

**STUDIES OF CELL DEATH IN PARKINSON'S
DISEASE USING ORGANOTYPIC CELL
CULTURES**

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DECLARATION

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PUBLICATIONS AND PRESENTATIONS

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

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ABBREVIATIONS

AA	-ascorbic acid
AA(A/D)C	-aromatic l-amino acid decarboxylase
Ab	-antibody
ACh	-acetylcholine
AChE	-acetylcholinesterase
AD	-Alzheimer's disease
AIF	-apoptosis-inducing factor
α -syn	-alpha synuclein
ALDH	-aldehyde dehydrogenase
AMPA	-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPT	-alpha-methyl-p-tyrosine
ANOVA	-analysis of variance
AP	-antero posterior
APTP	-1-amino-4-phenyl-1,2,3,6-tetrahydropyridine
APP+	-1-amino-4-phenyl-pyridinium
ARs	-aldose reductase
ART	-artemin
ATP	-adenosine triphosphate
Bax	- Bcl-2-associated protein X
BDNF	-brain-derived neurotrophic factor
bFGF	-basic fibroblast growth factor

BG	-basal ganglia
BJAB	-B lymphoma cell line
BMPs	-bone morphogenic factors
BSA	-bovine serum albumin
BSS	-balanced salt solution
Ca ²⁺	-calcium
cAMP	-3'-5'-cyclic adenosine monophosphate
CGC	-cerebellar granule cells
CoQ(10)	-coenzyme Q(10)
COMT	-catechol-O-methyl transferase
CNS	-central nervous system
Cu-SOD	-copper superoxide dismutase
cGMP	-Guanosine 3,5-cyclic monophosphate
DA	-dopamine
DAergic	-dopaminergic neurons
D1	-dopamine receptor, type 1
D2	-dopamine receptor, type 2
DAB	-3,3'-diaminobenzidine-Tetrahydrochloride
DAPI	-4,6-diamino-2-phenolindol dihydrochloride
DAT	-dopamine transport
DBS	-deep brain stimulation
DCF	-2',7'-dichlorofluorescin
DIV	-days in vitro

DMEM	-modified basal medium, Eagle
DMSO	-dimethyl sulfoxide
DLBD	-dementia with LB disease
DOPAC	-3,4-dihydroxyphenylacetic acid
DOPAL	-dihydroxyphenylacetaldehyde
DOPET	-3, 4-dihydroxyphenylethanol
DPX	-DePex mounting medium
DTC	-dithiocarbamate
DV	-dorso ventral
E	-embryonic
EDTA	-ethylenediaminetetraacetic acid
ELISA	-enzyme-linked immunosorbent assay
En-1	-engrailed
Enk	-enkephalin
Epo	-erythropoietin
ERKs	-extracellular signal-regulated kinases
ETC	-electron transport chain
FADD	-Fas-associated death domain
FGF8	-fibroblast growth factor-8
FD	-fluorodopa
FJC	-fluoro jade C
Foxa2	-Forkhead box protein A2
FP	-floor plate

FPD	- familial Parkinsons Disease
GABA	-gamma-aminobutyric acid
GADPH	- glyceraldehyde-3-phosphate dehydrogenase
GBSS	-Geys balanced salt solution
Gbx2	-gastrulation brain homeobox 2
GDNF	-glial-derived neurotrophic factor
GDF5	-growth/differentiation factor 5
GFAP	-glial fibrillary acidic protein
GLU	-glutamate
GM-CSF	-granulocyte macrophage colony-stimulating factor
GP	-globus pallidus
GP _e	-external segment of the globus pallidus
GP _i	-internal segment of the globus pallidus
GPx	-glutathione peroxidase
GSSG	-oxidised glutathione
GSH	-glutathione
h/hr	-hour
H&E	-haematoxylin and eosin
HBSS	-Hank's balanced salt solution
HB-EGF	-heparin-binding epidermal growth factor
Hesc	-human embryonic stem cells
HL-60	-human promyelocytic leukemia cell line
HP100	- H ₂ O ₂ -resistant cell line

