



<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.

INVESTIGATING THE THERAPEUTIC POTENTIAL OF THE CB1 CANNABINOID RECEPTOR IN HUNTINGTON'S DISEASE

Emma L. Daniel

A thesis submitted in fulfilment of the requirements for the degree of Doctor of
Philosophy in Pharmacology, The University of Auckland, 2009.

ABSTRACT

The degeneration of neurons in the striatum, cortex and other diffuse brain regions in Huntington's disease (HD) is a progressive process, leading to debilitating symptoms and ultimately death. Despite the identification of the *huntingtin* gene, whose genetic mutation underlies the disease, the pathophysiology of HD remains ill-defined and there are currently no drugs in clinical use which effectively delay the progression of the disease. Recently, the CB1 cannabinoid receptor (CB1R) has gained attention as a therapeutic target in neurodegenerative diseases such as Parkinson's, and investigations have also begun in animal models of HD. However, these studies have yielded mixed reports of the efficacy of cannabinoids in this context. This may relate to the finding that CB1R binding is dramatically reduced early in the human disease. This thesis undertook the investigation of the therapeutic potential of the CB1R through the development of a PC12 cell model of HD. This model was found to recapitulate expression and aggregation of mutant huntingtin, loss of CB1Rs early in the pathological process, and cell death.

Using the Alamar Blue assay, it was determined that the CB1R agonists HU210 and WIN55,212-2, produced a small but significant reduction in huntingtin-associated cell death (6-8%). This rescue was dependent upon the activation of the G α i G-protein subtype and ERK 1/2, which was necessary but not sufficient to modulate cell survival. Conversely, compounds which increased cAMP formation exacerbated cell death and this correlated with enhanced formation of mutant huntingtin aggregates. Interestingly, the protective CB1R agonist HU210 also increased cellular aggregate load (6%). While the reduction of cell death by HU210 had been mediated by G α i-coupled CB1Rs, the enhancement of aggregate formation was likely to have occurred through CB1Rs coupling to G α s. The enhancement of huntingtin aggregation was generally associated with toxicity. Therefore the ability of the CB1R to promiscuously couple to G α s, and this detrimental aggregation pathway, may limit its therapeutic usefulness and account for the modest survival effects described here. However, compounds which are selective for G α i activation by the CB1R, or compounds which target protective downstream effectors, could represent novel therapeutic strategies to alleviate neurodegeneration in HD.

ACKNOWLEDGEMENTS

Many people have contributed both directly and indirectly to this thesis. My supervisors Associate Professor Michelle Glass and Professor Mike Dragunow laid the foundations for this research many years before my project began, and their enthusiasm, passion and brilliance for this work has continued throughout my project. I thank them for their patience, encouragement, and excellence, as well as their friendship and guidance. Dr. Scott Graham has also been a valuable mentor and friend over the course of my PhD. His methodical approach and extensive knowledge of all things molecular are traits I have both benefited from and aspire towards in my own research. I would also like to sincerely thank the Marsden Fund of the Royal Society of New Zealand, The University of Auckland, and The Neurological Foundation. This work could not have been undertaken without their generous financial support.

The other members of the Receptor Signalling Lab have also had an undeniable impact on the work contained in this thesis. Megan Dowie, Natasha Grimsey, Catherine Goodfellow and Sandie Fry have each played the role of colleague, with whom to discuss methods and troubleshoot, and friend, with whom to discuss life outside of the lab over a glass of wine. I have learned a lot from each of these lovely ladies and their friendship made the lab a great place to be. I would also like to acknowledge Pritika Narayan for her friendship and incredible patience in teaching me to operate Discovery 1. Other staff and students in the Pharmacology department have also contributed to a wonderful four years, and I look forward to future collaborations and continued friendship.

Finally I would like to acknowledge my family and friends, who have supported me in every step of this scientific journey. Thank you especially to Mum and Devon for listening to my excited ramblings or disappointed grumbings over the many dinners you cooked for me. Thank you to Dad for always supporting me and for insisting on calling my cell cultures 'sea monkeys' to put it all into perspective. And thank you to John for being my rock and for believing in me always.

