LEABHARLANN CHOLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH | TRINITY COLLEGE LIBRARY DUBLIN
Ollscoil Átha Cliath | The University of Dublin

Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Influence of anti-inflammatory interventions in the Kainic acid model of hippocampal excitotoxicity

Thesis submitted for the degree of Doctor of Philosophy at the University of Dublin, Trinity College

2010



Lorna C. Gleeson

Supervisor: Andrew Harkin

School of Pharmacy and Pharmaceutical Sciences

Trinity College Institute of Neuroscience

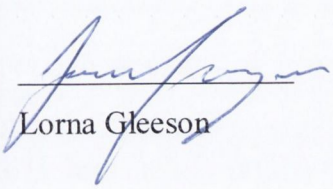
Trinity College

Dublin 2.

TRINITY COLLEGE
2 7 AUG 2010
LIBRARY DUBLIN
THESIS
8906

I Declaration

This thesis is submitted by the undersigned for the degree of Doctor of Philosophy at the University of Dublin, Trinity College and has not been submitted to any other university as an exercise for a degree. I declare that this thesis is entirely my own work and I give permission to the library to lend or copy this thesis upon request



Lorna Gleeson

II Acknowledgements

I would like to acknowledge the Irish Research Council for Science Engineering and Technology and Dr Harkin for their support over the duration of this thesis.

I'd like to say a big thanks to Tom for all his advice and support throughout the 3 (and a bit) years.

I have to also say a big thanks to Jen, for all her help, especially when I was working from home, you were like my foothold in the office, and when email failed me you were always there to pass messages on for me-which was always greatly appreciated.

Eadaoin, I would also like to thank you for being so harsh, though thorough is more how I would describe it! I'd say you're as delighted as me to see me submit this thesis, as it means no more pestering from me about anything to do with stats, BDNF, formatting or anything to do with writing up-im sorry!

Thanks to everyone who proofread any of this thesis and helped me throughout the 3 years-Jen, the other Jen, Katie, Valentina, Aine, Karen, Ronan and Daragh.

Most importantly I have to say a huge thank you to Natacha. You were there for every *in vivo* study, for every injection, all the behaviour- the good, the bad and the ugly. You were a great support during the last 3 years, in both the lab and outside of it, I can safely say I would not have completed this thesis without your help- *Nous sommes maintenant nettoyeurs professionnels*!

And to Ronan, Thank you for cooking my dinners, walking the dog, minding me when I was sick and then again when I broke that vertebrae, for listening to me complain all the time and helping me whenever you could with this thesis.

III Abstract

Excitotoxicity is implicated as a mechanism of neuronal cell death in a range of neurodegenerative disorders. Such toxicity can be induced experimentally by systemic administration of the glutamate kainate receptor agonist, kainic acid (KA). Specifically, KA induces seizures, inflammation, apoptosis and neuronal cell loss in the hippocampus, following systemic administration to rats. All changes are evident within 24 hours of KA administration and over this period, the model is representative of acute neurodegeneration. The aims of the work described in this thesis were to (i) characterise the inflammatory and apoptotic changes which occur in tandem with cell loss in a time course fashion, (ii) to determine if the inflammatory changes underlie KA-induced apoptosis and cell loss and (iii) to determine if modulation of central noradrenaline (NA) could influence KA-induced neurodegeneration. (i) Animals receiving KA showed seizures and related stereotyped behaviours within 3 hours. Expression of IL-1β, TNF-α and IFN-γ were increased in the hippocampus 4, 12 and 24 hours following KA administration. This was accompanied by an increase in expression of the microglial activation marker CD11b, the apoptotic marker caspase 3 and a reduction in cresyl violet staining of viable hippocampal neurons, 24 hours post challenge. (ii) To determine if KA-induced cell loss may be driven by the induction of inflammatory markers in the hippocampus, animals were pre-treated with the anti-inflammatory glucocorticoid, dexamethasone. Pre-treatment with dexamethasone, 1 hour prior to KA administration significantly attenuated the excitotoxin-induced increase in IFN-γ, CD11b and caspase 3 expression, but failed to influence KA-induced seizures or increased DNA fragmentation and hippocampal neuronal cell loss. Thus whilst the inflammatory related changes may contribute to apoptosis, the data suggest that microglial activation and IFN-γ expression do not account for KA-induced neuronal loss. The kynurenine pathway (KP) is a major metabolic pathway of the essential amino acid tryptophan. Indoleamine 2,3-dioxygenase (IDO) is the rate limiting enzyme in the KP. Under inflammatory conditions, IDO is induced by mediators such as IFN-γ, enhancing TRP utilization. In order to determine the degree to which activation of the KP may influence KA-induced excitotoxicity and neuronal loss, the effects of pre-treatment with the IDO inhibitor, 1-methyl tryptophan (1-MT) was assessed in the model. 1-MT failed to influence KA-induced seizures but significantly enhanced KA-induced hippocampal IFN-γ, CD11b, IDO and caspase 3 expression, and attenuated KA-induced DNA fragmentation and hippocampal cell loss.

Thus direct inhibition of IDO and the KP may afford protection against KA-induced hippocampal cell loss and apoptosis, but not inflammation. (iii) NA has been reported to have both innate anti-inflammatory and neuroprotective properties. Here it was determined if the selective noradrenaline reuptake inhibitors- desipramine (DMI) and reboxetine (RBX), could influence neuroinflammatory and degenerative changes induced following KA administration to rats. Pre-treatment with DMI significantly attenuated the KA-induced increase in expression of the hippocampal inflammatory markers and of the growth factor brain derived neurotrophic factor (BDNF) but enhanced KA-induced DNA fragmentation. Pre-treatment with RBX produced a similar response in enhancing KA-induced DNA fragmentation, yet by contrast to DMI, RBX enhanced seizure behaviour, hippocampal iNOS expression and attenuated both the KA-induced increase in BDNF expression, and protein concentrations in the hippocampus. Thus NA transporter inhibition appears to exacerbate KA-induced apoptotic damage, while decreasing the expression of the protective neurotrophin BDNF in the hippocampus. In order to further examine the relationship between transporter inhibition, extracellular NA concentrations and excitotoxin-induced hippocampal injury, pre-treatment with the β-adrenoceptor antagonist propranolol (PRP) was assessed alone and in combination with RBX, following KA administration. PRP attenuated the ability of RBX to enhance KA-induced seizure behaviour, DNA fragmentation and iNOS expression. In line with the anti-inflammatory and neuroprotective properties of NA, signalling via the β_2-adrenoceptor has been associated with neuroprotection. Thus the effect of the β_2 adrenoceptor agonist- clenbuterol on KA-induced inflammation and apoptosis was determined in the hippocampus. Pre-treatment with clenbuterol resulted in an amelioration of KA-induced IFN-γ and iNOS expression. Expression and concentrations of the protective neurotrophin-BDNF and glial fibrillary acidic protein, a marker of astrocytic activation, were increased following pre-treatment with clenbuterol. The results of this study indicate that clenbuterol is anti-inflammatory and anti-apoptotic following KA-induced neuroinflammation and further displays neuroprotective properties by increasing BDNF expression in the hippocampus. Overall the results support a modulatory role for NA in the regulation of KA-induced excitotoxicity, and suggest that the β_2-adrenergic receptor is at least partially responsible for the neuroprotective properties attributed to the modulation of NA transmission centrally.

IV Table of Contents

V TABLE OF FIGURES ..... **XI**

VI TABLE OF TABLES ..... **XV**

VII LIST OF ABBREVIATIONS ..... **XVI**

1.0 INTRODUCTION **1**

1.1 Cell types that participate in neurodegeneration 3

1.2 Excitatory amino acid neurotransmission and neurodegeneration 5

1.3 Ionotropic glutamate receptors 6

1.4 Excitotoxicity 9

1.5 Excitotoxicity and neurodegeneration 10

1.6 Apoptosis and neurodegeneration 12

1.7 Caspases 12

1.7.1 Caspase 3 15

1.8 The role of inflammation in neurodegeneration 18

1.9 The role of pro-inflammatory cytokines in neurodegeneration 19

1.9.1 Inducible nitric oxide synthase (iNOS) 24

1.10 The kynurenine pathway (KP) 25

1.10.1 Indoleamine 2, 3-dioxygenase 26

1.10.2 The neuroprotective branch of the KP 27

1.10.3 The neurotoxic branch of the KP 28

1.11 Excitotoxicity, neuroinflammation and cell death in hippocampal neurodegeneration 29

1.12 The role of neurotrophic factors in neurodegeneration 33
1.12.1 Brain derived neurotrophic factor (BDNF) 33
1.12.2 Nerve Growth Factor 36
1.12.3 Glial derived growth factor 37
1.12.4 Transforming growth factor β 38

1.13 Kainic acid-induced excitotoxicity – a model of acute neurodegeneration 38

1.14 Kainic acid-induced neuroinflammation 41

1.15 Noradrenaline 42
1.15.1 Synthesis, storage and release 42
1.15.2 Adrenergic receptors 45

1.16 NA and neurodegeneration 47

1.17 Aims and objectives 50

MATERIALS AND METHODS 52

2.0 MATERIALS 53

2.1 Methods 57
2.1.1 Animal husbandry 57
2.1.2 Drug Treatments 57
2.1.3 Kainic acid (KA) treatment 58
2.1.4 Behavioural assessment following KA administration 58
2.1.5 Preparation of tissue and serum 58
2.1.6 Tissue sectioning, cresyl violet staining and cell counting 59
2.1.7 Analytical Methods: mRNA 60
2.1.8 Terminal deoxynucleotidyltransferase-mediated UTP nick end labelling (TUNEL) 64
2.1.9 Protein quantification 65
2.1.10 Activated Caspase 3 assay 65
BDNF Enzyme linked immunosorbent assay 66
2.1.11 66

2.1.12 Determination of tryptophan and KP metabolite concentrations by high performance liquid chromatography (HPLC) 68
2.1.13 Statistical Analysis of Results 71

CHAPTER 1 72

3.0 INTRODUCTION 73

3.1 Experimental protocol 77

3.2 Results 78
3.2.1 KA-induced seizure behaviours 78
3.2.2 Time dependent changes in hippocampal cell loss following KA administration 79
3.2.3 Time dependent changes in DNA fragmentation following KA administration 82
3.2.4 Time dependent changes in apoptotic markers following KA administration.... 84
3.2.5 Time dependent changes in neuroinflammatory markers following KA administration. 85
3.2.6 Time dependent changes in the expression of enzymes of the KP in the hippocampus following KA administration. 87
3.2.7 Time dependent changes in the concentrations of tryptophan, and kynurenine pathway metabolites in the hippocampus and serum following KA administration. 89
3.2.8 KA provokes seizure related behaviours in a dose dependant manner 91
3.2.9 KA induces hippocampal cell loss in a dose dependent manner 92
3.2.10 KA provokes a dose dependent increase in hippocampal caspase 3, IFN-γ and IDO expression. 93

3.3 Discussion 96

CHAPTER 2 101

4.0 INTRODUCTION 102

4.1 Experimental protocol 107

4.2 Results 108
4.2.1 Pre-treatment with DEX does not alter KA-induced seizure behaviour 108
4.2.2 Pre-treatment with DEX fails to attenuate KA-induced hippocampal cell loss 109
4.2.3 Pre-treatment with DEX has no effect on KA-induced DNA fragmentation in the CA3 and CA1 110
4.2.4 Pre-treatment with DEX attenuates KA-induced caspase-3 expression in the hippocampus 113
4.2.5 Pre-treatment with DEX attenuates KA-induced hippocampal IFN-γ, CD-11b and IDO expression. 114
4.2.6 Pre-treatment with DEX has no effect on KA-induced tryptophan, and KP metabolites in the hippocampus and serum, following KA administration. 116
4.2.7 Pre-treatment with 1-MT does not alter KA-induced seizure behaviour 118
4.2.8 Pre-treatment with 1-MT attenuates KA-induced hippocampal cell loss 119
4.2.9 Pre-treatment with 1-MT attenuates KA-induced DNA fragmentation in the hippocampus 120
4.2.10 Pre-treatment with 1-MT potentiates KA -induced caspase 3 expression 123
4.2.11 Pre-treatment with 1-MT influences KA-induced IFN-γ and IDO expression in the hippocampus 124
4.2.12 Pre-treatment with 1-MT does not affect KA-induced changes in tryptophan and KP metabolites in the hippocampus and serum, following KA administration 126

CHAPTER 3 135

5.0 INTRODUCTION 136

5.1 NA has innate anti-inflammatory and neuroprotective properties 136

5.2 Pharmacological manipulation of NA prevents cell death 137

5.3 NA confers neuroprotection through the promotion of pro-survival pathways 138

5.4 NA promotes the expression of protective neurotrophins 139

5.5 Experimental protocol 147

5.6 Results 148

5.6.1 Pre-treatment with DMI does not alter KA-induced seizure behaviour 148
5.6.2 Pre-treatment with DMI does not influence KA-induced hippocampal cell loss 149
5.6.3 Pre-treatment with DMI influences KA-induced DNA fragmentation in the hippocampus 150
5.6.4 Pre-treatment with DMI does not influence KA-induced caspase-3 expression but affects caspase 3 activity, in the hippocampus 153
5.6.5 Pre-treatment with DMI attenuates KA-induced hippocampal IFN-γ, IDO and iNOS, but not CD-11b or GFAP expression. 154
5.6.6 Pre-treatment with DMI attenuates KA-induced BDNF mRNA expression without influencing the KA-induced increase in hippocampal BDNF concentrations..156
Pre-treatment with RBX does not alter KA-induced seizure behaviour 157
5.6.7.....157
5.6.8 Pre-treatment with RBX does not affect KA-induced hippocampal cell loss....158
5.6.9 Pre-treatment with RBX does not significantly affect KA-induced DNA fragmentation in the hippocampus 159
5.6.10 Pre-treatment with RBX does not significantly influence KA-induced caspase-3 expression or caspase 3 activity, in the hippocampus 162
5.6.11 Pre-treatment with RBX does not influence KA-induced IFN-γ, IDO, CD-11b or GFAP expression but potentiates KA-induced iNOS expression in the hippocampus 163
5.6.12 Pre-treatment with RBX attenuates KA-induced BDNF expression and BDNF concentrations 165

5.7 Experimental protocol 167
5.7.1 Pre-treatment with PRP and RBX in combination reduces KA-induced seizure behaviours.....167
5.7.2 Pre-treatment with RBX or PRP treatment, alone or in combination, does not affect KA-induced hippocampal cell loss.....169
5.7.3 Pre-treatment with RBX and PRP influences KA-induced DNA fragmentation.... 170
5.7.4 Pre-treatment with PRP and RBX alone or in combination do not influence KA-induced caspase 3 expression in the hippocampus 173
5.7.5 Effect of PRP or RBX alone and in combination on KA-induced IFN-γ, IDO, iNOS, CD-11b and GFAP expression in the hippocampus.....174

5.7.6 Pre-treatment with RBX but not PRP attenuates KA-induced BDNF expression... 177

5.8 Experimental protocol 179

5.8.1 Pre-treatment with CLN does not alter KA-induced seizure behaviour 179

5.8.2 Pre-treatment with CLN does not influence KA-induced hippocampal cell loss.... 180

5.8.3 Pre-treatment with CLN attenuates KA-induced DNA fragmentation in the hippocampus 181

5.8.4 Pre-treatment with CLN does not influence KA-induced caspase-3 expression, but significantly attenuates KA-induced caspase 3 activity in the hippocampus 184

5.8.5 Pre-treatment with CLN affects KA-induced hippocampal IFN-γ, IDO, iNOS, CD11b and GFAP expression. 185

5.8.6 Pre-treatment with CLN augments NGF expression, KA-induced BDNF expression and BDNF concentrations in the hippocampus 187

5.9 Discussion 191

5.9.1 DMI attenuates KA related inflammation yet augments KA-induced apoptosis in the hippocampus 192

5.9.2 RBX augments KA-induced apoptosis associated with an inhibition of BDNF expression in the hippocampus 195

5.9.3 PRP attenuates KA-induced inflammatory and degenerative changes and counteracts the RBX-induced augmentation of apoptosis in the hippocampus 199

5.9.4 CLN has neuroprotective properties in the KA model of hippocampal excitotoxicity 202

6.0 GENERAL DISCUSSION 206

7.0 FUTURE DIRECTIONS 216

VIII BIBLIOGRAPHY 218

IX PUBLICATIONS 279

V Table of Figures

Figure 1.1 The glutamatergic synapse (Gass and Olive, 2008) 8
Figure 1.2 Excitotoxic neuronal death (Fan and Raymond, 2006) 10
Figure 1.3 Caspase signalling and its modulation (Lavrik et al., 2005) 14
Figure 1.4 IL-1 signalling pathway (Kariko *et al.,* 2004) 23
Figure 1.5 Nitric oxide synthases catalyze the production of NO and L-citrulline from L-arginine, O_2, and NADPH-derived electrons (R&D systems) 24
Figure 1.6 An outline of the KP (MacKay *et al.,* 2004) 26
Figure 1.7 Hippocampal circuitry 30
Figure 1.8 BDNF synthesis, processing, sorting, transport and secretion in neurons (Thomas and Davies, 2005) 34
Figure 1.9 BDNF signalling pathways (Sossin and Barker, 2007) 36
Figure 1.10 Structure of Kainic acid. (Biscoe *et al.*, 1976) 39
Figure 1.11 The biosynthesis of NA (Adapted from Kuhar *et al.*, 1999) 43
Figure 1.12 Noradrenergic pathways in the brain (Rudoy and Bockstaele, 2005) 45
Figure 3.1 KA-induced seizure behaviour 78
Figure 3.2 Representative cresyl violet stains indicating hippocampal cell loss (a) 12 hours and (b) 24 hours following KA administration, indicated by arrows 80
Figure 3.3 KA induces a reduction in cell number in the (a) CA3 (b) CA1 and (c) dentate gyrus of the hippocampus 81
Figure 3.5 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus 12 and 24 hours following administration 83
Figure 3.6 KA induces the expression of caspase 3 but not Bcl2 in the hippocampus 84
Figure 3.7 KA induces the expression of hippocampal IL-1β, TNF-α, IFN-γ and iNOS in a time dependant manner. 86
Figure 3.8 KA alters the expression of IDO, KAT-II and KMO in the hippocampus in a time dependant manner 88
Figure 3.9 KA provokes a time dependent increase in hippocampal and circulating tryptophan concentrations 90
Figure 3.10 Dose dependent effects of KA on seizure related behaviours 91
Figure 3.11 Dose related effects of KA on cell loss in the (a) CA3 and (b) CA1 of the hippocampus 92

Figure 3.12 KA induces hippocampal caspase 3, IFN-γ and IDO expression in a dose dependent manner 94
Figure 4.1: KA-induced seizure behaviour is not affected by pre-treatment with DEX 108
Figure 4.2 KA-induced hippocampal cell loss in the CA3 and CA1 is not attenuated by prior treatment with DEX 109
Figure 4.3 KA-induced DNA fragmentation in the CA3 and CA1 is not ameliorated by pre-treatment with DEX. 111
Figure 4.4 KA-induced DNA fragmentation is not affected by pre-treatment with DEX 112
Figure 4.5 KA-induced hippocampal caspase-3 expression is attenuated by pre-treatment with DEX 113
Figure 4.6 KA-induced hippocampal IFN-γ, CD-11b and IDO expression is attenuated following pre-treatment with DEX 115
Figure 4.7 No significant changes were seen in hippocampal tryptophan concentrations, circulating tryptophan and KP metabolites. 117
Figure 4.8 KA-induced seizures are not affected by pre-treatment with 1-MT 118
Figure 4.9 KA-induced hippocampal cell loss is attenuated by treatment with 1-MT 119
Figure 4.10: KA-induced DNA fragmentation in the CA3 and CA1 is attenuated by pre-treatment with 1-MT. 121
Figure 4.11: KA provokes an increase in TUNEL intensity in the (a) CA3 and (b) CA1 of the hippocampus, which is attenuated in the CA3 by treatment with 1-MT. 122
Figure 4.12: KA-induced hippocampal caspase 3 expression is augmented following pre-treatment with 1-MT 123
Figure 4.13: KA-induced hippocampal IFN-γ and IDO expression is augmented following pre-treatment with 1-MT 125
Figure 4.14: KA-induced changes in hippocampal tryptophan concentrations, circulating tryptophan and KP metabolites, are unchanged following pre-treatment with 1-MT 127
Figure 5.1 KA-induced seizure behaviour is not affected by pre-treatment with DMI 148
Figure 5.2 KA-induced hippocampal cell loss in the CA3 and CA1, is not attenuated by prior treatment with DMI 149
Figure 5.4 KA provokes an increase in TUNEL intensity in the (a) CA3 and (b) CA1 of the hippocampus, which is enhanced by prior treatment with DMI in the CA3. 152
Figure 5.5 KA-induced hippocampal caspase-3 expression, and activity is not influenced, by pre-treatment with DMI 153

Figure 5.6 KA-induced hippocampal IFN-γ and iNOS expression is attenuated, but IDO, CD11b and GFAP expression is unchanged following pre-treatment with DMI 155
Figure 5.7 KA-induced hippocampal BDNF mRNA expression was attenuated, and BDNF protein was unchanged, following pre-treatment with DMI 156
Figure 5.8 KA-induced seizure behaviour is not affected by pre-treatment with RBX 157
Figure 5.9 KA-induced hippocampal cell loss in the CA3 and CA1 is not attenuated by prior treatment with RBX 158
Figure 5.11 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus which is not significantly affected by RBX 161
Figure 5.12 KA-induced hippocampal caspase-3 expression is unaltered, but caspase 3 activity is augmented, following pre-treatment with RBX 162
Figure 5.13 KA-induced hippocampal iNOS expression is augmented, but IFN-γ, IDO, CD-11b and GFAP expression is unchanged, following pre-treatment with RBX 164
Figure 5.14 KA-induced hippocampal BDNF expression and BDNF protein is attenuated following pre-treatment 165
Figure 5.15 RBX augments KA induced seizure behaviours, an effect which is ameliorated by the co-administration of PRP and RBX 168
Figure 5.16 KA-induced hippocampal cell loss in the CA3 and CA1 is unchanged following pre-treatment with RBX, PRP or RBX and PRP in combination 169
Figure 5.18 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus which is influenced by pre-treatment with RBX and PRP 172
Figure 5.19 KA-induced hippocampal caspase 3 expression was not influenced by pre-treatment with PRP and RBX alone or in combination 173
Figure 5.20 KA-induced hippocampal iNOS and CD-11b expression was attenuated, and IFN-γ, IDO and GFAP expression was unchanged following pre-treatment with RBX and PRP 176
Figure 5.21 RBX attenuates KA-induced BDNF expression, an effect which is unaffected by co-administration of PRP and RBX 177
Figure 5.22 KA-induced seizure behaviour is affected by pre-treatment with CLN 179
Figure 5.23 KA-induced hippocampal cell loss in the CA3 and CA1 is not attenuated by prior treatment with CLN 180
Figure 5.25 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus which is attenuated by prior treatment with CLN 183

Figure 5.26 KA-induced hippocampal caspase-3 expression is not influenced, but caspase 3 activity is significantly ameliorated, by pre-treatment with CLN 184
Figure 5.27 KA-induced IDO and iNOS expression is attenuated, GFAP expression is augmented, and CD11b and IFN-γ expression is unaffected, following pre-treatment with CLN 186
Figure 5.28 KA failed to induce hippocampal NGF expression alone, but pre-treatment with CIN resulted in an increase in NGF expression, following KA administration 187
Figure 5.29 KA-induced hippocampal BDNF expression and BDNF protein shows a trend towards an increase following pre-treatment with CLN. 188
Figure 6.1 Mechanism of action of DEX 208
Figure 6.2 Mechanism of action of DMI, RBX and CLN 210

VI Table of Tables

Table 1.1 Comorbidity of Neurodegenerative disorders and Depression 2
Table 1.2 Classification of adrenergic receptors 46
Table 2.1 Racine scale 58
Table 2.2 List of genes used in single RT-PCR studies with the gene expression assay and genbank ref sequence numbers 63
Table 2.3 Excitation wavelengths and filter settings for TUNEL staining 65
Table 3.1 Summary of Time course results 95
Table 3.2 Summary of Dose response results 95
Table 4.1 Summary of DEX results 128
Table 4.2 Summary table 1-MT results 128
Table 5.1 Summary of DMI results 189
Table 5.2 Summary table of RBX results 189
Table 5.3 Summary table of RBX and PRP results 190
Table 5.4 Summary of CLN results 190

VII List of abbreviations

1-MT	1 methyl tryptophan
3-HAA	3-hydroxyanthranilic acid
3-HAO	3-hydroxyanthranilic acid oxygenase
3-HK	3-hydroxykynurenine
5-HT	serotonin
ABI	Applied Biosystems
AD	Alzheimer's disease
AIDS	Acquired immune deficiency syndrome
ALS	Amyotrophic lateral sclerosis
AMPA	a-amino-3-hydroxy-5-methylisoxazole
Aβ	Amyloid beta
ANOVA	Analysis of variance
AP-1	activator protein-1
Apaf-1	apoptotic protease-activating factor-1
Asp	Aspartate
ATP	Adenosine triphosphate
BAX	Bcl-2 associated X protien
BBB	blood brain barrier
BCA	bicinchoninic acid
BCG	Bacillus Calmette-Guerin
Bcl-2	B cell lymphoma-2
BDNF	Brain derived neurotrophic factor
Bid	BH3 interacting domain
BP	Band Pass Filter
BSA	Bovine serum albumin

C	Celcius
Ca	Calcium
CA1	Cornu ammonus 1
CA3	Cornu ammonus 3
CAD	caspase-activated deoxyribonuclease
CaMKII	calmodulin dependent kinase II
cAMP	cyclic adenosine monophosphate
CD	Cluster differentiation
cDNA	complementary DNA
CED	Caenorhabditis elgans Death gene
c-FLIP	cellular FLICE-like inhibitory protein
CHAPS	3-[(3-Cholamidopropyl) dimethylammonio]-1-
Cl	Chloride
CLN	Clenbuterol
CNS	Central nervous system
$CoCl_2$	Cobalt Chloride
COMT	catechol-O-methyl transferase
Con-A	Concanavalin-A
COX	Cyclooxygenase
CPP-32	32-kDa putative cysteine protease
CRE	cAMP-response element
CREB	cAMP-response element binding protein
CRP	C-reactive protein
Ct	Threshold cycle
D	Dextro
DAG	Diacylglycerol
dATP	deoxyadenosine triphosphate

DBH	dopamine b-hydroxylase
DEPC	Diethyl Pyrocarbonate
DEX	Dexamethasone
DG	Dentate gyrus
dH2O	Distilled water
DIABLO	Direct IAP Binding Protein with Low PI
DISC	death inducing signalling complex
DMI	Desipramine
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribnucleic acid
DOMA	aldehyde- dihydroxymandelic acid
DOPA	dihydroxyphenylalanine
DPX	Dibutyl phthalate xylene
DSP-4	N-(2- chloroethyl)-N-ethyl-2 bromobenzylamin
DTT	Dithiothreitol
EAE	Experimental allergic encephalomyelitis
EC	Entorhinal cortex
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
EPSP	Excitatory post synaptic potential
ERK	Extracellular receptor kinase
FADD	Fas-Associated protein with Death Domain
FAM	6-carboxy fluorescein
FDA	Food and drug administration
Fe	Iron
FLICE	FADD-like IL-1β-converting enzyme

FRET	Fluorescence resonance energy transfer
G protein	Guanine nucleotide protein
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Gas2	Growth arrest-specific protein
GDNF	Glial cell line-derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
Gi	Inhibitory G protein
GluT	Glutamate transporter
Gq	
Gs	Stimulatory G protein
GSH	Glutathione
GSK	Glycogen synthase kinase
H202	Hydrogen peroxide
HCl	Hydro Chloride
HD	Huntington's disease
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HFT	Main Dichroic Beam Splitter
HPA	Hypothalamic-pituitary-adrenal
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
i.p	Intra peritoneal
IAP	Inhibitors of apoptosis
ICAD	Inhibitor of caspase-activated DNase
ICAM-1	Intercellular cell adhesion molecule 1
ICE	Interleukin-1β converting enzyme
i.c.v	Intracerebroventricular

IDO	Indoleamine 2, 3-dioxygenase
IFN-γ	Interferon gamma
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IP3	Inositol triphosphate
IκB	inhibitory kappa B
IKK	IκB kinase
JNK	Jun N-terminal Kinase
K	Potassium
K3-H	kynurenine 3-hydroxylase
KA	Kainic acid
KAR	Kainic acid receptor
KAT	Kynurenine amino transferase
KCl	Potassium Chloride
kDa	kilo Dalton
kg	kilograms
KH2PO4	Potassium dihydrogen Phosphate
KMO	Kynurenine 3-mono-oxygenase
KP	Kynurenine pathway
KYNA	Kynurenic acid
KYNase	Kynureninase
L	Levo
LC	Locus coeruleus
LDH	Lactose dehydrogenase
LTD	Long-term depression
LDP	Long-term potentiation
L-KYN	L-kynurenine

L-NAME	Nω-nitro-L-arginine methyl ester
LP	High Pass Filter
LPS	Lipopolysaccharide
L-TRP	L- trypotophan
MAO	Monoamine oxidase
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein
MeOH	Methanol
Mf	mossy fibres
Mg	Magnesium
mg	milligrams
MGB	Minor groove binders
mGluR	metabotropic glutamate receptor
MHC	major histocompatibility complex
ml	millilitres
mNBA	meta-nitrobenzoylalanine
MPP	1-methyl-4-phenylpyridinium
mRNA	messenger ribonucleic acid
MS	Multiple sclerosis
Na	Sodium
NA	Noradrenaline
Na_2CO_3	Sodium carbonate
Na_2HPO_4	Sodium Phosphate
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodium Bicarbonate

NET	Noradrenaline transporter
NFQ	Non-fluorescent quencher
NFκB	Nuclear factor kappa B
NGF	Nerve growth factor
NK	Natural killer
NMDA	N-methyl-D-aspartate
NMDAR	NMDA receptor
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
NRI	noradrenaline reuptake inhibitor
O_2	Oxygen
O_{2^-}	Superoxide
OCT	Optimum cutting temperature
OH	Hydroxide
ONOO	Peroxynitrite
Pab	Polyclonal antibody
PARP	poly (ADP-ribose) polymerase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD	Parkinson's disease
PDA-UV	Photo diode array ultra-violet
PI3-K	Phosphatidylinositol 3′ kinase
PKA	cAMP-dependent protein kinase
PKC	Phosphokinase C
PLC	Phospholipase C
PNS	Peripheral nervous system

PPAR	Peroxisome proliferators-activated receptor
PRP	Propranolol
QUIN	Quinolinic acid
Rb	Retinoblastoma
RBX	Reboxetine
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
Rpm	Revolutions per minute
RT-PCR	Real time PCR
RyR	Ryanodine receptor
s.c	sub cutaneous
Sc	Schaffer Collateral
SCC	Sodium chloride citrate
SEM	Standard error of the mean
Ser	Serine
Smac	Second Mitochondria-derived Activator of Caspases
tBid	Truncated Bid
TCA	Tricyclic
TDO	Trypotophan 2, 3-dioxygenase
TdT	Terminal Deoxynucleotidyl Transferase
TGF	Transforming growth factor
Th	T helper cells
TLE	Temporal lobe epilepsy
TNF	Tumor necrosis factor
TNFR	TNF receptor
trk-B	Tropomyosin-related kinase-B

TRP	Tryptophan
TUNEL	Terminal deoxynucleotidyltransferase-mediated UTP nick end labelling
UK	United Kingdom
UV	Ultraviolet
VGCC	Voltage-gated calcium channels
VMA	3-methoxy- 4-hydroxymandelic acid
WDS	Wet dog shakes
WHO	World health organisation
XIAP	X-linked inhibitor of apoptosis
µl	microlitres

1.0 Introduction

Neurodegenerative diseases are defined as hereditary and sporadic conditions which are characterized by progressive nervous system dysfunction. They can be classified into chronic and acute neurodegeneration, according to the factors involved, such as age, genotype and protein dysfunction, which contribute to chronic neurodegeneration, whereas processes such as excitotoxicity, inflammation, deoxyribonucleic acid (DNA) damage, apoptosis and oxidative stress underlie both acute and chronic neurodegeneration. Neurodegenerative diseases are a major global health burden in the Western world particularly within the ageing population. The World Health Organization (WHO) predicts that by 2050, 30 million people will be affected by Alzheimer's disease (AD) in Europe and the USA alone, with other diseases such as Parkinson's disease (PD), Huntington's disease (HD), fronto-temporal dementia, and amyotrophic lateral sclerosis also presenting a great socio-economic burden (WHO, 2002). Research over the last ten years has identified many key markers of various neurodegenerative disorders, for example, it is now well established that there is a familial counterpart to each of the major classes of neurodegenerative disease, most of which are inherited in an autosomal dominant pattern, and that abnormal processing of misfolded proteins is a key event in many neurological disorders (Forman *et al.*, 2004). Current available treatments for various neurodegenerative diseases, such as AD, PD and HD only focus on one aspect of these diseases and do not address the array of mechanisms that are disrupted in these conditions, thus highlighting the need for newer, more comprehensive treatments.

Neuropsychiatric disorders such as depression and anxiety have been shown to be linked to neurodegenerative conditions. Hippocampal atrophy is implicated in depression where some studies have found significant bilateral volume deficits in depressive disorders (MacQueen *et al.*, 2003; Sheline, 1996; Sheline, 1999; Colla *et al.*, 2007). A correlation between age of onset of depression and hippocampal volume was seen, with those suffering late onset depression having smaller hippocampi, especially the right hemisphere (Steffens *et al.*, 2000). This atrophy could potentially be due to neuronal loss through exposure to repeated episodes of hypercortisolemia and glial loss, leading to glutamate neurotoxicity. Also stress-induced reduction in neurogenesis and neurotrophic factors leave the hippocampus vulnerable to damage (Lee *et al.*, 2002). Hippocampal atrophy is also a hallmark of AD (Convit *et al.*, 1997; de Leon *et al.*, 1996) and post mortem studies

have indicated a volume loss of 25% to 50% in AD patients (Sheline *et al.*, 2002), suggesting a link between AD and depression. There is a strong body of evidence that suggests comorbidity of depression with neurodegenerative conditions such as AD, but also schizophrenia, stroke, epilepsy and PD (Asaal, 2002; Sweet *et al.*, 2004; Kessler, 1993; Mulholland *et al.*, 1996; Whyte *et al.*, 2002; Robertson and Trimble 1983, O'Donoghue *et al.*, 1999; Lieberman, 2006).

Table 1.1 Comorbidity of Neurodegenerative disorders and Depression

AD and depression	Asaal, 2002; Sweet *et al.*, 2004; Lee *et al.*, 2003; Lyketsos *et al.*, 2004
PD and depression	Lieberman, 2006; Reijnders *et al.*, 2007; Willner, 1997
Epilepsy and depression	Robertson and Trimble, 1983; O'Donoghue *et al.*, 1999; Baker *et al.*, 1996; Edeh and Toone, 1987; Jacoby *et al.*, 1996
Stroke and depression	Whyte *et al.*, 2002; Belbo *et al.*, 1999; Gainotti *et al.*, 1999

Chronic neurodegeneration is a slow process that occurs over time and is characterised by a number of degenerative events that culminate in neuronal cell loss. Chronic neurodegeneration predominantly affects the ageing population with the incidence of these disorders increasing dramatically with advancing age (WHO, 2002). The mechanisms involved in chronic neurodegeneration involve neuroinflammation, excitotoxicity, mitochondrial dysfunction, production of free radicals, and disruptions in the production of neurotransmitters, neurotrophic factors, calcium homeostasis, metal ions and nitric oxide. Many of these events are secondary processes to core changes in the processing of misfolded proteins such as β amyloid (Aβ), Tau, α-synuclein and Huntington, and their subsequent aggregation causing the formation of senile plaques, neurofibrillary tangles and lewy bodies (Forman *et al.*, 2004). There is now significant evidence that brain inflammation contributes to the pathology of neurodegenerative diseases, and that interventions to inhibit the onset of this inflammation provide protection against the onset or reduction of the symptoms of the disease (Klegeris *et al.*, 2007; Zipp and Aktas, 2006; Lucas *et al.*, 2006; Brown and Bal-Price, 2003; Block *et al.*, 2007). Excitotoxicity and subsequent activation of apoptosis and oxidative stress all contribute to neurodegeneration. Disruptions in calcium homeostasis via excessive release of glutamate can initiate a chain

of events that result in alterations in apoptosis and oxidative stress (mechanisms which are discussed in depth below), leading to subsequent cell death (Arundine and Tymianski, 2003).

Acute neurodegeneration refers to conditions in which neurons are rapidly damaged and cell death occurs within a short period of time. This encompasses stroke, brain injury, epilepsy and cerebral or subarachnoid haemorrhage. As the work described in this thesis refers to a model of acute neurodegeneration, the emphasis throughout is placed on the mechanisms underlying acute neuronal degeneration and their regulation.

1.1 Cell types that participate in neurodegeneration

Neurons are intrinsic cells of the central nervous system (CNS) which are generally classified according to the number of branches that extend from the cell body. Unipolar neurons consist of only one branch and are commonly found in invertebrate nervous systems. Bipolar neurons are generally involved in sensory transmission and carry signals from each of the senses to the various areas of the brain responsible for the manifestation of the sensory modalities. Multipolar neurons are the most common in the CNS and consist of many, short branched dendrites. Examples of these neurons are pyramidal cells of the hippocampus, cells of the motor cortex and purkinje cells of the cerebellum (for review see: Feldman *et al.,* 1997). Neurons communicate with each other via the release of neurotransmitters. Their excitable membranes extend over the surface of a cell, and following excitation, this signal can be transferred to adjacent neuronal cells or the cells of effector organs.

Glial cells were first described by Virchow (1860) who observed that they make up connective tissue that lies between neural elements, in a manner different from other connective tissue. He coined the term "neural-glia" which literally translates from greek as "nerve glue". Glial cells occupy the spaces between neurons and outnumber them by 10:1, but only occupy half of the brain volume as they are much smaller in size than neurons (Feldman *et al.,* 1997). There are three main types of glial cell; oligodendrocytes, astrocytes and microglia. Oligodendrocytes are involved in synthesis of myelin which insulates neuronal axons and promotes rapid signalling along the axon. Astrocytes and microglia are involved in neuroinflammation and are frequently implicated in mechanisms

mediating acute neuronal degeneration and are further discussed as individual cells types below.

Astrocytes are subdivided into two categories; protoplasmic and fibrous astrocytes. The protoplasmic astrocytes have a large nucleus, abundant granular cytoplasm and numerous thick processes that extend from the cell body which flatten out to form pedicles which adhere to blood vessels and neurons. These astrocytes are found in grey matter and are closely associated with synapses (Stevens, 2003). The fibrous (or fibrillary) astrocytes are located among the bundles of myelinated nerve fibres that make up white matter. They consist of long, thin, smooth, infrequently branched extensions which form pedicles and attach to capillaries and neurons (Feldman *et al.,* 1997). The cytoplasm of astrocytes consists of aggregations of densely packed slender protein filaments called glial fibrillary acidic protein (GFAP) which is commonly used as a phenotypic marker of astrocytes (Liem and Messing, 2009). Astrocytes are highly permeable and mop up excess potassium ions which can accumulate in the extracellular space during increased neuronal activity. They are also major sources of extracellular matrix proteins, adhesion molecules and neurotrophic factors, which may contribute to neuronal growth, migration and survival (Stevens, 2003).

Microglia are the smallest of the glia and have small, elongated nuclei which are surrounded by a small amount of cytoplasmic fluid. They have a few short and twisted branches that are covered in numerous pointed spines. Microglia compose about 10% of CNS glia and are located mainly in the grey matter and spinal cord, but may also be found in white matter (Stevens, 2003; Feldman, 1997). They are the immune regulators of the brain and can elicit inflammatory responses, and phagocytose cellular debris in response to injury or degenerative lesions (Stevens, 2003).

Acute forms of neuronal injury can result in neuronal loss that occurs hours and even days after the initial event, due to the subsequent release of endogenous factors including neurotransmitters and inflammatory mediators from both neurons and glial cells in response to the primary injury (Allan and Rothwell, 2001). Rapid ischemic necrosis occurs following acute neurodegenerative events. It is primarily the result of anaerobic inactivation of mitochondrial oxidative phosphorylation, subsequent cellular de-energisation and massive influx of Ca^{2+} and other ions from the activation of voltage-

dependent ion channels and ligand-dependent channels (e.g., glutamate receptor operated channels). Calcium triggers degradative events, which ultimately cause widespread destruction of cellular components (Siesjo, 1995), resulting in relatively rapid necrosis. Mitochondrial dysfunction is also related to rapid release of cytochrome c and subsequent apoptosis (Niizuma *et al.*, 2009). In addition inflammatory events have been implicated in both acute and chronic neurodegeneration, with a rapid induction of inflammatory processes such as the activation of microglia, release of cytokines, acute phase proteins and complement. The role of each of these events is detailed in turn below. Strategies to prevent such events may represent a clinically feasible neuroprotective approach following acute neuronal insult.

1.2 Excitatory amino acid neurotransmission and neurodegeneration

Glutamate is the most abundant excitatory neurotransmitter in the mammalian brain and is involved in up to 70% of synaptic transmission in the central nervous system (CNS). Glutamine and alpha-ketoglutarate are proposed as the major precursors of glutamate. They are taken into the pre-synaptic terminal in a sodium (Na^+) dependent manner, and are transported to the mitochondria where they are transaminated to glutamate (Daikhin and Yukoff, 2000). Glutamate is stored in the pre-synaptic terminal by vesicular glutamate transporters following the hydrolysis of adenosine triphosphate (ATP).

When glutamate is released into the synaptic cleft it can bind to both pre and post-synaptic receptors. It is removed from the cleft by three main mechanisms; (i) it is taken back up into the synapse via glutamate transporters (GluT), (ii) it can diffuse away from the cleft, (iii) it is taken up by glial cells, where it is broken down into glutamine and α-ketoglutarate which re-enter the pre-synaptic terminal for re-synthesis of glutamate (Meldrum, 1999).

Glutamate is released from pre-synaptic vesicles by a calcium (Ca^{2+}) dependent mechanism, resulting in an excitatory post synaptic potential (EPSP) (Meldrum, 2000). It can also be "released" during ischemic conditions by the reverse action of GluT due to a reduced potassium K^+/Na^+ gradient (Levy *et al.*, 1998). The release of glutamate is regulated by a wide array of pre-synaptic receptors, including group II and III metabotropic glutamate receptors, cholinergic receptors, GABA receptors and adenosine receptors (Meldrum, 2000).

