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**THE ROLE OF NEUROTROPHIC FACTORS IN
NEURODEGENERATIVE DISORDERS OF THE
HUMAN BRAIN**

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ABSTRACT

Neurotrophic factors are a family of polypeptides that promote the differentiation, growth and survival of numerous central nervous system neurons during development and adulthood. It has been proposed that alterations in neurotrophic factor protein or receptor expression may be involved in the pathogenesis of human neurodegenerative disorders. Recent research supports the therapeutic use of neurotrophic factors in neurodegenerative disorders. However, while information has been obtained regarding the structure and function of neurotrophic factors and their receptors (trk receptors) in the developing and mature rodent central nervous system, little research has been performed examining the expression and functional role of these factors in the normal and diseased human brain.

This thesis investigated the role neurotrophic factors and trk receptors play in the pathogenesis of human neurodegenerative disorders. Using immunohistochemical and *in situ* hybridisation techniques, the regional distribution and cellular localisation of neurotrophic factors and trk receptors was examined throughout both the adult rat and normal human brain. The expression of individual neurotrophic factors and trk receptors was also examined in human post mortem normal, Alzheimer's and Huntington's disease brain tissue, as well as in an animal model of apoptotic nerve cell death.

Individual neurotrophic factors exhibited a specific and heterogeneous regional pattern of distribution throughout the adult human brain. Neurotrophic factor expression was detected in several neuronal populations which exhibit selective vulnerability in various neurodegenerative disorders. Alterations in the expression of neurotrophic factors within specific regions of the human brain may result in neuronal atrophy, possibly via apoptotic mechanisms. A significant reduction in the level of brain-derived neurotrophic factor (BDNF) was observed within the hippocampus and temporal cortex of the Alzheimer's disease brain. A loss of neuroprotection afforded by BDNF may contribute to the progressive atrophy of neurons in Alzheimer's disease. The high-affinity trk receptors, trkA and trkB (full-length and truncated) were also altered within the Alzheimer's disease brain. TrkA receptor-immunoreactivity was observed in astrocytes in the CA1 region of the Alzheimer's disease hippocampus, some of which were associated with β -amyloid plaques. Truncated trkB receptors were found in high levels in senile plaques while the full-length trkB receptor was expressed in glial-like cells in the Alzheimer's disease hippocampus. The appearance of trkA and trkB receptors in astrocytes

and plaques in the Alzheimer's disease brain might be related to β -amyloid deposition and could be implicated in the development of Alzheimer's disease.

Alterations in insulin-like growth factor-I (IGF-I) protein expression were also observed within the Alzheimer's disease brain. IGF-I-immunoreactivity was expressed in a subpopulation of reactive astrocytes in the Alzheimer's disease temporal cortex. These observations may indicate that IGF-I is involved in the neuropathology of Alzheimer's disease. The induction of IGF-I in response to neuronal injury may be an attempt to inhibit mechanisms that result in delayed neuronal death.

In addition, neurotrophic factor expression was examined in the Huntington's disease brain. Glial cell line-derived neurotrophic factor (GDNF) and transforming growth factor- α (TGF- α) were significantly reduced within both the Huntington's disease globus pallidus and substantia nigra. Reduced GDNF and TGF- α levels within the Huntington's disease brain may produce a loss of local or target-derived neurotrophic support within the basal ganglia and contribute to the preferential degeneration of medium-sized spiny projection neurons within the Huntington's disease striatum.

Moderate hypoxic-ischemic (HI) injury was used as an animal model of apoptotic nerve cell death. In agreement with the observations made in the Alzheimer's disease brain, moderate HI injury resulted in the loss of BDNF within the rat hippocampus. In contrast, an increase in trkB (truncated) receptor expression was detected within glial cells in the rat brain. Alterations in BDNF and trkB receptor levels may lead to a loss of neuroprotection and the initiation of downstream mechanisms resulting in the induction of apoptotic processes. A cascade of events similar to those observed within the rat HI model may occur within human neurodegenerative disorders.

This study demonstrated that, while the neuropathogenesis of both Alzheimer's and Huntington's disease is complex, alterations in individual neurotrophic factor or trk receptor expression within selectively vulnerable cortical or subcortical regions may play a role in their pathophysiology. Furthermore, these results support the proposal that neurotrophic factors may be considered for the treatment of neurodegenerative disorders by protecting against neuronal cell loss and by increasing the function of surviving neuronal populations.

