



<http://researchspace.auckland.ac.nz>

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

AAV-vector Mediated Gene Delivery for Huntington's Disease: *An Investigative Therapeutic Study*

Adrian P. Kells

A thesis submitted in fulfillment of the requirements for the
Degree of Doctor of Philosophy in Pharmacology
at The University of Auckland, March, 2007.



**THE UNIVERSITY
OF AUCKLAND**
NEW ZEALAND

Te Whare Wānanga o Tāmaki Makaurau

Abstract

Progressive degeneration in the central nervous system (CNS) of Huntington's disease (HD) patients is a relentless debilitating process, resulting from the inheritance of a single gene mutation. With limited knowledge of the underlying pathological molecular mechanisms, pharmaceutical intervention has to-date not provided any effective clinical treatment strategies to attenuate or compensate the neuronal cell death. Attention has therefore turned to biotherapeutic molecules and novel treatment approaches to promote restoration and protection of selectively vulnerable populations of neurons in the HD brain.

Rapid advances in vectorology and gene-based medicine over the past decade have opened the way for safe and efficient delivery of biotherapeutics to the CNS. With numerous factors known to regulate the development, plasticity and maintenance of the mammalian nervous system many proteins have emerged as potential therapeutic agents to alleviate HD progression. This investigative study utilised gene delivery vectors derived from the non-pathogenic adeno-associated virus (AAV) to direct high-level expression of brain-derived neurotrophic factor (BDNF), glial cell-line derived neurotrophic factor (GDNF), Bcl-x_L or X-linked inhibitor of apoptosis protein (XIAP) within the rodent striatum. Maintenance of the basal ganglia and functional behaviour deficits were assessed following excitotoxic insult of the striatum by quinolinic acid (QA), a neurotoxic model of HD pathology.

Enhanced striatal expression of BDNF prior to QA-induced lesioning provided maintenance of the striosome-matrix organisation of the striatum, attenuating impairments of sensorimotor behaviour with a 36-38% increase in the maintenance of DARPP-32 / krox-24 expressing striatal neurons, reduced striatal atrophy and increased maintenance of striatonigral projections. Higher levels of BDNF however induced seizures and weight-loss highlighting the need to provide regulatable control over biotherapeutic protein expression. Continuous high-expression of BDNF or GDNF resulted in a downregulation of intracellular signal mediating proteins including DARPP-32, with AAV-GDNF not found to enhance the overall maintenance of striatal neurons. Neither of the anti-apoptotic factors provided significant protection of transduced striatal neurons but tended towards ameliorating QA-induced behavioural deficits, displaying behaviour – pathology correlations with the survival of parvalbumin-expressing neurons in the globus pallidus. The results of this thesis suggest BDNF as a promising putative biotherapeutic for HD, but emphasises the requirement to control expression following gene delivery, and for further elucidation of the physiological impact that enhanced expression of endogenous factors has on the host cells. Additionally the maintenance of neural networks beyond the caudate-putamen will be vital to ensuring efficient clinical outcomes for HD.

Acknowledgements

I wish to specifically convey my gratitude to a number of people who have assisted either directly or indirectly over the past four years to ensuring the completion of this thesis.

Specifically I would like to thank my supervisor Dr Bronwen Connor for having provided the opportunity to have conducted this research. Bronwen has always been optimistic towards all aspects of the research providing valuable rationality to study design and assessment, while always encouraging self belief to pursue my own initiatives.

Thanks to Professor Richard Faull, my co-supervisor. A radiating enthusiasm for life and Richard's passion to unravel the workings of the human brain was a constant inspiration. His interest in this study and vast knowledge of neuroanatomy always provided a fresh perspective to my evaluation.

To Andrew, Rebecca, Kevin and Elena, your company as fellow PhD students throughout the highs and lows of our complementary investigations has been truly invaluable, and I sincerely wish you each the best in all your future endeavours. Particular thanks to Rebecca for patiently guiding me around the invisible barriers of molecular biology, and to Andrew for assistance with animal modelling and numerous discussions of the subsequent neurogenic phenomena – a welcome distraction.