HRP	-horseradish peroxidase
HT1080	-human fibrosarcoma cell line
H ₂ O ₂	-hydrogen peroxide
H ₂ SO ₄	- sulphuric acid
5-HT	-serotonin
6-OHDA	-6-Hydroxydopamine
Fe ²⁺	-iron
IBMX	-isobutylmethylxanthine
IMVS	-Institute of Medical and Veterinary Science
IFN-γ	-interferon gamma
IL-1β	-interleukin-1β
IL-6	interleukin-6
iNOs	-inducible nitric oxide synthase
IPD	-idiopathic Parkinsons's disease
JNK	-Jun N-terminal kinase
LB	-Lewy body
L-dopa	-levodopa
LDH	-lactate dehydrogenase
Lmx1	-Lim-domain transcription factors
LPS	-lipopolysaccharide
MAPK	-Mitogen-activated protein (MAP) kinases
mDNs	-midbrain/mesencephalic dopaminergic neurons
M	-molar

MAO-B	-monoamine oxidase B
MHB	-mid-hindbrain organizer
MHJ	-midbrain–hindbrain junction
μm	-micromolar
μl	-microlitres
mg	-milligrams
mins	-minutes
MN9D	-midbrain-derived dopaminergic neuronal cell line
Mn-SOD	-magnesium superoxide dismutase
Mn–EBDC	-manganese ethylene-bis-dithiocarbamate
MPDP	-1-methyl-4-phenyl-2,3-dihydropyridinium
MPPC	-1-methyl-4-phenylpyridine
MPPP	-1-methyl-phenyl-propion-oxypiperidine
MPP ⁺	-N-methyl-1-4-phenylpyridinium ion
MPTP	-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Mw	-molecular weight
NA	-noradrenaline
NAC	-N-acetylcysteine
NDI1	-single-subunit nicotinamide adenine dinucleotide (reduced) dehydrogenase of <i>Saccharomyces cerevisiae</i>
NMDA	-N-methyl-D-aspartic acid
NADPH	-Nicotinamide adenine dinucleotide phosphate
NADH	-Nicotinamide adenine dinucleotide

NADH-DH	-NADH-dehydrogenase
3-NT	-3-Nitrotyrosine
n	-number
nM	-nanomolar
NGF	-nerve growth factor
NM	-neuromelanin
NO [•]	-nitric oxide
NTF	-neurotrophic factor
NRT	-neurturin
NSAIDs	-nonsteroidal anti-inflammatory drugs
NT-3	-neurotrophin-3
NT-4/5	-neurotrophin-4/5
NMDA	-N-methyl-D-aspartate
NF- κB	-nuclear factor κB
Nurr1	-Nuclear receptor related 1
O ₂	-oxygen
OH [•]	-hydroxyl radical
•O ₂ ⁻	-superoxide anion radical (superoxide)
ONOO ⁻	-peroxynitrite
OS	-oxidative stress
Otx2	-homeobox protein
PARP	-poly (ADP-ribose) polymerase
Pax-	-paired-like homeodomain proteins

PBS	-phosphate buffered saline
PC12 cells	-rat pheochromocytoma cell line
PD	-Parkinson's disease
P7	-postnatal day 7 (seven day old)
P75	-low affinity neurotrophin receptor
PBS	-phosphate buffered saline
PET	-positron emission tomography
Pitx3	-paired-like homeodomain transcription factor 3
PKC	-Protein Kinase C
PPX	-pramipexole
PSP	-persephin
Ref	-reference
ROI	-reactive oxygen intermediates
ROP	-ropinirole
ROS	-reactive oxygen species
RRF	-retrochubral field
SAPK	-stress-activated protein kinase
SD	-standard deviation
SEM	-standard error of the mean
sFas	- soluble Fas
Shh	- Sonic hedgehog
SH-SY5Y	-human dopaminergic cells
SK-N-SH	-human neuroblastoma cell line