TABLE OF CONTENTS

ABSTRACT	II
ACKNOWLEDGEMENTS	III
TABLE OF CONTENTS	IV
LIST OF TABLES	XII
LIST OF FIGURES	XIII
ABBREVIATIONS	XVI

1 CHAPTER ONE

Introduction	1
Huntington's Disease	1
Overview	1
Neurodegeneration and Symptoms	1
Huntingtin Gene	5
Molecular Pathophysiology	6
<i>Huntingtin function and interacting proteins.....</i>	<i>6</i>
<i>Transcriptional regulation</i>	<i>10</i>
<i>Cell survival and apoptosis</i>	<i>12</i>
<i>Cellular trafficking and endocytosis</i>	<i>12</i>
Key Features for the Development of HD Phenotype.....	15
<i>Huntingtin aggregation</i>	<i>15</i>
<i>N-terminal truncation and nuclear locali ation of huntingti</i>	<i>8</i>
<i>Huntingtin protein accumulation</i>	<i>19</i>
HD Models	19
<i>Lesion models</i>	<i>19</i>
<i>Full-length huntingtin models.....</i>	<i>20</i>
<i>Truncated huntingtin models</i>	<i>21</i>
Summary.....	23

CB1 Cannabinoid Receptors In HD	24
G-Protein Coupled Receptors: A Brief Overview.....	24
Cannabinoid Receptor Dysfunction In HD.....	24
CB1 Cannabinoid Receptor Structure.....	27
CB1 Receptor Localisation.....	27
Endocannabinoids	28
CB1 Receptor Signalling.....	29
<i>Adenylate cyclase</i>	29
<i>Calcium channels</i>	29
<i>Inwardly rectifying potassium channels</i>	30
<i>Raf-1/MEK/ERK cascade</i>	33
<i>c-Jun N-terminal kinase/ p38 Mitogen activated protein kinase</i>	36
<i>Immediate early genes</i>	36
CB1R transcriptional regulation: Normal and in HD.....	38
CB1 receptor trafficking: Normal and in HD.....	39
Summary.....	40
AIMS OF THESIS	42

2 CHAPTER TWO

Development of Cell Models and Assays For Investigating HD	44
Introduction.....	44
Materials and Methods	46
PC12 and HEK-293 Cell lines.....	46
<i>Routine maintenance</i>	46
<i>Freezing and thawing cells</i>	47
<i>Cell counting and plating</i>	47
Huntingtin Expression Constructs	48
<i>pBWN vector</i>	48
<i>Huntingtin gene insert</i>	48
<i>pBWN construct verification by genomic PCR</i>	49
GPCR Expression Constructs.....	51

<i>Endogenous/ transfected receptor verification by reverse transcriptase PCR</i>	51
<i>Generation of human CB1R and D1R receptor expression constructs</i>	53
<i>Transfection of Huntingtin and Receptor Constructs</i>	54
<i>Preparation of plasmid DNA for transfection</i>	54
<i>Transfection</i>	55
<i>Clonal isolation and selection</i>	55
<i>Validation of Expression of Transfected Huntingtin and Receptor Constructs</i>	55
<i>Tebufenozide inducer</i>	56
<i>'Live-labelling': Receptor immunocytochemistry</i>	56
<i>Imaging huntingtin and receptor expression</i>	57
<i>Validation of Alamar Blue Assay</i>	57
<i>Comparison of Aggregate Counting Assays</i>	58
<i>Confirmation of visually punctate species as aggregates</i>	58
<i>Comparison of high throughput assays for the quantification of mutant huntingtin aggregates</i>	59
<i>PolyQ Assay</i>	60
<i>Find Spots</i>	60
<i>Granularity</i>	61
<i>Cell Scoring</i>	61
<i>Statistical Analyses</i>	61
Results and Discussion	63
<i>Generation of Huntingtin- and Receptor-Expressing Cell Lines</i>	63
<i>pBWN construct verification</i>	63
<i>Reverse transcriptase-PCR shows endogenous expression of D2R but not CB1R or D1R by both HEK and PC12 cells</i>	63
<i>Huntingtin expression from pBWN is heterogeneous in HEK cell clones and homogeneous in PC12 cell clones</i>	64
<i>Immunocytochemistry shows expression of hCB1R and hD1R in transfected HEK and PC12 cells</i>	64
<i>Validation of Alamar Blue for Assay of Cell Death</i>	72
<i>The Alamar Blue assay is highly correlated with cell number allowing accurate quantification of cell death</i>	72