Dr Stephanie Hughes, for your counsel surrounding methodology and constructive feedback on written aspects of this thesis, I extend my gratitude. And to my remaining colleagues in the Neural Repair and Neurogenesis Laboratory and wider HRC Neuroscience collaboration that have provided advice, support and friendship, thank you.

To all the staff of the Animal Resource Unit, your always willing assistance to ensure the greatest of care was provided for the multitude of rats that enabled these investigations to be conducted has been greatly appreciated.

Finally to my Parents who have provided unwavering moral support throughout all of my university studies and have always encouraged me to pursue my own interests, without your loving support this thesis would not have been possible. And lastly to Petrea for your loyal friendship and patience in enduring with me through the final year of this thesis – this journey is now complete.

Financial support of this thesis was provided by an Auckland Medical Research Foundation research grant, and scholarships awarded by both the NZ Foundation for Research Science and Technology, and The University of Auckland.

Journal Publications

Research Articles

Kells AP, Henry RA, Connor B. (2007) **AAV-BDNF Mediated Attenuation of QA-Induced Neuropathology and Motor Function Impairment.** *Submitted to Gene Therapy.*

Kells AP, Henry RA, Connor B. (2007) **AAV-Mediated Expression of Bcl-x_L or XIAP Fails to Increase Neuronal Resistance against QA-induced Striatal Lesioning.** *Submitted to Experimental Neurology.*

Kells AP, Henry RA, Hughes SM, Connor B. (2006) **Verification of Functional AAV-mediated Neurotrophic and Anti-apoptotic Factor Expression.** *Journal of Neuroscience Methods.* 161(2): 291-300

Abstracts

Kells AP, Henry RA, Hughes SM, Faull, RLM, Connor B. (2007) **Attenuation of Functional Deficits in the QA Model of Huntington's Disease following AAV Vector Delivery.** *10th Annual Meeting of the American Society of Gene Therapy, Seattle, WA, USA.* Molecular Therapy 15: S209

Kells AP, Henry RA, Hughes SM, Faull, RLM, Connor B. (2006) **Protection against Huntington's Disease Progression: AAV-mediated Delivery of Biotherapeutics.** *9th Annual Meeting of the American Society of Gene Therapy, Baltimore, MD, USA.* Molecular Therapy 13: S96

Kells AP, Henry RA, Hughes SM, Faull, RLM, Connor B. (2005) **Investigating the Protective Effect of GDNF and Bcl-x_L Gene Delivery in a Rat Model of Huntington's Disease.** *9th International Conference on Neural Transplantation and Repair, Taipei, Taiwan.*

Table of Contents

Abstract	II
Acknowledgements	III
Journal Publications	IV
List of Figures	XII
List of Tables	XV
Abbreviations	XVI

Chapter 1	Review of Published Literature	1
------------------	---------------------------------------	----------

1.1	Huntington's Disease	1
1.2	Huntington's Disease: Neuropathology	2
1.3	Animal Models of Huntington's Disease	5
1.3.1	Transgenic models of Huntington's disease	5
1.3.2	Chemical neurotoxin models of Huntington's disease	6
1.4	Therapeutic Intervention for Huntington's Disease	8
1.5	<i>In vivo</i> Gene Delivery Vectors	10
1.5.1	Herpes simplex and adeno viral vectors	10
1.5.2	Adeno-associated viral vectors	11
1.5.3	Lentiviral vectors	13
1.6	Neurotrophic Factors as Biotherapeutic Agents for HD	13
1.6.1	Nerve growth factor	15
1.6.2	Brain derived neurotrophic factor	16
1.6.3	Glial cell-line derived neurotrophic factor	22
1.6.4	Ciliary neurotrophic factor	23
1.7	Apoptosis and Huntington's Disease Neurodegeneration	24
1.7.1	Anti-apoptotic Bcl-2 and Bcl-x _L proteins	29
1.7.2	Inhibitors of apoptosis	30
1.8	Summary	31