PUBLICATIONS RESULTING IN PART OR FULL FROM THIS THESIS

JOURNAL ARTICLES

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- B. Connor, D. Young, Q. Yan, R.L.M. Faull and M. Dragunow. 1997. Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Molecular Brain Research* (in press).
- B. Connor, E.J. Beilharz, C. Williams, P.D. Gluckman, R.L.M. Faull and M. Dragunow. 1997. Insulin-like growth factor (IGF-I) immunoreactivity in the Alzheimer's disease temporal cortex and hippocampus. *Molecular Brain Research* (in press).
- B. Connor and M. Dragunow. The role of neuronal growth factors in neurodegenerative disorders of the human brain (submitted).
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- M. Dragunow, M. Walton, B. Connor, Q. Yan, P. Lawlor, E. Sirimanne, C. Williams and P.D. Gluckman. Loss of brain-derived neurotrophic factor (BDNF) precedes apoptosis of CA1 pyramidal neurons after hypoxia-ischaemia. (in preparation).
- B. Connor, M. Walton, E. Sirimanne, C. Williams, P.D. Gluckman and M. Dragunow. TrkC receptors are altered in the rat hippocampus after moderate hypoxic-ischaemic injury (in preparation).

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TABLE OF CONTENTS

1. GENERAL INTRODUCTION	1
1.1 Alzheimer's disease	2
1.2 Nerve growth factor	10
1.3 Brain-derived neurotrophic factor	20
1.4 Neurotrophin-3	27
1.5 Glial cell line-derived neurotrophic factor	30
1.6 Transforming growth factor- α	34
1.7 Insulin-like growth factor I	38
1.8 The tyrosine kinase receptors	43
1.9 The low-affinity p75 receptor	44
1.10 The trkA receptor	48
1.11 The trkB receptor	52
1.12 The trkC receptor	56
1.13 Signal transduction mechanisms elicited by the trk receptors	57
1.14 Aims	61
 2. GENERAL METHODS	 63
2.1 Post mortem human brain tissue	63
2.2 Tissue preparation	63
2.3 Nissl staining	64
2.4 The Levine rat preparation	65
2.5 Rat perforant-path transection model	67
2.6 Immunohistochemistry	68
2.7 Control studies	70
2.8 Double label studies	71
2.9 In situ hybridisation	73
2.10 Western blotting - ECL technique	75
2.11 Analysis of Data	78

PART A: THE ROLE OF NEUROTROPHIC FACTORS IN THE NORMAL AND DISEASED HUMAN BRAIN _____ 80

3. IMMUNOHISTOCHEMICAL LOCALISATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR, GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR AND TRANSFORMING GROWTH FACTOR- α IN THE HUMAN BRAIN. _____ 81

3.1 Introduction _____	81
3.2 Methods _____	84
3.3 Results _____	87
3.4 Discussion _____	110

4. THE DISTRIBUTION OF NEURONAL CELL LOSS AND NEUROPATHOLOGY IN THE ALZHEIMER'S DISEASE BRAIN. _____ 121

4.1 Introduction _____	121
4.2 Methods _____	123
4.3 Results _____	126
4.4 Discussion _____	151

5. BRAIN-DERIVED NEUROTROPHIC FACTOR IS REDUCED IN ALZHEIMER'S DISEASE.162

5.1 Introduction _____	162
5.2 Methods _____	164
5.3 Results _____	168
5.4 Discussion _____	178

6. TRK RECEPTOR ALTERATIONS IN ALZHEIMER'S DISEASE _____ 182

6.1 Introduction _____	182
6.2 Methods _____	183
6.3 Results _____	187
6.4 Discussion _____	203

7. INSULIN-LIKE GROWTH FACTOR - I (IGF-I) IMMUNOREACTIVITY IN THE ALZHEIMER'S DISEASE TEMPORAL CORTEX AND HIPPOCAMPUS.	212
7.1 Introduction	212
7.2 Methods	214
7.3 Results	218
7.4 Discussion	224
8. THE EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) AND TRANSFORMING GROWTH FACTOR-α (TGF-α) IN THE HUNTINGTON'S DISEASED BRAIN	227
8.1 Introduction	227
8.2 Methods	233
8.3 Results	238
8.4 Discussion	260