Comparison of Aggregate Counting Assays.....	75
<i>The MetamorphTM Cell Scoring assay most accurately measures the formation of mutant huntingtin aggregates in PC12 cells</i>	75
Summary.....	81

3 CHAPTER THREE

Profiles and Mechanisms of Cell Death.....	82
Introduction.....	82
Materials and Methods	84
Cell Death and Aggregate Formation Profiles with Huntingtin Expression	84
Mechanisms of Cell Death	85
<i>Impact of mitochondrial energy disturbance on cell survival.....</i>	<i>85</i>
<i>Impact of increased histone acetylation on cell survival with chronic huntingtin expression</i>	<i>86</i>
<i>Changes in histone acetylation with chronic huntingtin expression</i>	<i>86</i>
Statistical Analyses	87
Results and Discussion.....	88
Cell Death and Aggregate Formation Profiles with Huntingtin Expression	88
<i>HEK cell death following huntingtin expression is mild and delayed</i>	<i>88</i>
<i>HEK cell aggregate formation following huntingtin expression is both expression time- and huntingtin concentration-dependent.....</i>	<i>90</i>
<i>PC12 cell death and aggregate formation following huntingtin expression was concentration-dependent</i>	<i>90</i>
<i>PC12 cell death and aggregate formation following huntingtin expression was expression time-dependent</i>	<i>92</i>
Mechanisms of Cell Death	97
<i>3-Nitropropionic acid shows additivity with mutant huntingtin-induced death in PC12 cells but not HEK cells</i>	<i>97</i>
<i>Valproic acid is protective against huntingtin-induced death in PC12 cells.....</i>	<i>102</i>
<i>Protection against huntingtin-induced death by valproic acid may be independent of histone deacetylase inhibition.....</i>	<i>103</i>
Summary.....	111

4 CHAPTER FOUR

Loss of CB1 Cannabinoid Receptors in HD.....	113
Introduction.....	113
Materials and Methods	115
Analysis of Receptor Levels with Chronic Huntingtin Expression	115
<i>Western blotting analysis of total receptor levels.....</i>	<i>115</i>
<i>Confocal analysis of surface and total receptor levels</i>	<i>116</i>
Analysis of Receptor Trafficking with Chronic Huntingtin Expression	117
Cannabinoid ligands	117
Dopaminergic ligands	117
<i>Validation of basal receptor trafficking.....</i>	<i>118</i>
<i>Validation of ligands for receptor internalisation</i>	<i>118</i>
<i>Concentration dependence and time course of receptor internalisation.....</i>	<i>118</i>
<i>Whole cell enzyme-linked immunosorbent assay (ELISA)</i>	<i>119</i>
Analysis of Receptor Levels with Chronic 3NP Treatment	120
<i>Western blotting analysis of total receptor levels.....</i>	<i>120</i>
Statistical Analyses	121
Results and Discussion.....	122
Total Receptor Expression Levels Following Chronic Huntingtin Expression.....	122
<i>Total CB1 receptor levels are decreased following chronic expression of mutant but not ‘wildtype’ huntingtin</i>	<i>122</i>
<i>Total D1 receptor levels are maintained following chronic expression of mutant huntingtin</i>	<i>124</i>
<i>Cell surface CB1 receptors are disorganised following chronic expression of mutant but not ‘wildtype’ huntingtin.....</i>	<i>131</i>
<i>Cell surface D1 receptors retain normal distribution following chronic expression of mutant huntingtin</i>	<i>131</i>
Validation of Basal Receptor Internalisation.....	136
<i>Both CB1 and D1 receptors internalise in the absence of exogenous ligand</i>	<i>136</i>
Validation of Ligands for Receptor Internalisation	137

<i>HU210 and dopamine are potent, efficacious ligands for the induction of CB1 and D1 receptor internalisation respectively.....</i>	<i>137</i>
Potency of Receptor Internalisation Following Chronic Huntingtin Expression ...	141
<i>The potency of internalisation is unchanged by chronic huntingtin expression for both CB1 and D1 receptors.....</i>	<i>141</i>
<i>The time course of internalisation is unchanged by chronic huntingtin expression for CB1 receptors but slowed for D1 receptors</i>	<i>145</i>
Total CB1 Receptor Expression Levels Following 3NP Treatment.....	149
Summary.....	153