1.9 Thesis Objectives	33
------------------------------------	-----------

Chapter 2 Adeno-Associated Viral Vectors: Plasmid Cloning, Vector Packaging and In Vitro Functional Testing	35
--	-----------

2.1 Overview	35
---------------------------	-----------

2.2 AAV Vector Development and Production Procedures	35
---	-----------

2.2.1 Molecular biology protocols	36
2.2.1.1 PCR Cloning	36
2.2.1.2 Agarose Gel Extraction	37
2.2.1.3 p-GEM [®] -T Easy Vector	38
2.2.1.4 Heatshock Transformation	38
2.2.1.5 Colony PCR	38
2.2.1.6 Restriction Enzyme Digestions	38
2.2.1.7 Ligation into the AAV Expression Cassette	39
2.2.1.8 Plasmid Amplification and Purification	39
2.2.1.9 DNA Sequencing	40

2.2.2 AAV plasmid construction	40
--------------------------------	----

2.2.3 Mammalian cell culture and analysis protocols	44
2.2.3.1 HEK 293 and HT-1080 Cell Culture	44
2.2.3.2 In Vitro Immunocytochemistry	45
2.2.3.3 Hoechst Nuclear Staining	45

2.2.4 AAV expression cassette transfection testing	46
--	----

2.2.5 AAV vector production	46
2.2.5.1 Packaging and Purification of AAV Vector Particles	46
2.2.5.2 Genomic Particle Titre	47

2.2.6 In vitro transduction testing	48
-------------------------------------	----

2.3 Functional AAV-Mediated Protein Expression Testing In Vitro	49
--	-----------

2.3.1 Isolating and culturing primary cells	49
2.3.2 AAV-BDNF	50
2.3.3 AAV-GDNF	50
2.3.4 AAV-Bcl-x _L	51
2.3.5 AAV-XIAP	52

2.4 Results	52
--------------------------	-----------

2.4.1 AAV plasmid construction	52
2.4.2 AAV vector production	54
2.4.3 AAV vector transduction	54
2.4.4 Functional protein expression	56
2.4.4.1 Brain Derived Neurotrophic Factor	56
2.4.4.2 Glial cell-line Derived Neurotrophic Factor	56

2.4.4.3	<i>Bcl-x_L</i>	59
2.4.4.4	<i>X-linked Inhibitor of Apoptosis Protein</i>	61
2.5	Discussion.....	64
2.5.1	AAV vector construction	64
2.5.2	Functional protein expression	65
2.5.2.1	<i>Differentiation Assays to Confirm Neurotrophic Factor Activity</i>	65
2.5.2.2	<i>Cell-Survival Assays to Confirm Anti-Apoptotic Protein Function</i>	68
2.5.3	Summary	69

Chapter 3 Rodent Model of Huntington's Disease: *Quinolinic Acid Lesion* 71

3.1	Introduction	71
3.2	Optimisation and Characterisation Procedures	71
3.2.1	Animals and surgeries	71
3.2.2	Behavioural assessment	72
3.2.3	Neuropathological analysis	72
3.3	QA Lesion Optimisation Analysis	73
3.3.1	Initial QA trial	73
3.3.2	Trial 2: Lower QA quantity	74
3.3.3	Trial 3: Behaviour testing – 30nmol QA	76
3.3.4	QA lesion model characterisation – 50nmol QA	76
	3.3.4.1 <i>Functional Behaviour Assessment of Unilateral QA Lesioned Rats</i>	77
	3.3.4.2 <i>Neuropathological Assessment of QA Lesion</i>	79
3.4	Discussion	82
3.4.1	QA-induced neuropathology	82
3.4.2	QA-induced behavioural impairments	83

Chapter 4 Neuroprotective Study Methods **85**

4.1	Overview	85
4.2	Neuroprotective Investigations	86
4.2.1	In vivo expression testing	86
4.2.2	Initial investigations: AAV-BDNF (high-titre), AAV-GDNF, AAV-Bcl-x _L	86
4.2.3	Follow-up investigations: AAV-BDNF (diluted), AAV-Bcl-x _L , AAV-XIAP	87
4.3	Animals	88

