSS.....</i>	<i>350</i>
<i>Estimation of Lesion Size – Rotometer</i>	<i>354</i>
8.3.2 HISTOLOGICAL OUTCOME	354
<i>Dopaminergic Response – TH Immunohistochemistry</i>	<i>354</i>
<i>General Pathology – H&E.....</i>	<i>361</i>
<i>Substance P Response – SP Immunohistochemistry.....</i>	<i>367</i>
<i>Astrocytic Response – GFAP Immunohistochemistry</i>	<i>367</i>
<i>Activated Microglial Response – ED-1 Immunohistochemistry</i>	<i>372</i>
8.5 CONCLUSIONS	390

CHAPTER 9: GENERAL DISCUSSION

9.1 CONCLUSIONS	408
------------------------------	------------

REFERENCES.....	410
------------------------	------------

LIST OF FIGURES AND TABLES

<i>Figure 1.1: Simplified flow diagram of the major basal ganglia pathways.</i>	<i>5</i>
<i>Figure 1.2: Synthesis and metabolism of dopamine.</i>	<i>9</i>
<i>Figure 1.3: The direct and indirect signalling pathways within the basal ganglia.</i>	<i>15</i>
<i>Figure 1.4: Dopamine influence on the direct and indirect basal ganglia signalling pathways.</i>	<i>18</i>
<i>Figure 1.5: Structure of the three mammalian tachykinins. Adapted from (Hokfelt et al., 2001).</i>	<i>35</i>
<i>Figure 2.1: The rotarod.</i>	<i>60</i>
<i>Figure 2.2: The bilateral asymmetry test.</i>	<i>62</i>
<i>Figure 2.3: The stepping tests – stepping time and step length.</i>	<i>63</i>
<i>Figure 2.4: The stepping tests - Initiation time and adjusting step test.</i>	<i>64</i>
<i>Figure 2.5: The open field.</i>	<i>69</i>
<i>Figure 2.6: The rotometer.</i>	<i>71</i>
<i>Figure 2.7: Regions of striatum and SN used in ELISA.</i>	<i>76</i>
<i>Figure 3.1: Cultured dopaminergic neurons.</i>	<i>84</i>
<i>Figure 3.2: 6-OHDA treatment in vitro - SP content following different 6-OHDA treatment times.</i>	<i>85</i>
<i>Figure 3.3: SP content following combined treatment of 6-OHDA with SP or the NK₁ receptor antagonist, NAT.</i>	<i>87</i>
<i>Figure 3.4: 6-OHDA treatment in vitro – LDH content following combined treatment of 6-OHDA with SP or the NK₁ receptor antagonist, NAT.</i>	<i>90</i>
<i>Figure 3.5: Comparison of SP and LDH content.</i>	<i>93</i>
<i>Figure 3.6: Linear regression between SP and LDH content on days 1 and 2.</i>	<i>93</i>
<i>Figure 4.1: Intrastratial 6-OHDA model of PD – Motor outcome as assessed by the rotarod.</i>	<i>105</i>
<i>Figure 4.2: Intrastratial 6-OHDA model of PD – Initiation time as assessed by the stepping tests.</i>	<i>106</i>
<i>Figure 4.3: Intrastratial 6-OHDA model of PD – Stepping time as assessed by the stepping tests.</i>	<i>109</i>
<i>Figure 4.4: Intrastratial 6-OHDA model of PD – Step length as assessed by the stepping tests.</i>	<i>110</i>

<i>Figure 4.5: Intrastriatal 6-OHDA model of PD – Contralateral forepaw adjusting steps as assessed by the stepping tests.</i>	113
<i>Figure 4.6: Intrastriatal 6-OHDA model of PD – Ipsilateral and contralateral latencies as assessed by the bilateral asymmetry test.</i>	116
<i>Figure 4.7: Intrastriatal 6-OHDA model of PD – Neurological outcome as assessed by the modified Neurological Severity Score.</i>	118
<i>Figure 4.8: Intrastriatal 6-OHDA model of PD – Behavioural outcome as assessed by the open field task.</i>	120
<i>Figure 4.9: Intrastriatal 6-OHDA Model of PD – Estimation of Lesion Size as Assessed by the Rotometer.</i>	122
<i>Figure 4.10: Intrastriatal 6-OHDA model of PD – Ipsilateral striatal TH immunoreactivity following 6-OHDA striatal lesions. TH stained sections (Bar = 90µm).</i>	124
<i>Figure 4.11: Intrastriatal 6-OHDA Model of PD – Ipsilateral striatal TH immunoreactivity following 6-OHDA striatal lesions. TH stained sections (Bar = 50µm).</i>	126
<i>Figure 4.12: Intrastriatal 6-OHDA model of PD – Contralateral striatal TH immunoreactivity following 6-OHDA striatal lesions. TH stained sections (Bar = 90µm).</i>	128
<i>Figure 4.13: Intrastriatal 6-OHDA model of PD – Substantia nigra TH immunoreactivity following 6-OHDA striatal lesions. TH stained sections.</i>	130
<i>Figure 4.14: Intrastriatal 6-OHDA model of PD – Ipsilateral substantia nigra TH immunoreactivity following 6-OHDA striatal lesions. TH stained sections (Bar = 200µm).</i>	132
<i>Figure 4.15: Intrastriatal 6-OHDA model of PD – Quantification of TH immunoreactive neurons within the ipsilateral substantia nigra.</i>	134
<i>Figure 4.16: Intrastriatal 6-OHDA model of PD – Cellular pathology of the ipsilateral striatum following 6-OHDA striatal lesions. H&E stained sections (Bar = 90 µm).</i>	136
<i>Figure 4.17: Intrastriatal 6-OHDA model of PD – Cellular pathology of the ipsilateral substantia nigra following 6-OHDA striatal lesions. H&E stained sections (Bar = 50µm).</i>	138
<i>Figure 4.18: Intrastriatal 6-OHDA model of PD – SP immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).</i>	141
<i>Figure 4.19: Intrastriatal 6-OHDA model of PD – SP immunoreactivity delineates the substantia nigra. SP stained section (Bar = 90µm).</i>	143