SN	-substantia nigra (A9)
SNpc	-substantia nigra pars compacta
SNpr	-substantia nigra pars reticulate
SOD	-superoxide dismutase
SP	-substance P
SPC	-Pierce peroxidase conjugated streptavidin tertiary
STN	-subthalamic nucleus
ST	-striatum
RPM	-revolutions per minute
TBARS	-thiobabituric acid reactive substances
TBS	-Tris buffered saline
TH	-tyrosine hydroxylase
TH-ir	-tyrosine hydroxylase immunoreactive
TGF- β	-transforming growth factor beta
TMB	-3,3',5,5'-Tetramethylbenzidine
TNF	-tumor necrosis factor
TNF α -R1	- tumor necrosis factor alpha receptor 1
TRADD	-TNFRSF1A-associated via death domain
TUNEL	-terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
Trk	- tyrosine receptor kinase
UV	-ultraviolet
VM	-ventral mesencephalon
VMAT	-vesicular monoamine transporter

VMB	-ventral midbrain
VTA	-ventral tegmental area (A10)
Wnt1	-Wingless-type MMTV integration site family
WT	-wild type
Zn-SOD	-zinc superoxide dismutase
Z-DEVD-fmk	-Caspase-3 Inhibitor
°C	-degrees Celsius

TABLE OF CONTENTS

CHAPTER 1:	1
<i>OVERVIEW OF PARKINSON'S DISEASE</i>	1
1A	
1.0 INTRODUCTION	2
1.1 Incidence and prevalence of PD)	2
1.2 Clinical Characteristics of PD	3
1.3 Neurochemical and neuropathological Features of PD	4
1.3.1 Cell loss in PD.....	6
1.3.2 Lewy Bodies in PD.....	6
1.4 Etiology of PD	6
1.4.1 Environmental Factors.....	6
1.4.2 Genetic Factors.....	9
1.4.3 Ageing and PD development.....	12
1.5 Mechanisms of Parkinsonian cell death	12
1.5.1 Oxidative stress (OS)	12
1.5.2 Iron.....	14
1.5.3 Neuromelanin (NM)	16
1.5.4 Glutathione (GSH)	17
1.5.5 Mitochondrial Dysfunction.....	19
1.5.6 Excitotoxicity.....	20
1.5.7 Nitric Oxide.....	22
1.5.8 Inflammation.....	23
1.6 Cell death in PD	25
1.6.1 Apoptosis.....	25
1.6.2 Apoptotic markers in PD.....	25
1.7 Current treatments and potential therapies for PD	27
1.7.1 Symptomatic Treatment.....	27
1.7.2 Cell transplantation	28

1.7.3 Gene therapy	29
1.7.4 Surgical methods	30
1B	
1.8 BASAL GANGLIA.....	33
1.8.1 Introduction.....	33
1.8.2 Striatum (ST)	34
1.8.2.1 Dopamine receptors (D1R & D2R)	35
1.8.3 Pallidal complex.....	36
1.8.3.1 Entopeduncular nucleus (medial globus pallidus)	36
1.8.3.2 Globus pallidus (lateral globus pallidus)	38
1.8.4 Substantia nigra (SN)	39
1.8.4.1 Substantia nigra pars compacta (SNpc)	39
1.8.4.2 Substantia nigra pars reticulate (SNpr)	40
1.8.5 Subthalamic nucleus.....	41
1.8.6 The basal ganglia circuitary.....	42
1.8.6.1 The direct and indirect pathway model.....	42
1.8.6.2 BG changes in PD.....	44
1 C	
THE NIGROSTRIATAL PATHWAY.....	49
1.9 Introduction.....	49
1.9.1 Ontogeny of Midbrain Dopaminergic neurons (mDA)	49
1.9.2 <i>In vivo</i> development of the nigro-striatal pathway.....	50
1.9.3 <i>In vivo</i> development of patch/matrix innervation.....	51
1.9.4 <i>In vitro</i> modelling of nigro-striatal pathway.....	52
1.10 FACTORS INFLUENCING THE DEVELOPMENT OF MESENCEPHALIC DOPAMINE NEURONS (mDNs)	54
1.10.1 Early development.....	54
1.10.2 Specification of mitotic mesencephalic precursors to the mDN fate.....	55
1.10.3 Postmitotic development of mDNs.....	56