1.3 Ionotropic glutamate receptors

These are ligand gated ion channels that mediate fast excitatory transmission. They consist of the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate acid (AMPA) and Kainic acid (also known as the Kainate receptor) receptors.

NMDA receptor: This receptor permits the entry of Na^+ and Ca^{2+} ions and allows the efflux of K^+ ions. The NMDA receptor pore is blocked by magnesium (Mg^{2+}) ions at resting membrane potentials, preventing receptor activation. This Mg^{2+} is removed upon sufficient membrane depolarisation (generally via AMPA receptor activation), allowing the influx of cations into the cell (Novak *et al.*, 1984). The NMDA receptor is unique, as it requires binding of the co-agonist glycine before depolarisation can occur. Receptor activity is modulated by polyamines and is inhibited by zinc ions. The NMDA receptor is involved in the process of learning and memory, is important in mediating neuronal plasticity and plays a key role in excitotoxicity – a leading mediator of acute neurodegeneration that is discussed below (Platt, 2007).

AMPA receptor: These receptors are responsible for mediating rapid excitatory transmission in the CNS. The AMPA receptor has two agonist binding sites and both must be occupied for receptor activation to occur. Binding to this receptor causes a change in membrane potential leading to EPSP and allowing the influx of K^+ and Na^+ ions. It has less affinity for glutamate than NMDA receptors and is also impermeable to Ca^{2+} ions.

KA receptor: The Kainic acid receptor (KAR) displays many of the characteristics of the AMPA receptor and until recently it was difficult to distinguish between the two receptors. KA receptors are widely distributed throughout the CNS and are located on both pre and post-synaptic terminals, thus modulating neurotransmitter release (Rodriquez-Moreno and Sihra, 2007). They possess two agonist binding sites and also allow the influx of K^+ and Na^+ ions following EPSP. Pre-synaptically they modulate neurotransmitter release from GABAergic and glutamatergic terminals. Post-synaptically they are involved in the control of neurotransmitter release and neuronal excitability. They are involved in learning and memory by supporting short and long-term potentiation (LTP) and long-term depression (LTD) (Doble, 1999). Over-activation of the KAR is implicated in excitotoxicity.

Metabotropic glutamate receptors (mGluR): mGluR's differ from ionotropic glutamate receptors in that they are coupled via second messenger systems to biochemical pathways and ion channels (Platt, 2007). They consist of seven *trans*membrane spanning domains similar to G protein coupled receptors. They have a modulatory role and upon binding of an agonist, activation of G proteins and the concurrent flow of ions through voltage-gated channels occur (Kunishima *et al.*, 2000). Prolonged release of glutamate or high concentrations of it in the synapse, result in the activation of mGluR's and this is associated with synaptic plasticity and excitotoxicity (Dingledine, 1999).

There are three groups of mGluRs which are divided according to their second messenger system and sequence homology. Group I mGluRs, mGluR1 and mGluR5, activate the Gq class of G proteins, which stimulate phospholipases to form inositol triphosphate (IP3) and diacylglycerol (DAG). Group I mGluRs are positively coupled to NMDA receptor function via phosphokinase C (PKC) (Gass, 2008). Group II, mGluR2 and mGluR3, and Group III, mGluR4, mGluR6, mGluR7, mGluR8, all activate Gi G proteins, which are negatively coupled to adenylyl cyclase and cause a decrease in intracellular levels of cyclic adenosine monophosphate (cAMP). Group II and III mGluR's represent the inhibitory autoreceptor and prevent excess glutamate release. mGluR3 and mGluR5 are localised on glial cells (Mudo *et al.*, 2007).

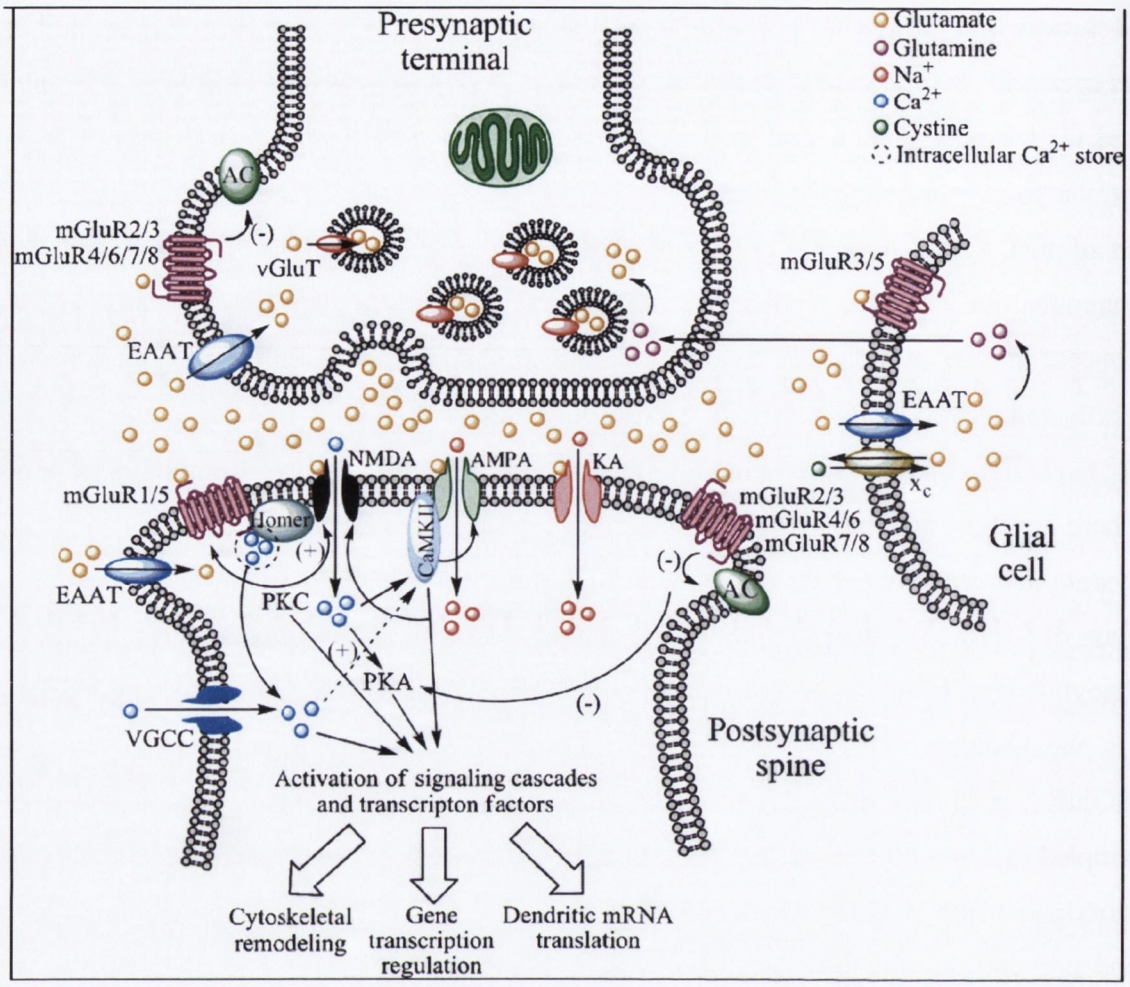


Figure 1.1 The glutamatergic synapse (Gass and Olive, 2008)

Glutamate is packaged into synaptic vesicles in the presynaptic terminal by vesicular GluT (vGluT). When an action potential arrives at the terminal, glutamate is released by exocytosis into the synaptic cleft where it binds to and activates ionotropic GluRs (NMDA, AMPA and KA receptors) localized on the postsynaptic neuron, which results in cation influx and subsequent activation of voltage-gated calcium channels (VGCCs) that propagate the action potential. The resulting cation influx can activate numerous second messenger systems, including protein kinase A (PKA), protein kinase C (PKC) and calmodulin dependent kinase II (CaMKII), which in turn interact with other signalling molecules or transcription factors which can modulate gene expression, local mRNA translation, or cytoskeletal remodelling. Glutamate can also be released into the extracellular space via non-exocytotic mechanisms such as the cystine-glutamate exchanger (xc) located on glial cells, and is taken up by excitatory amino acid transporters (EAAT), which are also known as GluT's.

1.4 Excitotoxicity

In 1957 Lucas and Newman demonstrated that feeding monosodium glutamate to mice led to necrotic lesions in the retina. This discovery led J.W Olney to examine this process in the brain and spinal cord (Olney, 1969). He demonstrated that glutamate-induced neuronal loss occurred throughout the brain, and that this was due to its interaction with its receptors during excitatory transmission in the CNS, and named this mechanism "excitotoxicity" (Olney, 1978).

Under normal conditions, glutamate is removed from the cleft by GluT's in a Na^+ dependent manner. Following energy failure there is a decreased Na^+ gradient in glial cells and this can lead to the failure of glutamate transport and removal of glutamate from the extracellular space and GluT's actually releasing glutamate into the extracellular space (Rossi *et al.*, 2000). The ATP-dependent conversion of glutamate to glutamine and α-ketoglutarate also fails, leading to an increase in extracellular glutamate concentrations (Lipton and Rosenberg, 1994).

Excitotoxicity occurs following an increase in levels of extracellular glutamate which is normally present in the 3-4μM range but, in excitotoxic conditions, can rise up to 16μM (Storm-Mathisen *et al.,* 1992). Elevated levels of glutamate result in persistent depolarisation of the neuron. Initial binding of glutamate to the AMPA receptor leads to depolarisation, allowing the influx of Na^+ ions which sustains this depolarisation. These events trigger the activation of the NMDA receptor following the removal of Mg^{2+} blockade, allowing the influx of Ca^{2+} ions into the cell. Continuous depolarisation causes a disturbance in osmotic balance of the cell. Na^+ entry causes chloride (Cl^-) ions to passively enter the cell to maintain ionic equilibrium (Rothman, 1985). This causes water to enter the cell until eventually cell lysis occurs and the cell contents are released (Doble, 1999).

Failure of the ATP-driven Na^+/K^+ and Na^+/Ca^{2+} exchangers is also attributed to persistent depolarisation. The consequence of this is a rise in intracellular Ca^{2+} (Koch and Barish, 1994). Activation of NMDA receptors also causes the release of Ca^{2+} from intracellular stores via the large amount of Ca^{2+} that enters the cell via the NMDA receptor, contributing to the production of IP3 by activating Ca^{2+} sensitive phospholipase C (PLC) isoforms, resulting in the activation and opening of IP3 receptors and/or ryanodine receptor (RyR) in the endoplasmic reticulum to release sequestered Ca^{2+} into the cytosol (Mody and

MacDonald, 1995; Berridge *et al.*, 2000). This increase in Ca^{2+} causes many events that trigger cell death, such as the activation of nucleases, which fragment DNA (Orrenius *et al.*, 1989) and the release of cytosolic proteases, which attack the cytoskeleton (Siman *et al.*, 1989), PKC, which disrupts cell function (Favaron *et al.*, 1990), and lipases such as phospholipase A_2, which attack the cell membrane (Doble, 1999). Mitochondria are involved in the regulation of intracellular calcium and increasing levels of Ca^{2+} cause mitochondria to produce reactive oxygen species (ROS) such as superoxide (O^{2-}) and hydrogen peroxide (H_20_2). Ca^{2+} also triggers the activation of nitric oxide synthase (NOS) and NO which reacts with O^{2-} to form the highly toxic compound peroxynitrite ($ONOO^-$) which in turn causes the nitration of proteins and oxidation of lipids, proteins and DNA (Dykens, 1994; Doble, 1999). This cellular degradation amplifies the excitotoxic process by causing further release of glutamate which acts on intact neurons, thus spreading the damage.

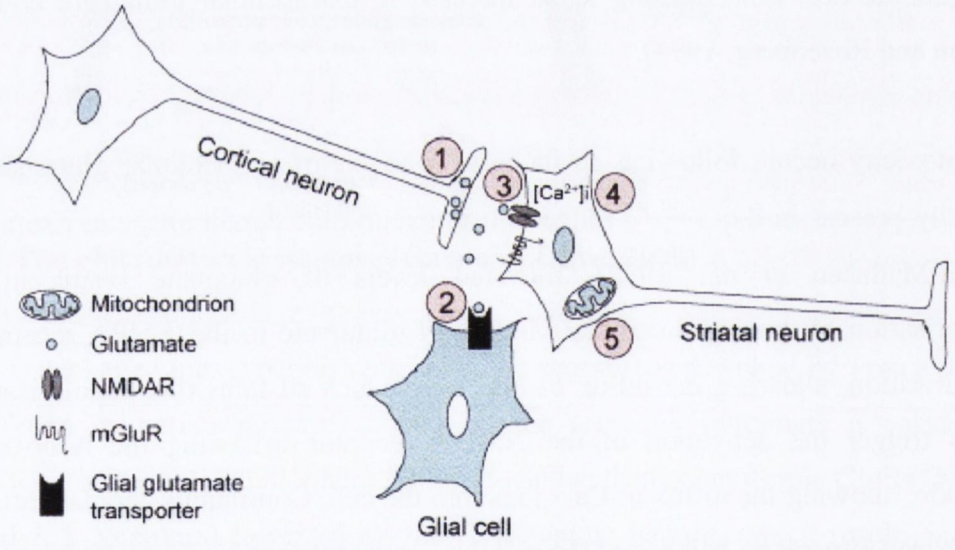


Figure 1.2 Excitotoxic neuronal death (Fan and Raymond, 2006)

This may result from a combination of (1) increased glutamate release from cortical afferents; (2) reduced uptake of glutamate by glia; (3) hypersensitivity of post-synaptic NMDA receptor's and/or augmentation of mGluR signalling; (4) altered intracellular calcium homeostasis; (5) mitochondrial dysfunction.

1.5 Excitotoxicity and neurodegeneration

Excitotoxicity is a factor that has been identified in the pathogenesis of many neurodegenerative disorders such as stroke, HD, AD, PD and epilepsy (Rothstein, 1996;

Wong *et al.*, 2002; Hynd *et al.*, 2004; Tannenberg *et al.*, 2004; Fujikawa, 2005; Yi and Hazell, 2006).

Ischemic stroke is the most common form of stroke (Rosamond *et al.*, 2007) which occurs following a cerebral occlusion. Cerebral blood flow is interrupted, causing ATP levels to fall drastically, leading to the excessive release of glutamate and excitotoxic damage throughout the infarct (Doyle *et al.*, 2008). Glutamate antagonists have been widely used in clinical trials for the treatment of stroke, although not all of these have proven to be successful (Ginsberg, 2008).

HD results in the loss of medium spiny neurons in the neostriatum which are involved in glutamate transmission (Graveland *et al.*, 1985). Some studies have demonstrated that intracerebral injection of glutamate, or its analogues, such as quinolinic acid (QUIN) or kainic acid, can induce a similar pattern of neuronal loss seen in HD (McGeer *et al.*, 1976). In support of this, studies have identified modifications to some of the components of glutamatergic neurotransmission in post-mortem studies of HD patients (Estrada-Sanchez *et al.*, 2008).

In AD, the over-expression of Aβ and Tau proteins appear to trigger the excessive activation of NMDA receptors and lead to excitotoxic damage (Harkany *et al.*, 2000; Amadoro *et al.*, 2006). This occurs following disruptions in the function of glutamine synthetase and GluT, both means of inactivating extraneuronal glutamate, which are oxidatively modified and dysfunctional in AD, leading to an increased opportunity for excitotoxicity to occur (Xiong *et al.*, 2004). Memantine, an NMDA receptor antagonist, approved by the Food and Drug Administration (FDA) drug for the treatment of AD, is thought to decrease excitotoxicity and increase neuronal function in the hippocampus (Parsons *et al.*, 1999).

Excitotoxicity is thought to occur in PD as a secondary response to a defect in the ATP-dependent Mg^{2+} blockade of the NMDA receptor, or to the activation of glutamatergic subthalamic nucleus neurons, resulting from dopamine depletion (Beal, 1998; Rodriguez *et al.*, 1998). It has been reported that NMDA antagonists can provide protection against 1-methyl-4-phenylpyridinium iodide (MPP^+)-induced neurotoxicity (Good *et al.*, 1998). Animal models of epilepsy include the kainic acid model of temporal lobe epilepsy, which

results in excitotoxic damage and typical seizure behaviours that are comparable to human temporal lobe epilepsy (Ben-Ari, 1985).

1.6 Apoptosis and neurodegeneration

Apoptosis is a well-ordered process of cell suicide which is necessary for embryonic development, immune regulation, tissue homeostasis and chemical-induced cell death (Nunez *et al.,* 1998; Cohen, 1997). It is characterised by DNA fragmentation, chromatin condensation, compaction of cytoplasmic organelles, dilation of the endoplasmic reticulum, decreased cell volume, membrane blebbing, cell shrinkage and disassembly into apoptotic bodies. These apoptotic bodies are engulfed by phagocytes and neighbouring cells, thus preventing the release of the intracellular contents, which could induce an inflammatory response (Thornberry and Lazebnik, 1998; Thornberry, 1998). Inappropriate apoptosis is implicated in the pathogenesis of a number of neurodegenerative diseases, such as AD, Stroke, HD and PD (Thompson, 1995; Nicholson, 1996). The family of caspases has been shown to play a pivotal role in the regulation and execution of this process.

1.7 Caspases

Caspases are synthesised in the cell as inactive enzyme precursors (zymogens). They consist of four distinct domains: an N-terminal pro-domain, a large subunit of about 20 kDa, a small subunit of about 10 kDa, and a linker sequence between the large and small domains, flanked by aspartate residues. Caspases are activated by proteolytic cleavage between domains, which remove the pro-domain and linker regions and allows the assembly of the large and small subunits into the active enzyme (Nunez *et al.,* 1998; Lavrik *et al.,* 2005). The name caspase derives from the words cysteine-dependent aspartate specific protease. Caspases were first implicated in apoptosis following the discovery of the CED-3 and CED-4 genes in the nematode *Caenorhabditis elgans*, which were required for the execution of cell death (Yuan and Horvitz, 1992; Yuan, 1993). It was demonstrated that the CED-3 gene was homologous to the mammalian interleukin-1β converting enzyme (ICE/ caspase 1). Although caspase 1 has no obvious role in apoptosis, this discovery provided the first indication that a large family of cysteine proteases played a critical role in inflammation and cell death (Yuan, 1993; Thornberry, 1998).

Initially caspases were classified as either "apoptotic" or "pro-inflammatory", but apoptotic caspases can be further sub-divided into "initiator" caspases (caspase 8, 9, 10) or "effector" caspases (caspase 2, 6, 7). A more conventional way of classifying the caspases is according to the length of the pro-peptide. Those with a long pro-domain activate by dimerisation (inflammatory caspases, initiator caspases and caspase 2); those with a short pro-domain activate by cleavage of the catalytic domain (caspase 3, 6 and 7) (Pop and Salvesen, 2009; Kumar 1999).

Initiator caspases require homodimerisation for activation. A pro-apoptotic signal culminates in caspase recruitment to activation platforms which bind to the caspase pro-domain. This results in an increase in local caspase concentration and facilitates dimerisation. Every initiator caspase has its own activation platform, for example the death inducing signalling complex (DISC) activates caspase 8 and 10, whereas the apoptosome activates caspase 9 (Riedl *et al.,* 2001; Pop, 2009). Activation of caspase 9 requires cytochrome c which is released from mitochondria in response to cytotoxic stress, oxidative stress and DNA damage. This in combination with deoxyadenosine triphosphate (dATP) binds to the apoptotic protease-activating factor-1 (Apaf-1) and creates a large apoptosome complex leading to the activation of pro-caspase 9 (Boatright *et al.,* 2003; Jiang and Wang, 2000).

There are two effector caspase cascades; the extrinsic or death receptor-mediated cascade, and the intrinsic or mitochondria-mediated cascade. The extrinsic pathway is further divided into two types of signalling pathways; type 1 is associated with high levels of DISC production, and thus an increased amount of caspase 8 activity, which leads to activation of the effector caspases-3 and 7. Type 2 involves the cleavage of the Bcl-2 family protein- BH3 interacting domain (Bid), by caspase 8, forming truncated Bcl-2 interacting protein (tBid), which in turn causes the release of cytochrome c from mitochondria. This as described above, results in the formation of the apoptosome, activating caspase 9, which in turn, activates caspase 3 and 7, which initiate the death cascade. The intrinsic pathway involves direct stimulation (for example by chemotherapeutic drugs) of the mitochondria to release cytochrome c, leading to formation of the apoptosome.

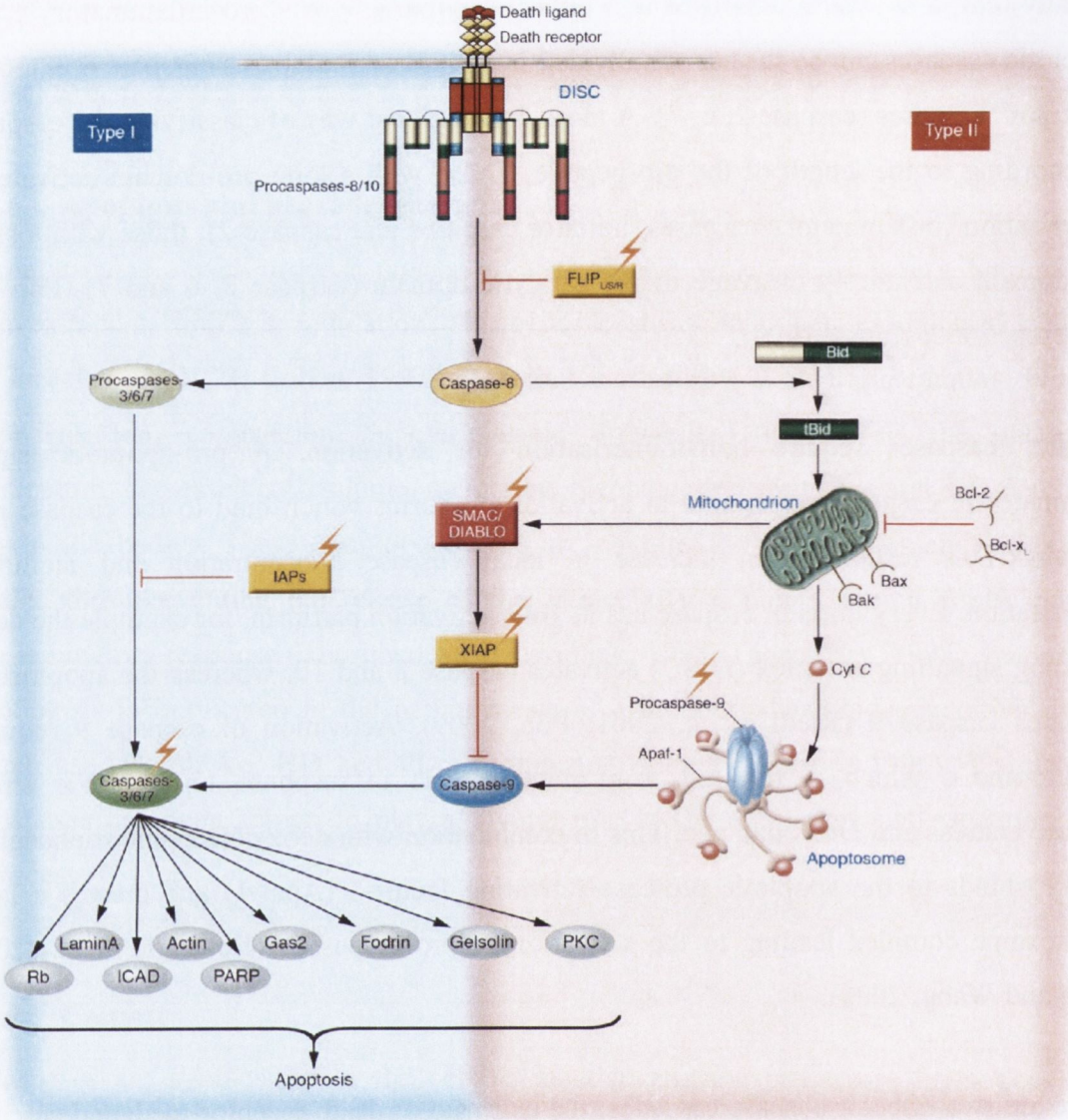


Figure 1.3 Caspase signalling and its modulation (Lavrik et al., 2005)

In the extrinsic pathway, DISC formation leads to caspase-8 activation. Two signalling pathways downstream from the receptor were established. In type I cells (shown in light blue) caspase-8 directly cleaves caspase-3, which starts the death cascade. In type II cells (shown in light red) an additional amplification loop is required, which involves tBid-mediated cytochrome *c* release from mitochondria and alterations in the Bax/Bcl-2 family, followed by apoptosome formation. Alternatively the mitochondrion releases Second Mitochondria-derived Activator of Caspases/Direct inhibitor of apoptosis (IAP) Binding Protein with Low PI (Smac/DIABLO) which neutralises the apoptosis inhibitory protein, x-linked inhibitor of apoptosis (XIAP). Initiation of the intrinsic pathway results in mitochondria-mediated apoptosome formation, followed by caspase-9 and -3 activation,

leading to destruction of the cell by affecting structural proteins such as lamin A, actin, fodrin and gelsolin, and cell survival proteins such as growth arrest-specific protein (Gas2), retinoblastoma (Rb) protein, inhibitor of caspase-activated DNase (ICAD) and poly (ADP)-ribose polymerase (PARP). Caspase action can be modulated on several levels. Activation of caspases at the DISC is inhibited by cellular FADD-like IL-1β-converting enzyme (FLICE)-like inhibitory protein (c-FLIP) proteins; activation of effector caspases is inhibited by inhibitor of apoptosis (IAP).

1.7.1 Caspase 3

Caspase 3 is also known as CPP32, Yama or apopain. It was discovered using the DNA sequence encoding CED-3 and the active site of caspase 1, to search an expressed sequence tag database, where a human sequence was identified for encoding a 32 kDa cysteine protease-named CPP32 (Fernandes-Alnemri *et al.,* 1994). At the same time two independent groups also identified it, naming it Yama (after the Hindu god of death) and apopain (Tewari *et al.,* 1995; Nicholson *et al.,* 1995). Caspase 3 plays a key role in the execution of apoptosis. It is distributed widely throughout the body, with high expression seen in lymphocytic cells, suggesting that it may play an important role in apoptosis in the immune system (Fernandes-Alnemri *et al.,* 1994). The active enzyme is comprised of two subunits- 17kDa and 12kDa- which are cleaved from the precursor protein at aspartate-28-serine-29 (Asp-28-Ser-29) and Asp-175-Ser-176 (Nicholson *et al.,* 1995). Caspase 3 and -1 are similar in overall structure, but caspase 3 has an S_4 subsite which is very different in size and chemical composition, accounting for their differences in specificity (Rotonda *et al.,* 1996).

During the execution phase of apoptosis, caspase 3 is responsible for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP), which share a common Asp-Xaa-Xaa-Asp (DXXD) motif (Lazebnik *et al.,* 1994). It also inactivates proteins that prevent apoptosis from occurring in living cells. An example of this is cleavage of an inhibitor (ICAD/DFF45) of a nuclease which is responsible for DNA fragmentation; caspase-activated deoxyribonuclease (CAD). In non-apoptotic cells, CAD exists as an inactive complex with ICAD. Caspase 3 inactivates ICAD, allowing CAD to operate as a nuclease and leads to DNA fragmentation (Enari *et al.,* 1998; Liu *et al.,* 1997). Caspase 3 also contributes to direct disassembly of cell structures. It cleaves the intermediate filament proteins in the lamina of the cell (lamins),

causing them to collapse, thus contributing to chromatin condensation (Takahashi *et al.*, 1996; Orth *et al.*, 1996).

Some of the caspases have been shown to be non-essential for apoptosis to occur. This was illustrated through the use of knock-out mice. Caspase 3 deficient mice were considerably smaller than their littermates, and died between 1 and 3 weeks of age. They exhibited a decreased presence of pyknotic clusters of apoptotic cells in the brain, which is typical of normal brain development. This demonstrates that caspase 3 is essential for the execution of mammalian cell death (Cohen, 1997; Kuida *et al.*, 1996).

Apoptosis contributes to cell death in neurodegenerative disorders. It has been shown that DNA single strand breakages are consistently found early in the progression of neurodegenerative diseases (Liu and Martin, 2001; Martin and Liu, 2002a, 2002b; Martin *et al.*, 2006; Martin *et al.*, 2007). In animal models of neurodegeneration, DNA single strand breakages accumulate at a time that corresponds with p53 activation and nuclear import (Liu and Martin, 2001; Martin and Liu, 2002). Apoptosis was shown to be the major form of neuronal cell death in Presenelin/amyloid precursor protein (PS/APP) mice which model Alzheimer's disease-like neurodegeneration. Pyknotic neurons in these mice exhibited the hallmarks of apoptosis including DNA fragmentation, caspase 3 activation and caspase-cleaved α-spectrin (Yang *et al.*, 2008). It has been demonstrated that traumatic brain injury and/or neurological disease-induced caspase activation, results in increased APP processing and may directly or indirectly affect Aβ levels, and that this in turn, may increase caspase activation (Allen *et al.*, 2001; Awasthi *et al.*, 2005; Mattson *et al.*, 1998). A study by Abrahamson and colleagues (2006) demonstrated that after traumatic brain injury, caspase inhibition suppressed elevations of Aβ. PD has been shown to be associated with increased markers of oxidative stress, mitochondrial dysfunction and apoptosis as identified by proteomic analysis (Chin *et al.*, 2008). The apoptotic pathways p53-glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-Bax pathway and the FAS receptor; Fas-Associated protein with Death Domain (FADD)-caspase 8-Bax pathway have been implicated in PD (for review see: Tatton *et al.*, 2003). Several observations identify apoptotic death in the striatum throughout the course of HD, with Huntingtin being a substrate for caspase 3 cleavage, and activating the Jun N-terminal Kinase (JNK) signalling pathway, leading to subsequent neuronal apoptosis (Liu, 1998: Sieradzan and Mann, 2001). Amyotrophic lateral sclerosis (ALS) is associated with increases in oxidative

stress, which leads to mitochondrial dysfunction and the subsequent activation of the intrinsic apoptosis cascade in these neurons (Kermer *et al.,* 2004). Increased expression of the pro-apoptotic protein Bax, with a concurrent decrease in the expression of Bcl-2, as well as DNA fragmentation have been found in transgenic mouse models of ALS, and in the spinal cord and the motor cortex of patients that had died from ALS (Sathasivam *et al.,* 2001; Troost *et al.,* 1995; Mu *et al.,* 1996; Ekegren *et al.,* 1999).

Degeneration of one or more nerve cell populations is a major feature in many acute and chronic neurological diseases. The intrinsic pathway depends on the integrity and function of mitochondria within the cell, whereas the extrinsic pathway is initiated by cell surface activation of cytokine receptors of the tumor necrosis factor (TNF) family (Reed, 2000). DNA damage (hereditary or induced), can increase expression of the tumor suppressor gene p53, increase calcium influx by overstimulation of glutamate receptors (excitotoxicity), cause damage to the components of the plasma membrane, the formation of free radicals (oxidative stress) and metabolic stress (hypoxia, hypoglycemia), which can cause mitochondrial changes resulting in the formation of pores in the mitochondrial membrane (permeability transition pores) and release of several mediators of apoptosis (cytochrome C, SMAC/Diablo, apoptosis-inducing factor) (Kermer *et al.,* 2004). Activation of the survival pathway involving the transcription factor nuclear factor kappa B (NF-κB) can protect cultured neurons against death induced by diverse stimuli, including trophic-factor withdrawal and exposure to excitotoxic, oxidative and metabolic insults, all of which are seen in both chronic and acute neurodegeneration. Gene targets that mediate the survival-promoting action of NF-κB may include manganese superoxide dismutase, Bcl-2 and IAP's. However, NF-κB activation in microglia can promote neuronal apoptosis by inducing production of free radicals and excitotoxins, so NF-κB may either prevent or promote neuronal death depending on specific neurodegenerative conditions (Aschner *et al.,* 1999).

Excitotoxicity has been shown to elicit cell death via apoptotic mechanisms. Excess glutamate release and subsequent interaction with its receptors causes alterations in intracellular ion concentrations, pH, protein phosphorylation and energy metabolism (Choi, 1992; Lipton and Rosenberg, 1994). Glutamate receptor stimulation results in an increase in cytosolic free Ca^{2+}, which causes an activation of Ca^{2+}-sensitive proteases, protein kinases/phosphatases and phospholipases, which initiate apoptotic cascades (Martin *et al.,* 1998). Choi and colleagues (1987a, 1987b) emphasized the role of Ca^{2+}

influx in glutamate neurotoxicity. They indicated through the use of ion substitution experiments that, although the removal of extracellular Na^+ eliminates the acute neuronal swelling in cortical cell cultures exposed to glutamate, neurons still undergo delayed degeneration unless extracellular Ca^{2+} is removed. Their observations suggested that an acute, Na^+- and Cl^--dependent component of excitotoxicity is characterised by immediate cell swelling, and a delayed cell degeneration that could be mimicked by the Ca^{2+} ionophore A23187, was another component of the excitotoxic process. They concluded that at lower glutamate exposures the Ca^{2+} component is the more significant cause of neuronal death. Increases in intracellular Ca^{2+} have been shown to contribute to mitochondrial dysfunction and thus lead to the activation of the intrinsic apoptotic cascade (Kermer *et al.,* 2004: Martin *et al.,* 1998: Arundine and Tymianski, 2003).

1.8 The role of inflammation in neurodegeneration

Diseases such as AD, PD, HD, multiple sclerosis (MS) and ALS have been shown to be associated with increased levels of inflammation and microglial activation (Nguyen *et al.,* 2002). Several disorders specific to the CNS such as schizophrenia, MS, and dementia have been shown to be associated with elevated levels of pro-inflammatory cytokines such as interferon-gamma (IFN-γ), TNF-α and interleukin (IL)-6 (Ganguli *et al.,* 1994, Mikova *et al.,* 2001, Teunissen *et al.,* 2003). Activated microglia have been implicated in the pathogenesis of AD and PD (Xiang *et al.,* 2006, Imamura *et al.*, 2003). Markers of inflammation such as intercellular cell adhesion molecule 1 (ICAM-1), which is expressed on vascular endothelial cells, immune cells and glial cells, and glial fibrillary acidic protein (GFAP), are both shown to be increased *in vivo* following brain damage (Lee and Benveniste 1999; Czeh *et al.*, 2006). Stress and depression have been shown to result in increased levels of IFN-γ, TNF-α and IL-6 and have raised the possibility that mood disorders may also be related to inflammatory processes.

Increased transcription of cytokines and altered Aβ processing was seen in the Tg2576 mouse model of AD following a challenge with bacterial lipopolysaccharide (LPS) (Sly *et al.,* 2001). Kitazawa *et al.,* (2005) demonstrated that repeated administration of LPS augmented CNS inflammation and Tau hyperphosphorylation in a transgenic model of AD. Many cytokines have been implicated in the development of AD, including IL-1. Its role is as yet unclear but it may exert its effect through its ability to induce the expression of inducible nitric oxide synthase (iNOS), which could indirectly increase neurotoxicity

(Hewett *et al.,* 1994). IL-1α and IL-1β have been shown to be potential risk factors for the development of AD, and may also be predictors of the age of onset (Meda *et al.,* 1995, Sciacca *et al.,* 2003).

Alterations in pro-inflammatory cytokine expression are associated with NMDA-induced excitotoxicity. IL-1β expression in glial cells is increased in the cortex and striatum following excitotoxic damage (Pearson *et al.,* 1999). Antagonism of the NMDA receptor suppresses IL-1β expression following cerebral ischemia (Jander *et al.,* 2000), suggesting that IL-1β potentially modulates ischemic damage via regulation of excitotoxicity. Intrastriatal application of IL-1ra can reduce NMDA-induced lesion size (Relton and Rothwell, 1992). Furthermore, direct application of IL-1β to the striatum of rats does not cause neuronal cell death itself, but enhances excitotoxic neuronal injury in both the striatum and cortex (Lawrence *et al.,* 1998; Stroemer and Rothwell 1998; Allan *et al.,* 2000). A relationship between increased pro-inflammatory cytokines and perinatal brain damage have been linked to adverse outcomes in several clinical and experimental studies (Nelson *et al.,* 1998: Yoon *et al.,* 1997; Dammann and Leviton, 2004; Debillon *et al.,* 2003; Dommergues *et al.,* 2000). Experimentally, infectious/inflammatory factors can induce brain damage by themselves, for example, systemic injection of LPS or IL- 1β to newborn mice or rats makes the brain much more sensitive to an excitotoxic insult (Dommergues *et al.,* 2000; Favrais *et al.,* 2007; Rousset *et al.,* 2008).

1.9 The role of pro-inflammatory cytokines in neurodegeneration

Interferon-γ: IFN-γ is a pro-inflammatory cytokine and a member of the IFN family, of which there are three members- IFN-α, IFN-β and IFN-γ. The nomenclature was derived from their ability to "interfere" with viral activity. IFN-γ favours the development of Th1 over Th2 cells, which in turn provide assistance to macrophages and has an inhibitory effect on B cell proliferation (Billiau and Matthys, 2009). Following immune activation, natural killer (NK) cells produce IFN-γ which primes mononuclear phagocytes for production of monokines, including TNF-α and IL-12. IFN-γ and TNF-α then act in synergy to augment the bacteriostatic potential of the phagocytes. Following this, a Th1 response mediated by IL-12 is mounted resulting in additional IFN-γ production by activated $CD4^+$ and $CD8^+$ T cells (Billiau and Matthys, 2009). The importance of IFN-γ in cell-mediated immunity was illustrated following administration of neutralizing antibodies

against IFN-γ, which abrogated resistance against *Listeria* infection (Buchmeier and Schreiber, 1985; Nakane *et al.,* 1989). IFN-γ production during the first two days of infection was shown to be critical for the development of protective antigen-specific T cells (Yang *et al.,* 1997) illustrating its ability to optimize effector cell function, and also to regulate the adaptive immune response to an infection.

This pro-inflammatory cytokine has also been implicated in autoimmune disease. In a mouse model of experimental allergic encephalomyelitis (EAE), administration of anti-IFN-γ antibodies augmented the damage seen in this model (Billiau *et al.,* 1988; Duong *et al.,* 1992, 1994; Willenborg *et al.,* 1996). It is well established that IFN-γ, and IFN-β to a lesser extent, is responsible for the induction of indoleamine 2,3 dioxygenase (IDO) in an array of cells such as macrophages, dendritic cells and microglia (Carlin *et al.*, 1989; Currier *et al.*, 2000; Fallarino *et al.*, 2002). IFN-γ induced anti-proliferation and pathogenic defensive mechanisms operate by activating IDO and enhancing tryptophan degradation through the kynurenine pathway (Alkondon *et al.*, 2004). By depleting tryptophan, IDO also contributes to innate host resistance against those pathogens that depend metabolically on the exogenous supply of tryptophan. Further details of the role of IDO in neuroinflammation and neurodegeneration are provided below. IFN-γ also leads to the induction of NOS and the production of NO which inhibits T cell proliferation and induces apoptosis (Yamazaki *et al.,* 2002).

Neuroinflammation has been reported to play a key role in many neurodegenerative disorders. It is protective by ensuring maintenance of virus latency after infection, yet deleterious by recruiting and activating microglia that secrete potentially damaging factors at sites of brain injury (Mastrangelo *et al.,* 2009). Increased IFN-γ levels were reported in the nigrostriatal dopaminergic regions (substantia nigra, caudate nucleus, and putamen) in parkinsonian patients when compared to control patients (Mogi *et al.,* 2007). Pronounced production of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ was observed when primary astrocytes or astrocytoma cells were stimulated with combinations of IFN-γ and TNF-α or IFN-γ and IL-1 beta (Blasko *et al.,* 2000).

Following infection in the brain, microglia act as innate producers of IFN-γ. Numerous reports have illustrated this following infection with *Toxoplasma gondii* (Suzuki *et al.,* 2005; Wang *et al.*, 2007; Kang and Suzuki, 2001). IFN-γ is a potent stimulator of

microglia /macrophages, and enhances astroglial reactivity in the corticectomized adult rat brain (Yong et al., 1991). In primary cultures of astrocytes, IFN-γ levels were increased following two different injury models; mechanical trauma and ischemia (Lau and Yu, 2001). IFN-γ has been implicated as an important mediator of the gliosis observed in pathologic conditions of the adult central nervous system associated with infiltrating lymphocytes. Recombinant IFN-γ alone can induce proliferation of human adult astrocytes *in vitro* and increase the extent of trauma-initiated gliosis in the adult mouse brain, an effect which is completely blocked with IFN-γ neutralizing antibody (Yong *et al.,* 1991).

Tumor necrosis factor-α: TNF-α is a 157 amino acid cytokine and is produced in response to injury and inflammatory or infectious stimuli by macrophages, lymphocytes, neutrophils, and structural cells, including fibroblast, smooth muscle cells (Balakumar and Singh, 2006), astrocytes and microglia (Kronfol and Remick, 2000). It is considered a pro-inflammatory cytokine, augmenting the immune response to help eliminate pathogens and assist the resolution of the inflammatory challenge (Kronfol and Remick, 2000). TNF-α has several effects, including cytotoxicity, antiviral activity, transcription factor activation, and immune response regulation (Bhardwaj and Aggarwal, 2003), which can lead to cell survival or apoptosis, depending on the receptor activated, and concentrations of TNF-α present. TNF receptors (TNFR) are divided into two main classes, TNFR1 (also called p55) and TNFR2 (also called p75), which have distinct and common signalling pathways. Signalling occurs predominantly via the TNFR1, which contains a death domain and is present in a variety of cell types including astroytes, neurons, oligodendrocytes and macrophages (Kuno *et al.*, 2006; Ohtori *et al.*, 2004; Buntinx *et al.*, 2004). Interestingly, TNFR1 activation can trigger a dual signalling cascade that in different cell types may lead to apoptosis, proliferation, differentiation, or survival (Bhardwaj and Aggarwal, 2003). In neurons and microglia, binding of TNF-α to the TNFR1 induces apoptosis (McDonald *et al.,* 2003). TNFR2 does not contain a death domain and is predominantly expressed on immune cells (Kuno *et al.*, 2006; Aggarwal, 2003).

High concentrations of this cytokine have been shown to result in an overwhelming inflammatory response that can lead to endotoxic shock and death. This can occur when large amounts of NO are released and cause vascular relaxation and a drop in blood pressure following iNOS induction. TNF-α causes the induction of adhesion molecules, cytokines, chemokines metallaproteases and inflammatory enzymes such as iNOS and

cyclooxygenase-2 (COX-2) (Zhang *et al.*, 1998; Aggarwal, 2003) and has been implicated in a variety of neurodegenerative disorders including AD, PD, MS and stroke (Rossi *et al.*, 2005; Sriram and O'Callaghan, 2007).