4.4	Stereotaxic Surgeries: AAV Vector Delivery and QA Injection	89
4.4.1	Operating procedure	89
4.4.2	Determination of initial AAV vector injections parameters	90
4.5	Functional Behavioural Analysis	92
4.5.1	Spontaneous exploratory forelimb use	92
4.5.2	“Corridor” task	93
4.5.3	Drug-induced rotational analysis	94
4.6	Quantitative Transgene ELISA.....	95
4.7	Immunohistochemical Analysis.....	96
4.7.1	Brain tissue collection and processing	96
4.7.2	DAB-staining immunocytochemistry protocol	97
4.7.3	Fluorescent immunocytochemistry analysis	98
4.7.4	Microscopy and stereology	99
4.7.4.1	<i>Striatal Neuron Stereology</i>	99
4.7.4.2	<i>Striatal Atrophy</i>	100
4.7.4.3	<i>Striatal TH-Staining Intensity</i>	100
4.7.4.4	<i>Maintenance of Striatonigral Projections</i>	100
4.7.4.5	<i>Pallidal Neuron Stereology</i>	100
4.7.4.6	<i>Confocal Microscopy</i>	100
4.8	Statistical Analysis.....	101

Chapter 5 Neurotrophic Factor Delivery: AAV-BDNF and AAV-GDNF **102**

5.1	Overview	102
5.2	Procedures	102
5.3	Animal Welfare.....	103
5.4	<i>In Vivo</i> AAV Vector Testing.....	104
5.5	BDNF and GDNF Expression Level	106
5.6	Functional Behaviour Assessment	107
5.6.1	Spontaneous forelimb use	108
5.6.2	Drug-induced rotational behaviour	111
5.6.2.1	<i>Apomorphine</i>	111
5.6.2.2	<i>Amphetamine</i>	113
5.6.3	Sensorimotor “corridor” task	114

5.7	Immunocytochemical Analysis.....	115
5.7.1	AAV-mediated transgene expression and QA lesioning of the striatum	116
5.7.1.1	<i>Initial Study – AAV-BDNF and AAV-GDNF Delivery</i>	117
5.7.1.2	<i>Study 2 – Reduced Titre AAV-BDNF Delivery</i>	119
5.7.1.3	<i>Stereological Analysis – DARPP-32</i>	120
5.7.1.4	<i>Striatal Atrophy</i>	122
5.7.2	Krox-24 expression	123
5.7.3	Analysis of transduced striatal cells	127
5.7.4	Dopaminergic innervations of the striatum	131
5.7.5	Cortical projection fibres in the striatum	133
5.7.6	Striatal projection nuclei	134
5.7.6.1	<i>Substantia Nigra</i>	134
5.7.6.2	<i>Globus Pallidus</i>	137
5.7.7	Pathological correlations with functional behaviour impairments	140
5.7.7.1	<i>Spontaneous Ipsilateral Forelimb Use Bias</i>	140
5.7.7.2	<i>Contralateral Sensorimotor Neglect</i>	141
5.7.7.3	<i>Apomorphine-Induced Rotations</i>	142
5.8	Discussion.....	144
5.8.1	AAV-mediated BDNF or GDNF expression prior to QA	144
5.8.1.1	<i>Undiluted AAV-BDNF</i>	144
5.8.1.2	<i>AAV-GDNF</i>	146
5.8.2	Reduced-titre AAV-BDNF behavioural and pathological protection	149
5.8.2.1	<i>Behavioural Protection</i>	149
5.8.2.2	<i>Neuropathological Protection</i>	151
5.8.2.3	<i>Pathological and Behavioural Correlations</i>	153
5.8.3	Conclusion	155

Chapter 6 Anti-Apoptotic Factor Delivery: AAV-Bcl-x_L and AAV-XIAP **156**

6.1	Overview	156
6.2	Procedures	156
6.3	<i>In Vivo</i> AAV Vector Testing.....	157
6.4	Bcl-x_L and XIAP Expression Level	160
6.5	Functional Behaviour Assessment	161
6.5.1	Spontaneous forelimb use	161
6.5.2	Drug-induced rotational behaviour	164
6.5.2.1	<i>Apomorphine</i>	165
6.5.2.2	<i>Amphetamine</i>	166