5 CHAPTER FIVE

Cannabinoid Rescue of HD Cell Death.....	156
Introduction.....	156
Materials and Methods	159
Cannabinoid Ligands.....	159
cAMP-Modulating Ligands.....	159
Dopaminergic Ligands	159
Analysis of Cannabinoid Effects on HD Cell Death	160
<i>Modulation of huntingtin-induced cell death profiles.....</i>	<i>160</i>
<i>Modulation of 3NP-induced cell death profiles.....</i>	<i>161</i>
Analysis of Cyclic Adenosine Monophosphate (cAMP) Levels	161
Analysis of Phosphorylated ERK Levels	162
<i>Western Blotting.....</i>	<i>162</i>
<i>Immunocytochemistry.....</i>	<i>163</i>
Statistical Analyses	164
Results and Discussion.....	165
Effect of Cannabinoids on Huntingtin-induced Cell Death Profile	165
<i>HU210 and WIN55,212-2 reduced mutant huntingtin-induced cell death</i>	<i>165</i>
Mechanism for HU210-Mediated Rescue of Huntingtin-induced cell death.....	171
<i>Elevation of cAMP levels exacerbated huntingtin-induced cell death</i>	<i>171</i>
<i>HU210-mediated rescue of huntingtin-induced cell death was not limited in magnitude by low basal cAMP levels</i>	<i>177</i>

<i>HU210-mediated rescue of huntingtin-induced cell death was not limited in magnitude by low levels of cell surface CB1 receptor</i>	180
<i>HU210-mediated rescue of huntingtin-induced cell death is dependent upon Gai and pERK</i>	183
<i>HU210 showed no significant alleviation of 3NP-induced death</i>	189
Summary.....	192

6 CHAPTER SIX

Cannabinoid Modulation Of Aggregate Formation in HD..... 195

Introduction.....	195
Materials and Methods	196
Effect of Cannabinoids, Dopaminergics and cAMP-Modulating Agents on Huntingtin Aggregate Formation.....	196
Statistical Analyses	196
Results and Discussion.....	197
Effect of Cannabinoids on Huntingtin Aggregate Formation	197
<i>HU210 enhanced the formation of mutant huntingtin aggregates</i>	197
Mechanism for Cannabinoid Effects on Huntingtin Aggregate Formation.....	203
<i>Activators of the cAMP pathway enhanced the formation of mutant huntingtin aggregates</i>	203
<i>HU210-mediated enhancement of aggregate formation proceeds through a different pathway to HU210-mediated alleviation of huntingtin-induced cell death</i>	209
Summary.....	218

7 GENERAL DISCUSSION

Overview.....	220
Aim One: Developing and characterising <i>in vitro</i> models of HD.....	221
Aim Two: Investigating Mechanisms for the Loss of the CB1 Receptor in HD.....	225
Aim Three: Investigating the CB1 Receptor as a Therapeutic Target in HD.....	226
Conclusion	228

8 APPENDICES

Gene and Plasmid Sequences	230
Huntingtin Constructs.....	230
25Q Htt-EGFP.....	230
97Q Htt-EGFP.....	231
pBWN Vector	232
Schematic of pBWN-97Q Htt Plasmid	237
GPCR Constructs	238
3x HA.11 CB1 Cannabinoid Receptor	238
3x HA.11 D1 Dopamine Receptor	239
pEF4 His5 Version A Vector	240
Schematic of pEF4a-3HA.D1R Plasmid	244

9 REFERENCE LIST

LIST OF TABLES

Table 1.1 The Vonsattel Scale for neuropathological classification of Huntington’s disease (Summarised from Vonsattel et al., 1985).	3
Table 1.2 Huntingtin interacting proteins and their functions.	8
Table 2.1 Cycling conditions for pBWN construct verification by genomic PCR.....	50
Table 2.2 Cycling conditions for endogenous receptor verification by RT-PCR.....	53
Table 2.3 Primers for endogenous receptor verification by RT-PCR.....	53
Table 2.4 Correlation between Alamar Blue, Hoechst fluorescence, and Discovery 1™ cell counts in PC12 cells.	72
Table 3.1 Conditions for investigating cell death and aggregate formation in HEK and PC12 cells.	84