1.10.4 Functional maturation and axonal pathfinding.....	59
1.11 Neurotrophins and the development of mDNs.....	62
1.11.1 Glial-cell-line-derived neurotrophic factor.....	63
1.11.2 GDNF family-ligands.....	64
1.11.2.1 Neurturin.....	65
1.11.2.2 Neublastin/artemin and persephin.....	65
1.11.3 Neurotrophins.....	66
1.11.4 Local factors.....	67
1.11.4.1 Transforming growth factor-beta.....	68
1.11.5 Low molecular weight compounds.....	68
1.11.6 Immunophilin ligands.....	69
1.12 Changes in cytokine levels, decline of essential NTFs and links with PD.....	70
1.12.1 Human evidence.....	70
1.12.2 Evidence from experimental PD models	71
CHAPTER 2:	73
<i>IN VIVO PARKINSONIAN MODELS: MPTP & ROTENONE.....</i>	73
2.0 INTRODUCTION.....	74
2.1 MPTP.....	74
2.2 Biochemistry of MPTP.....	75
2. ANIMAL MODELS OF PD USING MPTP.....	78
2.3.1 Factors influencing the neurotoxic action of MPTP.....	78
2.3.2 Behavioural changes.....	78
2.3.3 Histopathological changes.....	80
2.3.4 Neurochemical changes.....	81
2.3.5 Invertebrates.....	82
2.3.6 Genetic mouse models and MPTP.....	83
2.4 Mechanisms of MPTP neurotoxicity.....	83
2.4.1 Mitochondrial impairment.....	83
2.4.2 Energy Failure.....	85

2.4.3 Calcium homeostasis.....	85
2.4.4 Glutamate release.....	86
2.4.5 Reactive oxygen (ROS) and nitrogen species (NOS)	86
2.4.6 Cytokines and inflammatory processes.....	88
2.4.7 MPTP and apoptosis.....	90
2.5 DA agonists and MPTP.....	92
2.6 ROTENONE.....	95
2.6.1 Introduction.....	95
2.6.2 Biochemistry of rotenone.....	95
2.7 Rotenone-treated animal PD Models.....	96
2.7.1 Rodents.....	96
2.7.2 Invertebrates.....	98
2.8 Rotenone and PD Pathology.....	99
2.9 Rotenone and L-DOPA.....	100
2.10 Mechanisms of Rotenone toxicity.....	100
2.10.1 The Complex 1 Inhibitor.....	100
2.10.2 Rotenone and OS.....	102
2.10.3 Rotenone and Inflammation.....	124
2.10.4 Rotenone and Apoptosis.....	103
2.11 Other Parkinsonian-inducing neurotoxins.....	104
2.11.1 6-hydroxydopamine (6-OHDA)	104
2.11.2 Maneb.....	106
2.11.3 Paraquat.....	108
2.12 Summary.....	110
CHAPTER 3:	112
<i>IN VITRO MODELS OF PD.....</i>	112
3.0 Introduction.....	113
3.1 Dissociated cultures.....	113
3.2 Reaggregate cultures.....	115
3.3 Organotypic explant cultures.....	116

3.4 Organotypic slice cultures.....	117
3.5 <i>In Vitro</i> Systems And Their Relevance To PD.....	121
3.5.1 MPTP & <i>In Vitro</i> Studies.....	121
3.5.1.2 DAergic Neurons-Target Specific Toxicity.....	123
3.5.1.3 Complex 1 Inhibitor.....	124
3.5.1.4 Oxidative Stress (OS)	125
3.5.1.5 Inflammation: The Contribution of Microglia and Cytokines.....	127
3.5.1.6 Cell Death.....	129
3.5.1.7 DA Agonists & Antioxidants.....	132
3.5.2 Rotenone & <i>In Vitro</i> Studies.....	135
3.5.2.1 DAergic Toxicity.....	136
3.5.2.2 Oxidative Stress (OS).....	138
3.5.2.3 α -Synuclein.....	140
3.5.2.4 Complex I Inhibitor.....	141
3.5.2.5 Inflammation.....	142
3.5.2.6 Cell death via Apoptosis.....	144
3.5.2.7 DA Agonists and Antioxidants.....	147
3.6 ORGANOTYPIC CELL CULTURE: <i>IN VITRO</i> MODEL FOR PD.....	147
3.6.1 MPTP application in organotypic cell cultures.....	148
3.6.2 Rotenone and Organotypic Cell Culture.....	149
4.0 AIMS:.....	151
CHAPTER 5:	152
<i>MATERIAL & METHODS</i>.....	152
5.1 Animal care.....	153
5.1.1 Ethics.....	153
5.1.2 General.....	153
5.2 Organotypic slice cultures.....	153
5.2.1 Dissection of the ventral mesencephalon and striatum.....	153