Interleukin-1β: IL-1β is a pro-inflammatory cytokine that belongs to the IL-1 family of which IL-1α and IL-1ra are also members. There are two IL-1 receptors (IL-1R), IL-1RI and IL-1RII. The effects of IL-1β are mediated via activation of IL-1R1 and its accessory protein (IL-1RacP) that is necessary for signal transduction. Binding of IL-1β to IL-1R1 leads to NFkB and/or mitogen-activated protein kinase (MAPK) signalling (Fitzgerald and O'Neill, 2000). IL-1β can induce acute inflammation (cytokine cascade, high fever, C-reactive protein (CRP) production) in response to infection (Rijkers *et al.*, 2009). At the cellular level, the IL-1 response can be controlled by two mechanisms: 1) the ratio of IL-1RI/IL-1RII, and 2) the concentration of IL-1 agonists (IL-1α and IL-1β) versus that of IL-1ra, however high molar excess (100- to 1000-fold) of IL-1ra is needed to counteract the effects of IL-1β (Rijkers *et al.*, 2009).

Systemic IL-1 levels are usually very low, but are rapidly induced following inflammation (e.g. during systemic infection). Low basal expression of IL-1RI is found throughout the CNS, on all CNS cells (Allan and Rothwell, 2001) and following neuronal injury, IL-1 is rapidly produced by microglia and other CNS cells (Allan and Rothwell, 2003). Binding of IL-1β to the IL-1RI on any of the CNS cell types has both neuroprotective and neurotoxic effects. Neuroprotective effects take place when IL-1β is present in low concentrations, while under pathological conditions, when IL-1β levels are higher, processes leading to neurotoxicity are facilitated by IL-1β. This cytokine is also capable of inducing COX-2, chemokines and iNOS expression (Akama *et al.*, 2000; Laflamme *et al.*, 1999), and is implicated in a variety of neurodegenerative disorders including AD, MS and stroke (Lucas *et al.*, 2006).

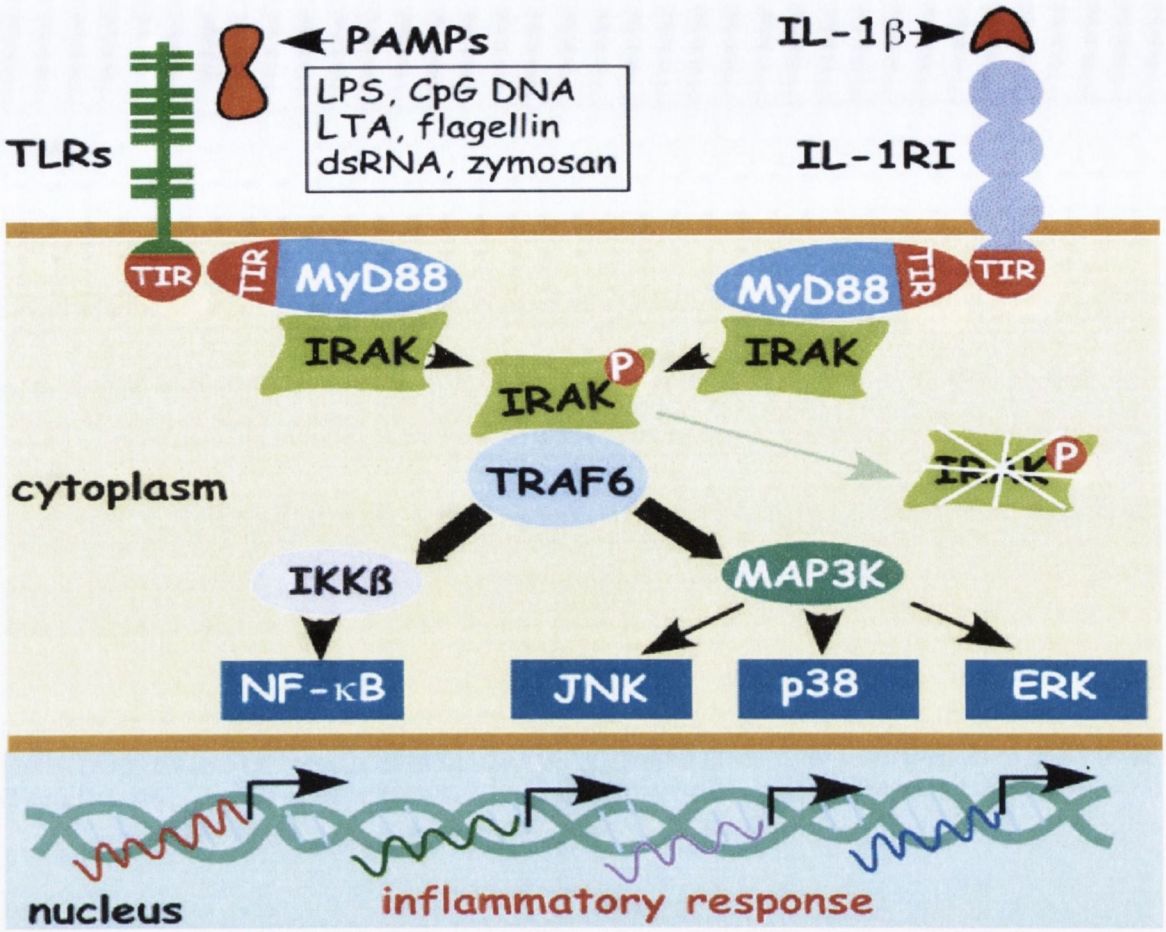


Figure 1.4 IL-1 signalling pathway (Kariko *et al.*, 2004)

Activation of Toll-like receptors (TLRs) and IL-1 receptor induces inflammation in immune cells via shared signaling cascades. TLRs expressed on immune cells (monocytes, macrophages, dendritic cells and microglia) recognize and respond to microbial infection. TLRs are activated by products containing pathogen-associated molecular patterns (PAMPs), derived from bacteria (LPS, lipotheichoic acid (LTA), DNA with non-methylated cytosine-guanine motifs (CpG DNA), flagellin), from yeast (zymosan), or from viruses (double-stranded RNA (dsRNA)). In response to activation by the corresponding ligands, TIR (Toll/IL-1 receptor) domains interact with the TIR domain of the signaling adaptor MyD88 (myeloid differentiation factor 88), which transduces the signal to a family of IL-1 receptor-associated kinases (IRAKs). Phosphorylation of IRAK, a serine-threonine kinase, by other IRAK family members induces cascades of signaling through TRAF6 (tumor necrosis factor receptor-associated factor 6). TRAF6 transduces the signal to IKKβ (IkappaB kinaseβ) and to MAP3K. This signaling results in transcriptional responses, mediated primarily by NF-κB, extracellular-signal regulated kinase (ERK) and stress-activated protein kinases, such as c-Jun N-terminal kinase (JNK) and p38, leads to expression of proinflammatory cytokines.

1.9.1 Inducible nitric oxide synthase (iNOS)

NO is synthesized by NOS via the oxidation of the amino acid- L-arginine, to form L-citrulline. There are three distinct isoforms of NOS; neuronal NOS or NOS1 which is constitutively expressed in neuronal tissue, endothelial NOS or NOS3 which is mainly expressed in vascular endothelial cells (Popp *et al.*, 1998; Ignarro *et al.*, 1999) and inducible NOS or NOS2. nNOS and eNOS are calcium/calmodulin dependent, whereas iNOS is calcium independent.

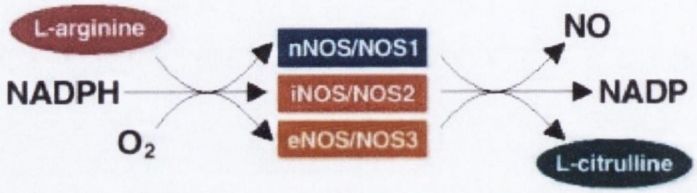


Figure 1.5 Nitric oxide synthases catalyze the production of NO and L-citrulline from L-arginine, O_2, and NADPH-derived electrons (R&D systems)

iNOS can be induced by a wide variety of stimuli-predominantly by pro-inflammatory cytokines, and once induced, it produces NO continuously until substrate depletion occurs (Kleinert *et al.*, 2004; Pannu and Singh, 2006). It is expressed for 4-8 days and produces 100-1000 times more NO than nNOS (Pannu and Singh, 2006). Increased production of NO can be protective, for example by eliminating infiltrating microorganisms and increasing blood supply to injured tissue, but overproduction of NO can be detrimental to the cell and is associated with many disease states, such as stroke, rheumatoid arthritis, diabetes and multiple sclerosis (Nathan, 1992; MacMicking *et al.*, 1997). Due to the reactive nature of NO, it readily binds to proteins containing heme-iron prosthetic groups, iron-sulphur clusters and reactive thiols, thus altering the activity of these proteins (Stamler, 1994). By binding to the iron sulphur cluster in mitochondria, it reduces oxidative phosphorylation which leads to energy depletion, a characteristic of neuronal cell death seen in many neuroinflammatory diseases (Yun *et al.*, 1997). In the presence of molecular oxygen, NO can auto-oxidize forming a number of potent nitrosylating agents, which can generate potentially carcinogenic nitrosamines and/or lead to the deamination of DNA. NO also readily reacts with the superanion free radical O_2^- to form the peroxynitrite

anion -ONOO $^{-}$, which further contributes to neuronal damage (Beckman and Koppenol, 1996).

Pro-inflammatory cytokines cause the induction of iNOS in several cells. Following cerebral ischemia, proinflammatory cytokines such as TNF-α, IL-1β and IFNγ are induced within a few hours, which influences the expression of iNOS in astrocytes, microglia, neurons and polymorphonuclear cells (Minc-Golomb *et al.*, 1996; Galea *et al.*, 1992; Simmons and Murphy, 1993; Simmons and Murphy, 1992). NFκB is an essential requirement for the expression of iNOS (Xie *et al.*, 1994) and the release of glutamate during cerebral ischemia has been shown to be involved in iNOS expression *in vitro* via calcium-dependent activation of NFκB (Cardenas *et al.*, 2000). Activation of NMDA receptors may lead to increased iNOS expression via the production of proinflammatory cytokines, or through the production of free radicals such as the superoxide anion, which also stimulates the induction of iNOS (Lafon-Cazal *et al.*, 1993).

1.10 The kynurenine pathway (KP)

This major metabolic pathway of the essential amino acid tryptophan is present in both the CNS and the periphery. The KP ultimately results in the formation of nicotinamide adenine dinucleotide (NAD^{+}) and niacin. NAD^{+} is an important electron transporter for oxidative phosphorylation in mitochondria and is essential for energy production and cell survival (Bender and McCreanor, 1982). Of the dietary tryptophan not involved in protein production approximately 95% undergoes metabolism through the KP. 1% of this is broken down into the neurotransmitter serotonin (Ruddick *et al.*, 2006). The KP takes place in various cell types within the brain including astrocytes, microglia, neurons, infiltrating macrophages and dendritic cells (Ruddick *et al.*, 2006), and can be partitioned into two major branches. One branch produces the neuroprotective kynurenic acid (KYNA) principally in astrocytes (Mackay *et al.*, 2006), whereas the other branch, mainly arising in microglia (Espey *et al.*, 1997), synthesises neurotoxic metabolites, quinolinic acid (QUIN) and 3-hydroxykynurenine (3-HK). KYNA is an antagonist to the α7-nicotinic acetlycholine receptor (α7-nAChR), the glycine co-agonist site of the NMDA receptor and the AMPA receptor (Guillemin *et al.*, 2001, Skoog, 2000, Chen *et al.*, 2007, Vignau *et al.*, 2004). The neurotoxic branch consists of free radical generators, 3-HK, 3-hydroxyanthranilic acid and QUIN.

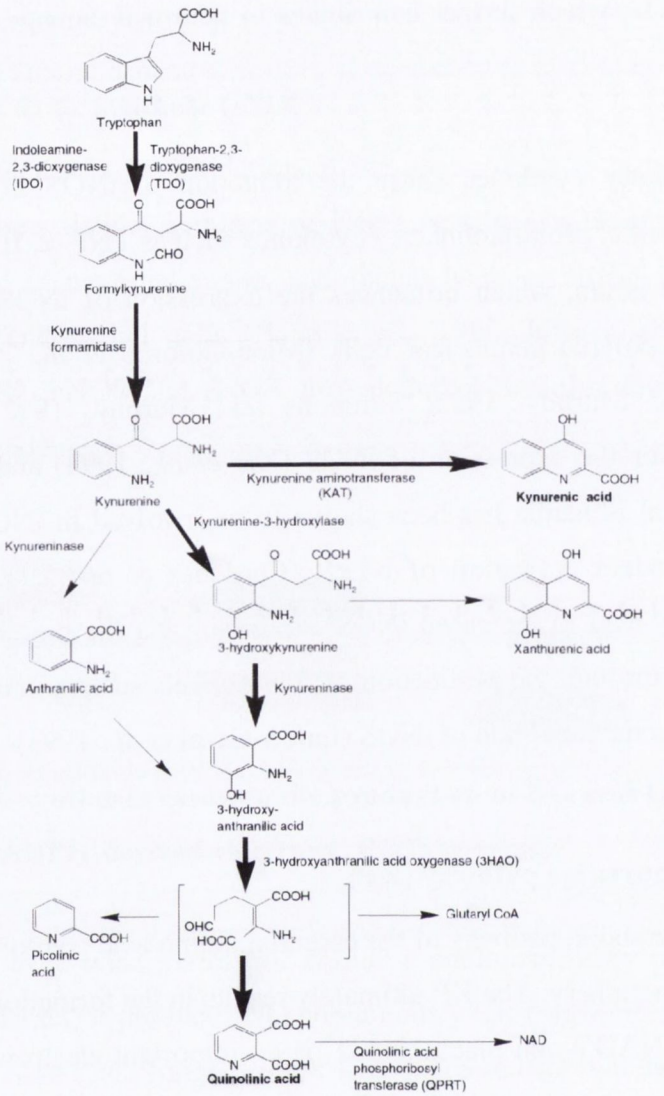


Figure 1.6 An outline of the KP (MacKay *et al.*, 2004)

Further details of the catalytic steps along the pathway are provided in the text below.

1.10.1 Indoleamine 2, 3-dioxygenase

IDO is the first and rate-limiting enzyme of the KP in the brain and periphery excluding hepatic tissue where trypotophan 2, 3-dioxygenase (TDO) is the catalyst of the pathways initial step. IDO is a heme-containing glycoprotein which catalyzes the conversion of L-tryptophan to formylkynurenine utilizing molecular oxygen and superoxide (Ruddick *et al.*, 2006). The expression of IDO is extensively distributed in the brain and peripheral tissues, with the pineal gland and choroid plexus of the brain showing high expression levels (Hansen *et al.*, 2000). The highest peripheral activity is found generally in tissues with a large mucosal surface such as the placenta, lungs and gut. Tissues with mucosal surfaces such as the lungs and gut tend to express IDO under normal conditions due to "constitutive" inflammation (Mellor, 2005). The inflammatory response to infection or

cancerous tissue can lead to increased IDO expression in other tissues including the brain. IDO has been shown to be an important element in antimicrobial resistance to bacteria and parasites (Sanni *et al.*, 1998; Silva *et al.*, 2002; Beatty *et al.*, 1994; MacKenzie *et al.*, 1998).

IDO catalyses a reaction that opens the tryptophan indole ring by an oxidation reaction forming formylkynurenine that is converted to L-kynurenine (L-KYN) by the enzyme formamidase (Ruddick *et al.*, 2006). L-KYN is the substrate for three enzymes; kynureninase (producing anthranilic acid), kynurenine amino transferase (KAT) leading to the side branch end product KYNA, and kynurenine 3-hydroxylase (K3-H) bringing the pathway towards the production of 3-HK, QUIN and NAD^+ (Guillemin *et al.*, 2001). Therefore L-KYN is an important branching point of the pathway diverging into the neurotoxic branch and the neuroprotective branch. Both 3-HK and anthranilic acid can yield 3-hydroxyanthranilic acid (3-HAA) which leads to the formation of QUIN through 3-hydroxyanthranilic acid oxygenase (3-HAO). QUIN phosphoribosyltransferase metabolises QUIN and further reactions lead to the generation of the pathway end product NAD^+.

1.10.2 The neuroprotective branch of the KP

KAT enzymes in the KP lead to the production of the neuroprotective metabolite KYNA. A wide variety of physiological effects induced by KYNA have been demonstrated both *in vitro* and *in vivo* including anticonvulsant properties and neuroprotective effects as it can confer protection against excitotoxic injury (Myint *et al.*, 2007). Studies have also shown that it may participate in sustaining physiological blood pressure and could play a role in the pathology of a number of neurological disorders such as HD, schizophrenia and epilepsy (Mellor, 2005; Guidetti *et al.*, 2006; Wu *et al.*, 2000; Skoog, 2000)

Perkins and Stone (1982) first described KYNA as a neuro-inhibitory compound, as it is a broad-spectrum antagonist of ionotropic excitatory amino acid receptors (at high/non-physiological concentrations). At physiological concentrations (low micromolar range) KYNA has been shown to antagonise both the α7-nAChR non-competitively, and the strychnine-insensitive glycine co-agonist site of NMDAR competitively in rodents and primates (Guillemin *et al.*, 2001, Skoog, 2000, Chen *et al.*, 2007, Vignau *et al.*, 2004). KYNA could therefore have a physiological role in both glutaminergic and cholingeric

neurotransmission. KYNA is predominantly produced in astrocytes (Skoog, 2000). Guillemin *et al.* (2001) demonstrated *in vitro* that astrocytes did not contain K3-H and subsequently only produced L-KYN and KYNA. During inflammation KYNA levels are elevated and small KYNA increases can inhibit the release of glutamate, thus preventing excitotoxicity from the over-activation of NMDA receptors (Stone and Addae, 2002).

1.10.3 The neurotoxic branch of the KP

3-HK is a free radical generator and the first metabolite of the QUIN branch found in nanomolar concentrations in the brain (Stone *et al.*, 2007). An increase in 3-HK is linked with neuroinflammation, potentiation of excitotoxicity, stroke, seizures and various neurodegenerative diseases such as HD. 3-HK can lead to the production of free radicals and H_2O_2 (Okuda *et al.*, 1998) and the production of superoxide anions leads to the oxidation of 3-HAA (Liochev and Fridovich, 2001).

K3-H (or kynurenine 3-mono-oxygenase (KMO)) is an NADPH-dependent flavin monooxygenase of the mitochondrial outer membrane, and converts L-KYN to 3-HK leading the pathway towards the generation of toxic metabolites. Tissue distribution studies in rodents showed it to be present everywhere in the brain with a low activity. Liver and kidney possess the highest specific activity for the enzyme (Erickson *et al.*, 1992). Further experiments by Carpenedo *et al.* (1994) using hippocampal slices found that inhibitors of K3-H, including (m-nitrobenzoyl)-alanine and Ro 61-8048 lower 3-HK and QUIN formation and perhaps provide more L-KYN for KYNA synthesis. This study concluded however that the possibility of neuroprotection mediated by KYNA was unlikely as the levels of KYNA recorded were far too low for glutamate receptor interaction.

QUIN is an NMDA receptor agonist and prolonged exposure (even at nanomolar concentrations) or high levels of QUIN have been seen to cause lipid peroxidation, free radical formation and cell death (Schwarcz *et al.*, 1983). Neurodegenerative diseases in particular HD and AD consistently correlate with raised concentrations of this degradation product (Stone, 1993 and 2001; Stone and Darlington, 2002; Guidetti *et al.*, 2006). 3-HAO metabolises 3-HAA to form α-amino-ω-carboxymuconic acid semialdehyde which can rearrange non-enzymatically into QUIN. Alternatively it can form picolinic acid. Cerebral tissue contains between 50 and 1000 nM of QUIN (Guillemin *et al.*, 2001).

QUIN has been shown to inhibit the uptake of glutamate by astrocytes, as well as promote its release from neurons, thus increasing glutamate concentrations in the synaptic cleft and further boosting its excitotoxic effect (Tavres *et al.*, 2002). The rat brain has been shown to produce ·OH radicals after QUIN exposure as well as peroxynitrite (formed by NO) in the early stages of toxicity (Rodriguez-Martinez *et al.*, 2000). It can induce astrocytes to release cytokines and chemokines thus augmenting inflammation locally and adding to excitotoxic damage (Miller *et al.*, 2006). Lipid peroxidation has been also reported *in vivo* and *in vitro* due to free radical formation. The basal amount of lipid peroxidation was seen to increase as much as 256% in the rat brain following exposure to 100µl of QUIN (Santamaria *et al.*, 1997).

1.11 Excitotoxicity, neuroinflammation and cell death in hippocampal neurodegeneration

The hippocampus is involved in learning and memory and is a structure that is particularly vulnerable to neuronal injury. It is a structure that is commonly associated with various neurodegenerative disorders, and hence is frequently studied to elucidate mechanisms underlying neuronal loss in these conditions.

The hippocampus is located in the medial temporal region of the brain. The name was donned by Giulio Cesare Aranzi in the sixteenth century, from the greek words *hippos* meaning horse, and *kampos* meaning sea monster as the structure resembled that of a seahorse (Gross, 1998). This structure has a very distinct shape and consists of three main areas; Ammon's horn (CA1-CA3 pyramidal cell layers) and the dentate gyrus, and is sometimes referred to as a trisynaptic circuit. This circuit is comprised of three sequential glutamatergic synapses: axons of layer II neurons in the entorhinal cortex project along the perforant pathway and innervates the granule cells of the dentate gyrus, mossy fibre axons of the granule cells project to the proximal dendrites of the CA3 pyramidal cells, and the Schaffer collateral axons of the CA3 project into the stratum radium of the CA3, where the apical dendrites of the CA1 are located (Amaral and Witter, 1989, Miller and O'Callaghan, 2005, Gutierrez, 2003). In the dentate gyrus, the perforant path and mossy fibres have been shown to innervate an array of GABAergic neurons, as well as glutamatergic mossy cells of the hilus, thus it is thought that the primary targets of mossy fibres are these GABAergic "interneurons", and they play an inhibitory role in hippocampal transmission (Ascady *et*

al., 1998). The hippocampus has a high density of kainate (KA) receptors, which are a subtype of the ionotropic glutamate receptors (Lerma, 2001). These receptors have been shown to mediate frequency-dependent transmission in mossy fibre cells, CA3 pyramidal cell synapses and in CA1 interneurons (Kamiya, 2002). As well as releasing glutamate, granule cells also release dynorphin, enkephalin, zinc, brain derived neurotrophic factor (BDNF) and peptides such as somatostatin, neuropeptide Y, neurokinin B and cholecystokinin (McGinty *et al.,* 1983; Gall *et al.,* 1981; Wenzel *et al.,* 1997; Gall and Lauterborn, 1992; Tonder *et al.,* 1994; Schwarzer *et al.,* 1995).

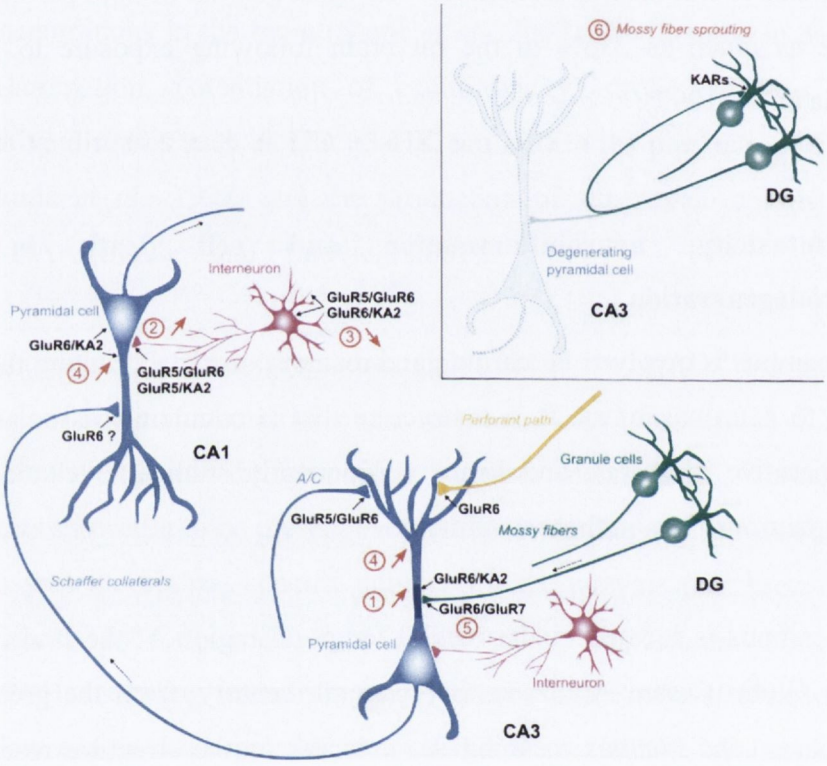


Figure 1.7 Hippocampal circuitry

The hippocampus is composed of distinct areas arranged in a closed circuit. External inputs are conveyed to the hippocampus through superficial layers of the entorhinal cortex (EC layers II and III). EC layer II projects to the dentate gyrus (DG) and the CA3 through the perforant path (pp). In turn, the dentate gyrus (DG) projects to the CA3 through the mossy fibers (mf) which projects to the CA1 through the Schaffer Collateral (sc). The CA1 projects outside the hippocampus to the deep layers of the entorhinal cortex (layer V). Additionally, the CA1 receives direct information from the EC layer III and the CA3 is massively interconnected (Vincent and Mulle, 2009).

The hippocampus is mainly involved in memory and learning. The formation of memories and learning involves inputs from the cortex to the hippocampus. The entorhinal cortex receives inputs from all the senses and other cortical areas, and passes these signals on to the hippocampus via the perforant pathway (Miller and O'Callaghan, 2005). The role of the hippocampus in learning is demonstrated by patients with gross destruction of medial temporal lobe structures, who display severe impairment of subsequent ability to learn (anterograde amnesia) and impairment of previously acquired learned behaviour (retrograde amnesia) (Scoville and Milner, 1957; Penfield and Milner, 1958). Evidence from lesion, behavioural, electrophysiological and gene activation studies suggest that the hippocampus has distinct sub-region functions within it. The dentate gyrus is thought to process metric spatial representation. The CA3 is related to spatial pattern association and short-term memory. The CA1 is involved in temporal pattern association and intermediate-term memory (Kesner *et al.,* 2004; Miyashita, 2004).

The hippocampus is implicated in a number of acute and chronic neurodegenerative diseases. Acute neurodegenerative conditions such as stroke and epilepsy induce increases in excitotoxicity, inflammation and apoptosis, which have been shown to mediate neurodegeneration within this structure. Sub regions of the hippocampus are particularly sensitive to ischemic damage, with numerous reports detailing the degeneration of the CA1 following ischemic insult (Schmidt-Kastner *et al.,* 1997; Jourdain *et al.,* 2002; Nikonenko *et al.,* 2003). An episode of 10–15 min of transient ischemia leads to selective cell death in CA1 pyramidal neurons and in the hilus after 48-72 h of reperfusion. Dentate gyrus granule cells and CA3 pyramidal cells largely recover, as do most hippocampal interneurons (Schmidt-Kastner and Freund, 1991). After transient focal ischemia, results showed that morphological alterations of the synaptic elements in stratum radiatum of the CA1 were observed (Kovalenko *et al.,* 2006). The spine synapse density in CA1 stratum radiatum showed a tendency to decrease 2 h after reperfusion. Along with some swelling of the endoplasmic reticulum in presynaptic terminals, dendrites, and spines, swelling of mitochondria and dendrites, and the appearance of membranous structures in the terminals, the post synaptic density thickness increased significantly (von Lubitz and Diemer, 1983; Schmidt-Kastner and Freund, 1991; Kovalenko *et al.,* 2006). Short periods of ischemia led to a significant decrease of mitochondrial capacity to accumulate Ca^{2+} in the hippocampus, an effect which was unchanged following reperfusion. This can initiate cell degenerative pathways, such as the opening of mitochondrial permeability transition pore or apoptosis

initiation, and indeed, might represent an important mechanism of ischemic damage to neurons within the hippocampus (Racay *et al.,* 2009). Transient cerebral ischemia significantly increased generation of ROS and modulated superoxide dismutase activity, following this, reperfusion evoked apoptosis in the form of mitochondrial cytochrome c release, Bcl-2 and caspase-9 expression, in the hippocampus. It also resulted in significant increases in hippocampal levels of TNF-α and increased expression of COX-2, iNOS, and intercellular adhesion molecule-1 (ICAM-1) (Collino *et al.,* 2008).

The hippocampus is a structure that is largely affected in epilepsy. Due to the GABAergic and glutamatergic innervation of the hippocampus, it makes it a prime target for seizure-induced degeneration. Administration of KA to rats represents a model of human temporal lobe epilepsy (TLE) and acute status epilepticus is accompanied by neurodegeneration in the CA1 and CA3 sector of the Ammon's horn and of interneurons in the hilus of the dentate gyrus (Tsunashima *et al.,* 1997). Changes within the pyramidal cell layer are accompanied by alterations in GABAergic subunit mRNA expression, an effect which is determined by concomitant neurodegenerative processes within the hippocampus (Tsunashima *et al.,* 1997). Mossy fibre sprouting is thought to be responsible for epileptogenesis in the KA induced model (Xu *et al.,* 2006). Caspase-3 expression was determined in the hippocampus of electrically kindled rats (Chen *et al.,* 2009). Increased glutamate concentration has been found in epileptogenic foci and may induce local over-excitation and excitotoxicity; this may be due to reduced extra-cellular clearance of glutamate by EAAT-1-5. EAAT-1 and EAAT-2 are mainly expressed on astroglial cells for the reuptake of glutamate from the extra-cellular space and it was reported by Sarac and co-workers (2009), that there was a downregulation of EAAT-1 and EAAT-2 in the hippocampus and temporal lobe of 12 patients with TLE. This may be an adaptive response to neuronal death or it may be a causative event contributing to neuronal death. Seizure activity has been linked with increased inflammation in the hippocampus, with levels of IL-1β mRNA and protein increased in a number of seizure models (Donnelly *et al.,* 2001; Heida and Pittman, 2005; Crespel *et al.,* 2002).

Due to the vast glutamatergic innervation of the hippocampus, it is particularly sensitive to excitotoxic damage. Distinct populations of hippocampal neurons are targeted by ischemia, excitotoxicity, oxidative stress, and inflammation, and are responsible for their damage and death (Nikonenko *et al.,* 2009). The hippocampus was chosen as the brain structure of

interest in the studies carried out here, due to its sensitivity to KA-induced damage and its involvement in a number of neurodegenerative disorders.

1.12 The role of neurotrophic factors in neurodegeneration

1.12.1 Brain derived neurotrophic factor (BDNF)

BDNF is a member of the neurotrophin family, along with nerve growth factor (NGF), neurotrophin-3 and neurotrophin-4, which are a group of homodimeric proteins, each exerting a range of trophic effects on neurons in the peripheral and central nervous system (Lessmann and Brigaski, 2009). BDNF was discovered in 1982 when it was purified from porcine brain and was shown to promote the survival of dorsal root ganglion neurons (Barde *et al.,* 1982). The BDNF gene is comprised of four 5' exons, which are associated with distinct promoters, and one 3' exon responsible for encoding mature BDNF protein (Metsis *et al.,* 1993; Timmusk *et al.,* 1993). BDNF consists of a non-covalently linked homodimer containing a signal peptide following the initiation codon, and a pro-region containing an N-linked glycosylation site. Pro-BDNF is converted to mature BDNF by pro-hormone convertases such as furin (Chao and Bothwell, 2002). BDNF signal transduction is associated with binding to the tropomyosin-related kinase-B (trk-B) receptor. Receptor activation occurs following ligand-induced receptor dimerization, which leads to kinase activation, resulting in autophosphorylation of multiple tyrosine residues, thus creating binding sites for intracellular target proteins. These target proteins bind to the activated receptor via SH2 domains (Patapoutian and Reichardt, 2001; Barbacid, 1994) and lead to activation of an array of signalling pathways such as the MAPK pathway and the phosphorylation of cAMP-response element binding protein (CREB) (Segal, 2003). BDNF also binds to the p75 receptor which is associated with several intracellular signal transduction pathways such as jun kinase and NFκB (Dechant and Barde, 2002).

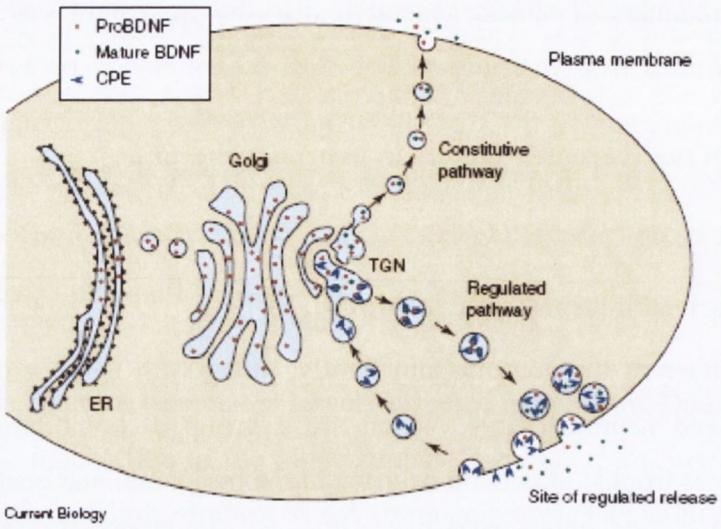


Figure 1.8 BDNF synthesis, processing, sorting, transport and secretion in neurons (Thomas and Davies, 2005)

BDNF is synthesized in the endoplasmic reticulum (ER) as a 32 kDa precursor protein (proBDNF) that moves though the Golgi apparatus to the trans Golgi network (TGN), from where it passes into the constitutive and regulated secretory pathways. Binding of BDNF to the lipid-raft-associated sorting receptor carboxypeptidase E (CPE) in the TGN is necessary for sorting into secretory vesicles of the regulated pathway. These vesicles are subsequently transported to appropriate sites for activity-dependent secretion. Most BDNF in the regulated secretory pathway is transported to postsynaptic dendrites and spines, but it also undergoes anterograde axonal transport and activity-dependent transfer from pre- to postsynaptic sites. The contents of these vesicles are eventually released upon triggering signals for regulated secretion.

Electrical stimuli that induce long-term potentiation in the hippocampus have been shown to increase the expression of BDNF and NGF (Patterson *et al.*, 1992; Castren *et al.*, 1993). Neural activity can differentially regulate distinct BDNF 5' exons. For example, following kainic acid-induced seizures, exons I-III but not exon IV are augmented (Lauterborn *et al.*, 1996, Tao *et al.*, 2002). CREB which is induced by a range of stimuli including antidepressants and exercise, has been shown to modulate exon III transcription.

BDNF and trkB are widely distributed in the CNS, with particularly high levels of BDNF in the hippocampus (Conner *et al.*, 1997). BDNF has been shown to play a role in survival and growth of neurons (Huang and Reichardt, 2001), enhance neurogenesis, enhance

learning and memory via its actions on the hippocampus, and to enhance excitatory (glutamatergic) synapses and weaken inhibitory (GABAergic) synapses (Binder and Scharfman, 2004). BDNF has also been shown to play a role in epilepsy. BDNF mRNA and protein is upregulated in the hippocampus following seizure activity (Ernfors *et al.*, 1991; Nibuya *et al.*, 1995). A redistribution of BDNF mRNA from the CA3 cell bodies to their apical dendrites is seen in the hippocampus following a seizure (Bregola *et al.*, 2000, Simonato *et al.*, 2002). Infusion of anti-BDNF and the use of BDNF knock-out mice have been shown to inhibit the induction of seizures in animal models of epileptogensis (Binder *et al.*, 1999; Kokaia *et al.*, 1995). Conversely, it has been shown that direct application of BDNF *in vivo* was shown to induce seizures (Scharfman *et al.*, 2002).

Distinct actions of BDNF mediated by p75 receptor activation include promotion of myelination (Cosgaya *et al.*, 2002), neuronal migration (Carter *et al.*, 2003), neuronal process retraction (Cahoon-Metzger *et al.*, 2001; Gehler *et al.*, 2004), and neuronal apoptosis (Bamji *et al.*, 1998; Boyd and Gordon, 2002; Troy *et al.*, 2002). Overexpression of proBDNF has been shown to be associated with increased neuronal death (Teng *et al.*, 2005).

Administration of the excitotoxin kainic acid has been shown to increase BDNF mRNA and protein (Zafra *et al.*, 1990; Isackson *et al.*, 1991; Gall *et al.*, 1991), an effect which is related to the coupling between neuronal excitation and trophic factor expression. This effect is thought to play a protective role against seizure-mediated excitotoxicity (Gall *et al.*, 1993; Schmidt-Kastner *et al.*, 1996) however, recent reports have shown that status epilepticus induces a rapid down-regulation of Trk-B that accompanies neuronal damage in the dentate gyrus (Unsain *et al.*, 2008), suggesting a potential apoptotic role for BDNF and/or pro-BDNF following seizure activity.

BDNF is implicated in neurodegenerative diseases, with numerous reports of decreased BDNF in the hippocampus with AD, and in the substantia nigra with PD (Phillips *et al.*, 1991; Ferrer *et al.*, 1999; Howells *et al.*, 2000). The protein Huntingtin, which is implicated in HD, upregulates BDNF transcription, and thus loss of this protein down-regulates BDNF transcription and subsequently there is a loss of trophic support to striatal neurons resulting in death of these neurons (Zuccato *et al.*, 2001). Exogenous administration of BDNF directly into the brain has antidepressant-like effects in multiple

animal models of depression (Siuciak *et al.*, 1997; Shirayama, 2002). Chronic antidepressant treatment has been shown to upregulate BDNF expression (Xu *et al.*, 2003; Torregrossa *et al.*, 2005). The cAMP pathway and cAMP response element binding protein (CREB) are implicated in antidepressant action and BDNF is a target of this pathway. It is proposed that cAMP is upregulated following antidepressant treatment and this in turn upregulates BDNF and CREB which increase neuronal survival and neural plasticity (D'Sa and Duman 2002).

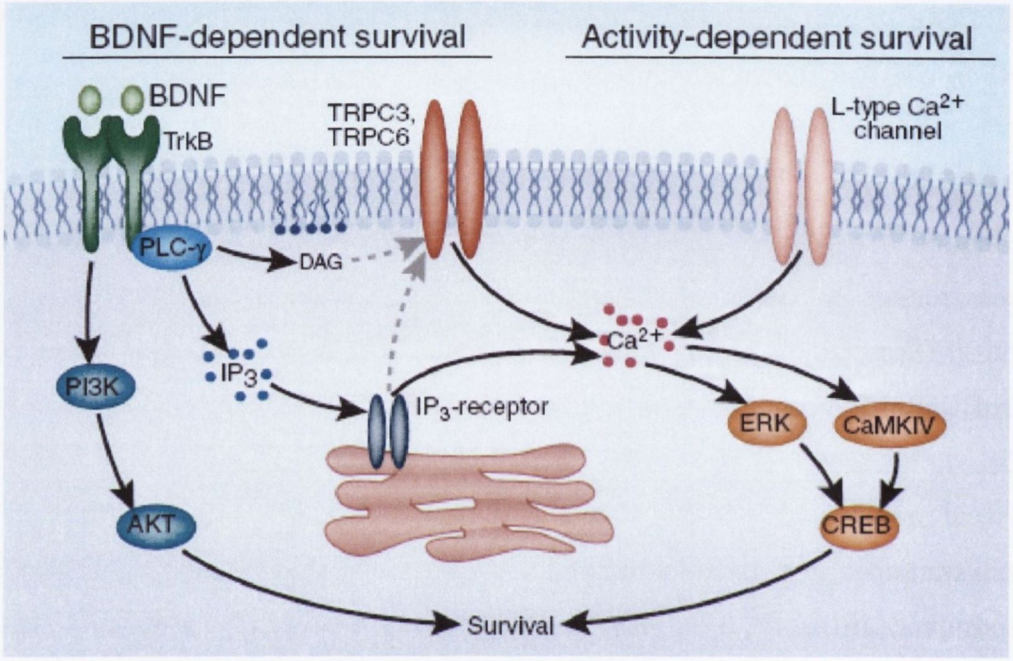


Figure 1.9 BDNF signalling pathways (Sossin and Barker, 2007)

BDNF-induced and activity-dependent survival mechanisms rely on convergent Ca^{2+}-dependent signaling pathways. The binding of BDNF to TrkB results in the activation of PLC-γ and the production of DAG and IP3. DAG directly activates TRP family channels, whereas IP3 may trigger store-operated Ca^{2+} release mechanisms. Ca^{2+} influx via TRPC3 and TRPC6 channels activates Erk and CaMKIV, which in turn activate CREB transcriptional pathways that collaborate with Akt-dependent pathways to support neuronal survival.

1.12.2 Nerve Growth Factor

The role of nerve growth factor (NGF) as a target-derived trophic agent was established in the peripheral nervous system (PNS) in 1968 by Levi-Montalcini and Angeletti. Following this, NGF was shown to be a key player in PNS development and to help maintain the viability of peripheral sympathetic neurons (Petruska and Mendell, 2004; Ruit *et al.*,

1990). NGF has been shown to play a role in the CNS, particularly in promoting survival and maintaining the phenotype of cholinergic neurons (Schweitzer, 1989; Sofroniew *et al.*, 2001). Several studies have also demonstrated that NGF can acutely modulate glutamate transmission in the rat hippocampus and neocortex (Knipper *et al.*, 1994a, 1994b; Carmignoto *et al.*, 1997). As with BDNF, NGF is synthesized as a larger precursor protein, pro-NGF, which requires further processing prior to secretion as a mature protein. It also contains a signal peptide following the initiation codon, a pro-region and a mature sequence (Binder *et al.*, 2007). Secretion of NGF is regulated within the CNS and is calcium-dependent (Lessmann *et al.*, 2003). Stimulation of NGF release has been demonstrated via glutamate receptor activation or high potassium concentrations, an effect which is dependent on intracellular calcium release and is mediated by sodium influx via voltage-gated sodium channels and non-NMDA glutamate receptors (Blochl and Thoenen, 1996). NGF has been reported to be neuroprotective in a number of models both *in vivo* and *in vitro* (Nguyen *et al.*, 2009; Tabakman *et al.*, 2005; Melchior *et al.*, 2003; Mitchell *et al.*, 1999). The precursor form of NGF, proNGF, can be secreted by cells and act via a dual receptor system of p75NTR and the type I transmembrane protein sortilin to mediate apoptosis (Nykjaer *et al.*, 2004). ProNGF interacts via its pro-domain with sortilin, whereas interactions with p75NTR are most likely mediated by the mature domain. However, binding of proNGF to both receptors on the cell surface is necessary to generate high-affinity sites and mediate apoptosis (Nykjaer *et al.*, 2004).