<u>Figure 4.20:</u> <i>Intrastriatal 6-OHDA model of PD – SP immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).</i>	144
<u>Figure 4.21:</u> <i>Intrastriatal 6-OHDA model of PD – Semi-quantification of SP expression within the striatum and substantia nigra.</i>	147
<u>Figure 4.22:</u> <i>Intrastriatal 6-OHDA model of PD – GFAP immunoreactivity within the striatum following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).</i>	149
<u>Figure 4.23:</u> <i>Intrastriatal 6-OHDA Model of PD – GFAP Immunoreactivity within the substantia nigra following a 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).</i>	151
<u>Figure 4.24:</u> <i>Intrastriatal 6-OHDA model of PD – ED-1 immunoreactivity within the striatum following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).</i>	154
<u>Figure 4.25:</u> <i>Intrastriatal 6-OHDA model of PD – ED-1 immunoreactivity within the substantia nigra following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).</i>	156
<u>Figure 4.26:</u> <i>Intrastriatal 6-OHDA model of PD - Inflammatory response in the striatum surrounding the needle tract. H&E, ED-1, GFAP and SP stained sections (Bar = 90µm).</i>	159
<u>Figure 4.27:</u> <i>Intrastriatal 6-OHDA Model of PD – Blood brain barrier dysfunction as assessed by albumin immunoreactivity.</i>	160
<u>Figure 5.1:</u> <i>Chronic Captopril treatment – Motor function as assessed by the rotarod.</i>	177
<u>Figure 5.2:</u> <i>Chronic Captopril treatment – Initiation of movement as assessed by the stepping tests.</i>	179
<u>Figure 5.3:</u> <i>Chronic Captopril treatment – Stepping time as assessed by the stepping tests.</i>	181
<u>Figure 5.4:</u> <i>Chronic Captopril treatment – Step length as assessed by the stepping tests.</i>	183
<u>Figure 5.5:</u> <i>Chronic Captopril treatment – Contralateral forepaw adjusting steps as assessed by the stepping tests.</i>	184
<u>Figure 5.6:</u> <i>Chronic Captopril treatment – Ipsilateral and contralateral latencies as assessed by the bilateral asymmetry test.</i>	187
<u>Figure 5.7:</u> <i>Chronic Captopril treatment – Neurological outcome as assessed by the modified Neurological Severity Score.</i>	190
<u>Figure 5.8:</u> <i>Chronic Captopril treatment – Behavioural outcome as assessed by the open field task.</i>	191
<u>Figure 5.9:</u> <i>Chronic Captopril treatment – Estimation of lesion size as assessed by the rotometer.</i>	193