**PART B: THE ROLE OF NEUROTROPHIC FACTORS IN AN ANIMAL MODEL OF
APOPTOTIC NERVE CELL DEATH _____ 267**

**9. THE ROLE OF NEUROTROPHIC FACTORS IN HYPOXIC ISCHAEMIC-INDUCED
NEURONAL CELL DEATH _____ 268**

- 9.1 The hypoxic-ischaemic rat model - neuropathology _____ 269
- 9.2 The effect of neuronal injury on BDNF and trkB receptor mRNA expression in the
rat hippocampus and neocortex _____ 270
- 9.3 The expression of the trkC receptor after brain injury _____ 271
- 9.4 Conclusion _____ 272

**10. IMMUNOHISTOCHEMICAL LOCALISATION OF BRAIN-DERIVED NEUROTROPHIC
FACTOR AND THE TYROSINE KINASE RECEPTORS, TRKA, TRKB FULL-LENGTH
AND TRKB TRUNCATED IN THE CENTRAL NERVOUS SYSTEM. _____ 273**

- 10.1 Introduction _____ 273
- 10.2 Methods _____ 275
- 10.3 Results _____ 278
- 10.4 Discussion _____ 299

**11. THE TEMPORAL AND SPATIAL PATTERN OF BDNF AFTER MODERATE AND
SEVERE HYPOXIC-ISCHEMIC INJURY IN REGIONS OF THE RAT HIPPOCAMPUS. 307**

- 11.1 Introduction _____ 307
- 11.2 Methods _____ 309
- 11.3 Results _____ 311
- 11.4 Discussion _____ 321

12. ALTERATION OF TRKB RECEPTOR EXPRESSION IN THE MODERATE RAT HYPOXIC-ISCHEMIC MODEL.	326
12.1 Introduction	326
12.2 Methods	328
12.3 Results	330
12.4 Discussion	338
13. TRKC RECEPTOR LEVELS ARE ALTERED IN THE RAT HIPPOCAMPUS AFTER MODERATE HYPOXIC-ISCHEMIC INJURY.	343
13.1 Introduction	343
13.2 Methods	344
13.3 Results	346
13.4 Discussion	350
14. GENERAL DISCUSSION	353
APPENDIX I	363
APPENDIX II	373
APPENDIX III	382
APPENDIX IV	383
REFERENCES	384

LIST OF FIGURES

Figure 3.1	BDNF-immunoreactivity in the normal post mortem human brain. _____	90
Figure 3.2	BDNF-immunoreactivity in the normal post mortem human brain. _____	91
Figure 3.3	GDNF-immunoreactivity in the normal post mortem human brain. _____	96
Figure 3.4	GDNF-immunoreactivity in the normal post mortem human brain. _____	97
Figure 3.5	Western blot analysis of the GDNF protein using protein samples from post mortem human hippocampus and temporal cortex. _____	99
Figure 3.6	TGF- α -immunoreactivity in the normal post mortem human brain. _____	103
Figure 3.7	TGF- α -immunoreactivity in the normal post mortem human brain. _____	104
Figure 3.8	TGF- α -immunoreactivity in the normal post mortem human brain. _____	105
Figure 3.9	Western blot analysis of the TGF- α protein using protein samples from post mortem human temporal cortex. _____	107
Figure 4.1	Neuronal viability within the CA1 hippocampal region of the normal and Alzheimer's disease post mortem human brain. _____	127
Figure 4.2	Graphs comparing the mean number of viable neurons as determined by Nissl staining in the hippocampus and temporal cortex of normal and Alzheimer's disease post mortem human brains. _____	128
Figure 4.3	Scattergrams comparing subject age and the mean number of viable neurons in the hippocampus and temporal cortex of normal and Alzheimer's disease post mortem human brains. _____	130
Figure 4.4	β -amyloid-positive plaques within regions of the post mortem human Alzheimer's disease hippocampus and temporal cortex. _____	132
Figure 4.5	Graphs comparing the mean number of β -amyloid-positive plaques in the hippocampus and temporal cortex of normal and Alzheimer's disease post mortem human brains. _____	133
Figure 4.6	Scattergrams comparing the mean number of β -amyloid-positive plaques and viable neurons in the hippocampus and temporal cortex of Alzheimer's disease post mortem human brains. _____	134
Figure 4.7	Scattergrams comparing the mean number of β -amyloid-positive plaques and viable neurons in the hippocampus and temporal cortex of normal post mortem human brains. _____	135
Figure 4.8	Tau-immunopositive neurofibrillary tangles within the post mortem human Alzheimer's disease hippocampus. _____	138