1.12.3 Glial derived growth factor

Glial cell line-derived neurotrophic factor (GDNF) was originally purified from rat B-49 glial cell's conditioned medium and was characterised as a potent neurotrophic factor for cultured dopaminergic neurons from developing substantia nigra (Lin *et al.*, 1993). GDNF is distantly related to the transforming growth factor-β superfamily (Poulsen *et al.*, 1994). Expression of GDNF is widespread throughout the CNS and PNS, as well as outside the nervous system (Choi-Lundberg and Bohn, 1995). GDNF is upregulated in glial cells and macrophages following cerebral ischemia and spinal cord injury (Satake *et al.*, 2000; Ikeda *et al.*, 2002). GDNF is a typical secretory protein first synthesised as an inactive 211 amino acids long proGDNF. The secreted proGDNF is then proteolytically cleaved, and the mature GDNF protein of 134 amino acids is formed (Saarma and Sariola, 1999). The structure of GDNF is similar to that of the TGF-β family, with c-terminal cysteines of

mature GDNF, Cys131 and Cys133, which participate in the formation of the ring structure due to two cysteine knots by linking with Cys68 and Cys72, respectively. The processing and secretion, as well as post-transcriptional regulation of GDNF, has to date, not been fully elucidated. However, GDNF is likely to be secreted both with and without processing by furin-like proteases, and the pro-domain and C-terminal cysteines of GDNF play important roles in its processing and secretion in cultured astrocytes and C6 cells (Oh-Hashi *et al.,* 2009).

1.12.4 Transforming growth factor β

Transforming growth factor β (TGFβ) regulates essential cellular functions such as cellular proliferation, differentiation and apoptosis. The TGFβ family of polypeptides comprises a group of highly conserved dimeric proteins with a molecular weight of approximately 25 kDa (Roberts and Sporn, 1993). TGF-β protein is produced by nearly all normal cells and functions through a complex cell surface receptor system (Massague, 2000). Signalling by TGFβ is initiated by an oligomeric receptor complex consisting of two transmembrane subunits, the type I and type II receptors (Schmierer and Hill, 2007; Rahimi and Leof, 2007; Siegel and Massague, 2003) Activation of the receptor complex leads to further propagation of TGFβ signalling to downstream signalling cascades, which include the Smads, MAPK and phosphatidylinositol 3′ kinase (PI3K) (Moustakas and Heldin, 2005; Javelaud and Mauviel, 2005). In the nucleus, activated Smad complexes, along with co-activators and cell-specific DNA-binding factors, regulate gene expression and ultimately cell cycle and tissue repair (Massague and Chen, 2000). As TGF-β is a potent growth inhibitor, loss of TGF-β activity has been implicated in the pathogenesis of ovarian cancer, pancreatic cancer, colon cancer, and squamous cell carcinoma (Wang *et al.,* 2000; Hahn *et al.,* 1996; Markowitz *et al.,* 1995; Garrigue-Antar *et al.,* 2005). It is also implicated in ischemic brain injury as a potential therapeutic target (Buisson *et al.,* 2003). It is thought to be neuroprotective via its ability to suppress apoptosis, upregulating type-1 plasminogen activator inhibitor and potentiating the effect of tissue-type plasminogen activator on NMDA receptor-mediated excitotoxicity (Vivien and Ali, 2006).

1.13 Kainic acid-induced excitotoxicity – a model of acute neurodegeneration

Kainic acid (KA) is a pyrolidine derivative that was originally isolated from the seaweed *Dignea simplex*. It was used traditionally in Japanese folk medicine as an ascaricidal

preparation (Tamura, 1954, Takemoto, 1978) and translates from Japanese as "Monster from the sea". The role of KA in neuroexcitation was demonstrated following the application of KA to rat cortical neurons. It was seen to directly excite these neurons and also potentiate the effects of L-glutamate (Shinozaki and Konishi, 1970). Further studies illustrated the excitatory activity of KA, which ranged from 10-200-fold greater than L-glutamate, following application to vertebrate spinal neurons (Johnston *et al.*, 1974, Biscoe *et al.*, 1976).

The excitatory and neurotoxic effects of KA were first investigated by Olney (1971) when he examined the effects of peripherally administered acidic amino acids and structurally related compounds. He noted that profound swelling of dendrites, an early indicator of toxic damage in the retina, was consistent with the depolarising effects of this excitatory amino acid. The binding of KA to its receptor triggers events that, at certain concentrations, can lead to the development of excitotoxicity.

Structural analysis has identified a structural specificity for the neuroexcitotoxic effects of KA (Biscoe *et al.*, 1976). KA consists of a pyrolidine ring, two carboxycilic acid moieties and an isopropyl side chain.

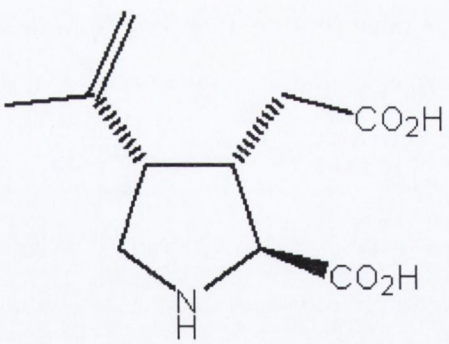


Figure 1.10 Structure of Kainic acid. (Biscoe *et al.*, 1976)

The importance of the isopropylene side chain is demonstrated by the attenuation of neuroexcitatory activity following the reduction of the double bond or a reversal of its spatial orientation (Coyle, 1983). A reduction in KA activity is seen following blockade of the pyrolidine ring by alkylation or esterification of the carboxyl terminus, or by substituting a ketone group for a methylene group-which only retains 10% of the activity of the parent compound (Coyle, 1983).

KA is typically used as a model of human temporal lobe epilepsy due to the behavioural and pathological similarities. Following KA administration systemically or directly into the brain, the rat exhibits abnormal behaviours such as freezing and staring, forelimb clonus and generalised seizures for a 4-6 hour time period (Schwarcz *et al.*, 1978). These seizures are frequently scored according to a scale devised by Racine (1972) with the following criteria: Stage 1-staring, freezing, mouth and facial movements, Stage 2-head nodding and wet dog shakes, Stage 3-forelimb clonus, Stage 4-forelimb clonus with rearing, Stage 5-rearing *and* falling, generalised tonic-clonic seizure.

KA receptors are found in all major brain areas in the rat but higher affinity sites are found predominantly in forebrain regions (London and Coyle, 1979). A dense band of KA receptors are found on mossy fibre axon terminals in the hippocampus (Foster *et al.*, 1981). Persistent seizures result in degeneration of neurons throughout the brain but specifically in the limbic system (Meldrum *et al.*, 1974). This may be due to the fact that continuous seizures may enhance neuronal vulnerability to glutamate and excitotoxicity especially in the limbic system, where there is a high density of KA receptors (Foster *et al.,* 1981). Ionophoretic studies have shown hippocampal CA3 pyramidal cells to be the most vulnerable to KA-induced neuronal damage (de Moutiguy and Tardif, 1981). This may be due to direct glutamatergic input from the dentate gyrus granule cells (Nadler *et al.*, 1980). Cell loss is seen not only in the CA3, but also the CA1 and dentate gyrus (Hu *et al.*, 1998, Yoo *et al.*, 2006, Tokuhara *et al.*, 2006).

It is unclear which of the two elements, seizure activity or glutamate release drive neurodegeneration in response to KA administration. Some studies suggest that damage occurring away from the site of injection is not likely to be due to the presence of KA in the affected areas, but results from the seizures that are induced by KA administration (Ben-ari *et al.*, 1980). Anticonvulsants prevent the development of cortical seizure activity, but do not protect against hippocampal neurotoxicity (Zaczek *et al.*, 1980). This may be due to their effects on GABAergic and glutamatergic neurotransmission (MacDonald and Barker, 1979).

The KA model of excitotoxicity is widely used for a number of reasons. It can be administered systemically as it readily crosses the blood brain barrier, where the alternative excitotoxins NMDA and QUIN do not. It is well established that KA results in alterations

to cellular and molecular mechanisms including cell loss and apoptosis, throughout the brain, but particularly in the hippocampus (Ben-Ari, 1985; Nadler *et al.*, 1980; Shetty and Turner, 2000).

1.14 Kainic acid-induced neuroinflammation

It has been reported that kainic acid induces neuroinflammation. Upon excitotoxic brain injury, proinflammatory cytokines are expressed by activated microglia (Barone and Feuerstein, 1999) and inhibition of microglial activation and proliferation by minocycline, an inhibitor of microglial activation, was shown to reduce excitotoxic spinal cord neuronal cell death (Tikka *et al.*, 2001). The peripheral administration of KA to rats was shown to induce a transient and biphasic expression of IL-1β mRNA in numerous brain regions (Minami *et al.*, 1990; Eriksson *et al.*, 2000; Minami *et al.*, 1991; Eriksson *et al.*, 1999). *In situ* hybridisation demonstrated an induction of IL-1β mRNA in the cerebral cortex, hippocampus, thalamus and hypothalamus after KA administration (Minami *et al.*, 1990). Intrahippocampal administration of KA resulted in the rapid induction of IL-1β in the dentate gyrus (Vezzani *et al.*, 1999). Addition of KA to cultured microglia resulted in their activation. Conditioned media from this preparation was administrated intracerebroventricularly (i.c.v) to rats, resulting in increases in IL-1β and iNOS expression both *in vitro* and *in vivo* (Zheng *et al.*, 2009). It is well known that NF-κB activation plays a critical role in microglial production of proinflammatory mediators such as- TNF-α, IL-1β and iNOS (Rasley *et al.*, 2002; Moriyama *et al.*, 2006). Upon stimulation, NF-κB is activated by inhibitory kappa binding protein (IκB) kinase (IKK) complex, in a manner dependent on the IKKβ catalytic subunit (Karin, 1999). Previous studies have reported that microglia activate NF-κB in excitotoxic brain injury (Matsuoka *et al.*, 1999; Acarin *et al.*, 2000). KA-induced neuronal death was reduced in IKKβ knock-out mice, and this was accompanied by suppressed glial cell activation, and subsequent suppression of IL-1β and TNF-α expression (Cho *et al.*, 2008). This study suggests that the induction of inflammatory mediators contributes to KA-induced hippocampal neuronal cell death.

Behan and Stone (2000) provided the first evidence that the KP may play a role in the KA model of excitotoxicity. Through the use of the KMO and kynureninase inhibitor- meta-nitrobenzoylalanine (mNBA), they demonstrated that by blocking the production of QUIN,

it was possible to reduce neuronal damage induced by KA. There appears to have been no subsequent investigations of the role of the KP in the KA model, and no evidence to demonstrate a relationship between KA administration and IDO expression, thus providing a rationale to investigate this relationship further.

1.15 Noradrenaline

Many studies provide evidence for the innate anti-inflammatory and neuroprotective effects of noradrenaline (NA), using a number of different models. Evidence suggests that NA can inhibit inflammatory processes in the CNS via its actions on microglia and also on astrocytes (For review see: Feinstein *et al.*, 2002; Galea *et al.*, 2003). NA displays neuroprotective properties such as modulation of neurotrophic factors, inhibition of apoptotic pathways and reduction of oxidative stress. Its neuroprotective effects are thought to be mediated via its ability to affect cAMP signalling and subsequent downstream signalling pathways such as MAPK and PI3K (Chen *et al.*, 2007). In various models of excitotoxicity and ischemia, NA confers neuroprotective properties (Burda *et al.*, 2009; Gustafson *et al.*, 1990; Baptiste *et al.*, 2002). The potential of NA as an endogenous transmitter which may be recruited to influence degenerative events following neuronal injury is reviewed below.

1.15.1 Synthesis, storage and release

NA is a monoamine neurotransmitter found throughout the sympathetic nervous system. NA is formed by the conversion of L-tyrosine to dihydroxyphenylalanine (DOPA) catalysed by the cytosolic enzyme tyrosine hydroxylase - this step is the rate limiting step and is inhibited by NA, thus providing a negative feedback loop. The next step in the pathway is the conversion of DOPA to dopamine, which is catalysed by the cytosolic enzyme DOPA decarboxylase. Dopamine is then converted to NA by the enzyme dopamine β-hydroxylase (DBH) (Rang *et al.*, 1999; Feldman *et al.*, 1997).

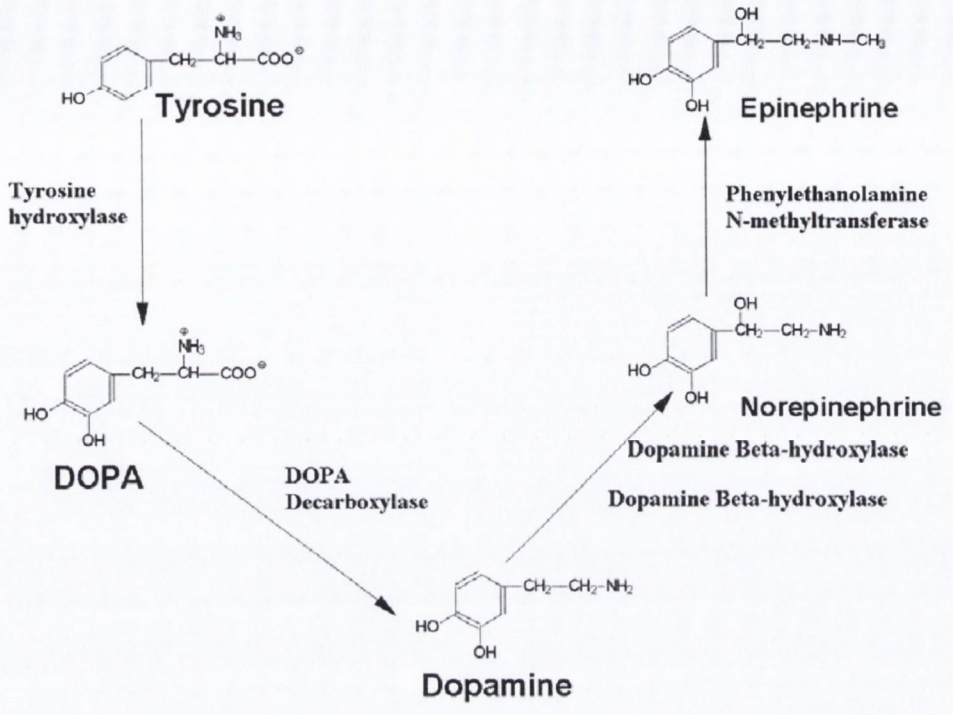


Figure 1.11 The biosynthesis of NA (Adapted from Kuhar *et al.*, 1999)

NA is stored in vesicles in chromaffin cells and at nerve terminals. Cytoplasmic levels are very low under normal circumstances but concentrations in the vesicles range from 0.3-1 mol/l. Noradrenergic vesicles also contain chromogranin A and ATP, which are both released with NA (Rang *et al.*, 1999). Depolarisation of the nerve terminal membrane opens calcium channels, resulting in the release of Ca^{2+} which promotes the discharge of synaptic vesicles (Feldman *et al.*, 1997). NA can regulate its own release by acting on presynaptic receptors in an auto-inhibitory feedback loop (Starke *et al.*, 1989). NA reuptake is facilitated by the Na^{+}- K^{+} ATPase pump which provides the energy to pump NA across the membrane, against the concentration gradient (Krueger, 1990). There are two distinct uptake mechanisms, uptake-1 is a high affinity system, located in neurons, and is relatively selective for NA. Uptake-2 is a low affinity extraneuronal system, which transports adrenaline, isoprenaline and NA but at a higher maximum rate (Iversen, 1974). The NA transporter (NET) acts as a cotransporter of Na^{+} and Cl^{-} with NA, using the Na^{+}-K^{+} gradient as its energy source. Changes in this gradient can affect the functioning of uptake-1 and can reverse it, thus releasing NA into the synapse (Rang *et al.*, 1999). The NET is located presynaptically on neurons and functions to clear NA from the synaptic cleft. It is a low capacity transporter and can become saturated in the presence of high levels of NA, thus leading to a build up of NA in the synaptic cleft (Eisenhofer *et al.*,

2001). The NET is a useful pharmacological target in the treatment of depression and other disorders associated with deficits in noradrenergic signalling. NET inhibitors such as reboxetine and desipramine selectively block the NET and cause an increase in synaptic availability of NA (Zhao *et al.*, 2009).

There are two principle enzymes involved in the metabolism of NA - monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). MAO is found within cells, bound to the surface membrane of mitochondria and is abundant in noradrenergic nerve terminals. It converts NA to its corresponding aldehyde - dihydroxymandelic acid (DOMA) which is converted by COMT to the final metabolite of NA and adrenaline-3-methoxy-4-hydroxymandelic acid (VMA) (Rang *et al.*, 1999).

The nuclei of origin for the NA system are located in the pons and medulla. They are composed of the locus coeruleus (LC), the lateral tegmental area and the dorsal medullary group (Linvall and Bjorklund, 1983). The LC is the most important noradrenergic nucleus with its axons innervating almost all parts of the telencephalon and diencephalons, including the neocortex, hippocampus, amygdala, septum, thalamus and hypothalamus (Rogawski, 1985).

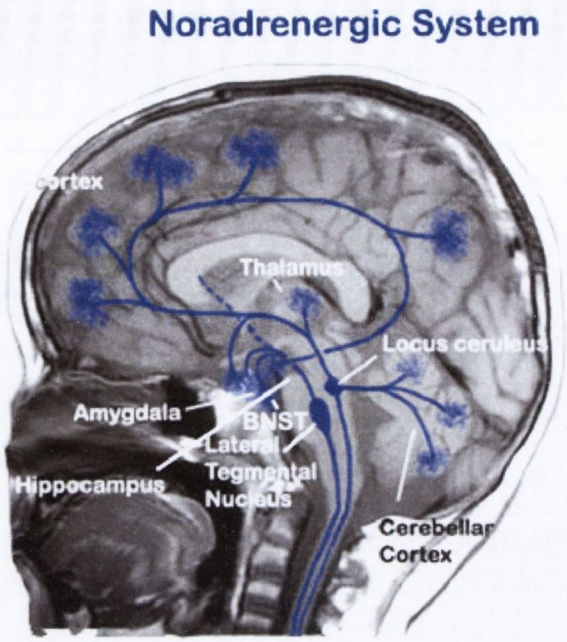


Figure 1.12 Noradrenergic pathways in the brain (Rudoy and Bockstaele, 2005)

1.15.2 Adrenergic receptors

Noradrenergic receptors (adrenoceptors) are divided into two major classes - α and β adrenoceptors (Ahlquist, 1948). These all belong to the G-protein coupled receptor superfamily which were classified according to rank order of potency of a range of sympathomimetic compounds. The α receptors are subdivided into α_1 and α_2, which was originally based on anatomical rather than pharmacological evidence. The α receptors located on peripheral sympathetic nerve terminals were termed α_2 to distinguish them from the postsynaptic α_1 receptors (Langer, 1974). It was later determined that although the subtypes of receptor displayed distinct selectivity profiles, some of Langers "presynaptic" receptors were also located postsynaptically, and thus it was suggested the receptors be classified according to their pharmacological properties (Berthelsen and Pettinger, 1977). Lands *et al.,* (1967) proposed the division of the β receptor into two subtypes, according to the rank order of potencies to various sympathomimetics. Today, it is know that there are three subtypes of β receptors- β_1, β_2 and β_3 (Krief *et al.,* 1993).

Table 1.2 Classification of adrenergic receptors

	Signalling Pathway	Brain regions
α_1 receptors	G_q/G_{11} form. Once activated-this G-protein activates phospholipase C which leads to the production of the fat soluble messenger - diacylglycerol, and the water soluble messenger - IP_3 (Summers and McMartin, 1993).	Specific actions of the α_1 receptor mainly involves smooth muscle contraction. It cause vasoconstriction in many blood vessels including those of the skin, gastrointestinal system, kidney and brain
α_2 receptors	Activation of the α_2 receptor is coupled to the inhibitory G (G_i) protein which results in the inhibition of adenylate cyclase, induces the phosphorylation of MAPK and inhibits the cAMP dependent phosphorylation of CREB (Alblas *et al.*, 1993). It also suppresses the release of NA. α_2 receptors have been shown to inhibit glutamate release and cause cell hyperpolarisation via direct action on calcium and potassium channels (Pralong and Jones, 1993; Pralong and Magistretti, 1995).	They are widely distributed throughout the peripheral and the central nervous system.
β_1 receptors	They are coupled to the stimulatory G (G_s-) protein, and stimulates the activation of adenylate cyclase (Pralong *et al.*, 2002).	In the brain, high concentrations of this receptor are found in forebrain structures such as the cerebral cortex, caudate and hippocampus (Minneman *et al.*, 1979).
β_2 receptors	β_2 receptors are also coupled to G_s proteins and increase cAMP signalling (Ordway *et al.*, 1987).	They are distributed throughout the CNS but high concentrations of this receptor are found in the cerebellum (Rainbow *et al.*, 1983; Minneman *et al.*, 1979). □2 adrenoceptors are more highly expressed on microglia and cells of the immune system and produce greater amounts of cAMP upon stimulation than □1 adrenoceptor (Mori et al., 2002).
β_3 receptors	They are coupled to G_s proteins and increase cAMP signalling (Feldman *et al.*, 1997)	Highest levels in hippocampus, cerebral cortex and striatum and lower in hypothalamus, brainstem and cerebellum (Summers *et al.*, 1995).

cAMP mediates its effects through cAMP-dependent protein kinases (PKAs). PKAs can modulate the activities of various intracellular enzymes by phosphorylation of serine and threonine residues, thereby increasing or decreasing the target enzyme activity (Lodish *et al.*, 2000). More than one PKA can mediate the action of cAMP and this can differ between cell types, hence the elevation of cAMP can exert differential effects dependent on the cell type (Lodish *et al.*, 2000). PKA's can also induce expression of genes that encode cAMP-response element (CRE). This occurs when the catalytic subunit of the PKA translocates to the nucleus where it can phosphorylate the transcription factor CRE-binding (CREB) protein which interacts with a co-activator CBP/300 to initiate gene transcription of CRE-encoding genes (Lodish *et al.*, 2000).

1.16 NA and neurodegeneration

Deficiencies in NA transmission, originating from cells of the locus coeruleus, are implicated in the pathogenesis of a range of neurodegenerative disorders, namely AD and PD. NA plays a key role in the modulation of endogenous compensatory and protective mechanisms such as promoting neurotrophic factors, alterations in oxidative metabolism, anti-excitotoxic mechanisms and neurotransmitter and receptor alterations (Marien *et al.*, 2004). Consequently, strategies that increase extracellular NA have been shown to be anti-inflammatory and neuroprotective.

As previously described inflammatory events have been shown to contribute to the onset, progression of damage and cell death in numerous neurological disorders including multiple sclerosis (MS), AD, PD, and AIDS dementia (Asensio and Campbell, 2001; Lee and Benveniste, 1999; Rothwell and Luheshi, 2000). The threshold for inflammation in the brain is lower than in the periphery (Lassman *et al.*, 1991) and it has been proposed that neurotransmitters act as local endogenous modulators of central inflammation (Feinstein *et al.*, 1993; Beneveniste *et al.*, 1995). NA has been shown to have potent anti-inflammatory properties and plays an important role in the maintenance of an immunosuppressive environment within the brain (Feinstein *et al.*, 2002). It has been reported that NA, via binding to β adrenoceptors and subsequent activation of cAMP signalling pathways, can inhibit a number of inflammogens such as iNOS, adhesion molecules, class II major histocompatibility complex (MHC), IL-1β, TNF-α and IFN-γ (Feinstein *et al.*, 2002). Adrenoceptors are expressed on glial cells and NA is thought to regulate their function (Mori *et al.*, 2002). *In vitro* studies have demonstrated that NA inhibits pro-inflammatory cytokine production from microglia, and that NA protects neurons from microglial-induced cell death (Mori *et al.*, 2002; Madrigal *et al.*, 2005). Exposure of mouse astrocytes to NA reduced expression of class II MHC and intracellular adhesion molecule (ICAM), via binding to and activation of β_2 adrenoceptors, an effect which was mimicked by administration of the β adrenoceptor agonist, isoproterenol, cAMP analogues, and forskolin which directly activates adenylate cyclase (Frohman *et al.*, 1988; Ballestas and Benveniste, 1997). iNOS is expressed by both astrocytes and microglia in various disease states such as demyelinating diseases and cerebral ischemia (Degroot *et al.*, 1997; Endoh *et al.*, 1993; Iadecola, 1997). Exposure of rat astrocytes to NA reduced LPS-induced iNOS expression, an effect also mediated by β adrenoceptors (Feinstein, 1998).

NA also displays anti-inflammatory properties *in vivo*. Central depletion of NA with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) has been shown to augment the inflammatory response to $A\beta_{1-42}$ in rat cortex (Feinstein *et al.,* 2002) and it has been shown that Alzheimer-type pathogenesis is seen following NA depletion in amyloid precursor protein transgenic mice (Kalinin *et al.,* 2007). Also, central depletion of NA using DSP-4 exacerbates cortical inflammatory responses to Aβ, leading to increases in pro-inflammatory mediators such as iNOS, IL-1β and IL-6 (Heneka *et al.,* 2002). Administration of β agonists such as isoproterenol and terbutaline, have been shown to suppress EAE symptoms, thus supporting a protective role for NA and/or cAMP in this model (Chelmicka-Schorr *et al.,* 1989; Wiegmann *et al.,* 1995).

NA modulates inflammation by a number of mechanisms. Activation of cAMP signalling as previously discussed is one of the main mediators of the actions of NA, but other transcription factors appear to participate in this mechanism, such as NFκB, CREB, CREB binding protein and CCAAT enhancer binding protein (Galea and Feinstein, 1999). NA has been shown to increase levels of IκB, which binds to and maintains NFκB in its inactive state in the cytosol, thus preventing NFκB signalling and the induction of pro-inflammatory mediators (Gavrilyuk *et al.,* 2002). The iNOS promoter, as well as the promoters present in the genes of a large number of inflammatory mediators, contains a binding site for the NFκB transcription factor (Vodovotz *et al.,* 1996; Xie *et al.,* 1993, 1994). Thus the effects of NA may be due, in part, to a reduction in the overall levels of NFκB activation. A generalized mechanism for NA transcriptional activation in the CNS may not exist but multiple pathways may contribute to an overall anti-inflammatory phenotype (Feinstein *et al.*, 2002).

NA has been shown to be neuroprotective via its promotion of pro-survival signalling pathways and increasing levels of protective neurotrophins. LC-lesions have been shown to exacerbate metabolic deficiency and hippocampal neuronal damage following cerebral ischemia in rats, an effect which is reversed following administration of the α_2 adrenoceptor antagonist, idazoxan (Gustafson *et al.,* 1989, 1990). NA increases the expression of the chemokine, monocyte chemoattractant protein (MCP-1) in astrocytes, which has neuroprotective effects against excitotoxic damage. MCP-1 reduced NMDA-dependent glutamate release, glutamate dependent Ca^{2+} entry and ATP loss, and lactose dehydrogenase (LDH) release attributable to NMDA or glutamate (Madrigal *et al.,* 2009).

Incubation of cortical neurons with LPS-treated conditioned media resulted in increased production of iNOS and induced cell death. Co-administration of LPS and NA potently blocked the induction of iNOS and promoted cell survival (Madrigal *et al.,* 2005). Post-conditioning with NA under certain conditions, is able to prevent delayed neuronal death initiated not only by ischemia but also by administration of KA (Burda *et al.,* 2009).

The neurotrophic influence of NA has been examined in a model of rat mesencephalic cells in culture, in which low concentrations of NA were remarkably effective in promoting long-term survival and function of dopaminergic neurons. Interestingly, in this model intracellular ROS were drastically reduced by treatment with NA, indicating that NA has antioxidant properties (Troadec *et al.,* 2001, 2002). NA has been shown to induce expression of peroxisome proliferators-activated receptor (PPAR) (Klotz *et al.*, 2003). This family of nuclear hormone receptors can bind PPAR elements to activate transcription and have been shown to be neuroprotective (Heneka *et al.,* 2000; Smith *et al.,* 2004; Iwashita *et al.,* 2007). Several studies have shown PPAR activation to increase antioxidant enzymes (Inoue *et al.,* 2001; Girnun *et al.,* 2002; Tao *et al.,* 2003; Pesant *et al.,* 2006). Exposure of neurons to Aβ causes depletion of intracellular glutathione (GSH) levels, and NA has been shown to increase the expression of GSH peroxidase and reduce the expression of gamma-glutamylcysteine synthetase, which together lead to an increase of GSH levels, an effect considered to be neuroprotective (Yen and Hsieh, 1997; Griffith and Mulcahy, 1999; Madrigal *et al.,* 2007).

NA can indirectly modulate neuroprotection via its activation of the cAMP signalling pathway. cAMP has also been reported to improve neuronal survival by phosphorylation and inhibition of glycogen synthase kinase 3β (Li *et al.,* 2000), an outcome which has anti-apoptotic effects. Administration of pituitary adenylate cyclase-activating polypeptide to cultured cerebellar granule cells promoted cell survival and inhibited DNA fragmentation and caspase 3 activity (Vaudry *et al.,* 2000). Increasing synaptic availability of NA through the use of the NA reuptake inhibitor (NRI), desipramine, promotes neuroprotection in neural stem cells via modulation of Bcl-2 expression (Huang *et al.,* 2007).

Application of NA to primary hippocampal neurons results in a dose and time-dependent release of BDNF protein, an effect mediated by the phosphatidylinositol 3′ kinase (PI3-K) and MAPK signalling pathways. It is thought that initial binding of NA to adrenoceptors

stimulates CREB activation, which subsequently activates TrkB, and PI3-K and MAPK pathways, stimulating transcription and synthesis of more BDNF (Chen *et al.,* 2007). *In vivo* studies have shown that increased levels of neurotransmitters such as NA, dopamine and serotonin, can induce neuronal BDNF expression within the cerebral cortex and hippocampus (Fawcett *et al.,* 1998; Ivy *et al.,* 2003), indicating that BDNF and monoamines promote signals in the brain that cooperatively modulate neuronal survival, neuroplasticity and neurogenesis (for review see Marien *et al.,* 2004). NA has been shown to regulate BDNF protein levels in astrocytes (Juric *et al.,* 2006) due to the expression of adrenoceptors on astrocytes which activate cAMP signal transduction or Ca^{2+} dependent protein kinases (for review see Hertz *et al.,* 2004). Neurotrophins such as NGF and BDNF activate the expression of Bcl-2 by a CREB-dependent transcriptional mechanism (Riccio *et al.,* 1999) and BDNF has been shown to inhibit caspase 3 activation and subsequent apoptosis, in an *in vivo* model of hypoxic-ischemic brain injury (Han *et al.,* 2000). Consequently, NA's regulation of neurotrophic factors provides neuroprotection by promoting neuronal survival and inhibiting apoptotic pathways.

1.17 Aims and objectives

The overall objective of this thesis was to examine the neuroprotective potential of pharmacological agents which influence endogenous noradrenergic transmission in an animal model of KA-induced excitotoxicity. In tandem, using anti-inflammatory agents, the role of the inflammatory response and KP activation were assessed in the model. Specifically the aims of this project were to determine:

1. time course and dose dependent effects of systemic KA administration on hippocampal neuronal cell loss, apoptosis and neuroinflammation in the rat

2. the potential of anti-inflammatory therapies, and/or inhibitors of KP activation, as a strategy to reverse KA-induced changes in behaviour, tryptophan metabolism and hippocampal neuronal cell loss

3. the effect of indirect modulation of endogenous NA by inhibition of NA uptake and direct activation of noradrenergic transmission by stimulation of β-adrenergic receptors on hippocampal inflammatory and neurodegenerative changes observed in the animal model of KA-induced excitotoxicty.

Materials and Methods

2.0 Materials

Animals

Wistar Rats	Bioresources, TCD
Lab rat diet	Red Mills

Treatments

Kainic acid	Accent Scientific
Minocycline Hydrochloride	Sigma
Dexamethasone	Sigma
1-Methyl-DL- tryptophan	Aldrich
Desipramine HCL	Sigma
Reboxetine	Pharmacia & Upjohn
Clenbuterol	Tocris
Propranolol	Sigma

Cresyl Violet staining

Cresyl Violet acetate	Sigma
Xylene	BDH
DPX	BDH
Gelatine	Fluka
Chromium (III) potassium sulphate	Reagecon
Tissue-tek O.C.T compound	Sakura
Twin frosted microscope slides	Ultima
Coverslips	Ultima
Acetic acid	Riedel-de Haen
Cork discs	Raymond A Lamb

HPLC Materials

Acetonitrile	Sigma
Distilled HPLC grade H_2O	Sigma
Glacial acetic acid	M&B, UK
3-Hydroxyanthraniliate	Sigma Aldrich
3-Hydroxykynurenine	Sigma Aldrich

L-Kynurenine	Sigma Aldrich
N-Methyl serotonin	Sigma Aldrich
L-Tryptophan	Sigma Aldrich
Kynurenic acid	Sigma Aldrich
Quinolinic acid (N-ω-methyl-5-hydroxy-tryptamine oxalate salt)	Sigma
Zinc acetate	Sigma
0.1ml conical insert	Labquip Ltd
2ml Vial	Lapquip Ltd
0.45μm nylon filter	Cronus

Standard assay kits

BCA Protein assay kit	Thermo Scientific
BDNF ELISA kit	Promega

Terminal deoxynucleotidyltransferase-mediated UTP nick end labeling (TUNEL) staining

Dako pen	Dako
Fluorometric TUNEL kit	Promega
Hoescht dye	Invitrogen
Nail varnish	Generic drugstore
Vecta shield	Vector laboratories

Molecular Reagents

Absolute ethanol	Sigma
Biosphere filter tips (1000, 200 and 100 μl)	Sarstedt
High capacity cDNA archive kit	Applied Biosystems
Molecular grade water	Sigma
Optical adhesive covers	Applied Biosystems
PCR tubes	Sarstedt
RNA*later*™	Ambion
RNase away	Invitrogen
RNase-free 1.5ml and 2ml microfuge tubes	Ambion
RNAse Zap wipes	Ambion

Total RNA isolation kit	Macherney-Nagel
TaqMan gene expression assays	Applied Biosystems
TaqMan universal PCR master mix	Applied Biosystems
96-well optical reaction plates	Applied Biosystems

Chemicals

β-Mercaptoethanol	Sigma
Calcium Chloride	BDH
3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)	Sigma
Dimethyl Sulfoxide	Sigma
Diethyl Pyrocarbonate (DEPC)	Sigma
Dithiothreitol (DTT)	Sigma
Ethylenediaminetetraacetic acid (EDTA)	Sigma
Glucose	Sigma
Glycerol	Sigma
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Sigma
Hydrochloric acid	BDH
Isopenthane (2 methly-butane)	Aldrich
Magnesium Sulphate	Sigma
Methanol (MeOH)	BDH
Potassium Chloride (KCl)	Merck
Potassium dihydrogen Phosphate (KH_2PO_4)	BDH
Sodium Bicarbonate ($NaHCO_3$)	BDH
Sodium carbonate (Na_2CO_3)	BDH
Sodium chloride (NaCl)	Sigma
Sodium Phosphate (Na_2HPO_4	Sigma
Triton-X	Sigma
Tris-HCL	Sigma
Tween-20	Sigma

Laboratory Plastics and hardware

Pipettes tips (1ml & 500µl)	Sarstedt Inc

Plastic syringes (1ml)	Becton Dickenson
Polystyrene round bottomed tubes (5ml)	Becton Dickenson
Microtubes (1.5ml & 0.5ml)	Sarstedt Inc
Eppendorf tubes	Sarstedt Inc
27 gauge needles	Tyco Healthcare
Filter paper	Whatman
Slide storage boxes	Raymond A Lamb
Slide carriers	Electron Microscopy Sciences

2.1 Methods

2.1.1 Animal husbandry

Male Wistar rats (200-300 grams) were obtained from the Bioresources Unit in Trinity College Dublin and housed in hard-bottomed polypropylene cages with wood shavings as bedding. Cages did not contain any environmental enrichment. Animals were housed 4 to a cage under standard laboratory conditions, with an ambient temperature of 20-24° Celsius (C) and a 12 hour light/dark cycle (lights on 08.00, lights off: 20.00). All animals were assessed for health and well-being on a daily basis, and had free access to water and standard laboratory food. To reduce stress, animals were exposed to frequent non-experimental handling (which consisted of handling the rats as they would be on the day of experiment, without receiving any injections) at least 3-days prior to experiments being performed. All animal experiments were performed under a license granted by the Minister for Health and Children (Ireland) under the Cruelty to Animals Act 1876, incorporating the European Community Directive, 86/609/EC.

2.1.2 Drug Treatments

Kainic acid (KA) was dissolved in 0.89% weight/volume (w/v) saline at a concentration of 10mg/ml in an injection volume of 1ml/kg. It was made up fresh on the day of administration. Dexamethasone was dissolved in 0.89% (w/v) saline with 0.2% volume/volume (v/v) Tween-20, at a concentration of 1mg/ml, and was heated until dissolved. The drug was administered i.p one hour prior to KA challenge. 1 D, L-Methyl tryptophan (1-MT) was dissolved in 0.89% (w/v) saline with 0.2% (v/v) Tween-20, at a concentration of 50mg/kg, and heated until dissolved. The drug was administered i.p at 16 and 1 hours prior to KA challenge, with a post-treatment 30 mins after KA challenge. For both dexamethasone and 1-MT 0.2% (v/v) Tween-20 in saline was used as vehicle control. Desipramine was prepared in 0.89% (w/v) saline at a concentration of 15mg/ml with mild heat application until dissolved. The drug was administered i.p one hour prior to KA challenge. Reboxetine was dissolved in 0.89% (w/v) saline at a concentration of 15mg/ml. The drug was administered i.p one hour prior to KA challenge. Propranolol was dissolved in 0.89% (w/v) saline at a concentration of 10mg/ml. The drug was administered i.p one hour prior to KA challenge. Clenbuterol was dissolved in 0.89% (w/v) saline at a concentration of 1mg/ml, and was frozen in 1 ml aliquots. The drug was reconstituted with

saline to a concentration of 0.5mg/ml and was administered i.p one hour prior to KA challenge. In each case above 0.89% (w/v) saline was used as vehicle control.

2.1.3 Kainic acid (KA) treatment

Animals were singly housed 1 week prior to experiments and handled everyday. It was essential to have animals singly housed in order to monitor their behaviour accurately, and to minimise stress during seizure activity. Increases in stress-related hormones such as cortisol were taken into account, and all control animals were singly housed in order to control for this phenomenon. Animal weights were recorded. KA (10mg/kg) or saline was administered either by the intraperitoneal (i.p), or subcutaneous (s.c) route of injection.

2.1.4 Behavioural assessment following KA administration

Animals were scored for one minute every ten minutes over a 3 hour observation period following KA administration as per scale below (Racine, 1972) and the stage of seizure-like behaviours were recorded.

Table 2.1 Racine scale

Stage 1	Face/whisker twitching	Staring	Freezing	
Stage 2	Head bobbing	Repetitive scratching	Circling	Wet dog shakes
Stage 3	Forelimb Clonus	Erect tail	Lordonic posturing	
Stage 4	Forelimb clonus with rearing			
Stage 5	Forelimb clonus with rearing ***and falling***	Generalized motor convulsion		

Animals were assigned to a particular stage of seizure related behaviour based on the pattern of behaviours evident as detailed in the table above. Once one behaviour per stage was experienced, each rat was assigned a stage number.

2.1.5 Preparation of tissue and serum

Animals were euthanised by stunning and decapitation, 24 hours after KA treatment. The brains were quickly removed and dissected over a glass plate over wet ice. The left

hemisphere, taken for histochemistry was snap frozen in iso-penthane, which was surrounded by dry-ice. Frozen brains were wrapped in tin-foil and kept on dry-ice until being stored at -80°C. The remaining right hemisphere was dissected free-hand to isolate the hippocampus and frontal cortex. From the right hemisphere, half of the hippocampus and frontal cortex were taken for RNA isolation. For this purpose the samples were placed in RNase-free tubes containing 300µL of RNA*later*™ solution and stored for two weeks at 4°C. Samples were then transferred to fresh RNase-free tubes and frozen at -80°C until RNA extraction took place. The remainder of the hippocampus and frontal cortex from the right hemisphere was transferred into fresh eppendorf tubes and snap-frozen on dry ice. The samples were then stored at -80°C. Division of the hippocampus into two parts was done in a manner that allowed each half to contain part of each of the sub structures within the hippocampus, and this division was carried out in the exact same manner for each rat, in order to be consistent thoughout each study.

Alternatively, the remainder of the hippocampus was cross-chopped, placed in Krebs DMSO (136 mM NaCl, 2.54 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4.7H_2O$, 16 mM $NaHCO_3$, 10 mM glucose, 10% DMSO, pH 7.4) for protein analysis, and stored at -80°C. Trunk blood was obtained following decapitation by exsanguination. The blood was collected into 15 ml falcon tubes and centrifuged at 2000 revolutions per minute (rpm) for 15 minutes at 4°C. The resultant serum was removed and stored in 500µl aliquots, at –80°C.

2.1.6 Tissue sectioning, cresyl violet staining and cell counting

For cryostat sectioning the left hemisphere was snap frozen in isopenthane and stored at -80°C. Glass slides were coated with subbing solution (0.5% gelatine (weight/volume), 0.05% chrome alum (weight/volume) in dH_2O for 10 seconds at 60°C and allowed to dry overnight at room temperature. This provided a suitable surface to which sections could adhere. On the day of the sectioning the half brain was equilibrated to -20°C for 20 - 30 minutes. The olfactory bulbs and cerebellum were removed from the left hemisphere and it was placed onto a cork disk (cerebellum facing down) using a drop of water to hold the brain in place. The cork was then secured to a pre-cooled chuck using a drop of water. The brain was covered in O.C.T Tissuetek™ and allowed to equilibrate for one hour. Coronal sections (10 µm) were cut, stained with toluidene blue for 2 minutes and viewed under the light microscope (Nikon Labophot, Nikon Instech Co., Ltd., Kanagawa, Japan) for

identification of the hippocampal formation. For each animal, 21 sections (10 μm) were cut onto 7 subbed slides (3 sections per slide), allowed to dry for 10 minutes and stored at -20°C until required. The depth to which each brain was sliced was recorded in order to maintain consistency between slices. For Cresyl violet and TUNEL staining, slides were chosen according to depth and slice number, so that they were the same for each rat in the study. The identity of each slide was then concealed, and each slide was assigned a randomised code.

5% Cresyl violet acetate solution was made up with distilled water with a few drops of glacial acetic acid added. The solution was stirred for one hour, filtered and stored at room temperature. Slides were passed through cresyl violet, dehydrated through a series of alcohols, and stopped in xylene. The slides were cover-slipped using DPX resin as a mountant medium. They were allowed to dry and stored at room temperature.