5.2.2	Mounting the slices.....	155
5.2.3	Slice culture by the roller-tube technique.....	156
5.3	Fixation of cultures.....	158
5.3.1	Immunoperoxidase antigen retrieval	158
5.3.2	Immunohistochemistry.....	159
5.3.3	Co-localisation of TH and active Caspase-3.....	160
5.4	Lactate Dehydrogenase (LDH) ELISA.....	160
5.6	Quantification and statistical analyses of cell counts.....	161
CHAPTER 6:	170
<i>DOPAMINERGIC NEURONS GROWN IN ORGANOTYPIC SLICE CULTURES, TH-ir CELLS ARE DEPENDENT ON TARGET REGION-STRIATUM.....</i>		
6.0	INTRODUCTION.....	171
6.1	AIMS.....	171
6.2	METHODS.....	171
6.3	RESULTS	
6.3.1	TH-ir neurons of the ventral mesencephalon in culture.....	172
6.3.2	TH-ir cell growth in the Co-cultures.....	173
6.3.3	The influence of trophic ST target on VM TH-ir cells.....	174
6.3.4	Glial cells contribute to the regulation of DAergic outgrowth.....	174
6.4	DISCUSSION.....	186
6.4.1	General findings.....	186
6.4.2	Advantage and disadvantages of the culture system-organotypic slice culture system.....	186
6.4.3	The influence of target region-Striatum on TH-ir neurons.....	188
6.4.4	The growth of TH-ir neurons with increasing culture duration.....	189
6.4.5	Possible influences on TH-ir cell development.....	189
6.4.6	Role of GFAP-ir cells in TH-ir neuronal development.....	190

CHAPTER 7:	193
<i>THE EFFECTS OF NEUROTOXINS: MPTP & ROTENONE ON TH-ir CELLS GROWTH IN ORGANOTYPIC SLICE CULTURES</i>	193
7.0 INTRODUCTION	194
7.1 AIMS	194
7.2 METHODS	194
7.2.1.1 MPTP Effects on 7 Day old VM & ST co-cultures.....	195
7.2.1.2 Rotenone Effects on 7 Day old VM & ST co-cultures.....	196
7.2.2.1 MPTP Effects on 14 Day old VM & ST co-cultures.....	197
7.2.2.2 Rotenone Effects on 14 Day old VM & ST co-cultures.....	198
7.2.2.3 Effects of MPP ⁺ on 14 Day old VM & ST co-cultures.....	199
7.2.4 Cytotoxicity of Neurotoxins	199
7.2.5 Neuronal Degeneration: Fluoro Jade C (FJ-C)	200
7.2.6 Apoptotic Cell Death: Caspase-3	200
7.3 RESULTS	201
7.3.1 Neurotoxin treatment of VM and ST co-cultures.....	202
7.3.2 Dose-dependent death of TH-ir cells-MPTP.....	202
7.3.3 MPTP toxicity varies with the time of co-culture exposure.....	214
7.3.4 Dose-dependent death of TH-ir cells-Rotenone.....	215
7.3.5 Rotenone toxicity varies with the time of co-culture exposure.....	216
7.3.6 TH-IR CELL DEATH BY MPTP AND ROTENONE	226
7.3.6.1 Cytotoxicity of MPTP and rotenone by LDH assay.....	226
7.3.6.2 Fluoro Jade C (FJ-C).....	226
7.3.6.3 MPTP and rotenone induced Caspase-3 activation.....	229
7.4 DISCUSSION	235
7.4.1 Dose and time dependent toxicity of neurotoxins.....	235
7.4.2 Toxicity of neurotoxins on Dopaminergic co-cultures varies with the age of co-cultures.....	238
7.4.3 Glial cells play an important role in DAergic degeneration.....	240
7.4.3 Rotenone vs MPTP neurotoxicity.....	243
7.4.4 MPTP and rotenone activate caspase-3.....	244