Figure 4.9	Graphs comparing the mean number of tau-positive neurofibrillary tangles in the hippocampus and temporal cortex of normal and Alzheimer's disease post mortem human brains. _____	139
Figure 4.10	Scattergrams comparing the mean number of tau-immunopositive neurofibrillary tangles and viable neurons in the hippocampus and temporal cortex of Alzheimer's disease post mortem human brains. _____	140
Figure 4.11	Scattergrams comparing the mean number of tau-immunopositive neurofibrillary tangles and viable neurons in the hippocampus and temporal cortex of normal post mortem human brains. _____	141
Figure 4.12	GFAP-immunoreactivity within the CA3 hippocampal region of the normal and Alzheimer's disease post mortem human brain. _____	145
Figure 4.13	Graphs comparing the mean number of GFAP-immunopositive glial cells in the hippocampus and temporal cortex of normal and Alzheimer's disease post mortem human brains. _____	146
Figure 4.14	Scattergrams comparing the mean number of GFAP-immunopositive glial cells and viable neurons in the hippocampus and temporal cortex of Alzheimer's disease post mortem human brains. _____	148
Figure 4.15	Scattergrams comparing the mean number of GFAP-immunopositive glial cells and viable neurons in the hippocampus and temporal cortex of normal post mortem human brains. _____	149
Figure 4.16	Scattergrams comparing the mean number of GFAP-immunopositive glial cells and β -amyloid-positive plaques in the hippocampus and temporal cortex of Alzheimer's disease post mortem human brains. _____	150
Figure 4.17	Diagram of a proposed self-propagating neurodegenerative cascade within the Alzheimer's disease brain involving β -amyloid protein, amyloid precursor protein, activated astrocytes and cytokines. _____	161
Figure 5.1	BDNF-immunostaining in the normal and Alzheimer's disease post mortem human hippocampus and temporal cortex. _____	171
Figure 5.2	BDNF-immunostaining in the hilar region of the normal and Alzheimer's disease post mortem human hippocampus. _____	172
Figure 5.3	Graph comparing the mean density of BDNF-immunoreactivity within the hilar region of the dentate gyrus in the Alzheimer's disease and normal human hippocampus. _____	173
Figure 5.4	Graphs comparing the mean number of BDNF-immunopositive cell bodies within the CA1 hippocampal subregion and the temporal cortex of Alzheimer's disease and normal post mortem human brains. _____	174
Figure 5.5	Western blot analysis of the BDNF protein using the Amgen polyclonal primary antibody. _____	175

Figure 5.6	Western blot analysis of the BDNF protein using the Santa Cruz polyclonal primary antibody. _____	177
Figure 6.1	TrkA-immunostaining in the hippocampus of normal and Alzheimer's disease post mortem human brains. _____	188
Figure 6.2	The association of trkA-immunopositive glial cells with β -amyloid-positive plaques in the hippocampus and temporal cortex of Alzheimer's disease post mortem human brains. _____	189
Figure 6.3	TrkB (full-length and truncated)-immunostaining in the hippocampus of normal and Alzheimer's disease post mortem human brains. _____	191
Figure 6.4	The association of trkB truncated receptor-immunoreactivity with β -amyloid-positive plaques in the hippocampus and temporal cortex of Alzheimer's disease post mortem human brains. _____	192
Figure 6.5	TrkA mRNA expression within the hippocampus and temporal cortex of post mortem human Huntington's disease and Alzheimer's disease brains as detected by <i>in situ</i> hybridisation. _____	200
Figure 6.6	Graphs comparing the mean density of trkA mRNA expression in the hippocampus and temporal cortex of Alzheimer's disease post mortem human brains with the mean density of trkA mRNA expression within normal and Huntington's disease post mortem human brains. _____	201
Figure 6.7	Western blot analysis of the trkA, trkB (full-length) and trkB (truncated) receptors in post mortem human hippocampal protein samples. _____	202
Figure 6.8	Diagram comparing the effect of altered BDNF and trkB truncated receptor protein expression in the Alzheimer's disease hippocampus and temporal cortex with the BDNF-activated signal transduction cascade seen in the normal human brain. _____	211
Figure 7.1	The expression of IGF-I-immunoreactivity within the hippocampus and temporal cortex of normal and Alzheimer's disease post mortem human brains. _____	219
Figure 7.2	Graphs comparing the level of IGF-I-immunoreactivity within the temporal cortex and CA1 hippocampal region of post mortem human normal and Alzheimer's disease brains. _____	220
Figure 7.3	Graph showing the mean number of IGF-I-immunoreactive cells in the temporal cortex of post mortem human normal and Alzheimer's disease brains. _____	221
Figure 7.4	Graph comparing the percentage of IGF-I-immunopositive/ GFAP-negative, GFAP-positive/IGF-I-negative and IGF-I-immunopositive/GFAP-positive double labelled cells in the temporal cortex of post mortem human normal and Alzheimer's disease brains. _____	222