The hippocampus was examined at 400X magnification using a Nikon eclipse 200 microscope (Mason Technology, Dublin). The CA3, CA1 and dentate gyrus sub-regions of the hippocampus were counted using a 27mm graticule (Micron optical, Wexford, Ireland) with a scale bar denoting 0-25mm at 400X. The scale bar was lined up along the straightest part of each of these structures and was counted by eye using a hand-held clicker (Caufield, Galway, Ireland) as outlined by Heinrich-Noack (1998). This procedure was carried out blind and repeated three times to obtain average values for each sub-region.

2.1.7 Analytical Methods: mRNA

Real-time polymerase chain reaction: Real Time PCR (RT-PCR) is a technique used to quantify messenger RNA (mRNA) from minimal volumes of tissue or cultured cells. It is able to detect the accumulation of product during the reaction where it is measured at the exponential phase of the amplification technique. The following steps were carried out to perform PCR analyses on tissue samples generated from the animal experiments.

(1) ***Total RNA extraction:*** Isolation of total RNA from brain tissue was carried out using Nucleospin RNA II kits (Macherey-Nagel) as per manufacturer's protocol. Tissue dissected was placed in RNA later and stored at 4°C to allow the tissue sample to be fixed and disable any potential Rnases present. All instruments used were wiped with RNase Away and rinsed with DEPC water prior to use. About 30mg of tissue was homogenized in

lysis buffer (350µl RA1 buffer and 3.5µl β-mercaptoethanol) using a Polytron (Kinetatica AG, Littau, Switzerland) for several minutes until all the tissue was chopped up. Following this, the lysate was filtered through Nucleospin Filter units and centrifuged for 1 minute at 11,000g. 350µl of 70% ethanol was applied to the lysate and pipetted several times until dissolution occurred. The total lysate was loaded to a nucleospin II column and centrifuged for 30 seconds at 11,000 x g. The next step involved desalting the membrane of the column by applying 350µl membrane desalting buffer and centrifuging at 11,000 x g for 1 minute. Following this, 95µl DNase reaction mixture was added directly onto the membrane of the column, to digest any potential DNA (which would be present in any contaminating products), and incubated at room temperature for 15 minutes. 200µl of RA2 buffer (inactivates DNase) was added and the column was centrifuged for 30 seconds at 11,000g. The column was placed into a new collecting tube and 600µl of RA3 buffer was added to the column. This was centrifuged for 30s at 11,000 x g and a final wash with 250µl RA3 buffer took place. This was centrifuged for 2 minutes at 11,000g to allow the membrane to dry completely. The column was placed in a nuclease-free micro-centrifuge tube and RNA was eluted with 60µl RNase-free H_2O and finally centrifuged for 1 minute at 11,000g. RNA was stored at –80°C.

(2) ***Assessment of RNA quality and quantity:*** Total RNA was quantified using a Nanodrop spectrophotometer with 1µL of RNA. The purity was demonstrated by the ratio of $A_{260/280}$ where if the ratio fell between 1.8 and 2.0 it was deemed as pure RNA. Significant deviations of this ratio indicated contamination. Concentration measurements were taken of RNA present in each sample and RNA was equalized to the lowest concentration of RNA detected, using RNase-free water.

(3) ***cDNA preparation:*** Following RNA equalization, samples were assembled for cDNA production using the ABI High Capacity cDNA kit (Applied Biosystems) A master-mix solution containing reverse transcription buffer, dNTPs, random primers, multiscribe reverse transcriptase and RNase free H_20 was prepared and stored on ice. RNA was diluted to a range between 0.02-0.2 µg/µl. Equal volumes of the master mix were added to an equivalent volume of diluted RNA, e.g. 25µl of master mix was added to 25µl RNA in PCR tubes. The tubes containing samples were placed in a thermocycler and the program was set according to the laboratory protocol as follows; step 1 was set for 10 minutes at 25°C and step 2 set for 120 minutes at 37°C. When the thermocycler was finished with

performing the amplification stage, samples were removed and stored at –20°C or used immediately for real time PCR.

(4) Real-time PCR: Real-time PCR was performed using Taqman Gene Expression Assays (Applied Biosystems), which contain forward and reverse primers, and a 6-carboxy fluorescein (FAM)-labeled minor groove binders (MGB) Taqman probe to each gene of interest. The assay IDs for the genes examined are listed in Table 2.3. A 1 in 4 dilution of cDNA was prepared with RNase-free water. All real-time PCR was conducted using an ABI Prism 7300 instrument (Applied Biosystems). A 25µl volume was added to each well (10µl of diluted cDNA, 1.25 µl of primer, 1.25 µl of β actin and 12µl of Taqman® Universal PCR Master Mix). Electronic pipettes (EDP3 20-200µl, 2-20µl and 10-100µl) were used to ensure pipetting accuracy. Samples were assayed in duplicate in one run (40 cycles), which composed of 3 stages, 95°C for 10 minutes, 95°C for 15 seconds for each cycle (denaturation) and finally the transcription step at 60°C for 1 minute. β-actin was used as an endogenous control to normalize gene expression data, its expression was measured using gene expression assays containing forward and reverse primers (primer limited) and a VIC (calibration dye)-labelled MGB Taqman probe (Applied Biosystems). The stability of this gene was monitored for consistency between studies. Gene expression was calculated relative to the endogenous control samples and to the control sample giving an RQ value (2^{-DDCt}, where CT is the threshold cycle).

In relation to the various stages carried out during the PCR reaction, initially samples were heated to 95°C for 15 seconds, which allows the double stranded cDNA to denature. Hereafter, the temperature of the reaction decreases to allow annealing and extension of the cDNA, however the target probe must anneal to the single-stranded cDNA since it has a higher melting temperature than that of the target primers (Applied Biosystems). This probe contains a FAM/VIC dye and a proprietary non-fluorescent quencher (NFQ) dye. This quencher blocks the dye from emitting a fluorescent signal by fluorescence resonance energy transfer (FRET) technology (Applied Biosystems). Once the reaction temperature reaches 60°C the primers anneal to the strand of cDNA and it is extended by 5' nuclease activity of the Taq polymerase. This induces the release of the FAM/VIC-labelled probe causing the FRET between the dye and quencher to be broken, and the generation of a fluorescent signal. Due to the specificity of the probe and primers for the cDNA sequence, one fluorescent signal is produced for each new cDNA copy and measured during the annealing stage of the PCR cycle (60°C).

Table 2.2 List of genes used in single RT-PCR studies with the gene expression assay and genbank ref sequence numbers

Gene Symbol	Gene Name	Taqman Gene Expression Assay ID
IFN-γ	Interferon gamma	Rn00594078_m1
IL-1β	Interleukin 1 beta	Rn00580432_m1
TNF-α	Tumor Necrosis factor alpha	Rn99999017_m1
CD11b	Cluster designation 11b	Rn00709342_m1
Bcl-2	B cell lymphoma-2	Rn99999125_m1
Caspase-3	Caspase 3	Rn00563902_m1
IDO	Indolamine 2,3 digoxygenase	Rn00576778_m1
KAT II	Kynurenine aminotransferase	Rn00567882_m1
KMO	3-hydroxykynurenine	Rn00572149_m1
KYNase	Kynureninase	Rn00588108_m1
BDNF	Brain derived neurotrophic factor	Rn00560868_m1
iNOS	Inducible Nitric oxide synthase	Rn00561646_m1
GFAP	Glial fibrillary acidic protein	Rn00533303_m1

(5) ***Real-time PCR analysis:*** The ΔΔCT method (Applied Biosystems RQ software, Applied Biosystems, UK) was used to assess gene expression for all real-time PCR analysis. This method is used to assess relative gene expression by comparing gene expression of treated/experimental samples to a normal or untreated sample (control), rather than quantifying the exact copy number of the target gene. In this manner the fold-difference (increase or decrease) can be assessed between treated and control samples. The fold-difference is assessed using the cycle number (CT) difference between samples. Briefly, a threshold for fluorescence is set, against which CT is measured. To accurately assess differences between gene expression the threshold is set when the PCR reaction is in the exponential phase, when the PCR reaction is optimal or 100% efficient. Thus, samples with low CT readings demonstrate high fluorescence, indicating greater amplification and hence, greater gene expression. When PCR is 100% efficient a one-cycle difference between samples means a 2-fold difference in copy number (2^2), similarly a 5-fold difference is a 32-fold difference (2^5).

To measure this fold-difference relative to control, the CT of the endogenous control (β-actin) is subtracted from the CT of the target gene for each sample, thus accounting for any difference in cDNA quantity that may exist. This normalised CT value is called the (CT). The CT difference (ΔCT) of the control is subtracted from itself to give 0, and subtracted from all other samples, this is the ΔΔCT value. The ΔΔCT (cycle difference corrected for β-actin) is then converted into a fold-difference. As a one-cycle difference corresponds to a two-fold increase or decrease relative to control, 2 to the power of the ΔΔCT (difference in control and sample CT corrected for actin) gives the fold-difference in gene expression between the control and treated samples. The control sample always has a ΔΔCT value of 0, thus 0^2 gives a $2^{\Delta\Delta CT}$ of 1, against which all other samples are referenced.

2.1.8 Terminal deoxynucleotidyltransferase-mediated UTP nick end labelling (TUNEL)

To determine apoptotic cell death in hippocampal slices, DNA fragmentation was assessed using TUNEL staining according to the manufacturer's instructions (DeadEnd Colorimetric/Fluorometric Apoptosis Detection System; Promega Corporation, Wisconsin, USA). TUNEL staining measures the fragmented DNA of apoptotic cells by catalytically incorporating fluorescein-12-dUTP at 3'-OH DNA ends using the Terminal Deoxynucleotidyl Transferase enzyme (TdT). Fluorescein chains were directly visualised by fluorescence microscopy using appropriate excitation wavelength and filter sets (see Table 2.3).

Fluorometric TUNEL: 10 μm frozen tissue sections were permeabilised with Triton-X100 (0.2%; volume/volume) for 5 minutes at room temperature. Sections were washed for two 5 minute washes. Sections were incubated in equilibration buffer (200 mM $K(CH_3)_2$, 25 mM Tris-HCl, 0.2 mM DTT, 0.25 mg/ml BSA, 2.5 mM $CoCl_2$) for 10 - 15 minutes. A reaction buffer (fluorescein-12-dUTP (10 mM Tris-HCl pH 7.6, 1 mM EDTA), TdT enzyme in equilibration buffer) was applied for 60 minutes at 37°C to incorporate fluorescein labelled nucleotides at the 3′-OH ends of fragmented DNA. To stop the reaction, sections were incubated with sodium chloride citrate (SCC) (0.15 M NaCl, 0.17 M Na $H(C_3H_5\ O(COO)_3)$) for 15 minutes and sections were washed for 5 minutes 3 times in phosphate buffered saline (PBS). Tissue sections were counter-stained with Hoecsht dye and mounted onto glass coverslips using Vectasheild, a fluorescent mounting medium (Vector Laboratories, Peterborough, UK). Samples were stored in the dark at 4°C until

needed for analysis. Incorporated fluorescein was visualised concomitantly with the counter-stain fluorophore with a confocal microscope (Zeiss, LSM-510-META, Karl Zeiss, UK) using appropriate excitation wavelengths and filter sets (see Table 2.3).

Table 2.3 Excitation wavelengths and filter settings for TUNEL staining

Fluorophore	Peak excitation/emission wavelength (nm)	Beam splitters
Fluorescein (FITC)	488/520	HFT 488 BP 505 - 530
Hoechst 33258	345/487	HFT UV/488/543/633 LP 456 - 499

HFT= Main Dichroic Beam Splitter, BP= Band Pass Filter, LP= High Pass Filter, UV= Ultraviolet

2.1.9 Protein quantification

Bradford assay: Protein concentration of hippocampal tissue samples was assessed according to the Bradford method (Bradford, 1976). Standards were prepared from a stock solution of 1000 µg/ml bovine serum albumin (BSA; Sigma-Aldrich, Dorset, UK). This stock solution was serially diluted with ddH_2O to prepare a range of standards from 3.125 µg/ml to 1000 µg/ml. A blank of ddH_2O was also included. Standards (10 µl) and samples (10 µl) were plated in triplicate into a 96-well plate (Sarstedt). Bio-Rad dye reagent concentrate (1:5 dilution in dH_2O, 200 µl; Bio-Rad, UK) was added to standards and samples and incubated for 5 minutes at room temperature. The absorbance was assessed at 595 nm using a 96/98-well plate reader (EIA Multiwell reader). The protein concentration of samples was calculated from a regression line plotted (Prism 4) from the absorbencies of the BSA standards. Each sample was equalised to the lowest protein concentration with assay diluent.

2.1.10 Activated Caspase 3 assay

Sample preparation

Samples stored in Krebs DMSO were thawed over ice. Each sample was washed 3 times in Krebs solution containing calcium (136 mM NaCl, 2.54 mM KCl, 1.18 mM KH_2PO_4, 1.18

mM $MgSO_4.7H_2O$, 16 mM $NaHCO_3$, 10 mM glucose, 2 mM $CaCl_2$, pH 7.4) and a final volume of 550µl of Krebs solution containing calcium, was added. Samples were homogenised using a polytron tissue disruptor (Kinetatica AG, Littau, Switzerland) and a protein assay was carried out on the homogenate. Samples were then equalised to the lowest concentration using Krebs solution containing calcium, within the range of 1-3µg of protein. The equalised homogenate was then spun at 10,000g for 10 mins at 4°C. The supernatant was removed and kept on ice.

Assay setup

Caspase 3 activity was measured using a caspase 3 drug discovery kit (AK-703 a QuantiZyme assay system, Biomol, UK). Briefly Ac-DEVD-pNA substrate was diluted 1/5 with assay buffer and allowed to equilibrate to room temperature. Assay buffer (50mM HEPES, pH 7.4, 100mM NaCl, 0.1% CHAPS, 10mM DTT, 1mM EDTA, 10% glycerol) was added to each well in triplicate (25µl). pNA calibration standard was added in duplicate (100µl) and caspase 3 was added in triplicate as the positive control (25µl; 2U/µl). Samples and standards were added (25µl per well) and allowed to equilibrate to room temperature. The reaction was started by the addition of substrate to each well (50µl/200µM) and the plate was read at 405nm at one minute intervals for 10 minutes.

Calculations

Enzyme activity was calculated with reference to a conversion factor, calculated from the optical density of the calibration standard. The slope of each sample was determined over 1 minute (0-1) (mOD/min), and is divided by the conversion factor to give caspase activity over time. This was divided by the protein concentration of each sample, determined on the day of the assay. Values are expressed as pmol/min/µg protein.

2.1.11 BDNF Enzyme linked immunosorbent assay

(1) Sample preparation: Samples stored in Krebs DMSO were thawed over ice. Each sample was washed 3 times in Krebs solution containing calcium (136 mM NaCl, 2.54 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4.7H_2O$, 16 mM $NaHCO_3$, 10 mM glucose, 2 mM $CaCl_2$, pH 7.4) and a final volume of 550µl of Krebs solution containing calcium was added. Samples were homogenised using a Polytron tissue disruptor and a protein assay

was carried out on the homogenate. Samples were then equalised to the lowest concentration using Krebs solution containing calcium, within the range of 1-3μg of protein. The equalised homogenate was then spun at 10,000g for 10 minutes at 4°C. The supernatant was removed and kept on ice.

(2) Assay protocol: Enzyme-liked immunosorbent assays (ELISA) were used to examine the concentration of BDNF protein in brain tissue homogenate. A commercially available ELISA kit was used; BDNF E_{max} immunoassay ELISA system kit (Promega, Madison, USA).

For each ELISA, a 96-well plate was coated with capture antibody (Anti BDNF, diluted to a concentration of 1μg/ml in carbonate coating buffer (0.25M sodium bicarbonate, 0.25M sodium carbonate; 50μl/well) and incubated overnight at room temperature. The plate was then washed with wash buffer (0.05% Tween®20 in PBS) using an automated plate washer (Columbus Plus, Tecan, Austria) and blocked with a reagent diluent (1% BSA in PBS, 150μl/well), for 1 hour at room temperature. After washing, samples and standards were added (50μl/well) and incubated for 2 hours at room temperature. The plates were washed and incubated in detection antibody (Anti-human BDNF pAB, 50μl/well) for 2 hours at room temperature, then washed and reacted with Streptavidin-horseradish peroxidase (1:200 dilution in reagent diluent; 50μl/well) for 20 minutes. The plates were washed and substrate solution was added (50μl/well) and incubated in the dark for a minimum of 20 minutes. 1N HCL was used to stop the reaction (50μl/well) once colour development had occurred. The absorbences of samples and standards were read at 450nm on a spectrophotometric plate reader (Sunrise Basic, Tecan, Austria). Each sample was normailsed to its corresponding protein concentration. Standard curves were constructed for each plate and concentrations of BDNF in the samples were obtained directly from these curves.

2.1.12 Determination of tryptophan and KP metabolite concentrations by high performance liquid chromatography (HPLC)

HPLC analysis was conducted with a fully automated system (Shimadzu ADVP module). Injection volumes were 20µl into a reverse phase analytical column (C18 column, specific area surface 250mm x 4mm, particle size 50µm, Phenomenex). Two detectors were connected in series; a photo diode array ultra-violet (PDA-UV) detector (SPD-M10A VP, Shimadzu) and a fluorescent detector (RF-10A XL, Shimadzu). The HPLC protocol was adapted from that previously described (Herve *et al.*, 1996; Swartz *et al.*, 1990; Luo *et al.*, 2008; Xiao *et al.*, 2008; Pi *et al.*, 2009) and is described in brief below.

(1) Standards and mobile phase: The mobile phase consisted of 50nM glacial acetic acid, 100mM zinc acetate and 3% acetonitrile dissolved in double-distilled NANOpure water (or distilled HPLC grade H_2O). The pH was then adjusted to 4.9. Standard solutions of quinolinic acid, 3-hydroxykynurenine, 3-hydroxyanthraniliate, L-kynurenine, 3-nitro-L-tyrosine, L-tryptophan and kynurenic acid were made up to a concentration of 10mg/10ml in mobile phase. Each standard was individually diluted with mobile phase to 25ng/20µl and a volume of 150µl of each was pipetted into a 0.1ml conical insert and transferred into a 2ml vial prior to placement in the autosampler and determination of the retention times of each standard.

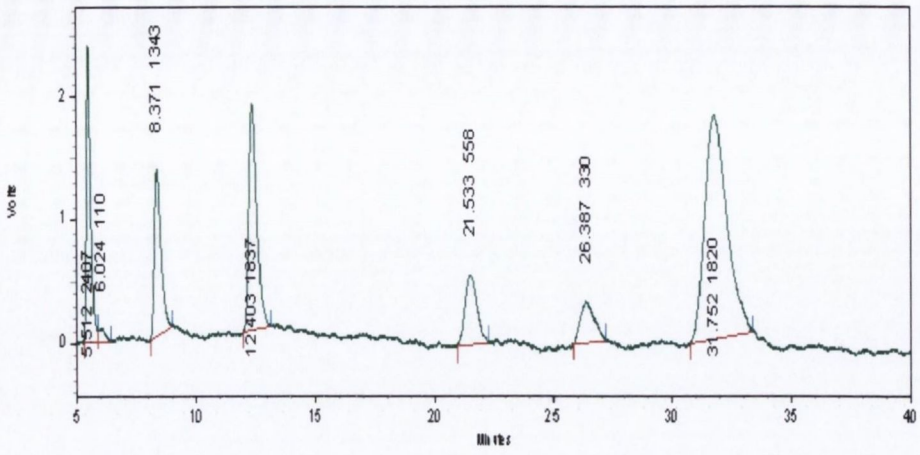


Figure 2.1 Sample chromatograms of a standard UV tryptophan and kynurenine metabolite mix

Standard mix showing retention times and peak heights of tryptophan and kynurenine metabolites (50ng/20µl) determined by UV detection (250nM). Retention times for 3-HK, 3-HAA, L-KYN, N-Methyl-5HT, L- tryptophan and KYNA were 5.51, 8.37, 12.40, 21.53, 26.387 and 31.75 minutes, respectively.

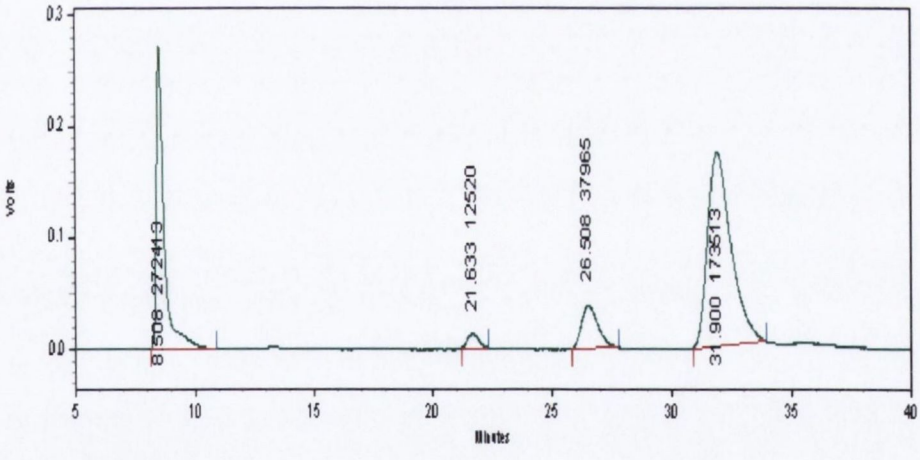


Figure 2.2 Sample chromatograms of a standard fluorescent tryptophan and kynurenine metabolite mix

Standard mix showing retention times and peak heights of tryptophan and kynurenine metabolites (50ng/20µl) determined by fluorescence detection (254nm excitation, 404nm emission). Retention times for 3-HAA, L-tryptophan, N-Methyl-5HT and KYNA were 8.5, 21.63, 26.5 and 31.9 minutes, respectively.

(2) ***Brain tissue and serum preparation for tryptophan and KP metabolite determinations***: Hippocampal tissue was weighed and placed directly into ice-cold 500µl of homogenising buffer (containing mobile phase, 7% perchloric acid and internal standard (50ng/20µl N-Methyl serotonin). Tissue was homogenised by sonication (MSE sonicator) for approximately 10 seconds in homogenising buffer for HPLC analysis of L- tryptophan and kynurenine metabolites coupled with UV and fluorescent detection. The samples were then centrifuged (Eppendorff Centrifuge 5804 R, rotor Eppendorff F45-30-11) at 15,000g for 20 minutes at 4°C. The supernatants were filtered into new 1.5ml eppendorfs, using a 1ml syringe and a 0.45µm nylon filter and frozen at -20°C until analysed. Preparation of serum samples involved 500µl of serum vortexed with 500µl of homogenising buffer and centrifuged and prepared as for brain samples described above.

(3) Quantification of tryptophan and KP metabolites: Supernatants were thawed at room temperature and then analysed by an automated HPLC system coupled with UV and fluorescent detection with an injection volume of 20µl and an acquisition delay of 4.8 minutes. The flow rate was 1ml/min (LC-10AT pump, Shimadzu) and the run time was 40 minutes. The PDA-UV detector was calibrated to analyze over a UV spectrum from 240nm to 370nm and the optimal wavelength for detection was set at 250nm. The fluorescent detection method utilised was 254nm (excitation) and 404nm (emission) (Pi *et al.*, 2009). Mobile phase was injected intermittently and a standard mix was injected every 5 samples in order to recalibrate the system and curtail divergences in the retention times during sampling. CLASS-VP software (Shizamdu) was used in the acquisition and integration of the chromatograms. The signal to noise ratio was set to 3 to 1. Peak heights of analytes compared with internal and external standards allowed quantification of concentrations. Peak heights obtained from the chromatograms were used to calculate ng of metabolite per g fresh weight of tissue.

2.1.13 Statistical Analysis of Results

All data were analysed using GraphPad Prism software, except in the case of the three-way ANOVA, where GB-STAT was used. Statistical comparisons were initially performed using a one-way (for KA-induced changes), two-way (for KA vs. Drug treatment), or three-way (for KA vs. RBX vs. PRP) analysis of variance (ANOVA), as indicated in the experimental sections. Behaviour data was analysed using a two-way repeated measures ANOVA (and a three-way repeated measures ANOVA for the RBX and PRP combination study) to examine drug effect over time. If significant changes were observed, the data was further analysed using Student Newman-Keuls or Dunnetts *post hoc* test as appropriate. Data was deemed significant when $P<0.05$. Outliers were identified as two standard deviations away from the mean. Results are expressed as means and standard error of the mean (SEM).

Chapter 1

Kainic acid-induced excitotoxicity and KP activation; Time course and dose related effects on KA-induced inflammatory and degenerative changes, and tryptophan metabolism in the hippocampus

3.0 Introduction

The KA model of excitotoxicity and acute neurodegeneration is a frequently employed animal model to determine mechanisms mediating excitotoxin-induced neuronal cell loss, and the processes by which such mechanisms are regulated. The main characteristics of this model are outlined as follows: (1) Systemic administration of KA results in seizure behaviour typified by wet dog shakes, forelimb clonus, and rearing and falling. These particular behaviours are present within the first three hours post KA, and spontaneous seizures occur throughout the duration of the animals life thereafter (Ben-ari, 1985). (2) Activation of the KA subtype of ionotropic glutamate receptors mediates the sustained seizure activity which is sufficient to induce cell loss in the hippocampus and hyper-excitability in surviving neurons (Dudek *et al.*, 1994; Ben-Ari, 1985, 2001; Dong *et al.*, 2003). (3) The mechanisms by which excitation of KA receptors causes neuronal loss involve an increase in the release of intracellular calcium and the activation of calcium-dependent apoptotic pathways (Wang *et al.*, 2005). Moreover, KA provokes an increase in the production of ROS and reactive nitrogen species (RNS) (Dugan *et al.*, 1995) which relates to mitochondrial dysfunction and enhanced oxidative load on cells, contributing to their demise. (4) In addition systemic administration of KA leads to activation of glial cells and an increase in inflammatory markers in the brain. All of these processes are evident especially in vulnerable brain regions where KA receptors are expressed in abundance including the sub-regions of the hippocampus; the CA1, CA3 and DG (Wang *et al.*, 2005).

Neuronal cell loss is a common feature associated with KA-induced excitotoxicity. Nadler and co-workers (1980) demonstrated that following intracerebroventricular (i.c.v) injection of KA, early signs of hippocampal pyramidal cell degeneration were detectable within one hour by electron microscopy. Initial degenerative changes occurred in the cell body and proximal dendritic shafts characterised by an increase in lysosomal bodies, increased background electron density, detachment of polyribosomes and dilation of cisternae in the endoplasmic reticulum. Degeneration of pyramidal cells ensued within hours of administration, where cells were observed to first shrink and then disintegrate. In the CA3, astrocytes adjacent to the cell body and mossy fiber layers began to swell. Pyramidal cell axons and boutons did not begin to degenerate until 24 hours following KA administration. Cresyl violet staining clearly represents the cytoarchitecture within the hippocampus, and enables visualisation and counting of the cells under the microscope. This approach is

widely employed to readily identify hippocampal cell loss following KA administration in laboratory rodents (Miltiadous *et al.,* 2009; Yoo *et al.,* 2006; Weise *et al.,* 2004).

The neurotoxic effect of KA is accompanied by reactive gliosis, resulting in proliferation and migration of astrocytes and microglia throughout the brain. Activated microglia are capable of secreting a range of inflammatory mediators, which contribute to and augment neuronal degeneration (Jorgensen *et al.,* 1993). Proinflammatory cytokines synthesised by microglia and astrocytes are released within 30 minutes of seizure onset (Minami *et al.,* 1990; Vezzani *et al.,* 1999, 2000). They are predominantly released in the hippocampus where they influence ongoing seizure activity, with an increase in the expression of proinflammatory cytokines during seizures associated with neuronal damage (Minami *et al.,* 1990; Vezzani *et al.,* 1999; Rizzi *et al.,* 2003). IL-1β has been shown to play a significant role in the inflammatory process following KA administration. The expression of IL-1β was shown to be upregulated at various time points following KA, in various brain regions such as the thalamus, cortex, hypothalamus and hippocampus (Eriksson *et al.,* 2000, Minami *et al.*, 1990). During excitotoxic insult, activated microglia increase the production of QUIN via the KP, and this may contribute to excitotoxin-induced damage. Behan and Stone (2000) investigated the effect of the mNBA, a potent inhibitor of the KP enzymes kynurenine hydroxylase (and kynureninase to a lesser degree), and reported a significant decrease in KA-induced hippocampal cell loss. By blocking these particular enzymes, the production of QUIN via the KP was halted, accompanied by a possible increase in the protective effects of KYNA. Such reports suggest that the KP is involved in KA- induced neuronal degeneration.

Necrosis is characterised by early swelling of organelles and rupture of the plasma membrane, whereas apoptosis is characterised by shrinkage of cell cytoplasm, chromatin condensation and DNA fragmentation. KA-induced cell death displays the morphological characteristics of necrosis (Fujikawa *et al.,* 2000a, 2000b) and apoptosis accompanied by increased caspase 3 activity in the hippocampus (Filipowski *et al.,* 1994; Pollard *et al.*, 1994; Henshall *et al.,* 2000; Kondratyev and Gale, 2000). It has been suggested that apoptosis is responsible for the neuronal degeneration that takes place following KA administration. In support of this, caspase 3/9 expression and activation (Gillardon *et al.,* 1997), cytochrome-c redistribution (Viswanath *et al.,* 2000; Henshall *et al.,* 2000), DNA fragmentation/laddering (Pollard *et al.*, 1994; Henshall *et al.,* 2000), induction of Bax

mRNA and reduction of the anti apoptotic Bcl-2 protein (Hsu *et al.,* 1997) are evident in the hippocampus following KA administration. Kondratyev and Gale (2000) reported that administration of the caspase 3 inhibitor- z-DEVD-fmk to rats reduces KA-induced neuronal cell death, DNA fragmentation and apoptotic morphology in the hippocampus and rhinal cortex suggesting that caspase 3 is necessary for KA-induced neurodegeneration.

The KA model of excitotoxicity is well established in the current literature (Yoo *et al.,* 2006, Kondratyev *et al.*, 2000, Sharma *et al.*, 2005, Faherty *et al.*, 1999, Coyle, 1987). However, the doses of KA used and times at which animals are euthanised vary greatly. There is also a high non-response rate, where some animals, upon receiving high doses of KA systemically, do not exhibit any seizure behaviour (Oprica *et al.*, 2003). Some studies have reported a 60-80% response rate to 10mg/kg of KA (Sperk *et al.*, 1985). Animals judged to "respond" are those that reach stage 4 or above on the Racine scale (1972). Although the convenience of this model is appealing, it is clear that it is subject to variability. It was therefore necessary to establish this model in our laboratory to ensure robust and reproducible effects of KA could be obtained.

Reported time points at which brain tissue is harvested following KA administration in rodent studies varies from 30 minutes (Milatovic *et al.*, 2002), 4-6 hours post KA (Ferrer *et al.,* 2001, Milatovic *et al.*, 2002), 24 hours post KA (Hunsberger *et al.*, 2005, Fujiki *et al.,* 2005, Milatovic *et al.*, 2002) to a number of days (Faherty *et al.,* 1999, Young-Mi Yoo *et al.*, 2006). As described above, the process of cell loss occurs within the first few hours post KA, with significant changes in morphology occurring 24 hours post KA. In order to identify and characterise molecular markers associated with apoptosis and inflammation following KA administration, earlier time points had to be considered due to the narrow time window in which the activation of some targets of interest can be detected. A time course study was devised where tissue was harvested from animals: 4, 12 and 24 hours following KA administration.

The optimal dose of KA to provoke seizures and excitotoxicity also vary across studies, from 7-15 mg/kg in adult rats (Reiss *et al.,* 2009; Kondratyev and Gale, 2000; Candelario-Jalil *et al.,* 2000; Narkilahti *et al.,* 2003, Sharma and Kaur, 2005). Doses of 12 mg/kg and 15 mg/kg have been used, but have resulted in significant fatality which is not a

satisfactory outcome. Thus in the current study a top-end dose of 10 mg/kg was used. The objective of this study was to find a non-lethal dose sufficient to induce cell loss, and to characterise concurrent molecular changes in the hippocampus. The following doses were chosen to determine the dose-related effects of KA: 2.5, 5, 10 mg/kg in the current study.

The null hypothesis of this study was that KA would not illicit an inflammatory or apoptotic response, and would not affect hippocampal cell loss, over a range of times and doses.

3.1 Experimental protocol

Animals were randomly divided into treatment groups and housed individually for one week prior to KA administration.

Time course: KA was prepared in 0.89% (w/v) saline and administered in an injection volume of 1ml/kg giving a dose of 10mg/kg (i.p). Controls received saline in an equivalent volume. Animals were observed for seizure related behaviours for 3 hours following KA administration as previously described in the Methods section (section 2.2) and animals were euthanised 4, 12 and 24 hours after KA administration.

Dose response: KA was prepared in 0.89% (w/v) saline to concentrations of 2.5, 5, 10mg/ml and administered to rats in an injection volume of 1ml/kg (i.p). Controls received saline in an equivalent volume. Animals were observed for seizure related behaviours for 3 hours following KA administration as previously described in the Methods section (section 2.2) and animals were euthanised 24 hours after KA administration.

All studies carried out in chapter one, utilised the i.p route of injection for KA, in all subsequent studies the s.c route was utilised.

3.2 Results

3.2.1 KA-induced seizure behaviours

KA (10mg/kg, i.p) was administered and animals were euthanised 4, 12 and 24 hours following drug administration. KA (10 mg/kg) induced an increase in seizure related behaviours. Wet dog shakes, forelimb clonus and forelimb clonus *with* rearing and falling were observed in each animal receiving KA.

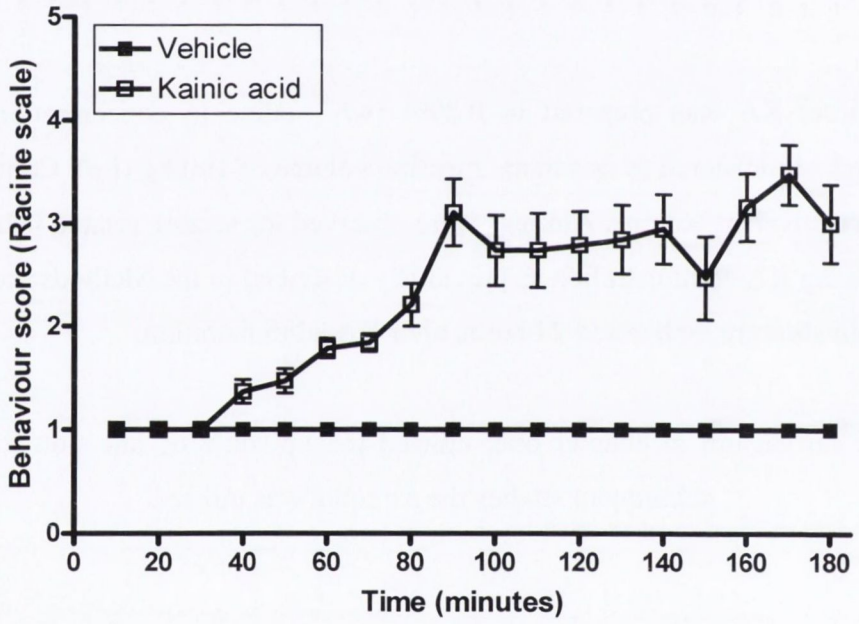


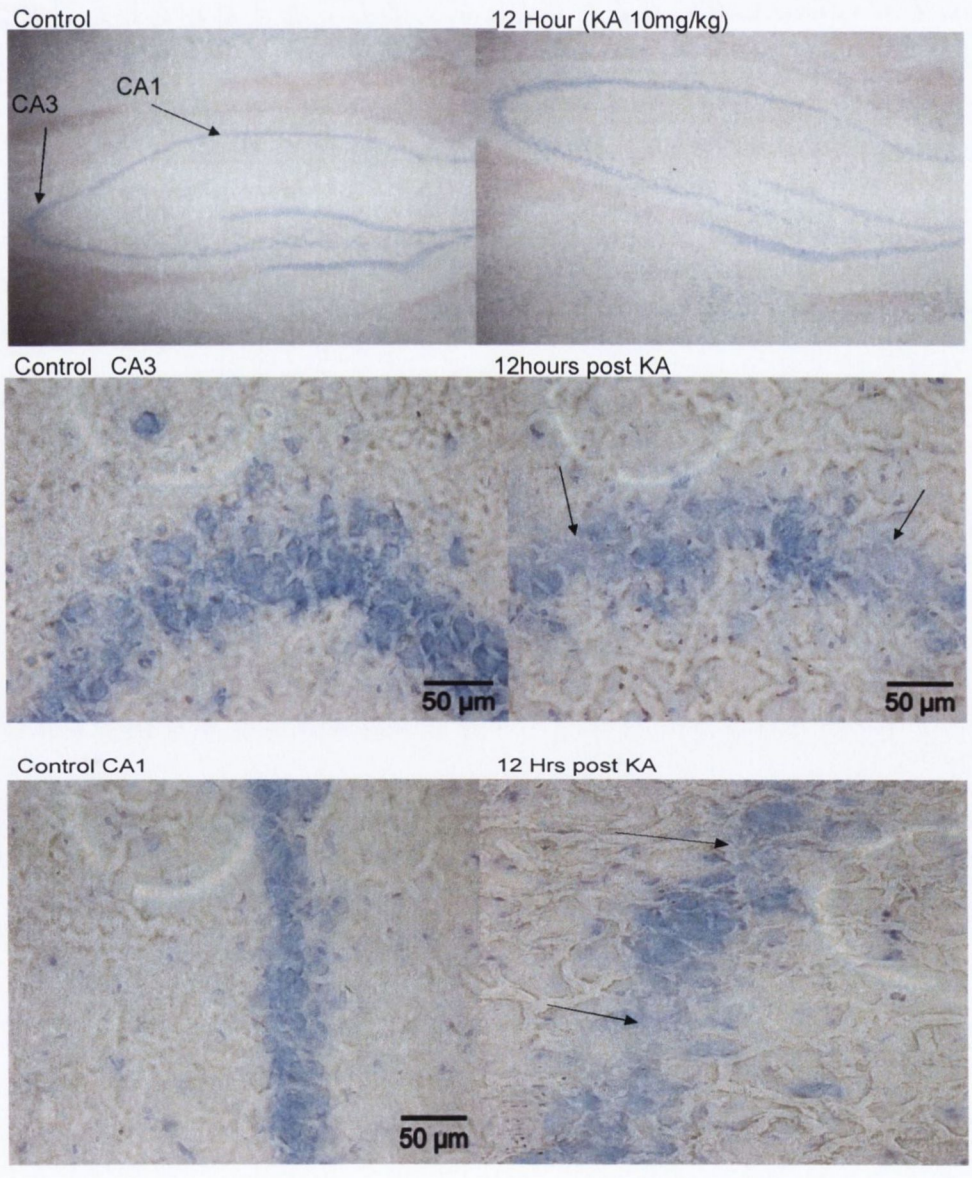
Figure 3.1 KA-induced seizure behaviour

Animals were scored 1-5 according to what stage they reached on the Racine scale for 1 minute every 10 minutes over a 3 hour observation period following KA administration. Data is expressed as mean±SEM over time. (n=6).

3.2.2 Time dependent changes in hippocampal cell loss following KA administration

KA (10mg/kg) was administered and animals were euthanised 12 and 24 hours following drug administration. Representative pictures of the hippocampus stained with cresyl violet showed a reduction in viable cells in the CA3 and CA1 in KA treated rats, at (a) 12 and (b) 24 hours post challenge as compared to saline controls. The first picture is a demonstration of the entire hippocampus at 20x magnification. There is a clear difference in cell morphology and number in the CA3 and CA1 of controls when compared to KA brains, where in controls the cells are round, dark and distinct, whereas hippocampal cells treated with KA are lighter, less distinct in shape and are clearly degenerated in some areas (indicated by arrows).

(a)



(b)

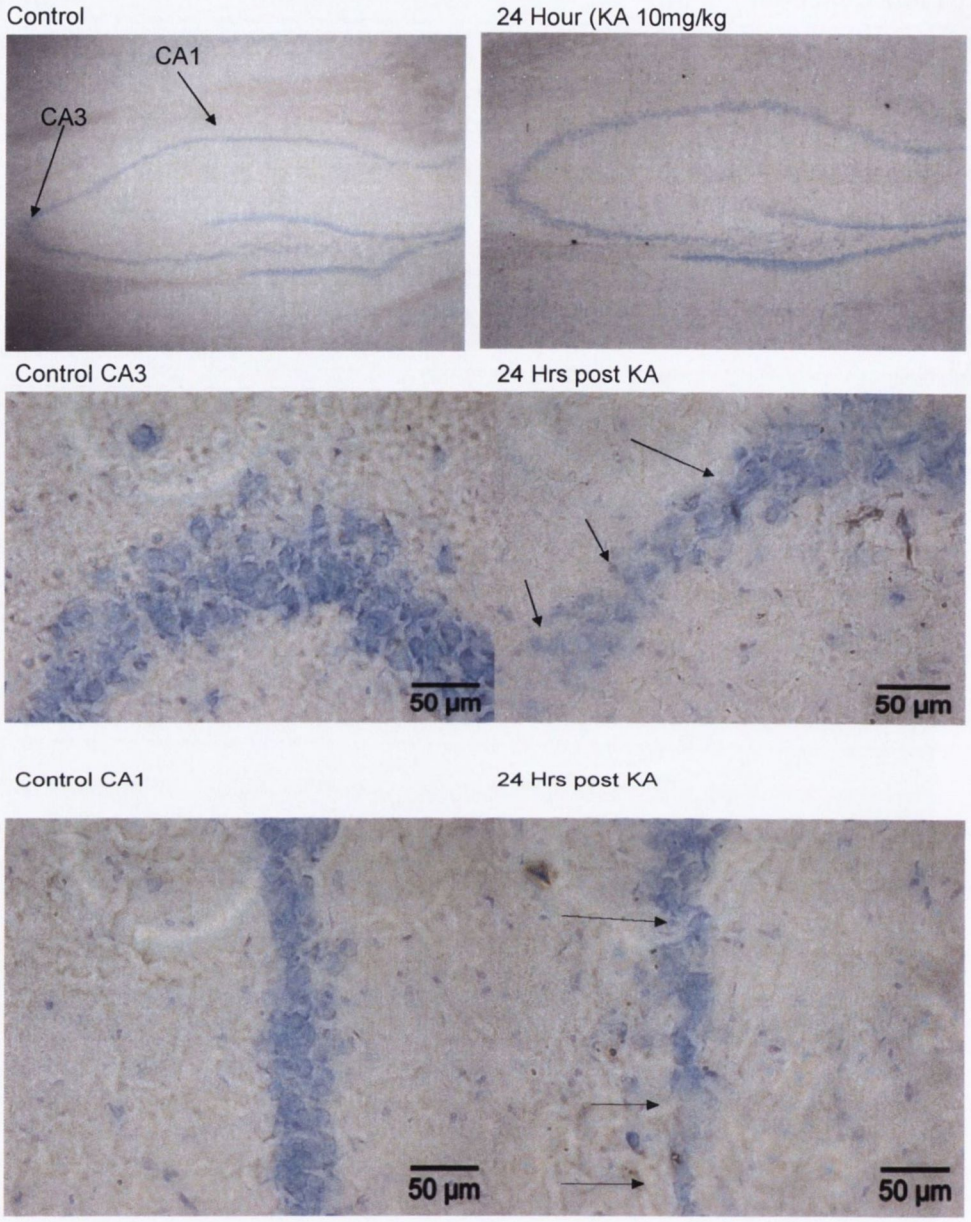


Figure 3.2 Representative cresyl violet stains indicating hippocampal cell loss (a) 12 hours and (b) 24 hours following KA administration, indicated by arrows

Photos (a) shows cell loss in CA3 and CA1 regions 12 hours following KA administration compared to saline treated controls indicated by arrows. Photos (b) shows cell loss in CA3 and CA1 fields 24 hours following KA administration when compared to saline treated controls.