6.5.3	Sensorimotor “corridor” task	167
6.6	Immunocytochemical Analysis.....	168
6.6.1	AAV-mediated transgene expression and QA lesioning of the striatum	168
6.6.1.1	<i>Study 1 – AAV-Bcl-x_L Delivery</i>	168
6.6.1.2	<i>Study 2 – AAV-Bcl-x_L and AAV-XIAP Delivery</i>	170
6.6.1.3	<i>Stereological Analysis – DARPP-32</i>	171
6.6.1.4	<i>Striatal Atrophy</i>	173
6.6.2	Krox-24 expression	174
6.6.3	Analysis of transduced striatal cells	176
6.6.4	Dopaminergic innervations of the striatum	178
6.6.5	Cortical projection fibres in the striatum	179
6.6.6	Striatal projection nuclei	181
6.6.6.1	<i>Substantia Nigra</i>	181
6.6.6.2	<i>Globus Pallidus</i>	184
6.6.7	Pathological correlations with functional behaviour impairments	187
6.6.7.1	<i>Spontaneous Forelimb Use</i>	187
6.6.7.2	<i>Sensorimotor Neglect</i>	188
6.6.7.3	<i>Apomorphine-Induced Rotations</i>	189
6.7	Discussion.....	190
6.7.1	AAV-mediated Bcl-x _L or XIAP expression prior to QA	190
6.7.1.1	<i>Neuropathological Changes</i>	191
6.7.1.2	<i>Behavioural Protection</i>	192
6.7.2	Comparison with previous studies	194
6.7.3	Conclusion	194

Chapter 7 General Discussion **196**

7.1	AAV-mediated Gene Delivery.....	196
7.2	Preventative Therapy for Huntington's Disease	198
7.3	Conclusion.....	204

Appendix A: Gene Sequences and DNA Plasmids **206**

A.1	cDNA Gene Sequences.....	206
A.1.1	Brain Derived Neurotrophic Factor	206
A.1.2	Glial cell-line Derived Neurotrophic Factor	207
A.1.3	Bcl-x _L	208
A.1.4	X-linked Inhibitor of Apoptosis	208

A.2	DNA Plasmid Maps	210
A.2.1	AAV Backbone Plasmid	210
A.2.2	AAV-Luciferase	211
A.2.3	AAV Helper Plasmids	212
A.2.3.1	AAV <i>rep</i> and <i>cap</i> genes	212
A.2.3.2	Adenoviral packaging genes	213
Appendix B:	General Materials & Protocols	214
B.1	Molecular Biology	214
B.2	Cell Culture.....	215
B.2.1	Tissue culture media	215
B.2.2	Cell counting	215
B.3	AAV Vector Genomic Titering	216
B.4	Immunocytochemistry Buffers.....	216
B.5	Antibodies	217
Literature	References	218

List of Figures

Chapter 1 Review of Published Literature

Figure 1-1	Potential mechanisms of mutant huntingtin induced cellular pathogenesis.....	4
Figure 1-2	Co-transfection rAAV packaging	12
Figure 1-3	TrkB signalling pathways controlling dendritic protein synthesis	18
Figure 1-4	Mutant huntingtin disrupts transcription of BDNF and vesicle transportation from the cortex to the striatum.	20
Figure 1-5	Potential involvement of Ca ²⁺ signalling in the induction of apoptosis in HD	26
Figure 1-6	Mitochondrial permeabilisation in intrinsic apoptosis.....	27
Figure 1-7	Model of neurotrophic factor and anti-apoptotic factor supplied protection.	32