CHAPTER 8:	246
<i>THE NEUROPROTECTIVE EFFECT OF GLIAL CELL-LINE DERIVED NEUROTROPHIC FACTOR (GDNF) ON TH-ir CELLS IN CO- CULTURES</i>	246
8.0 INTRODUCTION	247
8.1 AIMS:	247
8.2 METHODS	248
8.2.1 Dose-response of GDNF on TH-ir cells.....	248
8.2.2 TH-ir cells response to GDNF following neurotoxin treatment.....	250
8.2.3 TH-ir cell response to pre-treatment with GDNF followed by neurotoxin insult.....	250
8.2 Analysis of cell death.....	254
8.3 RESULTS	254
8.3.1 GDNF at varying doses did not induce a significant increase in TH-ir cells.....	254
8.3.2 GDNF treatment promotes increase in cell size and branching.....	254
8.3.3 Post GDNF treatment following MPTP and rotenone exposure.....	254
8.3.4 Pre-treatment with GDNF followed by MPTP and rotenone exposure.....	255
8.4 DISCUSSION	270
8.4.1 The neurotrophic effects of GDNF on DAergic neurons survival and development.....	270
8.4.2 Neuroprotection and regeneration of DAergic neurons by GDNF.....	271
8.4.3 GDNF neuroprotection against neurotoxins in slice cultures.....	272
 CHAPTER 9:	 273
<i>GENERAL DISCUSSION</i>	273
9.1 INTRODUCTION	274
9.2 ORGANOTYPIC SLICE CULTURE OF VM & ST	274
9.2.1 The advantages of using organotypic slice cultures.....	274

9.2.2 Disadvantages of using organotypic slice cultures.....	275
9.2.2 Summary of experimental findings.....	275
9.3 THE EFFECTS OF MPTP AND ROTENONE ON VM AND ST SLICE CULTURES.....	278
9.3.1 MPTP and rotenone.....	278
9.3.2 Summary of experimental findings.....	278
9.4 THE NEUROTROPHIC ROLE OF GDNF ON TH-ir NEURONS IN SLICE CULTURES.....	282
9.4.1 GDNF and DAergic neurons.....	282
9.4.2 Summary of experimental findings.....	283
9.5 Summary.....	288
9.6 Conclusion	289
<i>REFERENCES.....</i>	290
<i>APPENDICES.....</i>	400

LIST OF FIGURES AND TABLES

Figure 1.1: Diagrammatic representation of the age predilections for typical, sporadic Parkinson's disease and genetically determined Parkinsonism.....	13
Figure 1.2: Action sites of pharmacological therapies currently available for PD.....	32
Figure 1.3: The current model of the basal ganglia.....	47-48
Figure 1.4: Stages of MDN development.....	57
Figure 1.5: Signaling cascades in the MDN development.....	58
Figure 2.1: Schematic Representation of MPTP Metabolism.....	77
Figure 2.2: Schematic representation of MPP ⁺ intracellular pathways.....	87
Figure 2.3: Pathways implicated in MPTP-mediated toxicity.....	93-94
Figure. 2.4: Chemical structure of rotenone, paraquat, MPP ⁺ , and maneb.....	111
Figure 5.1: Schematic illustration of the preparation protocol of slice cultures from 4 to 5-day-old rats.....	163-164
Figure 5.2: Schematic diagram of the culturing process.....	165
Figure 5.3: Schematic representation of the biotin-avidin peroxidase procedure for the identification of TH-ir neurons.....	166-167
Figure 5.4: LDH ELISA.....	168-169
Figure 6.1: Dopaminergic (TH-ir) controls.....	175
Figure 6.2: Dopaminergic (TH-ir) neuronal growth in VM & ST co-cultures.....	176
Figure 6.3: Dopaminergic (TH-ir) neuronal growth in VM and ST co-cultures.....	177
Figure 6.4: TH-ir cell growth in co-cultures vs single cultures (SVM).....	178
Figure 6.5: Dopaminergic (TH-ir) neuronal growth in single VM cultures (SVM).....	179
Figure 6.6: Dopaminergic (TH-ir) neuronal growth in co-cultures vs SVM.....	180
Figure 6.7: GFAP growths in co-cultures vs single cultures.....	181