KA reduces hippocampal cell counts in the (a) CA3 region of the hippocampus 12 and 24 hours following administration. Cell counts in the (b) CA1 and (c) DG were also reduced but these reductions failed to reach significance, when compared to saline treated controls.

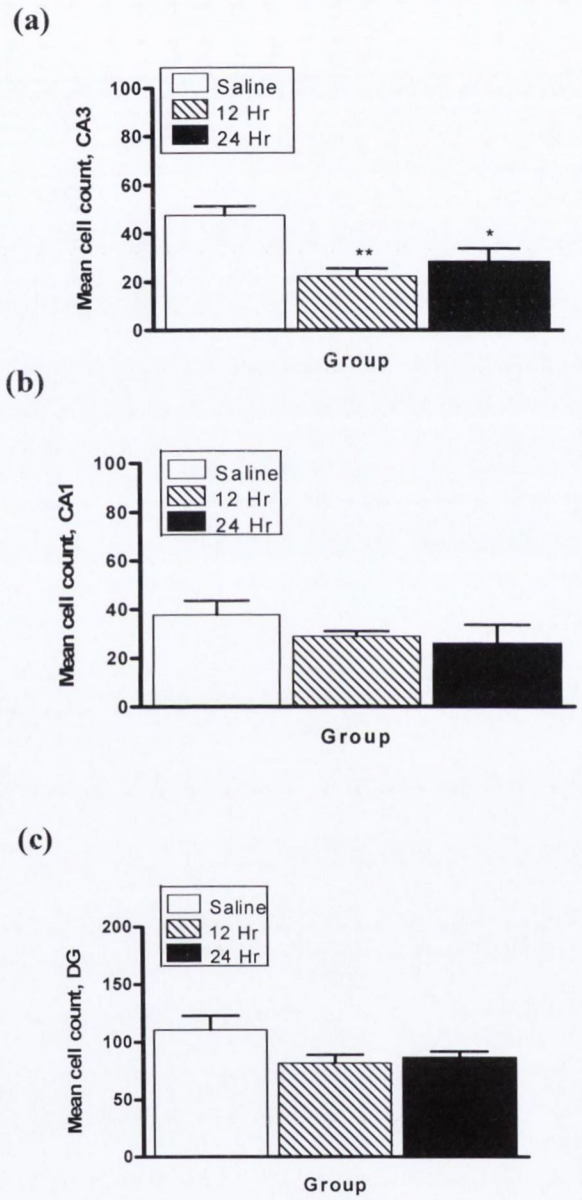


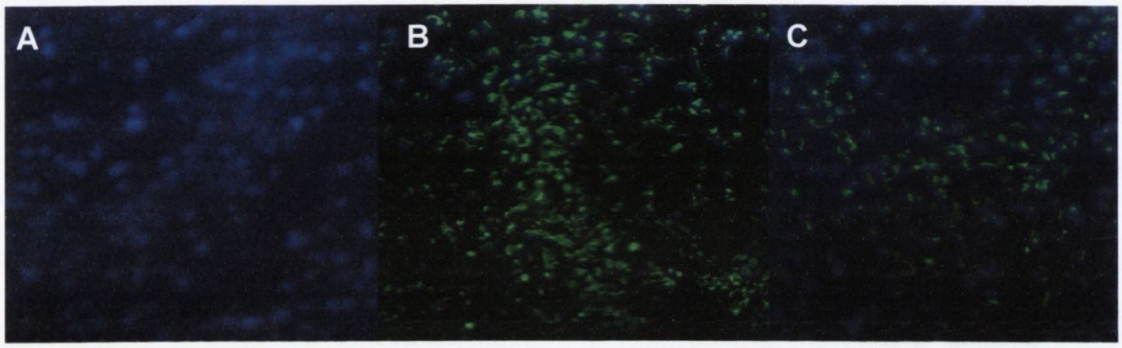
Figure 3.3 KA induces a reduction in cell number in the (a) CA3 (b) CA1 and (c) dentate gyrus of the hippocampus

One-way ANOVA showed an effect of KA on cell number in the CA3 [$F(2, 7) = 10.77$, $P<0.01$]. *Post hoc* comparisons revealed that KA provoked a significant reduction in cell number in the CA3 when compared to saline treated controls. Data is expressed as mean cell count ± SEM (n=3-4). *$P<0.05$; **$P<0.01$ vs saline treated controls (Dunnetts *post hoc* test).

3.2.3 Time dependent changes in DNA fragmentation following KA administration

KA (10mg/kg) was administered and animals were euthanised 12 and 24 hours following drug administration. TUNEL staining of hippocampal slices following KA administration resulted in an increased number of TUNEL positive cells in the (i) CA3 and (ii) CA1, indicating an increase in DNA fragmentation at 12 and 24 hours post KA.

(i) CA3



(ii) CA1

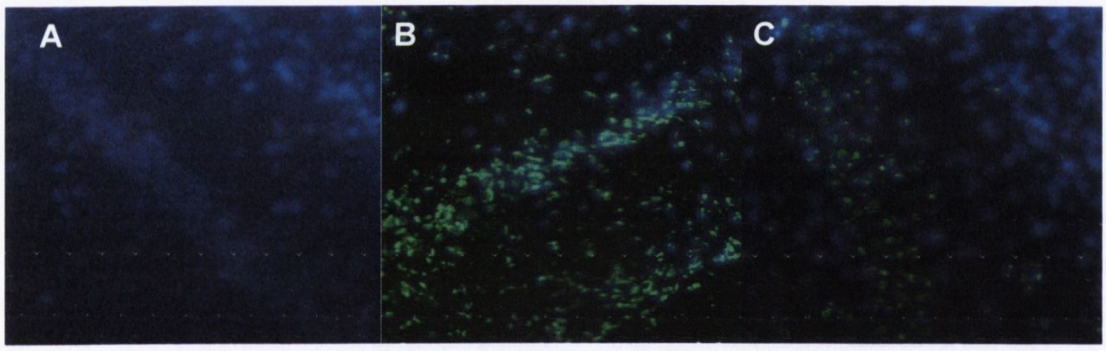
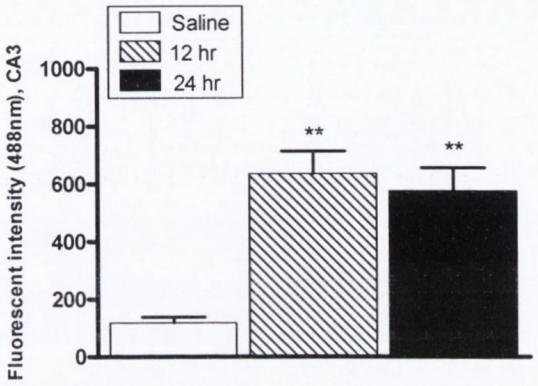


Figure 3.44 Kainic acid induces DNA fragmentation in a time-dependent manner in the (i) CA3 and (ii) CA1 of the hippocampus

TUNEL staining of the (i) CA3 and (ii) CA1, where green indicates TUNEL positive staining and blue is a Hoechst dye to indicate co-localisation of DNA fragmentation and hippocampal cells. (A) Saline treated (B) 12 hour (C) 24 hours post KA administration. (20X magnification)

Quantification of TUNEL staining intensity in hippocampal slices shows an increase in DNA fragmentation in the (a) CA3 and (b) CA1, 12 and 24 hours following KA administration.

(a)



(b)

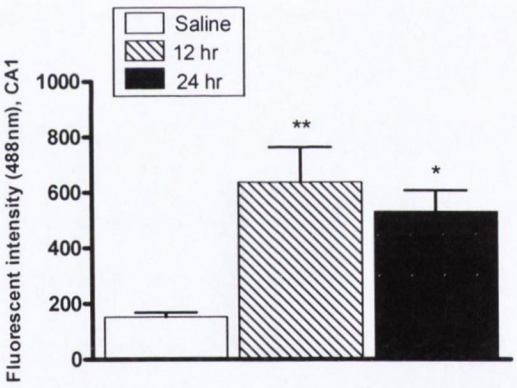


Figure 3.5 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus 12 and 24 hours following administration

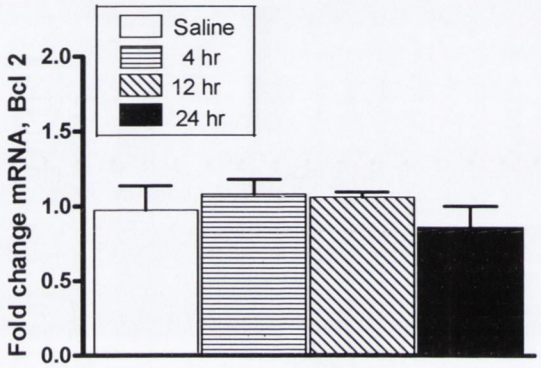
One-way ANOVA of mean TUNEL intensity showed an effect of KA in the CA3 [$F(2, 9) = 22.5$, $P<0.001$] and CA1 [$F(2, 9) = 8.9$, $P<0.01$]. *Post hoc* comparisons revealed that TUNEL intensity was increased 12 and 24 hours following KA administration when compared to saline treated controls. Data is expressed as mean intensity ± SEM (n=4). *P<0.05; **P<0.01 vs. saline controls (Dunnetts *post hoc* test)

Data for cell loss and TUNEL staining is not presented for the 4 hour treatment group as no cell loss is evident 4 hours following KA administration.

3.2.4 Time dependent changes in apoptotic markers following KA administration

KA (10mg/kg) was administered and animals were euthanised 4, 12 and 24 hours following drug administration. Hippocampal caspase 3 expression was increased 12 and 24 hours following KA administration when compared to saline controls. By contrast, no changes were apparent in Bcl 2 expression.

(a)



(b)

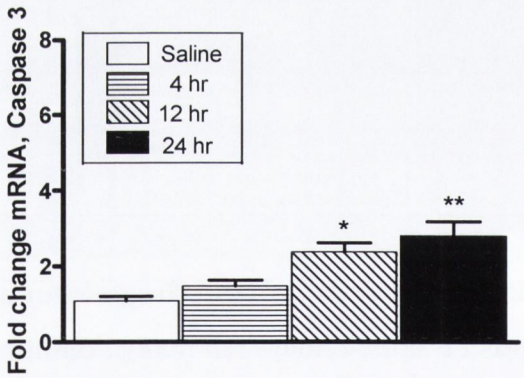


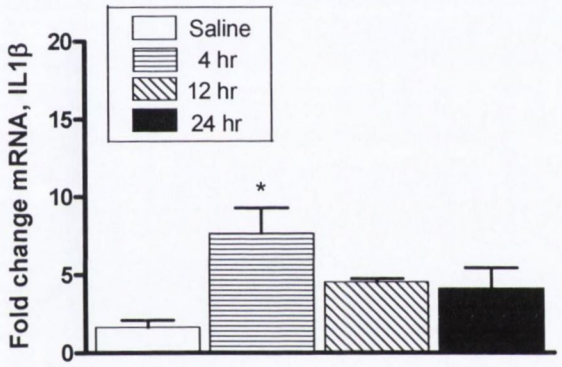
Figure 3.6 KA induces the expression of caspase 3 but not Bcl2 in the hippocampus

One way ANOVA of caspase 3 expression showed an effect of KA [$F(3, 15) = 8.07$, $P<0.01$]. *Post hoc* comparisons revealed that KA increased hippocampal caspase 3 expression, 12 and 24 hours following KA administration when compared to saline treated controls. Data is expressed as mean fold change ± SEM (n=4-6). *P<0.05, **P<0.01 vs. saline controls (Dunnetts *post hoc* test).

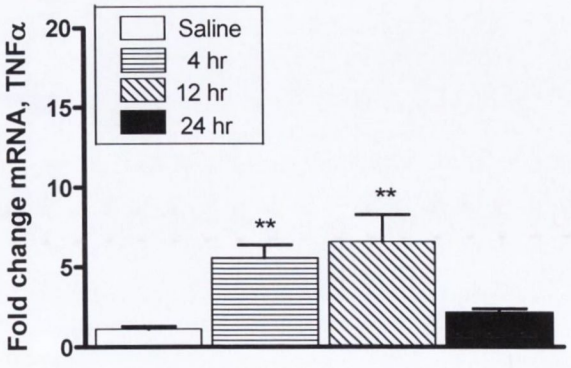
3.2.5 Time dependent changes in neuroinflammatory markers following KA administration.

KA (10mg/kg) was administered and animals were euthanised 4, 12 and 24 hours following drug administration. Hippocampal IL-1β and TNF-α expression was increased 5 fold, at 4, and 4 and 12 hours following KA administration respectively. Hippocampal IFN-γ expression was increased 5 to 10 fold, 12 and 24 hours following KA administration. In excess of a 20 fold increase in the expression of iNOS was found in the hippocampus 24 hours following KA administration.

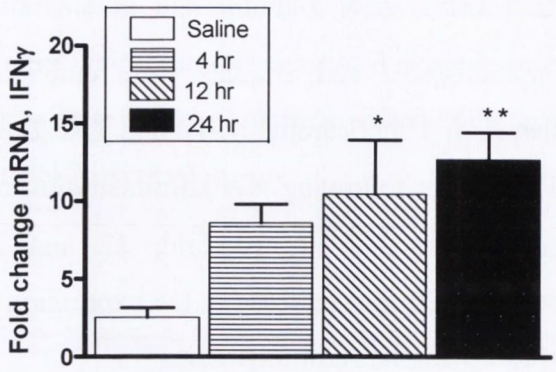
(a)



(b)



(c)



(d)

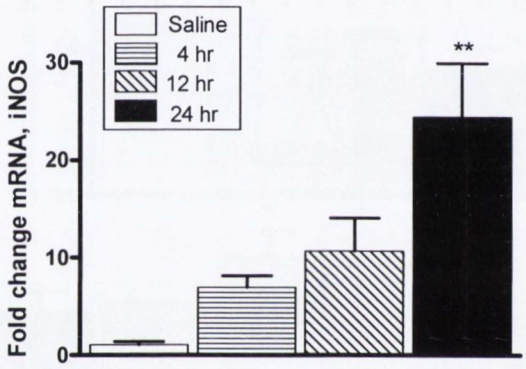


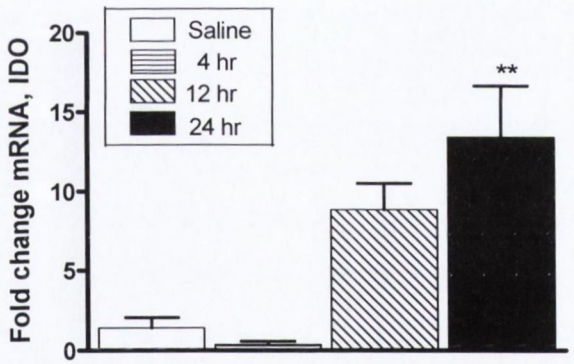
Figure 3.7 KA induces the expression of hippocampal IL-1β, TNF-α, IFN-γ and iNOS in a time dependant manner.

One way ANOVA showed an effect of KA on (a) IL1β [$F(3, 13) = 3.86$, $P<0.05$], (b) TNF-α [$F(3, 15) = 9.87$, $P<0.01$], (c) IFN-γ [$F(3, 14) = 4.8$, $P<0.01$] and (d) iNOS [$F(3, 11) = 6.58$, $P<0.01$]. *Post hoc* comparisons revealed that KA increased hippocampal IL-1β expression at 4 hours, TNF-α expression at 4 and 12 hours, IFN-γ expression at 12 and 24 hours, and iNOS expression at 24 hours following KA administration, when compared to saline treated controls. Data is expressed as mean fold change ± SEM (n=3-6). *$P<0.05$, **$P<0.01$ vs. saline controls (Dunnetts *post hoc* test).

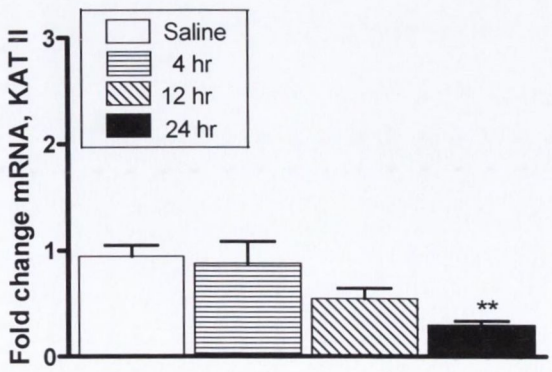
3.2.6 Time dependent changes in the expression of enzymes of the KP in the hippocampus following KA administration.

KA (10mg/kg) was administered and animals were euthanised 4, 12 and 24 hours following drug administration. There was a 7-12 fold increase in hippocampal IDO expression in the hippocampus 12 and 24 but not 4 hours after KA administration when compared to saline controls. Hippocampal KAT II expression was reduced 24 hours after KA administration, when compared to saline controls. There was a 2-3-fold increase in the expression of KMO and kynureninease in the hippocampus 24 hours following KA administration when compared to saline controls. Changes in KAT II, KMO and kynureninase were not evident 4 or 12 hours following KA administration.

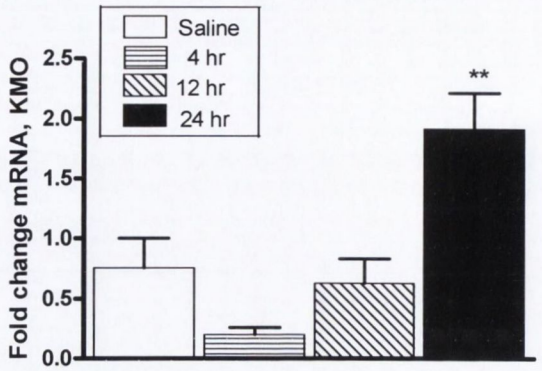
(a)



(b)



(c)



(d)

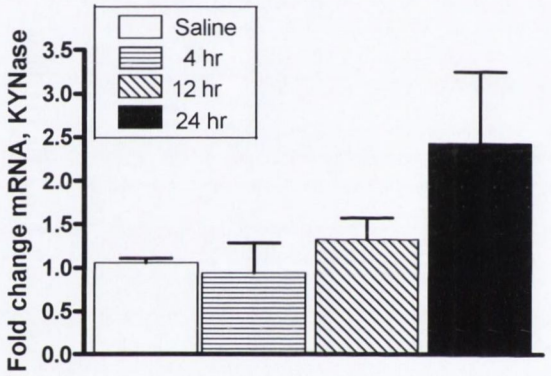


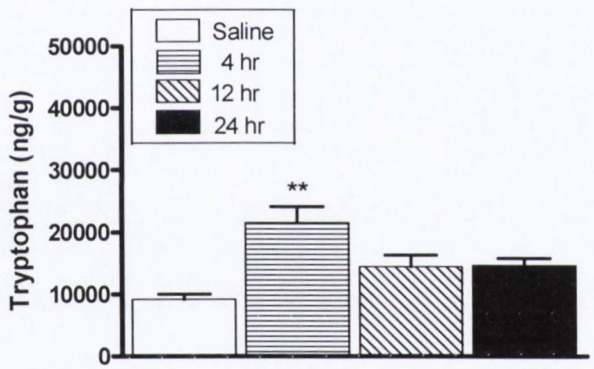
Figure 3.8 KA alters the expression of IDO, KAT-II and KMO in the hippocampus in a time dependant manner

One-way ANOVA showed an effect of KA on (a) IDO [$F(3, 20) = 6.64$, $P<0.01$], (b) KAT-II [$F(3, 20) = 7.32$, $P<0.01$] and (c) KMO [$F(3, 14) = 12.13$, $P<0.001$], but (d) kynureninase expression failed to achieve statistical significance. *Post hoc* comparisons revealed that KA increased hippocampal IDO and KMO expression 24 hours following KA administration, when compared to saline treated controls. By contrast hippocampal KAT-II expression was reduced 24 hours following KA administration when compared to saline treated controls. Data is expressed as mean fold change ± SEM (n=3-6). *$P<0.05$, **$P<0.01$ vs. saline controls (Dunnetts *post hoc* test).

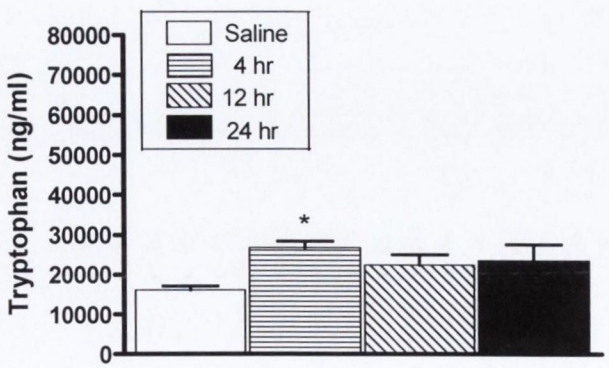
3.2.7 Time dependent changes in the concentrations of tryptophan, and kynurenine pathway metabolites in the hippocampus and serum following KA administration.

KA (10mg/kg) was administered and animals were euthanised 4, 12 and 24 hours following drug administration. Hippocampal tryptophan concentrations were increased 4 hours but not 12 or 24 hours following KA administration. Circulating tryptophan concentrations were also increased 4 hours but not 12 or 24 hours following KA administration. KYNA or related metabolites were not detectable in the hippocampus. No changes in circulating L-KYN or 3-HK were found following KA administration.

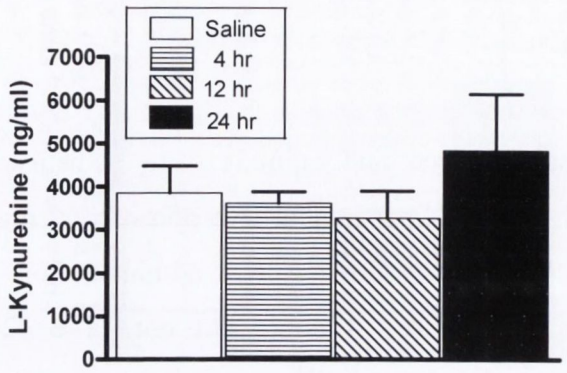
(a)



(b)



(c)



(d)

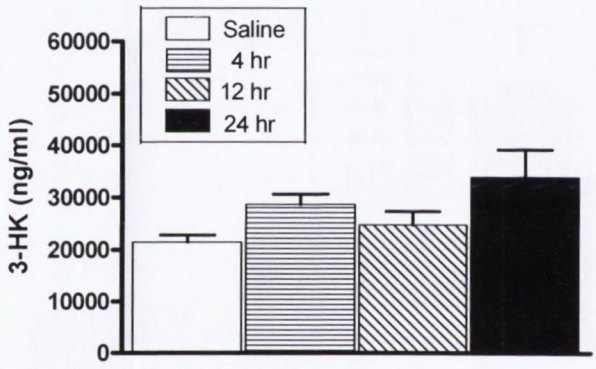


Figure 3.9 KA provokes a time dependent increase in hippocampal and circulating tryptophan concentrations

One-way ANOVA of (a) hippocampal [$F(3, 16) = 7.188$, $P<0.01$] and (b) circulating tryptophan [$F(3, 15) = 2.76$, $P<0.05$] concentrations showed an effect of KA. *Post hoc* comparisons revealed that KA increased hippocampal and serum tryptophan concentrations 4 hours following KA administration when compared to saline treated controls. One-way ANOVA of circulating (c) L-KYN and (d) 3-HK were not significant. Data are expressed as mean±SEM (n=4-6). *$P<0.05$; **$P<0.01$ vs. saline counterparts (Dunnetts *post hoc* test).

3.2.8 KA provokes seizure related behaviours in a dose dependant manner

KA (2.5, 5 and 10mg/kg) was administered and 24 hours later rats were euthanised. KA induced a dose dependent increase in seizure related behaviours in rats. 2.5 mg/kg slightly increased seizure behaviour, this response was stronger at a dose of 5 mg/kg but the largest increase in seizure behaviour was seen following a dose of 10 mg/kg KA. Wet dog shakes were noted in every treatment group, with forelimb clonus, with rearing *and* falling were observed in 1/4 rats in the 5mg/kg group and 3/4 rats in the 10 mg/kg group.

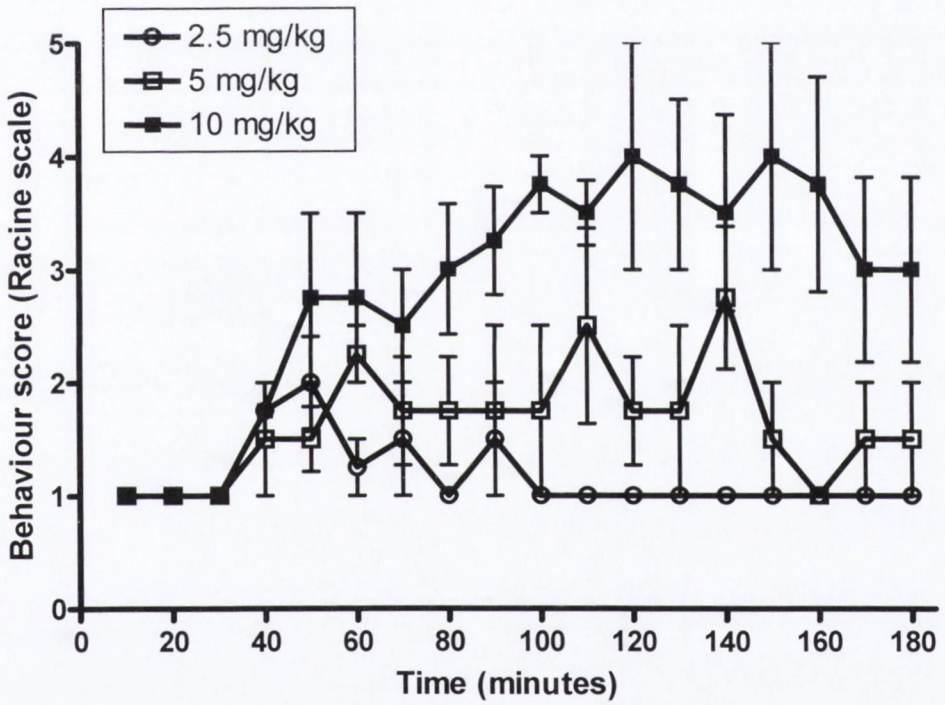


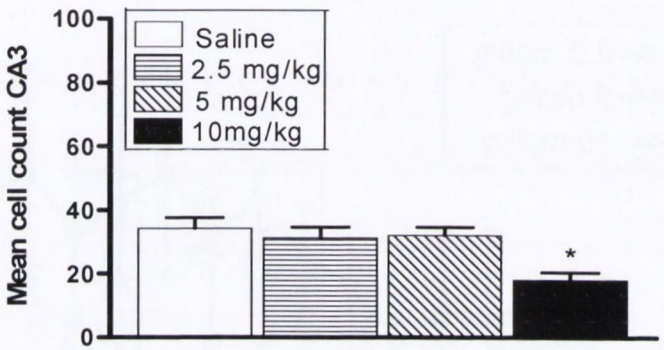
Figure 3.10 Dose dependent effects of KA on seizure related behaviours

Animals were scored 1-5 according to what stage they reached on the Racine scale. The animals were scored for 1 minute every 10 minutes over a 3 hour observation period following KA administration. Repeated measures ANOVA of behaviour scores showed an effect of KA [$F(2, 9) = 6.31$, $P<0.01$], Time [$F(17, 153) = 435$, $P<0.001$], and interaction between time and KA [$F(34, 153) = 2.77$, $P<0.001$]. Data is expressed as mean ± SEM over time. (n=4).

3.2.9 KA induces hippocampal cell loss in a dose dependent manner

KA (2.5, 5 and 10mg/kg) was administered and 24 hours later rats were euthanised. Counts of viable cells stained with cresyl violet showed a decrease in viable cells in the CA3 of the hippocampus, in rats treated with 10 mg/kg KA, when compared to saline controls. This effect was not evident at the lower doses of 2.5 or 5 mg/kg. Cell counts were also significantly decreased in the CA1 following KA (10 mg/kg) administration when compared to saline controls.

(a)



(b)

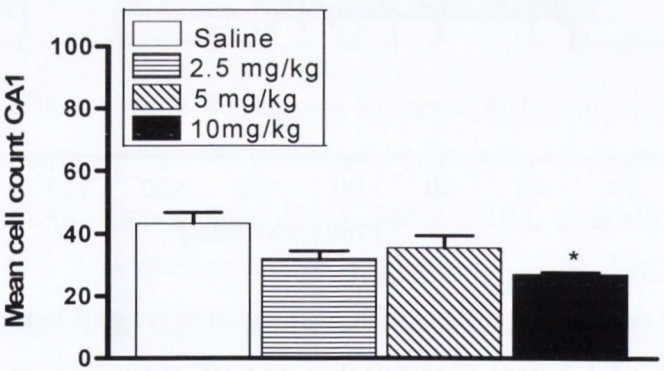


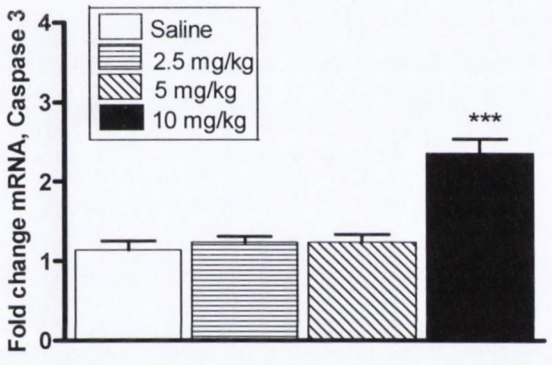
Figure 3.11 Dose related effects of KA on cell loss in the (a) CA3 and (b) CA1 of the hippocampus

One-way ANOVA showed an effect of KA in the (a) CA3 [$F(3, 11) = 4.96$, $P<0.05$] and (b) CA1 [$F(3, 10) = 5.22$, $P<0.05$]. *Post hoc* comparisons revealed that KA (10mg/kg) reduced cell number in the CA3 and CA1 when compared to saline treated controls. Data are expressed as mean cell count ± SEM (n=3-4). *$P<0.05$ vs. saline counterparts (Dunnetts *post hoc* test).

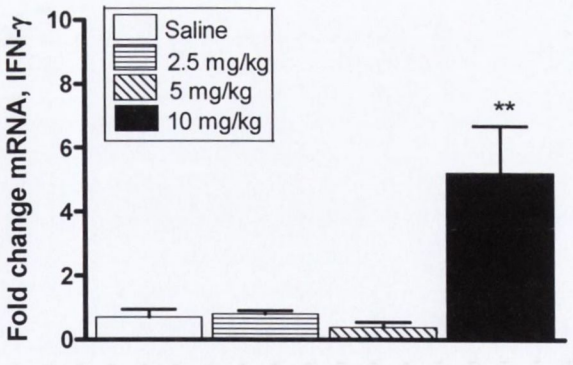
3.2.10 KA provokes a dose dependent increase in hippocampal caspase 3, IFN-γ and IDO expression.

KA (2.5, 5 and 10mg/kg) was administered and 24 hours later rats were euthanised. KA at 10mg/kg, but not 2.5 or 5mg/kg, induced a 2 fold increase in caspase 3, a 5 fold increase in IFN-γ and 20-25 fold increase in IDO expression, in the hippocampus 24 hours following administration when compared to saline treated controls.

(a)



(b)



(c)

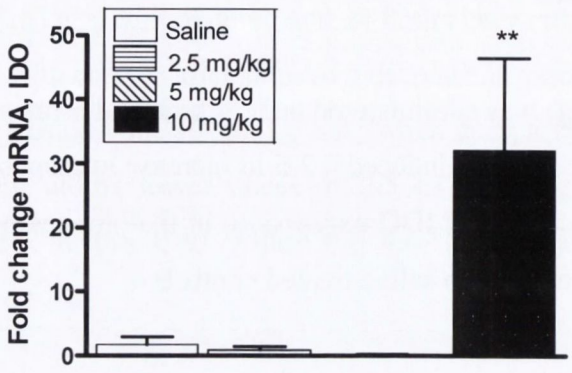


Figure 3.12 KA induces hippocampal caspase 3, IFN-γ and IDO expression in a dose dependent manner

One-way ANOVA showed an effect of KA on (a) caspase 3 [$F(3, 11) = 24.0$, $P<0.001$], (b) IFN-γ [$F(3, 10) = 12.2$, $P<0.01$], and (c) IDO [$F(3, 11) = 24.0$, $P<0.001$] expression. *Post hoc* comparison revealed that KA increased hippocampal expression of all 3 targets when compared to saline treated controls. Data is expressed as mean±SEM (n=3-4). **P<0.01; ***P<0.001 vs. saline counterparts (Dunnetts *post hoc* test).

Table 3.1 Summary of Time course results

Target	4 hour	12 hour	24 hour
Bcl-2	–	–	–
Caspase 3	–	–	↑
IL-1β	↑	–	–
TNF-α	↑	↑	–
IFN-γ	–	↑	↑
iNOS	–	–	↑
IDO	–	–	↑
KAT II	–	–	↓
KMO	–	–	↑
KYNase	–	–	↑
Hipocampal Tryptophan	↑	–	–
Serum Tryptophan	↑	–	–
Serum L-Kynurenine	–	–	–
Serum 3-HK	–	–	–

Table 3.2 Summary of Dose response results

Target	2.5 mg/kg	5 mg/kg	10 mg/kg
Caspase 3	–	–	↑
IFN-γ	–	–	↑
IDO	–	–	↑

3.3 Discussion

The results of the present study detail the time course and dose dependency of behavioural, inflammatory, apoptotic and cell loss changes in the hippocampus, following administration of the excitotoxin KA to rats. Systemic administration of KA provoked seizures and cell loss in the hippocampus, which were accompanied by inflammation and apoptosis. More specifically, expression of IL-1β, TNF-α, IFN-γ and iNOS were increased in the hippocampus 4, 12 and 24 hours post KA (10mg/kg) administration respectively, compared to saline treated controls. Moreover IDO production, which leads to the biosynthesis of kynurenine and other tryptophan metabolites, was up-regulated following KA administration. The increase in IFN-γ and IDO expression was accompanied by an increase in the expression of the apoptotic marker caspase 3 and increased DNA fragmentation, as determined by TUNEL staining 12 and 24 hours following KA administration. A reduction in Cresyl violet staining of viable hippocampal neurons was also evident in the CA3 region of the hippocampus 12 and 24 hours following KA administration. As lower doses of KA (<10mg/kg) did not provoke hippocampal cell loss, or changes in inflammatory or apoptotic markers, 24 hours following administration, a dose of 10mg/kg KA, with euthanisation at 24 hours post injection, was employed in all subsequent studies on the impact of novel anti-inflammatory or neuroprotective strategies in this animal model of excitotoxicity.

Typical KA-induced seizure behaviours were observed in the present investigation in accordance with reports from other laboratories (Ben-Ari, 1985; Racine, 1972). A dose of 2.5 mg/kg provoked an initial effect with some of the animals reaching stage 2 on the Racine scale, and then returning to baseline 60 minutes following KA administration. A dose of 5 mg/kg was sufficient to induce very mild seizure behaviour for a more sustained period, with some of the rats achieving stage 2 behaviours for the duration of the 3 hour observation period, however, this dose was not sufficient to induce stage 4 or 5 behaviour, which is the criteria for animals to achieve cell loss and the accompanying inflammatory changes (Castillo *et al.*, 2006; Benkovic *et al.*, 2004). Use of the Racine scale to score seizure related behaviours is critical, due to the varied response rate obtained following KA administration to rats. Typically responders are classified as those rats reaching stage 4 or 5 on the Racine scale (Castillo *et al.*, 2006; Benkovic *et al.*, 2004). Some studies have reported a response rate as low as 60% following KA administration to rats (Sperk *et al.*,

1985) which serve to highlight the importance of behavioural monitoring. The initial time course and dose response experiments carried out in this thesis used the i.p route of administration, which resulted in a variable response rate of 50%-85% (rats reaching stage 4-5 on the Racine scale) across experiments. In some cases due to the low response rate, additional animals were used to increase group sizes. The variability of the i.p route of injection may be explained by the dose being directly injected into the intestine, from where it passes out of the system. Subsequently, as a direct consequence of the variability obtained with the i.p route, the s.c was tried as an alternative and was found to be associated with a 100% response rate following KA (10mg/kg) administration. The improved response rate implies that the subcutaneous route leads to a more consistent delivery of KA to the brain. As KA-induced seizures are causally related to excitotoxin-induced cell loss, it is imperative that a consistent response to KA is obtained in any experiments which seek to determine the efficacy of novel anti-inflammatory or neuroprotective agents against excitotoxin-induced neuronal injury. The subcutaneous route was therefore adopted in all subsequent experiments.

Cresyl violet staining of cellular morphology in the hippocampus is widely employed to determine excitotoxin-induced damage in the hippocampus (Heo *et al.*, 2005, Hu *et al.*, 1998, Liu *et al.*, 2006, Morrison *et al.*, 1996). A decrease in the number of viable cells in the CA3 and CA1 sub-regions of the hippocampus was obtained following KA administration. By contrast, cell numbers in the DG were not significantly altered, consistent with reports from other laboratories (Vanoye-Carlo *et al.*, 2007, McLin and Steward, 2006), hence it was decided to focus on changes in the CA3 and CA1 only, in future studies. 24 hours following KA administration, cell loss is prevalent, with degeneration of pyramidal cells and axons evident at this time point (Nadler *et al.,* 1980). It was therefore decided that in future studies, tissue would be harvested 24 hours post KA administration. In addition to a reduction in the number of viable cells in the hippocampus, DNA fragmentation was evident following KA administration, determined by a marked increase in TUNEL positive staining consistent with previous reports from other laboratories (Yoo *et al.,* 2005; Faherty *et al.,* 1999). Such changes were apparent 12 and 24 hours following KA administration. Staining was not carried out 4 hours following KA administration as it was considered to be too early to determine cell loss in this model using a light microscope. Nadler *et al.,* (1980) described degenerative changes within one hour, however this was following i.c.v administration using an electron microscope.

KA-induced excitotoxicity has been reported to occur via both apoptotic and necrotic mechanisms (Ferrer *et al.*, 1995; Simonian *et al.*, 1996). Necrotic cell death involves severe mitochondrial swelling and plasma membrane rupture. However, it is well established that cerebral ischemia and status epilepticus produce what has been called "ischemic cell change" and "dark cell degeneration" (Brown and Brierley, 1972; Evans *et al.*, 1983). Ultrastructurally, these neurons show cellular shrinkage and condensation, nuclear pyknosis (shrinkage) with small, irregular chromatin clumps, and swelling of cytoplasmic organelles, including mitochondria and endoplasmic reticulum, all of which are *in vivo* evidence of neuronal necrosis (Colbourne *et al.*, 1999; Fujikawa *et al.*, 1999; Liu *et al.*, 1999; Portera-Cailliau, 1995, 1997; Sloviter *et al.*, 1996). KA-induced necrosis has been shown to occur in a number of studies (Fujikawa *et al.*, 2000; Nishiyama *et al.*, 1996; Bennett *et al.*, 1995). Evidence for necrosis in the study presented here is seen following cresyl violet staining. The images depicted demonstrate some evidence of nuclear and cellular shrinkage. A robust increase in the apoptotic marker-caspase 3 was observed in the hippocampus 12 and 24 hours following KA administration. Taken in conjunction with evidence for DNA fragmentation, these results suggest that apoptosis is occurring in the hippocampus following KA administration. By contrast expression of Bcl-2, a member of a family of proto-oncogenes which are involved in the regulation of programmed cell death and cell survival (Merry and Korsmeyer, 1997), was not altered in the hippocampus following KA administration. These results are consistent with some reports (Lopez *et al.*, 1999), where no changes were detected in Bcl-2 expression or protein at a number of time intervals following KA administration to rats. However, others have reported that caspase 3 and CPP-32 may play a significant role in the mechanism by which neurons die following seizures (Gillardon *et al.*, 1995, Henshall *et al.*, 2002). The results presented in this thesis suggest that the death receptor mediated cascade, which directly activates caspase 3, rather than the mitochondria mediated cascade, of which Bcl-2 is a key player, is involved in mediating cell loss following KA administration (Puig and Ferrer, 2002; Faherty *et al.*, 1999; Kondratyev and Gale, 2000). However further studies are warranted to clarify these mechanisms in the model.

KA induced time-dependent changes (ranging from 2 hours to 15 days post KA) in the expression of pro-inflammatory cytokines in the rat cerebral cortex, thalamus, hypothalamus, hippocampus and striatum, which is suggestive of a role for inflammation in KA-induced excitotoxicity and neuronal injury (Eriksson *et al.*, 2000, Minami *et al.*,

1990, Oprica *et al.*, 2003). In the present investigation, hippocampal IL-1β was significantly increased 4 hours post KA, but returned to almost basal levels at 12 and 24 hours. TNF-α mRNA was significantly increased at 4 and 12 hours, but not 24 hours following KA administration. IFN-γ expression was also increased at both 12 and 24 hours following KA administration. Increases in IL-1β have been shown to lead to the upregulation of NF-κB (Buss *et al.*, 2004), which increases the transcription of other pro-inflammatory cytokines, and is probably responsible for the upregulation of TNF-α and IFN-γ at later time-points. Moreover the expression of the pro-inflammatory mediator, iNOS, was significantly increased 24 hours following KA administration. The expression of pro-inflammatory cytokines was also determined in the cortex following KA administration and a similar pattern of expression was obtained to that observed in the hippocampus (data not shown). Moreover circulating IFN-γ concentrations were determined 24 hours following KA administration, however no changes were detected (data not shown) indicating that alterations in IFN-γ expression at this time are localised to the brain. This is in line with a previous study which examined if a systemic acute phase response occurred following central application of NMDA, and found that brain injury does not elicit an acute phase response (Wilcockson *et al.*, 2002).