Figure 7.5	The association of IGF-I-immunoreactivity with β -amyloid-positive plaques in the temporal cortex of Alzheimer's disease post mortem human brains. _____	223
Figure 8.1	GDNF-immunoreactivity within the internal and external segments of the normal and Huntington's disease globus pallidus. _____	241
Figure 8.2	TGF- α -immunoreactivity within the internal and external segments of the normal and Huntington's disease globus pallidus. _____	242
Figure 8.3	GDNF- and TGF- α - immunoreactive neurons within the normal post mortem human globus pallidus. _____	243
Figure 8.4	Graphs comparing the mean number of GDNF-immunopositive cell bodies within the external and internal segments of the globus pallidus in normal and Huntington's disease brains. _____	244
Figure 8.5	Graphs comparing the mean number of TGF- α -immunopositive cell bodies within the external and internal segments of the globus pallidus in normal and Huntington's disease brains. _____	245
Figure 8.6	GDNF-immunoreactivity within the pars reticulata and the pars compacta regions of the normal and Huntington's disease post mortem human substantia nigra. _____	249
Figure 8.7	TGF- α -immunoreactivity within the pars reticulata and the pars compacta regions of the normal and Huntington's disease post mortem human substantia nigra. _____	250
Figure 8.8	Graphs comparing the mean number of GDNF-immunopositive cell bodies within the substantia nigra pars reticulata and the pars compacta of normal and Huntington's disease brains. _____	251
Figure 8.9	Graphs comparing the mean number of TGF- α -immunopositive cell bodies within the substantia nigra pars reticulata and the pars compacta of normal and Huntington's disease brains. _____	252
Figure 8.10	Preabsorption and secondary control studies. _____	254
Figure 8.11	Co-expression of GDNF- and TGF- α -immunoreactivity in the normal post mortem human globus pallidus and substantia nigra. _____	255
Figure 8.12	Western blot analysis of the GDNF protein using protein samples from post mortem human substantia nigra. _____	257
Figure 8.13	Western blot analysis of the TGF- α protein using protein samples from post mortem human substantia nigra. _____	258
Figure 8.14	Diagram representing the major output projections from the basal ganglia. _____	259
Figure 10.1	TrkA-immunoreactivity in the neocortex of the adult rat brain. _____	279

Figure 10.2	TrkA-immunoreactivity in the olfactory bulb and the medial septum of the adult rat brain. _____	280
Figure 10.3	TrkA-immunoreactivity in the hippocampus and cerebellum of the adult rat brain. _____	281
Figure 10.4	TrkB-immunoreactivity in the thalamus, globus pallidus, Islands of Calleja and nucleus accumbens of the adult rat brain. _____	283
Figure 10.5	TrkB (full-length)- and trkB (truncated)- immunoreactivity in regions of the adult rat hippocampus. _____	284
Figure 10.6	BDNF-immunoreactivity in the amygdala, hippocampus, caudate nucleus and brainstem of the adult rat. _____	288
Figure 10.7	Comparison of trkB truncated-immunostaining between the control and the lesioned side of the rat hippocampus after a unilateral entorhinal cortical lesion. _____	289
Figure 10.8	Western blot analysis of the trkA, trkB (truncated) and trkB (full-length) receptors using samples from the rat hippocampus. _____	291
Figure 10.9	Western blot analysis of the BDNF protein using samples from the rat hippocampus. _____	292
Figure 11.1	BDNF-immunostaining in the control and lesioned hemispheres of the rat hippocampus after moderate HI injury. _____	313
Figure 11.2	Graphs showing the percentage change in mean density of BDNF-immunoreactivity in the rat hippocampus after moderate HI injury. _____	314
Figure 11.3	BDNF-immunostaining in the control and lesioned hemispheres of the rat hippocampus after severe HI injury. _____	316
Figure 11.4	Graphs showing the percentage change in mean density of BDNF-immunoreactivity in the rat hippocampus after severe HI injury. _____	317
Figure 11.5	Western blot analysis of the BDNF protein (Amgen) using samples derived from the hippocampus in the non-ligated hemisphere of a 21d old male Wistar rat. _____	319
Figure 11.6	Western blot analysis of the BDNF protein (Santa Cruz) using samples derived from the hippocampus in the non-ligated hemisphere of a 21d old male Wistar rat. _____	320
Figure 12.1	TrkB (full) mRNA expression within the postnatal 21d male Wistar rat hippocampus as detected by <i>in situ</i> hybridisation. _____	331
Figure 12.2	Graphs showing the percentage change in mean density of trkB (full) mRNA expression in the rat hippocampus after moderate HI injury. _____	332
Figure 12.3	Graphs showing the percentage change in mean density of trkB (all) mRNA expression in the rat hippocampus after moderate HI injury. _____	333