Figure 6.8: Glial fibrillary acidic protein (GFAP-ir) growth in VM & ST co-cultures.....	182
Figure 6.9: Glial fibrillary acidic protein (GFAP-ir) growth in single VM (SVM) cultures.....	183
Figure 6.10: Dopaminergic (TH-ir) & Glial fibrillary acidic protein (GFAP-ir) growth in VM & ST co-cultures.	184
Figure 6.11: Glial fibrillary acidic protein (GFAP-ir) growth in VM & ST co-cultures and SVM.....	185
Figure 7.1: Schematical diagram of the experiments.....	201
Figure 7.2: MPTP treated co-cultures at 7 days.....	203
Figure 7.3.1: Dopaminergic (TH-ir) neuronal growth following 1 week post MPTP treatment of 7 day co-cultures.....	204
Figure 7.3.2: Dopaminergic (TH-ir) neuronal growth following 2 week post MPTP treatment of 7 day co-cultures.....	205
Figure 7.3.3: Dopaminergic (TH-ir) neuronal growth following 3 week post MPTP treatment of 7 day co-cultures.....	206
Figure 7.4: MPTP treated co-cultures at 14 days.....	207
Figure 7.5.1: Dopaminergic (TH-ir) neuronal growth following 1 week post MPTP treatment of 14 day co-cultures.....	208
Figure 7.5.2: Dopaminergic (TH-ir) neuronal growth following 2 week post MPTP treatment of 14 day co-cultures.....	209
Figure 7.5.3: Dopaminergic (TH-ir) neuronal growth following 3 week post MPTP treatment of 14 day co-cultures.....	210
Figure 7.5.4: Dopaminergic (TH-ir) neuronal growth following 1 week post MPTP & MPP ⁺ treatment of 14 day co-cultures.....	211
Figure 7.6: 7 day & 14 day old co-cultures MPTP treated.....	212-213
Figure 7.7: Rotenone treated co-cultures at 7 days.....	217
Figure 7.8.1: Dopaminergic (TH-ir) neuronal growth following 1 week post rotenone treatment of 7 day co-cultures.....	218

Figure 7.8.2: Dopaminergic (TH-ir) neuronal growth following 2 week post rotenone treatment of 7 day co-cultures.....	219
Figure 7.8.3: Dopaminergic (TH-ir) neuronal growth following 3 week post rotenone treatment of 7 day co-cultures.....	220
Figure 7.9: Rotenone treated co-cultures at 14 days.....	221
Figure 7.10.1: Dopaminergic (TH-ir) neuronal growth following 1 week post rotenone treatment of 14 day co-cultures.....	222
Figure 7.10.2: Dopaminergic (TH-ir) neuronal growth following 2 week post rotenone treatment of 14 day co-cultures.....	223
Figure 7.10.3: Dopaminergic (TH-ir) neuronal growth following 3 week post rotenone treatment of 14 day co-cultures.....	224
Figure 7.11: 7 day & 14 day old co-cultures rotenone treated.....	225-226
Figure 7.12: Lactate Dehydrogenase Cytotoxicity Assay (LDH) of MPTP & Rotenone treated co-cultures.....	228-229
Figure 7.13: MPTP & rotenone treated co-cultures 1 week post treatment- Degenerating neurons-FJC.....	230
Figure 7.14: Co-localisation of TH-ir and caspase-3 in co-cultures.....	231
Figure 7.15: Co-localisation of TH-ir and caspase-3 in co-cultures following MPTP treatment at one week recovery period.....	232
Figure 7.16: Co-localisation of TH-ir and caspase-3 in co-cultures following rotenone treatment at one week recovery period.....	233
Figure 7.17: Co-localisation of TH-ir and caspase-3 in co-cultures following MPTP at one week recovery period.....	234
Figure 7.18: Co-localisation of TH-ir and caspase-3 in co-cultures following rotenone treatment at one week recovery period.....	235
Figure 8.1: Schematical diagram of the experiments.....	256
Figure 8.2: TH-ir cells in co-cultures treated with GDNF.....	256-257
Figure 8.3: TH-ir analysis of 7 day co-cultures treated at varying doses of GDNF.....	258