Chapter 2 Adeno-associated Viral Vectors

Figure 2-1	AAV expression plasmid	41
Figure 2-2	Transfection of AAV backbone plasmids	53
Figure 2-3	AAV vector transduction <i>in vitro</i>	55
Figure 2-4	Phenotypic differentiation of primary striatal cultures	57
Figure 2-5	AAV-BDNF induced calbindin expression in embryonic striatal cultures.....	57
Figure 2-6	Phenotypic differentiation of primary ventral mesencephalon cultures	58
Figure 2-7	AAV-GDNF induced TH expression in ventral mesencephalon cultures	58
Figure 2-8	AAV-Bcl-x _L reduced staurosporine-induced apoptosis	59
Figure 2-9	AAV-Bcl-x _L transduced cortical cells following staurosporine-induced apoptosis	60
Figure 2-10	AAV-XIAP reduced staurosporine-induced apoptosis	61
Figure 2-11	Enhanced survival of AAV-XIAP transduced HT-1080 cells	63

Chapter 3 Rodent Model of Huntington's Disease

Figure 3-1	Initial 100 and 50 nmol QA lesion assessment	74
Figure 3-2	QA lesion testing: 50 and 30 nmol intrastriatal QA injections	75
Figure 3-3	QA lesioning induced behavioural deficits	78
Figure 3-4	Pathological characterisation of the 50nmol QA lesion.....	80
Figure 3-5	Striatal interneurons following QA lesioning.....	81

Chapter 4 Neuroprotective Study Methods

Figure 4-1	Neuroprotective study timeline	85
Figure 4-2	Intrastriatal AAV vector delivery	91

Figure 4-3	Spontaneous exploratory forelimb use.....	92
Figure 4-4	Sensorimotor “corridor” task.....	94
Figure 4-5	Drug-induced rotational behaviour.....	95

Chapter 5 Neurotrophic Factor Delivery

Figure 5-1	Spread of undiluted AAV-BDNF transduction and BDNF expression.....	104
Figure 5-2	Spread of AAV-GDNF transduction and GDNF expression	105
Figure 5-3	Spread of the reduced-titre AAV-BDNF transduction and BDNF expression	106
Figure 5-4	ELISA quantification of BDNF and GDNF expression in the striatum	107
Figure 5-5	Spontaneous ipsilateral forelimb use asymmetry score	109
Figure 5-6	Spontaneous exploratory rearing activity.....	110
Figure 5-7	Apomorphine-induced rotational behaviour	112
Figure 5-8	Total apomorphine-induced rotations	113
Figure 5-9	Amphetamine-induced rotational behaviour	114
Figure 5-10	Preferential food selection in the sensorimotor “corridor” task	115
Figure 5-11	High-titre AAV-BDNF transduction and striatal cell maintenance	116
Figure 5-12	AAV-GDNF transduction and striatal cell maintenance	117
Figure 5-13	AAV-Luciferase transduction and striatal cell maintenance.....	118
Figure 5-14	Diluted AAV-BDNF administration and striatal cell maintenance.....	119
Figure 5-15	Two-site AAV-Luciferase injection and striatal cell maintenance.....	120
Figure 5-16	Maintenance of DARPP-32 positive striatal projection neurons.....	121
Figure 5-17	Atrophy of the lesioned striatum	123
Figure 5-18	Double-immunofluorescent labelling of striatal neurons.....	124
Figure 5-19	Krox-24 expression in the striatum.....	125
Figure 5-20	Maintenance of krox-24 expressing striatal neurons	126
Figure 5-21	Immunofluorescent analysis of AAV-Luciferase transduced cells	128
Figure 5-22	Immunofluorescent analysis of AAV-BDNF transduced cells in the striatum.....	129
Figure 5-23	Immunofluorescent analysis of AAV-GDNF transduced cells in the striatum.....	130
Figure 5-24	Tyrosine hydroxylase expression in the striatum	131
Figure 5-25	Maintenance of tyrosine hydroxylase striatal innervations.....	132
Figure 5-26	SMI32 expression in the cortex and striatum.....	133
Figure 5-27	Visual assessment of the substantia nigra in control AAV-Luciferase treated rats.....	135
Figure 5-28	Visual assessment of the substantia nigra in reduced-titre AAV-BDNF treated rats...	136
Figure 5-29	DARPP-32 innervations of the substantia nigra.....	137
Figure 5-30	Visual assessment of the globus pallidus following AAV-Luciferase treatment.....	138