Figure 12.4	TrkB (full-length) receptor-immunostaining in the control and lesioned hemisphere of the rat hippocampus after moderate HI injury. _____	335
Figure 12.5	Graphs showing the percentage change in mean density of trkB full-length receptor immunoreactivity in the rat hippocampus after moderate HI injury. ____	336
Figure 12.6	TrkB (truncated) receptor immunostaining in the control and lesioned hemisphere of the rat hippocampus after moderate HI injury. _____	337
Figure 12.7	Diagram comparing the combined effects of altered BDNF and trkB receptor protein expression in both the rat HI model and the human Alzheimer's disease brain. _____	342
Figure 13.1	TrkC receptor mRNA expression within the 21d old male Wistar rat hippocampus as detected by <i>in situ</i> hybridisation. _____	347
Figure 13.2	Graphs representing the percentage change in mean density of trkC receptor mRNA expression in the rat hippocampus after moderate HI injury. _____	348
Figure 13.3	TrkC receptor-immunostaining in the control and lesioned hemispheres of the rat hippocampus after moderate HI injury. _____	349

LIST OF TABLES

Table 3.1	Summary of post mortem data of normal human brain sections used in immunohistochemical studies. _____	84
Table 3.2	The regional distribution of brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and transforming growth factor- α (TGF- α) immunoreactivity in normal post mortem human brains. _____	108
Table 4.1	Summary of post mortem data of human Alzheimer's disease and normal brain sections used in cresyl violet cell viability and immunohistochemistry studies. _____	125
Table 5.1	Summary of post mortem data of normal and Alzheimer's disease brain tissue used in BDNF immunohistochemistry study. _____	167
Table 6.1	Summary of post mortem data of human Alzheimer's disease, Huntington's disease and normal brain sections used in immunohistochemistry and <i>in situ</i> hybridisation studies. _____	186
Table 6.2	Summary of trkA-immunopositive astrocytes in the CA1 hippocampal region and the continuity of dentate gyrus trkA-immunostaining in post mortem human Alzheimer's disease, Huntington's disease and normal brains. _____	194
Table 6.3	Summary of trkA-immunopositive plaques in the CA1 region of the hippocampus in Alzheimer's disease, Huntington's disease and normal post mortem human brains. _____	196
Table 6.4	Summary of trkA and β -amyloid double labelling in the hippocampus and cortical grey matter of the Alzheimer's disease temporal lobe. _____	197
Table 6.5	Mean number of trkB (truncated)-immunopositive senile plaques in the CA1 hippocampal region from post mortem human Alzheimer's disease, Huntington's disease and normal brains. _____	198
Table 7.1	Summary of post mortem data of human Alzheimer's disease and normal brain sections used in IGF-I immunohistochemical studies. _____	217
Table 8.1	Neuropathological progression of the neostriatum in HD. _____	229
Table 8.2	Summary of post mortem data of human normal, Alzheimer's and Huntington's disease brain sections used in GDNF and TGF- α immunohistochemistry studies. _____	236
Table 8.3	Summary of CAG gene repeats and neuropathological grades of the post mortem human Huntington's disease cases examined. _____	237
Table 10.1	The basal distribution of trkA, trkB full-length and trkB truncated receptor immunoreactivity in the adult rat brain. _____	293
Table 10.2	The basal distribution of BDNF-immunoreactivity in the adult rat brain. _____	296