LIST OF FIGURES

Figure 1.1 Schematic of the sequence of neuronal degeneration in the HD striatum.	4
Figure 1.2 A proposed mechanism for transcriptional dysregulation by mutant huntingtin.	11
Figure 1.3 A proposed mechanism for trafficking dysregulation by mutant huntingtin. ...	14
Figure 1.4 A proposed mechanism for amyloid fibrillation of mutant huntingtin.....	17
Figure 1.5 Autoradiogram of 3H-CP55940-labelled CB1 receptor loss in HD striatum.	26
Figure 1.6 Schematic of depolarisation-induced suppression of excitation or inhibition by cannabinoids.....	32
Figure 1.7 Schematic showing CB1 receptor activation of the Raf/ MEK/ ERK pathway....	35
Figure 1.8 Schematic showing the Rab GTPases potentially involved in CB1 receptor trafficking.....	41
Figure 2.1 Schematic of the pBWN expression system.....	49
Figure 2.2 Verification of pBWN construct in gifted PC12 cells and endogenous/ transfected receptor expression in HEK and PC12 cells.....	66
Figure 2.3 Wildtype and mutant huntingtin expression in transfected HEK and PC12 cells.	68
Figure 2.4 hCB1R and hD1R cell surface expression in transfected HEK and PC12 cells. ...	70
Figure 2.5 Validation of Alamar Blue assay.	73
Figure 2.6 Comparison of aggregate counting assays.	77
Figure 2.7 Photomicrograph of aggregate counting assays.	79
Figure 3.1 Cell death and aggregate formation profile with huntingtin expression in HEK cells.	93
Figure 3.2 Cell death and aggregate formation profile with huntingtin expression in PC12 cells.	95
Figure 3.3 Effect of the mitochondrial toxin 3-nitropropionic acid on huntingtin-induced death in HEK and PC12 cells.	100
Figure 3.4 Effect of the histone deacetylase inhibitor valproic acid on huntingtin- induced death in PC12 cells.....	105

Figure 3.5 Changes in histone acetylation with huntingtin expression in PC12 cells.	107
Figure 3.6 Effect of the histone deacetylase inhibitor valproic acid on histone acetylation in un-induced and huntingtin-expressing PC12 cells.	109
Figure 4.1 Western blot analysis of total CB1 and D1 receptor levels with chronic huntingtin expression in PC12 cells.....	125
Figure 4.2 Confocal imaging analysis of total CB1 and D1 receptor levels with chronic huntingtin expression in PC12 cells.....	127
Figure 4.3 Changes in surface CB1 and D1 receptor levels with chronic huntingtin expression in PC12 cells.....	132
Figure 4.4 Validation of basal receptor trafficking and ligands for receptor internalisation in PC12 cells.	139
Figure 4.5 Receptor internalisation concentration response following chronic huntingtin expression in PC12 cells.....	143
Figure 4.6 Receptor internalisation time course following chronic huntingtin expression in PC12 cells.....	147
Figure 4.7 Western blot analysis of total CB1 receptor levels with chronic 3NP treatment in PC12 cells.	151
Figure 5.1 Effect of HU210 and SR141716A signalling on huntingtin-induced death in PC12 cells.	167
Figure 5.2 Effect of WIN55,212-2 and BAY59-3074 signalling on huntingtin-induced death in PC12 cells.....	169
Figure 5.3 Validation of cAMP-modulating ligands and their effects on huntingtin-induced death in PC12 cells.....	172
Figure 5.4 Effect of dopamine and SCH23390 on huntingtin-induced death in PC12 cells.	175
Figure 5.5 Effect of co-application of forskolin and HU210 on huntingtin-induced cell death in PC12 cells.....	178
Figure 5.6 Effect of CB1 receptor up-regulation with SR141716A on subsequent HU210-mediated rescue of huntingtin-induced death in PC12 cells.	181
Figure 5.7 Effect of pertussis toxin and UO126 on HU210-mediated rescue of huntingtin-induced death and induction of pERK in PC12 cells.	187