<u>Figure 5.10:</u> Chronic Captopril treatment – TH immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. TH stained sections (Bar = 90µm).	195
<u>Figure 5.11:</u> Chronic Captopril treatment – TH immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. TH stained sections (Bar = 50µm).	197
<u>Figure 5.12:</u> Chronic Captopril treatment – TH immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. TH stained sections (Bar = 200µm).....	200
<u>Figure 5.13:</u> Chronic Captopril treatment – Quantification of TH immunoreactive neurons within the ipsilateral substantia nigra.	202
<u>Figure 5.14:</u> Chronic Captopril treatment - H&E stained ipsilateral substantia nigra following 6-OHDA striatal lesions. H&E stained sections (Bar = 50µm).	203
<u>Figure 5.15:</u> Chronic Captopril treatment - SP immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).....	206
<u>Figure 5.16:</u> Chronic Captopril treatment - SP immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).	208
<u>Figure 5.17:</u> Chronic Captopril treatment - GFAP immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).	211
<u>Figure 5.18:</u> Chronic Captopril treatment - GFAP immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).	213
<u>Figure 5.19:</u> Chronic Captopril treatment – ED-1 immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).	216
<u>Figure 5.20:</u> Chronic Captopril treatment – ED-1 immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).	218
<u>Figure 5.21:</u> Chronic Captopril treatment – Albumin immunoreactivity following 6-OHDA striatal lesions. Albumin stained sections.	221
<u>Figure 6.1:</u> Capsaicin treatment – Motor function as assessed by the rotarod.....	235
<u>Figure 6.2:</u> Capsaicin treatment - Initiation of movement as assessed by the stepping tests.....	236
<u>Figure 6.3:</u> Capsaicin treatment – Stepping time as assessed by the stepping tests.	239
<u>Figure 6.4:</u> Capsaicin treatment – Step length as assessed by the stepping tests.	240

<u>Figure 6.5: Capsaicin treatment – Contralateral forepaw adjusting steps as assessed by the stepping tests.</u>	243
<u>Figure 6.6: Capsaicin treatment – Ipsilateral and contralateral latencies as assessed by the bilateral asymmetry test.</u>	245
<u>Figure 6.7: Capsaicin treatment – Neurological outcome as assessed by the modified neurological severity score.</u>	247
<u>Figure 6.8: Capsaicin treatment – Behavioural outcome as assessed by the open field task.</u>	249
<u>Figure 6.9: Capsaicin treatment – Estimation of lesion size as assessed by the rotometer.</u>	250
<u>Figure 6.10: Capsaicin treatment – TH immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 90µm).</u>	252
<u>Figure 6.11: Capsaicin treatment – TH immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 50µm).</u>	253
<u>Figure 6.12: Capsaicin treatment – TH immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 200µm).</u>	255
<u>Figure 6.13: Capsaicin treatment – Quantification of TH immunoreactive neurons within the ipsilateral substantia nigra.</u>	256
<u>Figure 6.14: Capsaicin treatment: H&E stained ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. H&E stained sections (Bar = 50µm).</u>	257
<u>Figure 6.15: Capsaicin treatment – SP immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).</u>	259
<u>Figure 6.16: Capsaicin treatment – SP immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).</u>	260
<u>Figure 6.17: Capsaicin treatment – GFAP immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).</u>	262
<u>Figure 6.18: Capsaicin treatment – GFAP immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).</u>	263
<u>Figure 6.19: Capsaicin treatment – ED-1 immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).</u>	264
<u>Figure 6.20: Capsaicin treatment – ED-1 immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).</u>	265

<i>Figure 7.1: SP and NAT treatment – Motor function as assessed by the rotarod.....</i>	<i>279</i>
<i>Figure 7.2: SP and NAT treatment – Initiation of movement as assessed by the stepping tests.</i>	<i>281</i>
<i>Figure 7.3: SP and NAT treatment – Stepping time as assessed by the stepping tests.</i>	<i>284</i>
<i>Figure 7.4: SP and NAT treatment – Step length as assessed by the stepping tests.</i>	<i>285</i>
<i>Figure 7.5: SP and NAT treatment – Contralateral forepaw adjusting steps as assessed by the stepping tests.</i>	<i>287</i>
<i>Figure 7.6: SP and NAT treatment – Ipsilateral and Contralateral latencies as assessed by the bilateral asymmetry test.....</i>	<i>290</i>
<i>Figure 7.7: SP and NAT treatment – Neurological outcome as assessed by the modified Neurological Severity Score.....</i>	<i>292</i>
<i>Figure 7.8: SP and NAT treatment – Behavioural outcome as assessed by the open field task.....</i>	<i>294</i>
<i>Figure 7.9: SP and NAT treatment – Estimation of lesion size as assessed by the rotometer.....</i>	<i>296</i>
<i>Figure 7.10: SP and NAT treatment – TH immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 90µm).....</i>	<i>298</i>
<i>Figure 7.11: SP and NAT treatment – TH immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 50µm).....</i>	<i>300</i>
<i>Figure 7.12: SP and NAT treatment – TH immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 200µm).....</i>	<i>302</i>
<i>Figure 7.13: SP and NAT treatment – Quantification of TH immunoreactive neurons within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions.</i>	<i>304</i>
<i>Figure 7.14: SP and NAT treatment – H&E stained ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. H&E stained sections (Bar = 50µm).</i>	<i>306</i>
<i>Figure 7.15: SP and NAT treatment – SP immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).</i>	<i>309</i>
<i>Figure 7.16: SP and NAT treatment – SP immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).</i>	<i>311</i>
<i>Figure 7.17: SP and NAT treatment – Quantification of SP content within the striatum and substantia nigra brain regions at day 3 following 6-OHDA striatal lesions.</i>	<i>313</i>