LIST OF ABBREVIATIONS

Acd	nucleus accumbens
Ach	acetylcholine
AchE	acetylcholinesterase
AD	Alzheimer's disease
ad	anterodorsal thalamic nucleus
ALS	amyotrophic lateral sclerosis
am	anteromedial thalamic nucleus
βAP	β-amyloid protein
ApoE	apolipoprotein E
AP-1	activator protein-1
APP	amyloid precursor protein
av	anteroventral thalamic nucleus
BDHC	benzidine dihydrochloride
BDNF	brain-derived neurotrophic factor
BFCNs	basal forebrain cholinergic neurons
bFGF	basic fibroblast growth factor
BSA	bovine serum albumin
CERAD	consortium to establish a registry for Alzheimer's disease
cDNA	complementary deoxyribonucleic acid
ChAT	choline acetyltransferase
CNS	central nervous system
CNTF	ciliary neurotrophic factor
cRNA	complementary ribonucleic acid
DAB	3,3'-diaminobenzidine.4 hydrochloric acid
DG or dg	dentate gyrus
DNA	deoxyribonucleic acid

DS	Downs syndrome
DTT	dithiothreitol
EC	entorhinal cortex
ECL	electrochemiluminescence
EEG	electroencephalograph
EGF	epidermal growth factor
ENK	enkephalin
GABA	γ -amino-butyric-acid
GAD	glutamic acid decarboxylase
GAP	ras GTPase activating protein
GDNF	glial cell-line derived neurotrophic factor
GDNF-α	GPI-linked protein
GFAP	glial fibrillary acidic positive
GI	granule cell layer of the cerebellar cortex
GI	glomerular cell layer of the olfactory bulb
GPe	external segment of the globus pallidus
GPI	internal segment of the globus pallidus
GPI	glycosyl-phosphatidylinositol
gp110^{proto-trk}	glycoprotein (110kDa) for proto-oncogene for trkA receptor
gp140^{proto-trk}	glycoprotein (140kDa) for proto-oncogene for trkA receptor
gp95^{trkB}	glycoprotein (95kDa) for proto-oncogene for trkB receptor
gp145^{trkB}	glycoprotein (145kDa) for proto-oncogene for trkB receptor
gp145^{trkC}	glycoprotein (145kDa) for proto-oncogene for trkC receptor
H	neurologically-normal human post mortem brain
HD	Huntington's disease

HI	hypoxia-ischaemia
hl	hilar region of dentate gyrus
Hn	hypoglossal nucleus
hp	hippocampus
HRP	horseradish peroxidase
icj	Islands of Calleja
IEGs	immediate early genes
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IL-1	interleukin-1
io	inferior olive
IPI	internal plexiform layer of olfactory bulb
kDa	kilodalton
LTP	long-term potentiation
MAP kinase	mitogen-activated protein kinase
MEK	mitogen-sensitive MAP kinase kinases
mi	mitral cell layer of olfactory bulb
MI	myocardial infarction
MPTP	1-methyl-4-phenylpyridinium
mRNA	messenger ribonucleic acid
NADPH-d	NADPH-diaphorase
NGF	nerve growth factor
NGS	normal goat serum
NMDA	N-methyl-D-aspartate
NPY	neuropeptide-Y
NT-3	neurotrophic factor-3
O₂	oxygen

6-OHDA	6-hydroxydopamine
PBS	phosphate buffered saline
PD	Parkinson's disease
PHF	paired helical filaments
PI₃-kinase	phosphatidylinositol-3 kinase
Pj	Purkinje cell layer of cerebellar cortex
PLC-γ	phospholipase C- γ
PMSF	phenylmethylsulfonyl fluoride
Pn	pontine nucleus
pt	paratenial thalamic nucleus
PTPase	protein tyrosine phosphatase
rhBDNF	recombinant human BDNF
rhGDNF	recombinant human GDNF
rhNGF	recombinant human NGF
Rn	red nucleus
rt	reticular thalamic nucleus
SDS	sodium dodecyl sulfate
SH₂	src homology domains
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SP	substance P
SSC	standard saline citrate
TBST	Tris buffered saline containing Tween-20
tc	temporal cortex
TdT	terminal deoxynucleotidyl transferase
TEMED	tetramethylethylenediamine
TGF-α	transforming growth factor- α

TGF-β	transforming growth factor- β
trk receptor	tyrosine kinase-linked neurotrophic receptor
TUNEL	TdT-mediated dUTP-biotin nick end labelling
v/v	volume/volume
w/v	weight/volume