Figure 8.4: 7 day co-cultures treated at varying doses of GDNF.....	259
Figure 8.5: The neuroprotective effects of GDNF on TH-ir cells following MPTP exposure.....	260
Figure 8.6: The effects of GDNF post treatment following MPTP exposure.....	261
Figure 8.7: The neuroprotective effects of GDNF on TH-ir cells following rotenone exposure.....	262
Figure 8.8: The effects of GDNF post treatment following rotenone exposure.....	263
Figure 8.9: LDH ELISA of GDNF post treatment following MPTP exposure.....	264
Figure 8.10: LDH ELISA of GDNF post treatment following rotenone exposure.....	265
Figure 8.11: FJC-staining of GDNF Post-treatment on co-cultures following MPTP and rotenone exposure.....	266
Figure 8.12: The neuroprotective effects of GDNF Pre-treatment on TH-ir cells following MPTP and rotenone exposure.....	267
Figure 8.13: The effects of GDNF Pre-treatment following MPTP exposure.....	268
Figure 8.14: The effects of GDNF Pre-treatment following rotenone exposure.....	269
Table 1.1: Genes involved in Parkinsons Disease.....	11
Table 1.2: Summary of increased Oxidative stress (OS) in Idiopathic Parkinsons’s disease (IPD).....	15
Table 1.3: Summary of current PD treatment.....	33
Table 2.1: Characteristics of animal models of Parkinson’s disease.....	75
Table 2.2: Relative toxicity of MPTP in different animals.....	79
Table 2.3: MPTP-induced effects upon the DAergic system in different mouse strains.....	84

Table 3 : Summary of Experimental PD <i>In Vitro</i> models.....	136
Table 7.1: 7 day old co-cultures treated with MPTP.....	195
Table 7.2: 7 day old co-cultures treated with rotenone.....	196
Table 7.3: 14 day old co-cultures treated with MPTP.....	197
Table 7.4: 14 day old co-cultures treated with rotenone.....	198
Table 7.5: 14 day old co-cultures treated with MPTP & MPP ⁺	199
Table 8.1: Dose-response of GDNF on TH-ir cells.....	249
Table 8.2: Post treatment of Co-cultures with GDNF following MPTP/rotenone exposure.....	251
Table 8.3: Pre-treatment of Co-cultures with GDNF prior to MPTP/rotenone exposure.....	252

ABSTRACT

In this study we aimed to investigate the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone neurotoxins on dopaminergic (DAergic) neuronal survival using ventral mesencephalic (VM) organotypic cell culture derived from postnatal rat pups (P4-5) and immunocytochemistry for tyrosine hydroxylase (TH) as a marker of DAergic cells. In addition, we examined the neuroprotective effects of glial cell line-derived neurotrophic factor (GDNF) on TH-ir cells exposed to MPTP and rotenone as a possible treatment for PD.

The TH-ir cells in co-cultures with striatum (ST) as a target grew better than when VM was cultured alone and that TH-ir cells in co-cultures could be maintained without using conditioned and trophic media. We treated 7 day and 14 day co-cultures at different times with varying MPTP and rotenone concentrations and found 14 day old cultures were more vulnerable than 7 day old co-cultures to the effects of either neurotoxin with TH-ir cell numbers significantly lower in 14 day cultures compared to 7 day cultures. Both neurotoxins induced a dose-dependent TH-ir cell reduction in the co-cultures. In addition we compared the toxicity of MPTP and its active metabolite 1-methyl-4-phenylpyridinium (MPP^+) as the neurotoxic effects of MPTP on DAergic cells depends on its conversion to MPP^+ by astrocytes. We found no significant difference in TH-ir cell reduction in co-cultures treated with MPTP and MPP^+ . Rotenone was more toxic than MPTP with less TH-ir cell survival in the weeks post treatment. GDNF exposure produced increased cell size and significant increases in TH-ir cell branching in co-cultures in a dose-dependent manner. Post treatment of GDNF against MPTP and

rotenone provided significant neuroprotection as TH-ir cell survival was at the lower neurotoxin doses and not at the higher doses.