<u>Figure 7.18:</u> SP and NAT treatment – GFAP immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).	315
<u>Figure 7.19:</u> SP and NAT treatment – GFAP immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).	317
<u>Figure 7.20:</u> SP and NAT treatment – ED-1 immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).	320
<u>Figure 7.21:</u> SP and NAT treatment – ED-1 immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).	322
<u>Figure 8.1:</u> Comparison of NK ₁ antagonists L-333,060 and NAT - Motor function as assessed by the rotarod.	338
<u>Figure 8.2:</u> Comparison of NK ₁ antagonists L-333,060 and NAT - Initiation of movement as assessed by the stepping tests.	339
<u>Figure 8.3:</u> Comparison of NK ₁ antagonists L-333,060 and NAT - Stepping time as assessed by the stepping tests.	342
<u>Figure 8.4:</u> Comparison of NK ₁ antagonists L-333,060 and NAT - Step length as assessed by the stepping tests.	343
<u>Figure 8.5:</u> Comparison of NK ₁ antagonists L-333,060 and NAT - Contralateral forepaw adjusting steps as assessed by the stepping tests.	345
<u>Figure 8.6:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – Ipsilateral and contralateral latency scores for sensorimotor function as assessed by the bilateral asymmetry test.	348
<u>Figure 8.7:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – Neurological outcome as assessed by the modified Neurological Severity Score.	351
<u>Figure 8.8:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – Behavioural outcome as assessed by the open field.	353
<u>Figure 8.9:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – Estimation of lesion size as assessed by the rotometer.	355
<u>Figure 8.10:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – TH immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 90µm).	357

<u>Figure 8.11:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – TH immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 50µm).	359
<u>Figure 8.12:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – TH immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. TH stained sections (Bar = 200µm).....	362
<u>Figure 8.13:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – Quantification of TH immunoreactive neurons within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions.	364
<u>Figure 8.14:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – H&E stained ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. H&E stained sections (Bar = 50µm).....	365
<u>Figure 8.15:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – SP immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).....	368
<u>Figure 8.16:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – SP immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. SP stained sections (Bar = 25µm).	370
<u>Figure 8.17:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – GFAP immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm). .	373
<u>Figure 8.18:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – GFAP immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50µm).....	375
<u>Figure 8.19:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – ED-1 immunoreactivity within the ipsilateral striatum at day 21 following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm). .	378
<u>Figure 8.20:</u> Comparison of NK ₁ antagonists L-333,060 and NAT – ED-1 immunoreactivity within the ipsilateral substantia nigra at day 21 following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50µm).....	380
<u>Table 2.1:</u> The modified Neurological Severity Score.....	67

ABSTRACT

Parkinson's disease (PD) is one of the most common motor neurodegenerative diseases, affecting 1-2% of the world's population over the age of 65. It is characterised by a loss of dopamine neurons within the substantia nigra, which is an integral part of the basal ganglia (BG) where dopamine is the most important modulating neurotransmitter. As the BG is primarily involved with the execution of movement, the lack of dopamine input results in dysfunctional motor control. The current PD treatment, L-DOPA, improves these motor symptoms, however only provides patients 5 to 10 years of improved quality of life before debilitating side effects, often worse than the original symptoms, begin.

The neuropeptide substance P (SP) is found in high concentration in the substantia nigra, and BG in general, where it is involved in dopamine release. In the late stages of PD, SP content within the substantia nigra and BG is decreased, thus implicating SP in the pathophysiology of PD. However, SP production has not been examined in the early stages of PD when dopaminergic degeneration is first initiated. This thesis therefore sought to characterise the role of SP in dopaminergic degeneration in an experimental model of early PD, the 6-hydroxydopamine model in rats.

In contrast to the prevailing dogma that a decline in SP is associated with neurodegeneration in PD, this thesis demonstrates that SP is actually increased within the striatum in early PD, particular in perivascular tissue and within surviving dopaminergic neurons during the degenerative process. Increasing exposure of the dopaminergic neurons to SP, either by inhibition of substance P breakdown with Captopril or by direct injection

with SP, exacerbated the disease progression as indicated by more profound neurogenic inflammation, functional deficits and increased dopaminergic cell death. However, when SP was inhibited by treatment with a SP NK₁ receptor antagonist, dopaminergic neurons were conserved, the inflammatory response was reduced and motor function was returned to near normal levels.

We conclude that SP is increased in early PD, and that increased SP plays an important role in the degenerative process, specifically, in the genesis of BBB breakdown and initiation of neurogenic inflammation. Treatment with an NK1 antagonist may thus represent a novel therapeutic approach to early stage Parkinson's disease.