As hippocampal IFN-γ expression is increased, and IFN-γ is known to induce the rate limiting enzyme of the kynurenine pathway, IDO (Carlin et al., 1989), it was opportune to determine if hippocampal IDO expression is influenced following KA administration. IDO was found to be robustly induced in the hippocampus 24 hours following KA administration. Changes to other enzymes associated with kynurenine metabolism were less apparent, although increases in the expression of KMO and kynureninase and reductions in KAT-II were obtained. It is interesting to speculate as to whether such changes have any functional impact on the KA-induced excitotoxicity and neuronal cell loss. Down-regulation of the astroglial enzyme KAT II following KA administration may reflect a generalised inhibition of astrocytes in response to excitotoxicity. By contrast up-regulation of the microglial associated KMO and kynureninase suggest that the neurotoxic branch of the KP is activated. As IDO was induced, concentrations of tryptophan were determined in the hippocampus to determine if activation of the KP may enhance tryptophan utilization, leading to a depletion of tryptophan in this region. However hippocampal tryptophan concentrations were found to be elevated 4 hours following KA administration with no change observed 24 hours following drug administration to

correspond with the time at which IDO expression was found to be elevated. Previous studies have demonstrated that peak responses of cerebral tryptophan to LPS are around 8 hours, and to IL-1β are around 4 hours (Dunn, 1992), thus it is possible that KA-induced tryptophan levels peak between 4-8 hours, and return to basal levels by 24 hours post KA. Circulating tryptophan concentrations were also found to be elevated at 4 hours, but were unchanged 12 and 24 hours, following KA administration. In addition L-KYN and 3-HK concentrations were detectable in serum and were unchanged at all time intervals following KA administration.

The current data raises the possibility that administration of KA results in the induction of the KP, accompanied by increasing concentrations of circulating and brain tryptophan. Such a combination may lead to enhanced tryptophan metabolism through the KP, particularly through the neurotoxic branch of the pathway, and the possibility of increased production of the endogenous excitotoxin QUIN, which could further augment KA-induced excitotoxic damage. It would be of interest to quantify QUIN following KA administration in this model to further examine this possibility. Further investigation is warranted on the role of KP in excitotoxin induced neuronal injury.

IDO is primarily produced by activated macrophages and microglia in the brain (Yadav *et al.*, 2007). In neurodegenerative diseases such as AD, increased inflammatory cytokines and production of $A\beta_{(1-42)}$, has been shown to lead to the production of QUIN from macrophages and microglia via the KP (Guillemin and Brew, 2002; Guillemin *et al.*, 2003). The pathogenesis of neurodegenerative disease involves inflammation mediated by activated microglia, which respond to brain injury. There exists an ongoing debate as to whether microglia play a beneficial role through their ability to phagocytose injured cells- or induce detrimental effects through the release of pro-inflammatory cytokines and reactive oxygen species (Dheen, 2007; Hailer, 2008). In this model, production of QUIN by microglia would further stimulate hypersensitive glutamate receptors, and contribute to neuronal damage through the production of ROS. Thus the presence of activated microglia following KA administration possibly augments the damage, rather than ameliorating it.

Chapter 2

The potential of anti-inflammatory therapy and KP inhibition to reverse KA-induced inflammation, degenerative changes, and alterations in tryptophan metabolism in the hippocampus

4.0 Introduction

Neuronal inflammation has been implicated in both acute and chronic neurodegeneration and is characterised by the activation of astrocytes and microglia, which release an array of pro-inflammatory cytokines (e.g. IFN-γ, IL-1β, TNF-α and IL-6), chemokines, neurotransmitters (e.g. glutamate), and reactive oxygen species (e.g. NO). Such inflammatory mediators increase the permeability of the blood brain barrier (BBB), facilitating the infiltration of peripheral immune cells such as monocytes and lymphocytes (Simi *et al.,* 2007). These become activated and release additional inflammatory mediators, creating a positive feedback loop, contributing to neuronal damage (Das and Basu, 2008). Both pro and anti-inflammatory cytokines are released following experimental ischemia and hypoxia, excitotoxicity, and following epileptogenesis in rodents (Del Zoppo *et al.,* 2000; Barone and Feuerstein, 1999; Flanders *et al.,* 1998). Increased expression of pro-inflammatory cytokines such as TNF-α and IL-1β are detected as early as 1 hour post insult, and prior to neuronal death (Buttini *et al.,* 1994; Liu *et al.,* 1994; Wang *et al.,* 1994). Increased expression of cytokines is also found in the cerebrospinal fluid or in post-mortem brains of patients suffering stroke or brain injury, and the levels of expression often correlate to the amount of tissue damage and recovery rate (Griffin *et al.,* 1994; Krupinski *et al.,* 1996).

In vitro studies have shown that some pro-inflammatory cytokines can directly induce cell death (Zhao *et al.,* 2001; Reimann-Philipp *et al.,* 2001). Central or peripheral administration of pro-inflammatory cytokines such as IL-1β and TNF-α, augments excitotoxic and ischemic damage (Yamasaki *et al.,* 1995; Lawrence *et al.,* 1998; McColl *et al.,* 2007), whereas administration of anti-inflammatory cytokines such as TGFβ, IL-10 and IL-1ra ameliorates this damage (Relton and Rothwell, 1992; Prehn *et al.,* 1993; Henrich-Noack *et al.,* 1996; Spera *et al.,* 1998). In rodents, deletion of the genes encoding IL-1α and IL-1β reduces ischemic damage by approximately 80% (Boutin *et al.,* 2001), whereas deletion of IL-1ra exacerbates ischemic damage (Loddick *et al.,* 1997; Pinteaux *et al.,* 2006). Inactivation, deletion or inhibition of caspase 1 (which is required to cleave pro-IL-1β into its active form) has been shown to inhibit neuronal cell death (Hara *et al.,* 1997; Hayashi *et al.,* 2001; Schielke *et al.,* 1998). A similar trend is seen in studies involving the manipulation of endogenous cytokine expression. Inhibition of TNF-α ameliorates ischemic and excitotoxic damage in rodents (Barone *et al.,* 1997; Nawashiro *et al.,* 1997;

Mayne *et al.,* 2001), and over-expression of IL-1ra inhibits ischemic brain damage (Betz *et al.,* 1995; for review see: Allan and Rothwell, 2001). These data indicate that pro-inflammatory cytokines contribute to neuronal cell death, and anti-inflammatory cytokines are neuroprotective. However, in contrast to this, the pro-inflammatory cytokine, IL-6, ameliorates cell death in rodent models of excitotoxicity and ischemia (Toulmond *et al.,* 1992; Loddick *et al.,* 1998). Further studies by Campbell *et al* (1993) demonstrated that transgenic mice over-expressing IL-6 display marked neurodegeneration, indicating that chronic IL-6 is neurotoxic.

Inflammatory cytokines, in particular-IL-1β, have been shown to play a role in the pathogenesis of seizures. Seizure activity increases the expression of IL-1α, IL-1β, IL-6, IL-1ra and TNF-α in the brain (Jankowsky and Patterson, 2001). Intracerebral administration of IL-1 enhances KA-induced seizures, and central administration of IL-1ra has anticonvulsant properties (Vezzani *et al.,* 1999; Vezzani *et al.,* 2000). Direct application of KA to discrete brain regions results in seizure activity and cell death in remote brain regions that are connected by neuronal projections (Ben-Ari, 1985). It has been proposed that cytokines may function to propagate seizure activity signals throughout the brain and contribute to neurodegeneration (Vitkovic *et al.,* 2000).

Pro-inflammatory cytokines are involved in the induction of IDO, the rate limiting enzyme of the KP. IFN-γ is predominantly implicated in the induction of IDO (Schroecksnadel *et al.,* 2003; Takikawa *et al.,* 1999; Popov *et al.,* 2006). Two IFN-stimulated response elements and IFN-γ activated site sequences are located on the 5'-promoter region of the IDO gene (Hassanain *et al.,* 1993; Chon *et al.,* 1995; Konan and Taylor, 1996). Individuals carrying an allelic variant of the IFN-γ gene have increased levels of IFN-γ and also elevated ratios of plasma kynurenine/tryptophan indicative of increased IDO activity (Raitala *et al.,* 2005). TNF-α has also been implicated in the induction of IDO expression. O'Connor and colleagues (2009) demonstrated that TNF-α alone did not induce IDO expression in primary microglial cultures, but following co-administration with IFN-γ, it resulted in a robust synergistic effect on IDO expression. They also demonstrated that inhibition of TNF-α *in vivo* attenuates the up-regulation of IDO in Bacillus Calmette-Guerin (BCG) infected, IFN-γR-knockout mice. Thus a role for pro-inflammatory cytokines in the induction of IDO and activation of the KP has been described.

Alterations in the KP have been implicated in neurodegenerative, immunological and seizure disease, as well as cerebral malaria, severe ALS, neonatal asphyxia, and chronic inflammatory bowel disease (Stone and Darlington, 2002; Schwarcz and Pellicciari, 2002; Medana *et al.,* 2003; Ilzecka *et al.,* 2003; Ceresoli-Borroni and Schwarcz, 2001; Forrest *et al.,* 2002). QUIN, a KP metabolite, is an agonist of the NMDA receptor subtype containing the NR2A and NR2B subunits and is capable of inducing NMDA-related convulsions, excitotoxic and cytotoxic damage (Sas *et al.,* 2007; Stone, 2001; Schwarcz and Pellicciari, 2002) including lipid peroxidation via the production of ROS (Rios and Santamaria, 1991; Santamaria *et al.,* 2001). 3-HK has also been shown to have pro-excitotoxic properties as it is a free radical generator (Schwarcz and Pellicciari, 2002). A number of studies have indicated abnormally high levels of hydroxyanthranilate-3, 4-dioxygenase in the brain of epilepsy-prone EI mice-a mutant strain prone to convulsions, thus leading to increased levels of QUIN in this seizure model (Nakano *et al.,* 1992; Nakagawa *et al.,* 1995, 1998). KYNA is an NMDA receptor antagonist with anti-convulsant properties (Nemeth *et al.,* 2004; Vecsei *et al.,* 1992) and has been shown to be neuroprotective following KA and QUIN administration in animal models of excitotoxicity (Foster *et al.,* 1994). High levels of KYNA are capable of inhibiting glutamate release when administered directly into the rat brain, while a reduction in KYNA, induced by the administration of d-amphetamine to neonatal rats, enhances vulnerability to excitotoxic damage (Carpenedo *et al.,* 2001; Poeggeler *et al.,* 1998).

Activated microglia are capable of secreting QUIN *in vitro,* in amounts that easily exceed those known to be neurotoxic *in vivo* after prolonged exposure (Pemberton *et al.,* 1997), which could contribute towards KA-induced excitotoxic damage. Behan and Stone (2000) examined the hypothesis that QUIN produced from activated microglia plays a key role in the excitotoxic damage seen following KA administration. Through the use of the combined KMO and kynureninase inhibitor, mNBA applied directly to the hippocampus of anaesthetized rats, they demonstrated that by blocking the production of QUIN, it was possible to reduce neuronal damage induced by KA.

Dexamethasone (DEX) is a powerful glucocorticoid and anti-inflammatory drug. DEX has been shown to reduce circulating IFN-γ following infection with *Plasmodium chabaudi* in mice (Tsutsui and Kamiyama, 1998). It suppresses concanavalin-A (con-A) induced lympho-proliferation of IL-2 and IFN-γ (Isobe and Lillehoj, 1993) and suppresses sepsis-

induced NFκB and TNF-α in rats (Chang *et al.,* 1997). DEX has been shown to inhibit the production of mitogen-induced inflammatory mediators in cultured astrocytes (Schwartz *et al.,* 1998; Brenner *et al.,* 1993; Brenner *et al.,* 1994). Saito and colleagues (1994) investigated the role of DEX in the activation of the KP following stimulation with pokeweed mitogen in mice. They demonstrated that DEX attenuated mitogen-induced brain and lung increases in IDO activity, and elevations in plasma L-KYN levels. DEX was also able to reduce the accumulation of cerebral and circulating QUIN in this model suggesting that DEX may confer neuroprotection through its suppression of the KP associated with neuroinflammation and neurodegeneration. To test if neuroinflammation is causally related to acute neurodegeneration in the animal model of hippocampal excitotoxicity established in this thesis, the aim of this study was to reduce the neuroinflammatory response to KA by pre-treatment with DEX, and to examine the resultant effects on hippocampal cell loss.

1 methyl-D, L-tryptophan (1-MT), an analog of tryptophan, is a competitive inhibitor of IDO. It has been used widely in humans as an immunotherapeutic drug in rheumatoid arthritis, type 1 diabetes and multiple sclerosis (Seo *et al.*, 2004; Ueno *et al.*, 2007; Kwidzinski *et al.*, 2005), and is being developed as a vaccine adjuvant and an immunotherapeutic agent for combination with chemotherapy (Jia *et al.*, 2007). O'Connor *et al* (2009) examined the use of 1-MT in mice at a dose of 5mg/kg and reported that it can attenuate sickness behaviour in mice, induced by the systemic inflammogen LPS. 1-MT blocks human dendritic cell regulatory function *in vitro* (Munn *et al.,* 2002) and has been shown to block IDO mediated immune events such as suppression of antigen-specific CD4+ T cells, increasing levels of human chorionic gonadotrpohin in pregnancy to increase resistance to autoimmunity and improving severity scores in EAE mice in animal models of rheumatoid arthritis, type 1 diabetes and multiple sclerosis (Seo *et al.,* 2004; Ueno *et al.,* 2007; Kwidzinski *et al.,* 2005). O'Connor *et al* (2009) also demonstrated that administration of 1-MT does not significantly alter the expression of the pro-inflammatory cytokines, IFN-γ, TNF-α and IL-1β. It does, however suppress LPS-induced IDO expression which is coupled with a decrease in the kynurenine/tryptophan ratio in both the brain and the periphery. Jia *et al., (*2007) examined the pharmacokinetics of 1 methyl-D-tryptophan in rats and dogs over a wide range of doses from 12.5-1000 mg/kg and reported that a dose of 50mg/kg is well tolerated. An additional aim of the current study was to determine the effects of 1-MT in the established model of hippocampal excitotoxicity

particularly in light of KA-induced hippocampal IDO expression observed 24 hours following KA administration, and to determine the resultant effects of IDO inhibition on cell loss in the hippocampus.

The aims of the current study were to determine the effects of DEX and 1-MT on KA-induced neuroinflammation, apoptosis and cell loss in the hippocampus. The results show that KA-induced neuroinflammation may be attenuated by prior treatment with DEX, but these effects do not affect neuroprotection in the model. By contrast, treatment with 1-MT attenuates KA-induced hippocampal cell loss, but fails to influence neuroinflammatory markers, and in some cases enhances KA-induced expression of inflammatory markers, in the KA model.

The null hypothesis of the first study carried out here was that 1-MT would have no effect on the KP and subsequently, would not protect against hippocampal cell loss. The null hypothesis of the second study in this chapter was that DEX would have no effect on KA-induced inflammation and subsequently would not protect again KA-induced hippocampal cell loss.

4.1 Experimental protocol

Animals were randomly divided into treatment groups and housed individually for one week prior to KA administration.

DEX (1mg/ml) was dissolved in 0.2% (v/v) tween-20 and 0.89% (w/v) saline and administered in an injection volume of 1ml/kg giving a dose of 1mg/kg. It was injected i.p. 1 hour prior to KA (10mg/kg, s.c.). Controls received saline and 0.2% (v/v) tween-20 in an equivalent volume.

1 methyl-D, L-tryptophan (50mg/ml) was dissolved in 0.89% (w/v) saline with 0.2% (v/v) tween 20 and administered in an injection volume of 2ml/kg giving a dose of 50mg/kg. It was administered i.p. 16 and 1 hour prior to and 30 minutes following KA (10mg/kg, s.c.) administration. Controls received saline and 0.2% (v/v) tween-20 in an equivalent volume.

Seizure behaviour was monitored for 3 hours following KA administration as previously described and animals were euthanised 24 hours after KA administration.

4.2 Results

4.2.1 Pre-treatment with DEX does not alter KA-induced seizure behaviour

DEX (1mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. KA induced an increase in seizure behaviour according to the Racine scale, which was not affected following pre-treatment with DEX. Wet dog shakes were observed in both groups. Forelimb clonus with rearing, *and* falling was observed in both groups.

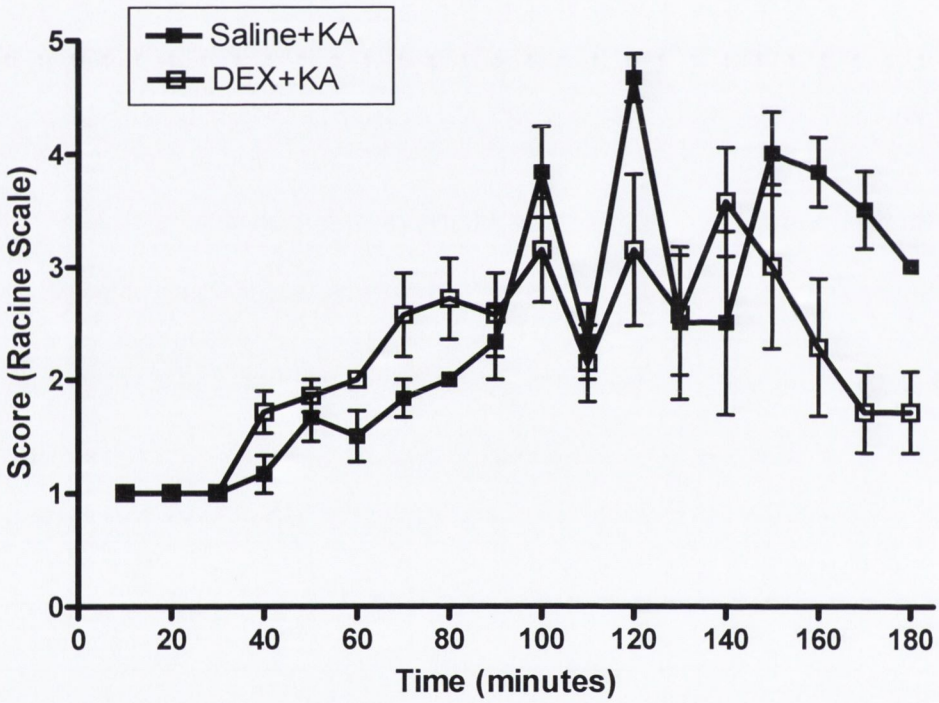
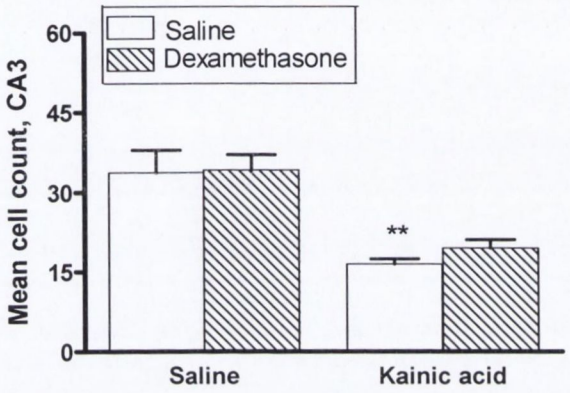


Figure 4.1: KA-induced seizure behaviour is not affected by pre-treatment with DEX
Animals were scored 1-5 according to what stage they reached on the Racine scale for 1 minute every 10 minutes over a 3 hour observation period following KA administration. Repeated measures ANOVA of behaviour scores showed an effect of time [$F(17, 187) = 11.77$, $P<0.001$] and an interaction between DEX and time [$F(17, 187) = 2.92$, $P<0.001$]. Pre-treatment with DEX did not significantly alter KA-induced seizure behaviour. Data is expressed as mean ± SEM over time. (n=6-7).

4.2.2 Pre-treatment with DEX fails to attenuate KA-induced hippocampal cell loss

DEX (1mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later, rats were euthanised. Counts of viable cells revealed a reduction in the number of viable cells in KA treated rats in the CA3 and CA1 of the hippocampus when compared to saline treated controls. Prior treatment with DEX did not affect the KA-induced reduction in viable cells.

(a)



(b)

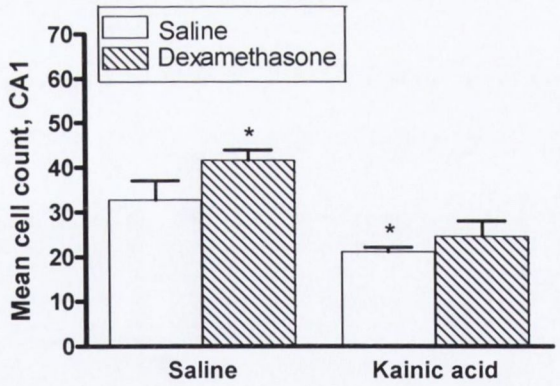


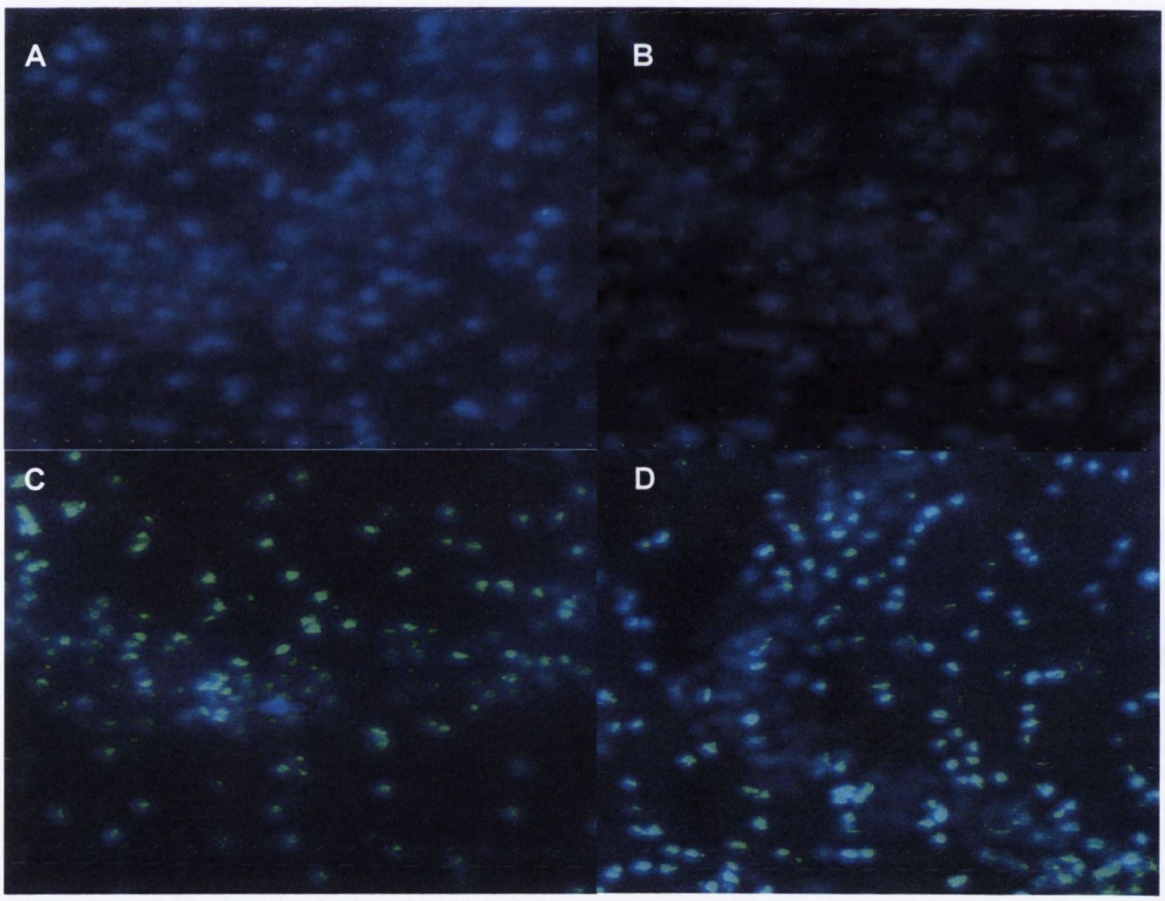
Figure 4.2 KA-induced hippocampal cell loss in the CA3 and CA1 is not attenuated by prior treatment with DEX

Two-way ANOVA showed an effect of KA on cell number in the (a) CA3 [$F(1, 20) = 43.7$, $P<0.001$], (b) CA1 [$F(1, 20) = 27.17$, $P<0.001$] and a DEX effect in the CA1 [$F(1, 20) = 4.96$, $P<0.05$] 24 hours following KA administration. No interaction effect was seen in either the CA3 or CA1. *Post hoc* comparisons revealed that KA provoked a significant reduction in cell number in the CA3 when compared to saline treated controls. Administration of DEX alone significantly increased cell count in the CA1. Data is expressed as mean±SEM (n=4-7). *$P<0.05$ vs. saline counterparts, **$P<0.01$ vs. saline counterparts (Neuman-Keuls *post hoc* test).

4.2.3 Pre-treatment with DEX has no effect on KA-induced DNA fragmentation in the CA3 and CA1

DEX (1mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later, rats were euthanised. TUNEL staining of hippocampal slices following KA administration resulted in an increased number of TUNEL positive cells in the (i) CA3 and (ii) CA1 indicating an increase in DNA fragmentation. KA-induced TUNEL staining was not affected by pre-treatment with DEX.

(i) CA3 of the hippocampus



(ii) CA1 of the hippocampus

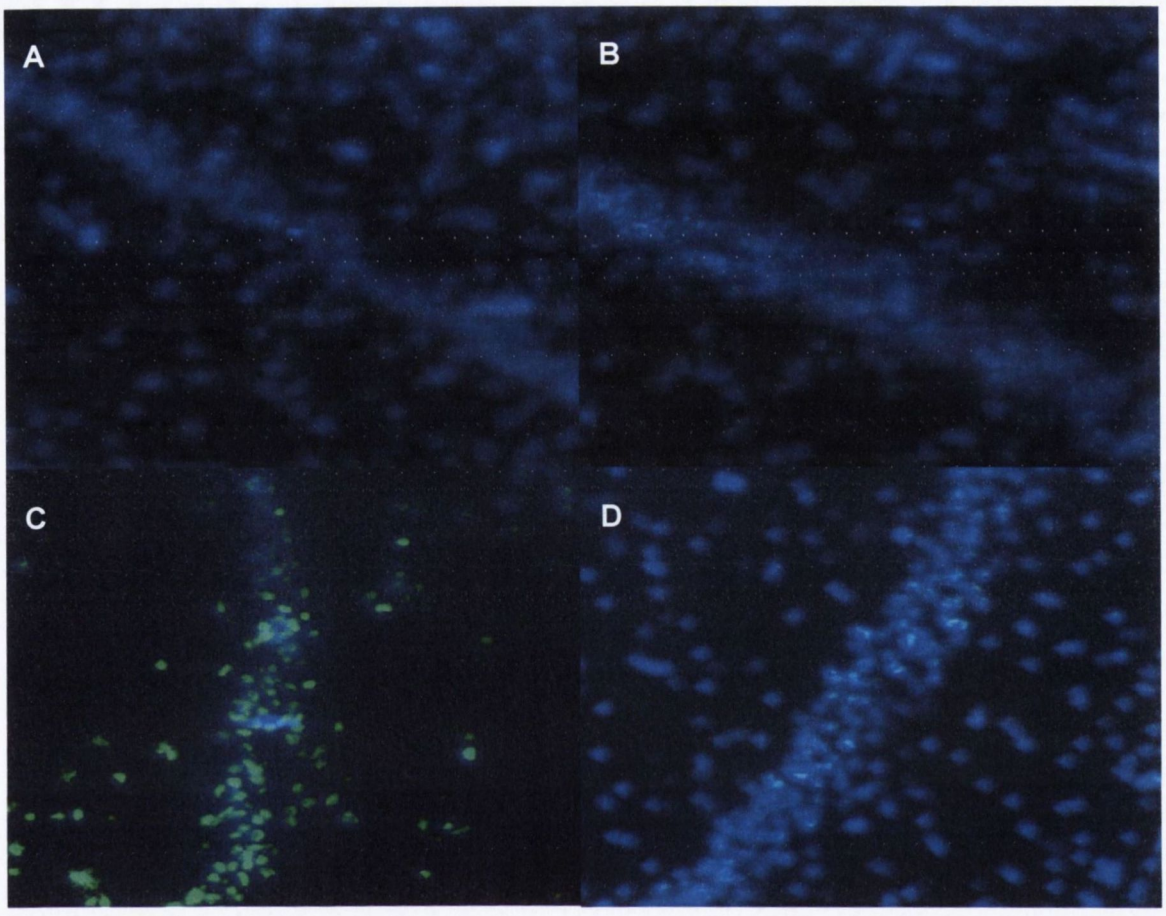
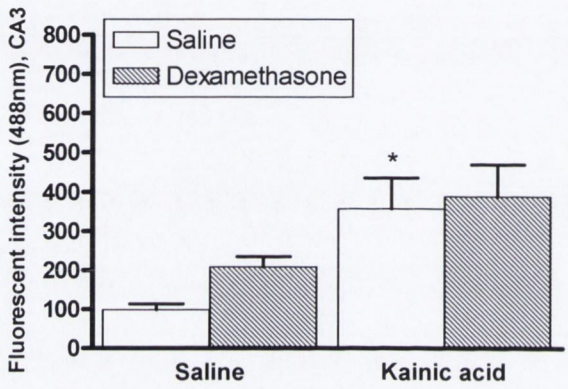


Figure 4.3 KA-induced DNA fragmentation in the CA3 and CA1 is not ameliorated by pre-treatment with DEX.

TUNEL staining of the (i) CA3 and (ii) CA1 where green indicates TUNEL positive staining and blue is a Hoechst dye to indicate co-localisation of DNA fragmentation with hippocampal cells. (A) Saline treated (B) DEX and saline (C) KA induces DNA fragmentation as indicated by TUNEL positive staining, which is (D) not ameliorated by pre-treatment with DEX. (20X magnification)

Quantification of TUNEL staining intensity in hippocampal slices, shows an increase in staining intensity, indicating DNA fragmentation in the (a) CA3 and (b) CA1 following KA administration. The increase in TUNEL staining following KA administration was not ameliorated by pre-treatment with DEX. However, DEX alone induced an increase in DNA fragmentation in the CA1.

(a)



(b)

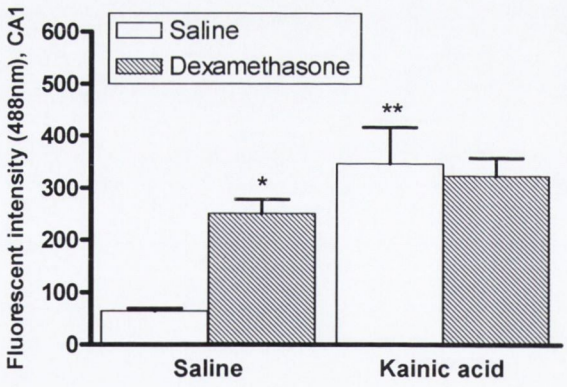


Figure 4.4 KA-induced DNA fragmentation is not affected by pre-treatment with DEX

Two-way ANOVA of mean TUNEL intensity showed (a) an effect of KA in the CA3 [$F(1, 23) = 12.87$, $P<0.01$] and (b) an effect of KA [$F(1, 19) = 13.34$, $P<0.01$] and an interaction between DEX and KA [$F(1, 19) = 7.43$, $P<0.05$] in the CA1. No effect of DEX alone was seen in either the CA3 or CA1. *Post hoc* comparisons revealed that TUNEL intensity was increased in the CA3 and CA1 following KA administration, when compared to saline treated controls. DEX alone also increased TUNEL intensity in the CA1 when compared to saline treated controls. Data is expressed as mean±SEM (n=4-7). *$P<0.05$; **$P<0.01$ vs. saline counterparts (Neuman-Keuls *post hoc* test).

4.2.4 Pre-treatment with DEX attenuates KA-induced caspase-3 expression in the hippocampus

DEX (1mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. KA induced a 10-fold increase in hippocampal caspase 3 expression, which was attenuated by prior treatment with DEX.

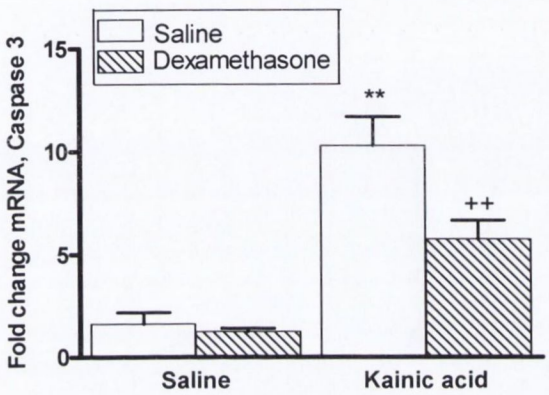


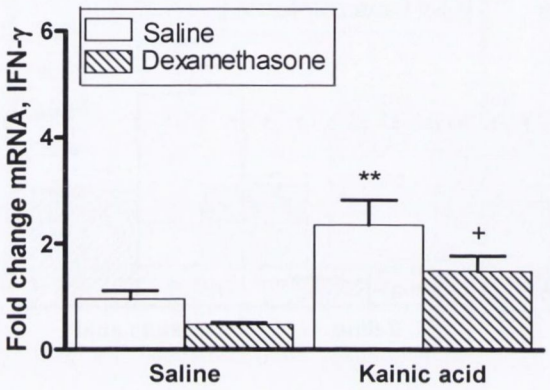
Figure 4.5 KA-induced hippocampal caspase-3 expression is attenuated by pre-treatment with DEX

Two-way ANOVA of fold change in expression showed an interaction between KA and DEX [$F(1, 20) = 5.1$, $P<0.05$], a DEX effect [$F(1, 20) = 6.94$, $P<0.05$] and a KA effect [$F(1, 20) = 50.74$, $P<0.001$]. *Post hoc* comparisons revealed that KA increased caspase 3 expression, when compared to saline treated controls. DEX attenuated the KA-induced increase in caspase-3 expression when compared to KA treatment alone. Data is expressed as mean ± SEM (n=4-7). $^{**}P<0.01$ vs. saline counterparts, $^{++}P<0.01$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

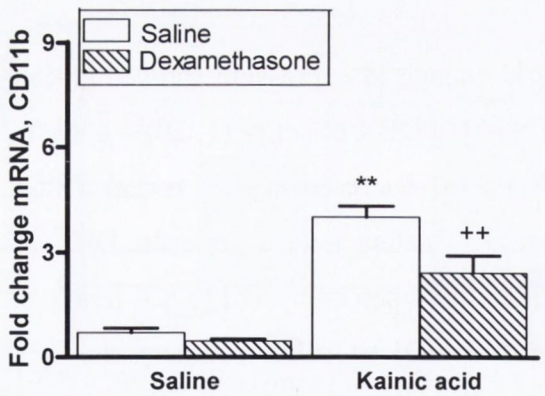
4.2.5 Pre-treatment with DEX attenuates KA-induced hippocampal IFN-γ, CD-11b and IDO expression.

DEX (1mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. KA induced a 3, 4 and 15-fold increase in hippocampal (a) IFN-γ, (b) CD-11b and (c) IDO expression respectively, when compared to saline counterparts. These increases were attenuated following pre-treatment with DEX.

(a)



(b)



(c)

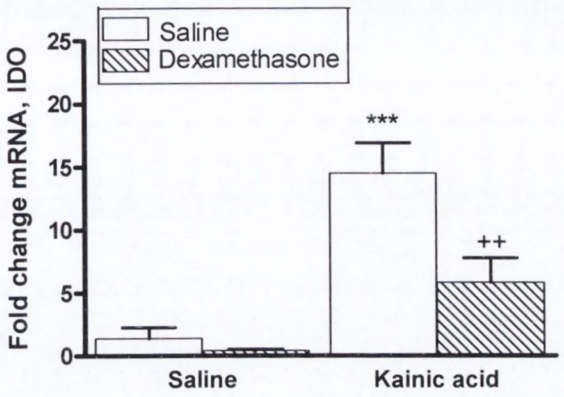


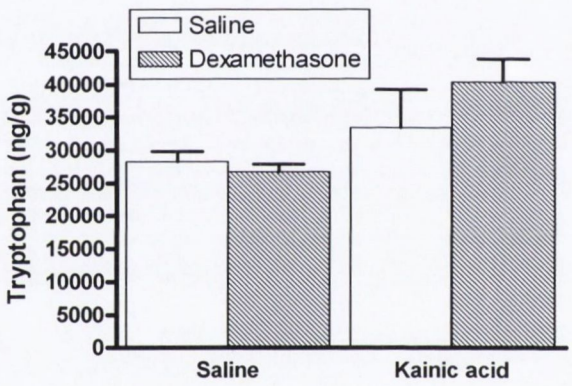
Figure 4.6 KA-induced hippocampal IFN-γ, CD-11b and IDO expression is attenuated following pre-treatment with DEX

Two-way ANOVA revealed (a) an effect of KA [F (1, 16) = 21.1, P<0.001] and a DEX effect [F (1, 16) = 6.49, P<0.05] on IFN-γ expression (b) an effect of KA [F (1, 20) = 59.85, P<0.001] and a DEX effect [F (1, 20) = 7.3, P<0.05] on CD11b expression and (c) an effect of KA [F (1, 15) = 5.18, P<0.05], a DEX effect [F (1, 15) = 8.28, P<0.05], and an interaction effect in IDO expression [F (1, 15) = 30.28, P<0.001]. No interaction effect was seen in IFN-γ or CD11b expression. *Post hoc* comparisons revealed that KA increased IFN-γ, CD-11b and IDO expression when compared to saline treated controls. DEX attenuated the KA-induced increase in IFN-γ, IDO and CD11b expression, when compared to KA treatment alone. Data is expressed as mean±SEM (n=4-8). **P<0.01; *** P < 0.001 vs. saline counterparts, $^{+}$P<0.05; $^{++}$P<0.01 vs. KA counterparts (Neuman-Keuls *post hoc* test).

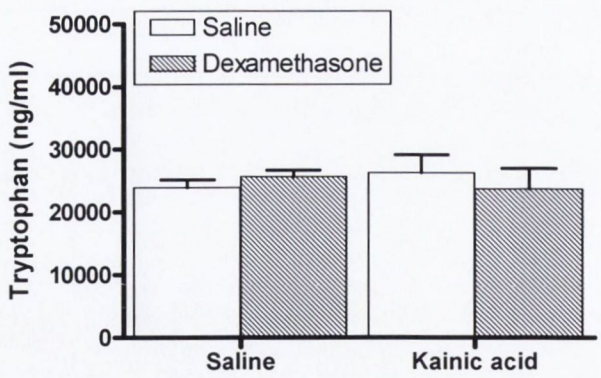
4.2.6 Pre-treatment with DEX has no effect on KA-induced tryptophan, and KP metabolites in the hippocampus and serum, following KA administration.

DEX (1mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. KA did not significantly increase (a) hippocampal tryptophan concentrations following pre-treatment with DEX, although a trend towards an increase was apparent. KP metabolites were not detectable in the hippocampus. There were no changes in (b) circulating tryptophan following DEX or KA treatments alone or in combination. KA had no effect on circulating (c) L-KYN, (d) 3-HK and (e) KYNA.

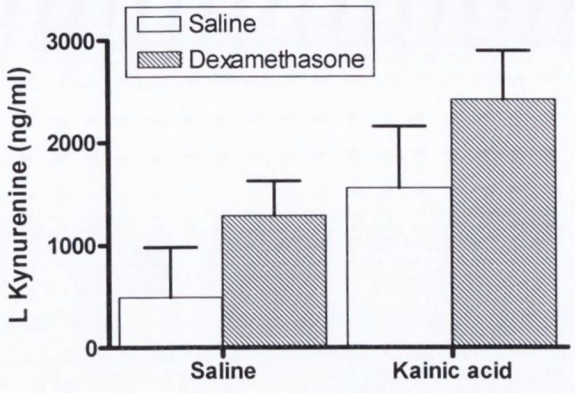
(a)



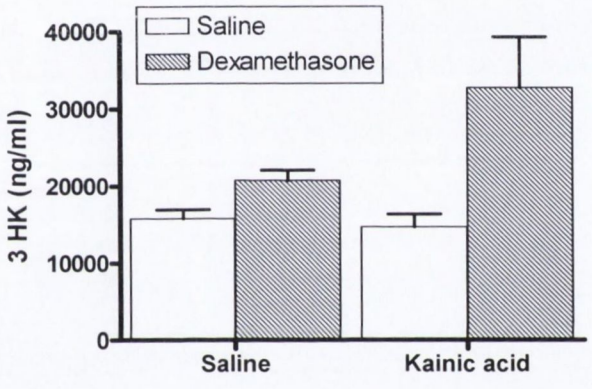
(b)



(c)



(d)



(e)

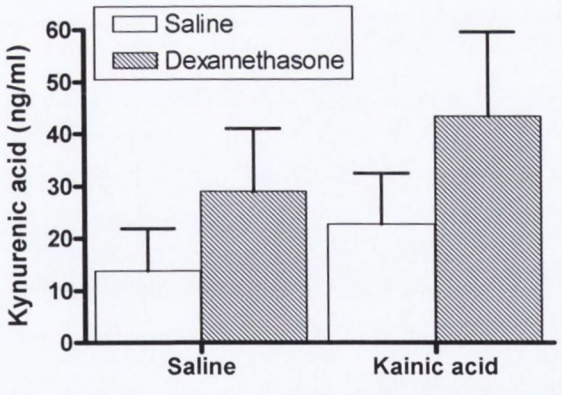


Figure 4.7 No significant changes were seen in hippocampal tryptophan concentrations, circulating tryptophan and KP metabolites.

Two-way ANOVA of (a) hippocampal tryptophan concentrations showed an effect of KA only [$F(1, 11) = 6.87$, $P<0.05$], (b) circulating tryptophan concentrations showed no significant KA, DEX or interaction effect, (c) L-KYN demonstrated a KA effect only [$F(1, 16) = 5.5$, $P<0.05$], d) 3-HK revealed a DEX effect only [$F(1, 15) = 8.8$, $P<0.01$], and (e) KYNA showed no significant KA, DEX or interaction effect. Data is expressed as mean±SEM (n=4-8). (Neuman-Keuls *post hoc* test).

4.2.7 Pre-treatment with 1-MT does not alter KA-induced seizure behaviour

Animals received 1-MT 16 and 1 hour prior to, and 30 minutes following KA administration, and rats were euthanized 24 hours later. KA induced an increase in seizure behaviour according to the Racine scale, which was not affected following pre-treatment with 1-MT. Wet dog shakes were experienced by both groups. Forelimb clonus, with rearing was experienced by both groups. Rearing *and* falling and generalised seizures were not experienced by either group. This may be due to the variability of the behavioural response and all groups experienced stage 4 behaviours, which meets the inclusion criteria.