Figure 5.8 Effect of HU210 and SR141716A signalling on 3NP-induced death in PC12 cells.	190
Figure 5.9 Schematic for the proposed CB1 receptor signalling pathways which modulate cell viability in the PC12 cell model of HD.	196
Figure 6.1 Effect of HU210 and SR141716A signalling on the formation of mutant huntingtin aggregates in PC12 cells.....	199
Figure 6.2 Effect of WIN55,212-2 and BAY59-3074 signalling on the formation of mutant huntingtin aggregates in PC12 cells.....	201
Figure 6.3 Effect of cAMP-modulating ligands on the formation of mutant huntingtin aggregates in PC12 cells.	205
Figure 6.4 Effect of dopamine and SCH23390 on the formation of mutant huntingtin aggregates in PC12 cells.	207
Figure 6.5 Effect of co-application of forskolin and HU210 on the formation of mutant huntingtin aggregates in PC12 cells.....	210
Figure 6.6 Effect of pre-treatment with pertussis toxin and UO126 on the formation of mutant huntingtin aggregates in PC12 cells.....	215
Figure 6.7 Schematic for the proposed CB1 receptor signalling pathways which modulate cell viability and huntingtin aggregation in the PC12 cell model of HD.....	217
Figure 7.1 Schematic for the proposed CB1 receptor signalling pathways which modulate cell viability and huntingtin aggregation in the PC12 cell model of HD.....	229

ABBREVIATIONS

2-AG	2-arachidonyl glycerol
3NP	3-nitropropionic acid
6E	Ecdysone receptor-responsive promoter
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
BAY59-3074	3-[2-Cyano-3-(trifluoromethyl)phenoxy] phenyl 4,4,4-trifluoro-1-butanesulfonic acid ester
BDNF	Brain derived neurotrophic factor
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
cDNA	Complimentary DNA
CMV	Cytomegalovirus
CRE	cAMP response element
DEPC	Diethylpyrocarbonate
dNTP	Dinucleotide triphosphates
DSE	Depolarisation-induced suppression of excitation
DSI	Depolarisation-induced suppression of inhibition
DTT	Dithiothreitol
EcR	Ecdysone receptor
EGFP	Enhanced green fluorescent protein
Enk	Enkephalin
EPSP	Excitatory postsynaptic potential
ER	Endoplasmic reticulum
ERK	Extracellular signal regulated kinase
FAN	Factor associated with neutral sphingomyelinase activation
GABA	γ -aminobutyric acid
GIRK	G-protein coupled inwardly rectifying potassium channel
GPCR	G-protein coupled receptor
GPe	Globus pallidus externus
GPi	Globus pallidus internus

G-protein	GTP-binding protein
HA	Haemagglutinin
HAP1	Huntingtin associated protein 1
HAT	Histone acetyl transferase
HD	Huntington's disease
HDAC	Histone deacetylase
HEK	Human embryonic kidney
HIP1	Huntingtin interacting protein 1
HU210	(6aR,10aR)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol
IBMX	Isobutylmethylxanthine
IEG	Immediate early gene
IP3	Inositol triphosphate
IT15	Interesting Transcript 15
JNK	c-jun N-terminal kinase
KA	Kainic acid
LB	Luria-Bertani
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
mRNA	Messenger RNA
MSN	Medium spiny neuron
NADA	N-arachidonyl-dopamine
NADP	Nicotinamide adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartic acid
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGK	Phosphoglycerate kinase
PI3K γ	Phosphatidylinositol-3-kinase γ
PKA	cAMP-dependent protein kinase A
PKB	Protein kinase B
PLC β	Phospholipase C β
PRD	Proline rich domain

polyQ	Polyglutamine
PPN	Pedunculo pontine nucleus
PTX	Pertussis toxin
QA	Quinolinic acid
Rp-cAMPS	Rp-Adenosine-3',5'-cyclic monophosphorothioate
RT-PCR	Reverse transcriptase PCR
RXR	Retinoid X receptor
RyR	Ryanodine receptor
SCH23390	7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol maleate
SFM	Serum-free media
SKF38393	1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol
SNC	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
Sp1	Stimulating protein 1
SR141716A	N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
SRE	Serum response element
STN	Subthalamic nucleus
TFZ	Tebufenozide, N-tert-butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide
TNFR	Tumour necrosis factor receptor
WIN55,212-2	(R)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt]
YAC	Yeast artificial chromosome
Δ^9 -THC	Δ^9 -tetrahydrocannabinol