Figure 5-31	Visual assessment of the globus pallidus following reduced-titre AAV-BDNF treatment.....	139
Figure 5-32	Survival of parvalbumin-positive neurons in the globus pallidus	140
Figure 5-33	Preferential forelimb use correlation with striatum and globus pallidus cell loss.....	141
Figure 5-34	Sensorimotor neglect correlation with striatal and pallidal neuronal cell loss.....	142
Figure 5-35	Correlation of apomorphine-induced rotational behaviour with striatal and pallidal neuronal cell loss	143

Chapter 6 Anti-apoptotic Factor Delivery

Figure 6-1	Single delivery site for AAV-Bcl-x _L transduction and subsequent Bcl-x _L expression	157
Figure 6-2	AAV-Bcl-x _L transduction and Bcl-x _L expression following two-site striatal delivery	158
Figure 6-3	Spread of AAV-XIAP transduction and subsequent XIAP expression	159
Figure 6-4	ELISA quantification of Bcl-x _L and XIAP expression in the striatum	161
Figure 6-5	Spontaneous ipsilateral forelimb use asymmetry scores.....	162
Figure 6-6	Spontaneous exploratory rearing activity.....	163
Figure 6-7	Apomorphine-induced rotational behaviour	164
Figure 6-8	Total apomorphine-induced rotations	165
Figure 6-9	Amphetamine-induced rotational behaviour	166
Figure 6-10	Preferential food selection in the sensorimotor “corridor” task	167
Figure 6-11	Single injection site AAV-Bcl-x _L transduction and striatal cell maintenance.....	169
Figure 6-12	Dual injection site AAV-Bcl-x _L transduction and striatal cell maintenance	170
Figure 6-13	Dual injection site AAV-XIAP transduction and striatal cell maintenance.....	171
Figure 6-14	Maintenance of DARPP-32 striatal projection neurons.....	172
Figure 6-15	Atrophy of the lesioned striatum	173
Figure 6-16	Krox-24 expression in the striatum.....	175
Figure 6-17	Maintenance of krox-24 expressing striatal neurons	176
Figure 6-18	Immunofluorescent double-labelling of AAV-Bcl-x _L transduced striatal neurons	177
Figure 6-19	Immunofluorescent double-labelling of AAV-XIAP transduced striatal neurons	178
Figure 6-20	Tyrosine hydroxylase expression in the striatum	179
Figure 6-21	SMI32 expression in the striatum.....	180
Figure 6-22	DARPP-32 innervations of the substantia nigra.....	181
Figure 6-23	Visual assessment of the substantia nigra in AAV-Bcl-x _L treated rats.....	182
Figure 6-24	Visual assessment of the substantia nigra in AAV-XIAP treated rats.....	183
Figure 6-25	Survival of parvalbumin-positive neurons in the globus pallidus	184
Figure 6-26	Visual assessment of the globus pallidus following AAV-Bcl-x _L treatment	185
Figure 6-27	Visual assessment of the globus pallidus following AAV-XIAP treatment	186

Figure 6-28	Preferential forelimb usage correlation with neuron loss in the striatum and globus pallidus	187
Figure 6-29	Sensorimotor neglect correlation with striatal and pallidal neuronal cell loss	188
Figure 6-30	Correlation of apomorphine-induced rotational behaviour with striatal and pallidal neuronal cell loss	189

List of Tables

Chapter 1 Review of Published Literature

Table 1-1	Neuronal phenotypes within the striatum.....	3
-----------	--	---

Chapter 2 Adeno-associated Viral Vectors

Table 2-1	Source of cDNA sequences and AAV Plasmids	41
Table 2-2	PCR primer sequences	42
Table 2-3	Genes-of-interest.....	43
Table 2-4	New England Biolabs (NEB) restriction enzymes and buffers	43
Table 2-5	Primary antibodies for <i>in vitro</i> immunocytochemistry	45
Table 2-6	Genomic titres of AAV vector stocks	54

Chapter 3 Rodent Model of Huntington's Disease

Table 3-1	QA lesion optimisation trials.....	72
-----------	------------------------------------	----

Chapter 4 Neuroprotective Study Methods

Table 4-1	Stereotaxic injection coordinates for AAV vector delivery	86
Table 4-2	First neuroprotective investigation timeline.....	87
Table 4-3	Second neuroprotective investigation timeline.....	87
Table 4-4	Allocation of animals	88
Table 4-5	Dilutions used for ELISA quantification of <i>in vivo</i> transgenic protein expression.	96
Table 4-6	Primary antibodies for DAB-staining immunocytochemistry.....	98
Table 4-7	Antibodies for co-immunofluorescent labelling	99

Abbreviations

3-NP	3-nitropropionic acid
AAV	Adeno-associated virus
ABTS	2,2-Azino-di-3-ethylbenzthiazoline sulfonate
Ad	Adenovirus
Amp	Ampicillin
ANOVA	Analysis of variance
A-P, M-L, D-V	Anterior-Posterior, Medial-Lateral, Dorsal-Ventral
BCA	Bicinchinonic acid
Bcl-x _L	Bcl-2-like protein long
BDNF	Brain derived neurotrophic factor
BSA	Bovine serum albumin
CAG	Cytosine-adenine-guanine
CBA	Chicken- β -actin
cDNA	Complementary DNA
ChAT	Choline acetyltransferase
CIP	Calf intestinal phosphatase
CMV	Cytomegalovirus
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
Ct	Cycle time
DAB	3-3 diaminobenzidine tetrahydrochloride
DARPP-32	Dopamine- and adenosine 3', 5'-monophosphate-regulated phosphoprotein of 32 kDa
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic acid
E14/15	Embryonic day 14/15
EDTA	Ethylenediaminetetraacetic acid
<i>E. coli</i>	<i>Escherichia coli</i>
FBS	Fetal bovine serum
GABA	Gamma-amino butyric acid
GDNF	Glial cell-line derived neurotrophic factor
GFR α 1	GDNF family receptor α -1
GPe	Globus pallidus external segment
GPI	Globus pallidus internal segment
HA	Hemagglutinin
HD	Huntington's disease
<i>Hdh</i>	Huntingtin gene

HEK293	Human embryonic kidney 293 cells
HIAP	Human inhibitor of apoptosis
HSV	Herpes simplex virus
HT-1080	Human osteosarcoma cells
IAP	Inhibitor of apoptosis protein
IMDM	Iscoe's Modified Dulbecco's Media
ITR	Inverted terminal repeats
LB	Luria-Bertani broth
Luc	Luciferase
LV	Lentivirus
MAPK	Mitogen-activated protein kinase
N171-82Q	Transgenic mice with 171aa N-terminal fragment of <i>Hdh</i> with 82 CAG repeats
NADPHd	Nicotinamide adenine diphosphate diaphorase
NAIP	Neuornal apoptosis inhibitor protein
NEB	New England Biolabs
NeuN	Neuronal nuclei
NGF	Nerve growth factor
NMDA	N-methly-D-aspartate
NOS	Nitric oxide synthase
NR2B	NMDA receptor 2B subunit
p75 ^{NTR}	p75 neurotrophin receptor
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Pen	Penicillin
PNS	Peripheral nervous system
poly-Q	poly-glutamine tract
QA	Quinolinic acid
R6/2	Transgenic mice with exon 1 of <i>Hdh</i> containing ~150 CAG repeats
rh	Recombinant human
RM ANOVA	Repeated measures analysis of variance
SNC	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
ssDNA	Single-stranded DNA
Strep	Streptomycin
STS	Staurosporine
TE	Tris-EDTA buffer
TH	Tyrosine hydroxylase
WPRE	Woodchuck hepatitis post-transcriptional regulatory element
XIAP	X-linked inhibitor of apoptosis