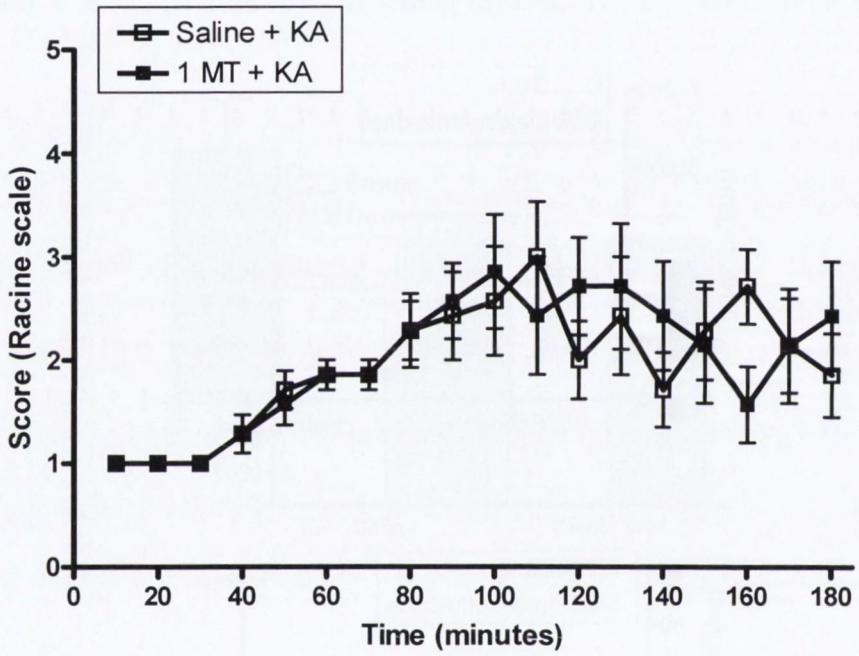


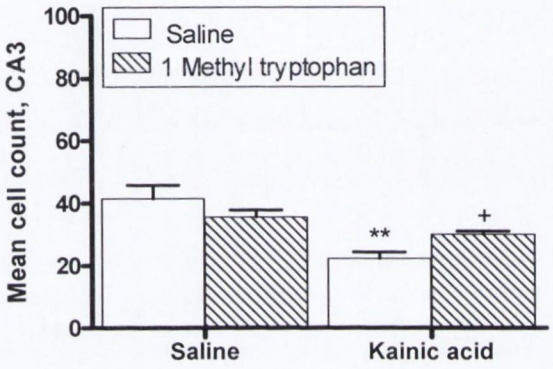
Figure 4.8 KA-induced seizures are not affected by pre-treatment with 1-MT

Animals were scored 1-5 according to what stage they were in using the Racine scale, for 1 minute every 10 minutes over a 3 hour observation period, following KA administration. Repeated measures ANOVA of behaviour scores showed an effect of time [$F(17, 204) = 6.36$, $P<0.001$]. No interaction between time and 1MT, or 1MT alone was seen. Pre-treatment with 1-MT did not significantly alter KA-induced seizure behaviour. Data is expressed as mean ± SEM over time. (n=7).

4.2.8 Pre-treatment with 1-MT attenuates KA-induced hippocampal cell loss

Animals received 1-MT 16 and 1 hour prior and 30 minutes following KA administration and rats were euthanized 24 hours later. Counts of viable cells revealed a reduction in the number of viable cells in KA treated rats in the CA3 and CA1 when compared to saline treated controls. Prior treatment with 1-MT attenuated the KA-induced reduction in viable cells.

(a)



(b)

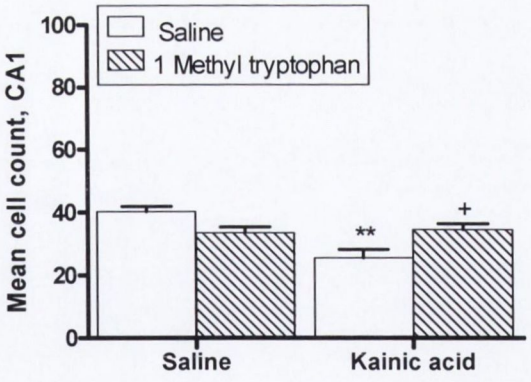


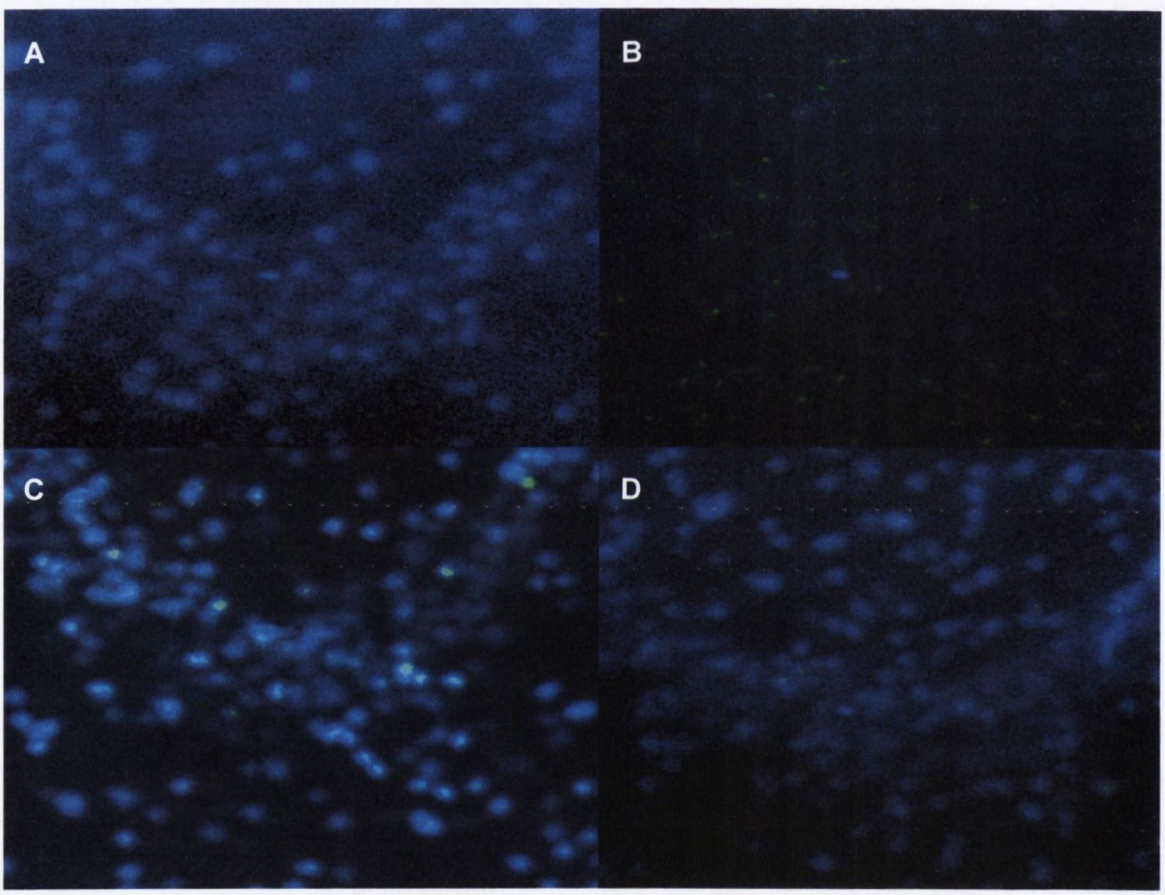
Figure 4.9 KA-induced hippocampal cell loss is attenuated by treatment with 1-MT

A two-way ANOVA showed an effect of KA, and interaction effect on cell number in the (a) CA3 [$F(1, 16) = 25.02$, $P<0.001$], [$F(1, 16) = 13.07$, $P<0.01$] and (b) CA1 [$F(1, 17) = 9.61$, $P<0.01$], [$F(1, 17) = 13.42$, $P<0.01$] respectively 24 hours following KA administration. No effect of 1-MT alone was seen in either the CA3 or the CA1. *Post hoc* comparisons revealed that KA provoked a significant reduction in cell number in the CA3 and CA1 when compared to saline treated controls. Pre-treatment with 1 MT attenuated this reduction in cell count. Data is expressed as mean±SEM (n=4-7). **$P<0.001$ vs. saline counterparts, $^{+}P<0.05$; $^{++}P<0.01$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

4.2.9 Pre-treatment with 1-MT attenuates KA-induced DNA fragmentation in the hippocampus

Animals received 1-MT 16 and 1 hour prior and 30 minutes following KA administration and rats were euthanized 24 hours later. TUNEL staining of hippocampal slices following KA administration resulted in an increased number of TUNEL positive cells in the (i) CA3 and (ii) CA1-indicating an increase in DNA fragmentation. This effect was attenuated by pre-treatment with 1-MT.

(i) CA3 of the hippocampus



(ii) CA1 of the hippocampus

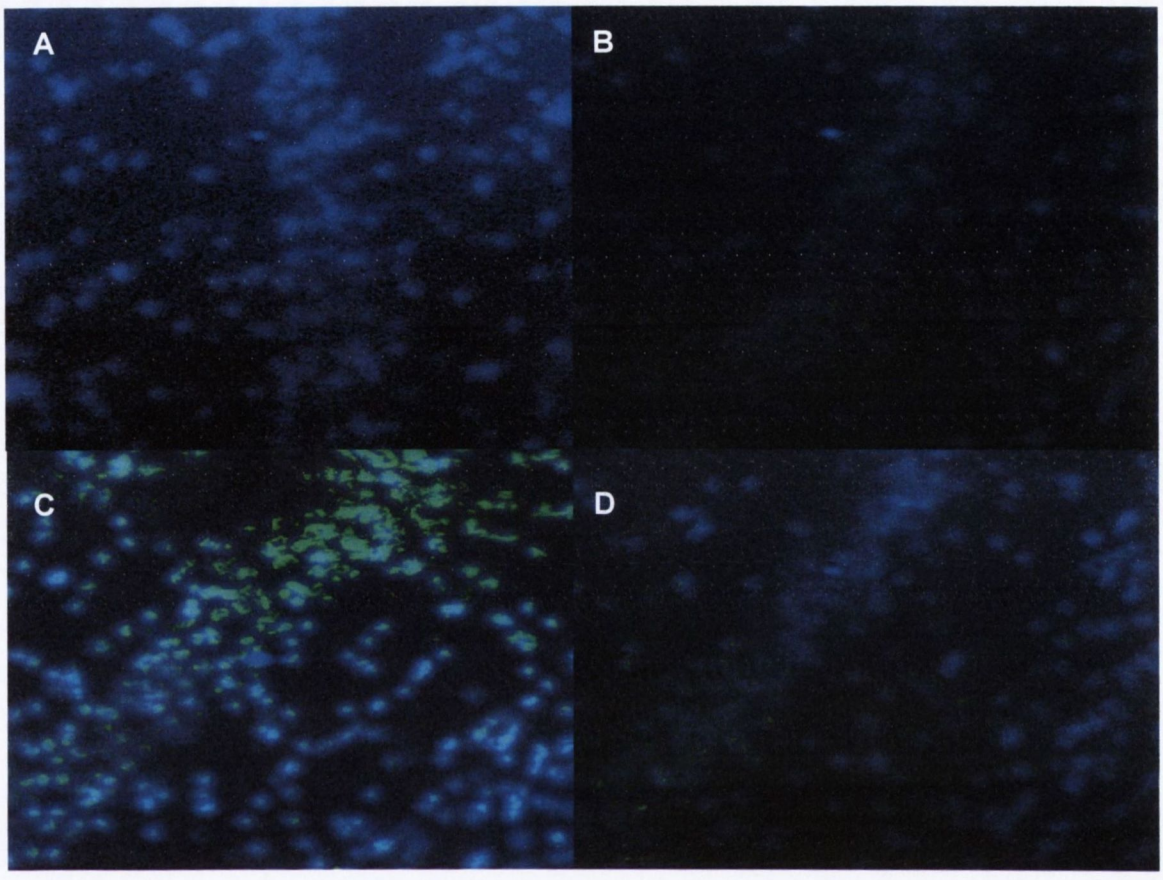
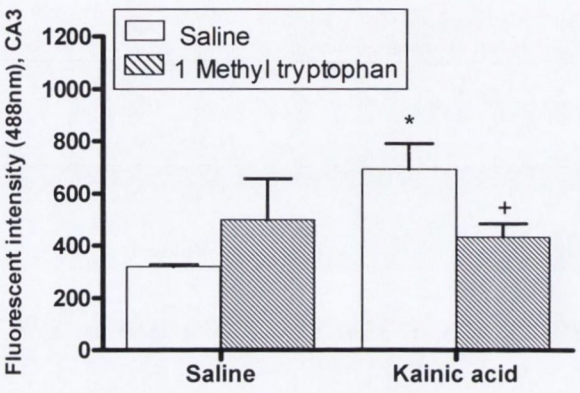


Figure 4.10: KA-induced DNA fragmentation in the CA3 and CA1 is attenuated by pre-treatment with 1-MT.

TUNEL staining of the (i) CA3 and (ii) CA1 where green indicates TUNEL positive staining and blue is a Hoechst dye to indicate co-localisation of DNA fragmentation with hippocampal cells. (A) Saline treated (B) 1-MT and saline (C) KA induces DNA fragmentation as indicated by TUNEL positive staining, which is (D) attenuated by pre-treatment with 1-MT (20X magnification)

Quantification of TUNEL staining intensity in hippocampal slices shows an increase in staining intensity indicating DNA fragmentation in the (a) CA3 and (b) CA1 following KA administration. The increase in TUNEL staining in the CA3 following KA administration, was attenuated by pre-treatment with 1-MT.

(a)



(b)

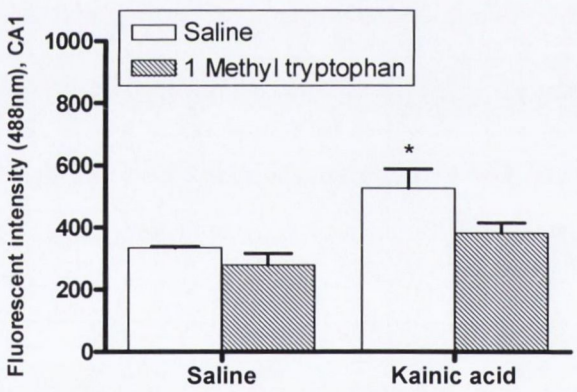


Figure 4.11: KA provokes an increase in TUNEL intensity in the (a) CA3 and (b) CA1 of the hippocampus, which is attenuated in the CA3 by treatment with 1-MT.

Two-way ANOVA of mean TUNEL intensity showed (a) an interaction effect only, between 1-MT and KA [$F(1, 16) = 4.79$, $P<0.05$] in the CA3 and (b) a KA effect only [$F(1, 15) = 7.36$, $P<0.05$] in the CA1, 24 hours following KA administration. *Post hoc* comparisons revealed that TUNEL intensity was increased in the CA3 and CA1 following KA administration when compared to saline treated controls. Pre-treatment with 1-MT attenuates KA-induced DNA fragmentation in the CA3, however this failed to achieve statistical significance in the CA1. Data is expressed as mean±SEM (n=4-7). *$P<0.05$ vs. saline counterparts, $^{+}P<0.05$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

4.2.10 Pre-treatment with 1-MT potentiates KA -induced caspase 3 expression

Animals received 1-MT 16 and 1 hour prior, and 30 minutes following KA administration, and rats were euthanised 24 hours later. KA induced a 2-fold increase in hippocampal caspase 3 expression, which was potentiated by pre-treatment with 1-MT.

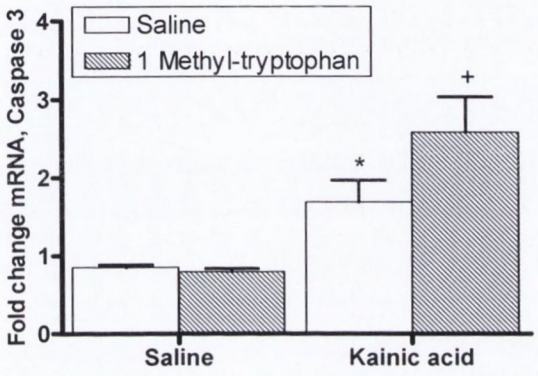


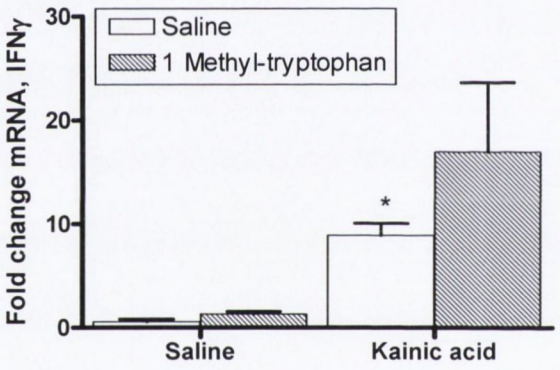
Figure 4.12: KA-induced hippocampal caspase 3 expression is augmented following pre-treatment with 1-MT

Two-way ANOVA of caspase-3 expression showed an effect of KA [$F(1, 20) = 20.47$, $P<0.001$], and a trend towards an interaction [$F(1, 20) = 4.01$, $P<0.059$]. No effect of 1-MT alone was seen. *Post hoc* comparisons revealed that hippocampal caspase-3 expression was increased following KA administration when compared to saline treated controls. 1-MT further enhanced KA-induced caspase-3 expression when compared to KA treatment alone. Data is expressed as mean±SEM (n=4-7). *$P<0.05$; vs. saline counterparts, $^{+}P<0.05$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

4.2.11 Pre-treatment with 1-MT influences KA-induced IFN-γ and IDO expression in the hippocampus

Animals received 1-MT 16 and 1 hour prior and 30 minutes following KA administration, and rats were euthanised 24 hours later. KA induced an 8 and 15-fold increase in hippocampal IFN-γ and IDO expression respectively. The KA-related induction IDO was potentiated by pre-treatment with 1-MT. A similar trend was seen in IFN-γ expression, however this failed to achieve statistical significance.

(a)



(b)

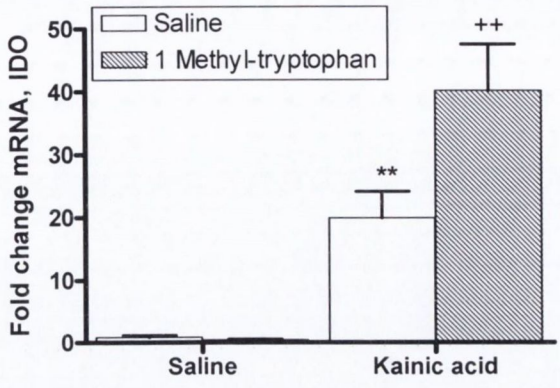


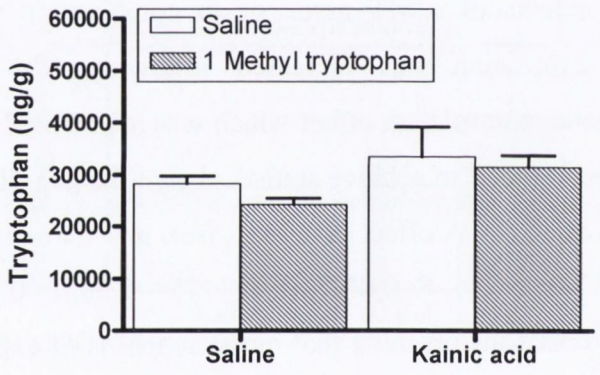
Figure 4.13: KA-induced hippocampal IFN-γ and IDO expression is augmented following pre-treatment with 1-MT

Two-way ANOVA of (a) IFN-γ expression showed an effect of KA only [F (1, 17) = 13.64, P<0.001]. No effects of 1-MT alone or in combination with KA, were seen. Hippocampal IFN-γ expression was increased following KA administration when compared to saline treated controls, an effect which was augmented by pre-treatment with 1-MT, however this result failed to achieve statistical significance. (b) a two-way ANOVA of IDO expression revealed a KA effect, a 1-MT effect and an interaction effect between KA and 1-MT [F (1, 17) = 53.44, P<0.001], [F (1, 17) = 6.06, P<0.05], [F (1, 17) = 6.51, P<0.05]. *Post hoc* comparisons revealed that hippocampal IDO expression was increased following KA administration when compared to saline treated controls. 1-MT augmented KA-induced IDO activity when compared to KA treatment alone. Data is expressed as mean±SEM (n=4-7). **P<0.01 vs. saline counterparts, $^{++}$P<0.01 vs. KA counterparts (Neuman-Keuls *post hoc* test).

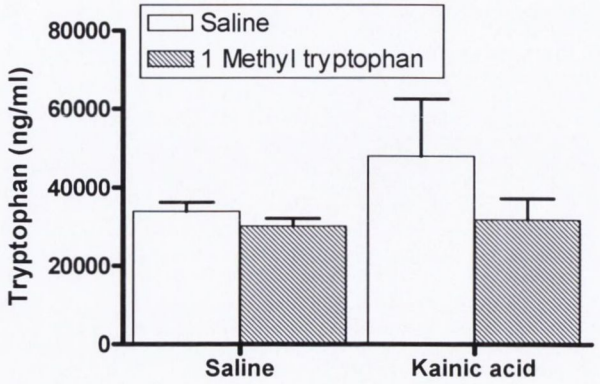
4.2.12 Pre-treatment with 1-MT does not affect KA-induced changes in tryptophan and KP metabolites in the hippocampus and serum, following KA administration.

Animals received 1-MT 16 and 1 hour prior and 30 minutes following KA administration and rats were euthanised 24 hours later. KA did not influence (a) hippocampal tryptophan concentrations alone or following pre-treatment with 1-MT. KP metabolites were not detectable in the hippocampus. Neither 1-MT nor KA alone or in combination significantly influenced circulating concentrations of (b) tryptophan (c) L-KYN and (d) 3-HK and (e) KYNA.

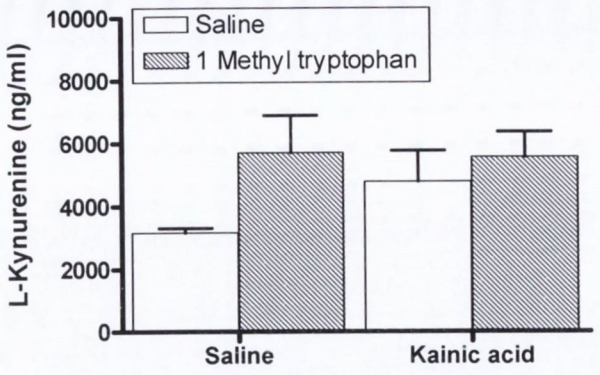
(a)



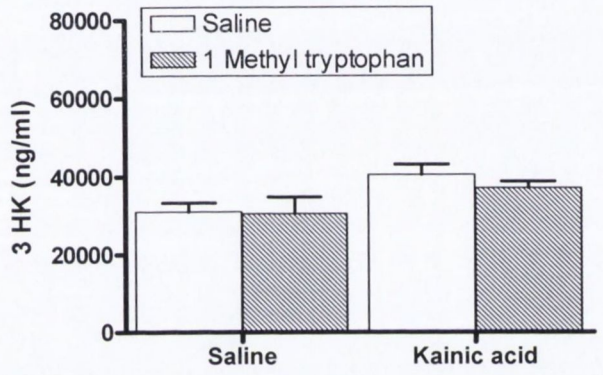
(b)



(c)



(d)



(e)

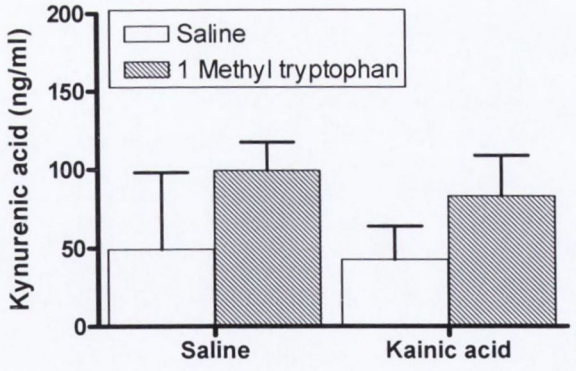


Figure 4.14: KA-induced changes in hippocampal tryptophan concentrations, circulating tryptophan and KP metabolites, are unchanged following pre-treatment with 1-MT

Two-way ANOVA of each of the analyte concentrations revealed no changes in (a) hippocampal tryptophan concentrations (b) serum tryptophan (c) serum L-KYN and (d) serum 3-HK and (e) serum KYNA. Data is expressed as mean±SEM (n=4-7).

Table 4.1 Summary of DEX results

Target	DEX and Vehicle	KA and Vehicle	DEX and KA
Hippocampal cell loss CA3	–	↓	–
Hippocampal cell loss CA1	–	↓	–
DNA fragmentation CA3	–	↑	–
DNA fragmentation CA1	↑	↑	–
Caspase 3	–	↑	↓
IFN-γ	–	↑	↓
CD11b	–	↑	↓
IDO	–	↑	↓
Hipocampal Tryptophan	–	–	–
Serum Tryptophan	–	–	–
Serum L-Kynurenine	–	–	–
Serum 3-HK	–	–	–
KYNA	–	–	–

Table 4.2 Summary table 1-MT results

Target	1 MT and Vehicle	KA and Vehicle	1 MT and KA
Hippocampal cell loss CA3	–	↓	↑
Hippocampal cell loss CA1	–	↓	↑
DNA fragmentation CA3	–	↑	↓
DNA fragmentation CA1	–	↑	↓
Caspase 3	–	↑	↑
IFN-γ	–	↑	–
IDO	–	↑	↑
Hipocampal Tryptophan	–	–	–
Serum Tryptophan	–	–	–
Serum L-Kynurenine	–	–	–
Serum 3-HK	–	–	–
KYNA	–	–	–

Summary of results where (–) indicates no significant change relative to either Vehicle or KA and Vehicle controls, where appropriate, and (↑/↓) indicates an increase/decrease relative to either Vehicle or KA and Vehicle controls, where appropriate.

Discussion

The current study determined if pre-treatment with DEX would elicit neuroprotective effects in the KA model of hippocampal excitotoxicity. In a similar manner to the previous study all animals receiving KA showed seizures and related stereotyped behaviours within 3 hours. Expression of IFN-γ, CD11b and IDO were increased in the hippocampus 24 hours following KA administration. The increase in the inflammatory markers was accompanied by an increase in expression of the apoptotic marker caspase 3, increase in TUNEL staining and a reduction in Cresyl violet staining of viable hippocampal cells, 24 hours post challenge. Pre-treatment with DEX significantly attenuated KA-induced increase in hippocampal IFN-γ, CD11b, IDO and caspase 3 expression, but failed to influence KA-induced seizures, increased TUNEL staining or hippocampal cell loss. Increased hippocampal tryptophan and circulating L-KYN and 3-HK concentrations were obtained following DEX treatment in combination with KA but not following either treatment alone. By contrast, pre-treatment with the IDO inhibitor 1-MT significantly enhanced KA-induced increase in hippocampal CD11b, IDO and caspase 3 expression, but failed to influence KA-induced seizures, and attenuated increased TUNEL staining and cell loss in the CA3 and CA1 subfields. No significant changes in hippocampal tryptophan or circulating tryptophan or KP metabolites were obtained following 1-MT treatment alone or in combination with KA.

Pre-treatment with DEX did not affect KA-induced seizure behaviours. Initially, KA treated rats pre-treated with DEX displayed greater stage 2 and 3 behaviours for the first 90 minutes, after this time point there was no discernible difference in stage 4 or 5 behaviours. A previous investigation reported that KA-induced WDS and seizure activity was potentiated by glucocorticoids, and attenuated in adrenalectomised rats, suggesting that seizure behaviour is regulated by glucocorticoids (Lee *et al.,* 1989). Conversely, it was reported that seizures induced by 4-aminopyridine (a pyridine derivative which chemically induces seizures in rodents) were symptomatically ameliorated by DEX (Fazekas *et al.,* 2006). The results presented here are consistent with the observations of Lee *et al.,* (1989) as there was a distinct increase in WDS behaviour (stage 2) following pre-treatment with DEX.

Pre-treatment with DEX did not afford protection against KA-induced hippocampal cell loss. *In vitro* studies of primary hippocampal cultures have previously demonstrated that DEX potentiates KA-induced neurotoxicity (Semba *et al.,* 1996). By contrast, Felszeghy and colleagues (2004) reported that DEX decreases CXCR4 receptor density in the penumbra, a protein involved in the regulation of apoptosis, and reduces astrogliosis following hypoxia/ischaemia in rat neonates, and proposed that DEX can afford protection in models of acute neurodegeneration. DEX has also been shown by a number of studies to induce apoptosis in its own right (Yan *et al.,* 2006; Laane *et al.,* 2007; Tazik *et al.,* 2009). Such effects were also observed in the current investigation as demonstrated by increased TUNEL staining in the CA3 and CA1 following DEX administration. Despite this induction of DNA fragmentation by DEX alone, it did not potentiate the KA-induced increase in DNA fragmentation. However KA-induced caspase 3 expression was significantly suppressed following pre-treatment with DEX. Similar results were obtained in neonatal rats by Wang and colleagues (2005), following administration of LPS, where treatment with DEX resulted in an inhibition of caspase 3 expression in the neonatal brain. DEX-induced apoptosis has been shown to involve early activation of caspase 3 *in vitro* (Laane *et al.,* 2007) and the current study reports that KA-induced caspase 3 expression is amenable to suppression by DEX. It has been reported that glucocorticoid-induced cell death occurs downstream from gene regulation, involving execution caspases and proteosome complex activation (Distelhorst, 2002). DEX-mediated cell death is associated with repression of survival factors such as AP-1, c-myc and NFκB (Baker *et al.,* 1996). Such mechanisms may account for an increase in DNA fragmentation, despite the reduction in caspase 3 expression.

DEX is potent inhibitor of gene transcription of a wide array of inflammatory cytokines, including a reduction in pro-inflammatory cytokine signal transduction and gene expression (Nadeau and Rivest, 2003; Mckay and Cidlowski, 1999). DEX has also been shown to inhibit microglial activation (Herber *et al.*, 2007) through the activation of the glucocorticoid receptor in these cells (Sierra *et al.*, 2008). In a pilot investigation the effects of microglial inhibitor, minocycline as a novel anti-inflammatory and putative neuroprotectant, were determined in the KA model. Many studies have previously reported neuroprotective and anti-inflammatory actions of minocycline (Chen *et al.,* 2000; Du *et al.,* 2001; Wells *et al.,* 2003; Diguet *et al.,* 2004; Hunter *et al.,* 2004; Metz *et al.,* 2004; Yong *et al.,* 2004). Minocycline has been shown to have anti-apoptotic properties and to be

neuroprotective in NMDA-mediated excitotoxicity *in vitro* (Wang *et al.,* 2004; Tikka and Koistinaho, 2001) and *in vivo* (Gagliardi, 2000). In this pilot study, minocycline was administered at a dose of 45 mg/kg given 16 hours and 1 hour pre-KA challenge, and 30 minutes post KA in accordance with previous studies in the literature (Yenari *et al.,* 2006; Kim *et al.,* 2009; Yrjanheikki *et al.,* 1999). Minocycline however had no effect on KA-induced changes including CD11b, the marker of microglial activation. For this reason it was decided to test DEX, a more potent anti-inflammatory agent, to investigate the role of inflammation in the KA model of excitotoxicity and hippocampal cell loss.

DEX suppressed KA-induced IFN-γ, CD-11b and IDO expression in the hippocampus. Due to the fact that IFN-γ is such a potent inducer of IDO expression (Schroecksnadel *et al.,* 2003; Takikawa *et al.,* 1999; Popov *et al.,* 2006), the reduction in IFN-γ expression may well be a contributing factor in the suppression of IDO. It has been previously reported that administration of DEX resulted in an attenuation of IDO in both the brain and lungs of mice, following systemic pokeweed mitogen administration (Saito *et al.,* 1994). Elevated expression of the microglial activation marker-CD11b following KA administration was suppressed by pre-treatment with DEX, indicating a plausible link between microglial activation and the enhanced expression of IFN-γ in this model. The inhibition of microglia by DEX is likely to have a knock-on effect reducing IFN-γ expression, which in turn suppresses the induction of IDO. In order to test this theory, protein concentrations of both IDO and IFN-γ would need to be examined, as it was not possible to measure the proteins of interest here, further work is required to clarify the proposed mechanism.

Pre-treatment with DEX does not alter hippocampal or circulating levels of tryptophan. In clinical studies, administration of DEX to healthy volunteers has been reported to result in a decrease in plasma levels of L-tryptophan (Maes *et al.,* 1990). It is possible that in this study, alterations in serum tryptophan may have occurred at an earlier time point-as was demonstrated in the time course study. Circulating L-KYN concentrations displayed a trend towards being increased when DEX was administered with KA only. DEX was previously shown to increase plasma levels of L-KYN following systemic pokeweed mitogen administration to mice (Saito *et al.,* 1994). To account for these changes it is not unreasonable to suggest that tryptophan concentrations were elevated at an earlier time point, thus leading to an increase in the production of KP metabolites and the generation of

L-KYN, 3 HK and KYNA in the serum 24 hours following KA administration. It would be of interest to investigate these markers at an earlier time point to clarify the temporal nature of these changes. Alternatively, another possibility is that the tryptophan levels determined here represent total tryptophan and do not reflect changes which may be occurring in free unbound tryptophan. Total tryptophan levels include albumin-bound tryptophan and free tryptophan levels, therefore assessment of the effective circulating concentration of tryptophan requires the determination of non-protein-bound tryptophan (Walser and Hill, 1993). Previous studies have reported no significant changes in total tryptophan but a significant change in free tryptophan following forced exercise in rats (Chaouloff *et al.,* 1985, 1986). By contrast, combined treatment of DEX with KA increased hippocampal tryptophan concentrations. Inhibition of KA-induced IDO by DEX may account for the build up of tryptophan concentrations in the hippocampus. A similar result was obtained in cortical tissue (data not shown).

In the current study 1-MT did not influence KA-induced seizure behaviour, with a similar behavioural profile observed with or without 1-MT. By contrast to DEX, 1-MT attenuated KA-induced hippocampal cell loss in the CA3 and CA1. A similar result was reported by Behan *et al.,* (2000) following the administration of the KMO and kynureninase inhibitor mNBA. 1-MT also protects against KA-induced DNA fragmentation in the CA3 and CA1. However, 1-MT augmented KA-induced caspase 3 mRNA in the hippocampus. It is possible that 1-MT induced protection against cell death occurs downstream from gene regulation, in a manner opposite to that of DEX described by Distelhorst (2002). It is also possible that changes in mRNA expression do not translate into the activated protein. Such mechanisms may account for an decrease in DNA fragmentation, despite the increase in caspase 3 expression.

KA-induced IFN-γ and IDO expression were significantly enhanced following pre-treatment with 1-MT. By contrast O'Connor *et al* (2009) investigated the use of 1-MT following LPS challenge, and reported no change in cytokine profile following 1-MT administration. As 1-MT is regarded as a competitive inhibitor of IDO enzymatic activity, it may not directly influence the expression of IDO. However, it is possible that compensatory actions subsequent to KP inhibition and a fall in the levels of kynurenine and its catabolites could result in the upregulation of IDO. IFN-γ may be a part of this counter regulatory circuit accounting for the up-regulation of KA-induced IDO expression

following 1-MT administration. However, the findings of O'Connor and colleagues (2009) indicate a suppression of IDO expression following 1-MT administration. The study presented here differs from their work in respect of the route of administration, dose and species. They employed the use of slow release pellets, designed to release 5 mg/day of the drug over 21 days, in 10-14 week old male ICR mice which could account for the differences in outcome between both studies. Determination of IDO protein would further help to verify the mechanisms proposed and the role of IDO in excitotoxin-induced hippocampal injury.

Trends towards an increase in circulating L-KYN and KYNA concentrations were observed following 1-MT administration without any significant effect on circulating or hippocampal tryptophan concentrations. As described earlier, determination of total tryptophan as opposed to free circulating tryptophan may lack the sensitivity required to detect changes to IDO activity.

There is a discrepancy in the literature, regarding the activity of the enantiomers of 1-MT, D and L 1-MT, which differ significantly in their effects on recombinant IDO activity *in vitro* (Peterson *et al.*, 1994). The racemic version (D, L 1-MT) has been utilised in a number of murine studies (O'Connor *et al.*, 2009; Szanto *et al.*, 2007). Lob and colleagues (2008) performed studies on the affinity of D and L 1-MT for IDO and the newly identified-IDO2-which is a novel tryptophan catabolising enzyme. IDO2 is potently inhibited by D-1-MT but is unaffected by L-1-MT. L-1-MT is specific for IDO1 (the natural form of IDO) (Metz *et al.*, 2007). Jia and co-workers (2007) reported the presence and inhibition of IDO2 in the rat brain following systemic administration of D-1-MT at a dose of 50 mg/kg. Thus the D isoform may produce results of greater significance in future studies than those observed with the racemate in the current study.

When taken together the experiments with DEX and 1-MT do not provide evidence in support of a neuroinflammatory basis for the acute neurodegeneration in the model yet provide some support for a role of the KP in mediating KA-induced hippocampal cell loss. Indirect inhibition of the KP, using DEX to inhibit pro-inflammatory cytokine production and induction of the KP, did not result in alterations in KA-induced cell loss or DNA fragmentation. DEX did however attenuate changes in the expression of inflammatory and apoptotic markers including IFN-γ, IDO, CD11b and caspase 3. Despite the suppression of

IFN-γ and IDO, and the subsequent alterations to metabolites of the KP, pre-treatment with DEX did not confer neuroprotection in the KA model of excitotoxicity. Whilst inflammatory related changes may contribute to apoptosis, the data suggest that microglial activation and IFN-γ expression do not account for KA-induced neuronal loss, and therefore, alternative mechanisms such as necrosis are likely to account for the neurodegeneration observed. By contrast, direct inhibition of the KP, using the competitive inhibitor of IDO, 1-MT, offered some protection against KA-induced cell loss and DNA fragmentation. It is clear that by ameliorating KA-induced apoptosis, it confers neuroprotection in this model of excitotoxicity. Despite these protective changes, 1-MT was not successful in suppressing IDO expression or significantly altering any of the KP metabolites. Further assessment of the role of the KP in the KA-induced model hippocampal neurodegeneration is warranted particularly with earlier and later time points following KA administration to clarify the role of KP metabolites in the neuroprotective properties of 1-MT in the model.

Chapter 3

Noradrenergic modulation as a strategy to influence KA-induced inflammatory and degenerative changes in the hippocampus

5.0 Introduction

It was postulated in this thesis that inflammation plays a key role in KA-induced hippocampal damage. However, the potent anti-inflammatory DEX was not found to be protective against neuronal loss in this model of hippocampal excitotoxicity, despite showing robust anti-inflammatory activity. By contrast, 1-MT was shown to be neuroprotective, despite enhancing KA-induction of pro-inflammatory markers in the hippocampus. A third and final strategy to determine innate linkages between inflammation and neurodegenerative processes in the KA model of hippocampal excitotoxicty examines the modulatory role of endogenous NA. The rationale for choosing NA as an endogenous target is provided below.

5.1 NA has innate anti-inflammatory and neuroprotective properties

The role of NA in the diseased brain has been a point of interest since it was first implicated in the pathogenesis of depression in the 1950s. Current literature supports the theory that a deficiency in NA originating from the LC, is involved in the pathogenesis of neurodegenerative disorders such as PD and AD (for review see: Marien *et al.,* 2004), and that raising endogenous concentrations of NA can have neuroprotective effects. A review of the evidence in support of such a role for NA is provided below.

A number of *in vitro* studies have demonstrated that NA can suppress the production of pro-inflammatory cytokines in astrocytes and microglia following β_2 adrenoceptor activation, and subsequent increases in cAMP signalling (Feinstein *et al.,* 1993; Nakamura *et al.,* 1998; Mori *et al.,* 2002; Dello Russo *et al.,* 2004). Direct application of NA to mixed glial cells induces the production of IL-1ra and IL-1RII, which negatively regulate the production of the pro-inflammatory cytokine IL-1β (McNamee *et al.,* 2009). Furthermore, *in vitro* application of IL-1ra suppresses IL-1β-induced TNF-α and iNOS production in astrocytes (Liu *et al.,* 1996). Co-administration of NA and LPS to microglia results in an inhibition of IL-1β production and reduces the ability of conditioned media to induce iNOS expression and cell death (Madrigal *et al.,* 2005). Moreover, NA and/or cAMP have been shown to reduce oxidative stress-dependent damage *in vitro*. Levels of GSH were used as a marker of oxidative stress, and dihydrorhodamine 123, a fluorescent indicator,

was also employed to determine ROS production *in vitro*. Spontaneous neuronal loss is reduced by low levels of NA in primary mesencephalic dopaminergic neuronal cultures (Troadec *et al.,* 2001). cAMP reduces the spontaneous loss of neurons, in primary mixed cultures of dopaminergic neurons and glial cells (Mourlevat *et al.,* 2003). In primary septal cholinergic neurons, NA administration reduces neuronal death induced by culture conditions which lead to low-level oxidative stress (Traver *et al.,* 2005).

In vivo depletion of NA was found to exacerbate cortical inflammatory responses to amyloid beta, causing the over-expression of iNOS, IL-1β and IL-6 in rats (Heneka *et al.,* 2002). The selective neurotoxin N-(2-chloroethyl)-N-ethyl-2 bromobenzylamine (DSP4) was used to lesion the LC of rats, and was shown to reduce basal levels of anti-inflammatory molecules such as NFκB inhibitory IκB proteins, and heat shock protein 70, which is a potent survival protein (Heneka *et al.,* 2003). Increasing the synaptic availability of NA, through the use of selective NRI's, results in the attenuation of LPS-induced increases in pro-inflammatory cytokines and increases in iNOS, CD40 and CD11b (markers of microglial activation) gene expression in the rat cortex (O'Sullivan *et al.,* 2009).

Thus it is clear that NA plays a key role in the pathogenesis of a number of disease states, and that its anti-inflammatory actions may contribute to a neuroprotective role within the CNS.

5.2 Pharmacological manipulation of NA prevents cell death

NA has been shown to ameliorate cell death in numerous studies. Madrigal *et al* (2005) demonstrated that NA protects neurons against the cytotoxic effects of microglial conditioned media. Furthermore, pre-treatment with NA significantly reduced Aβ-induced cytotoxicity (Madrigal *et al.,* 2007). Administration of the α_2-adrenergic antagonists, dexefaroxan and F 14413, to rats following unilateral olfactory nerve oblation, reduced neuronal cell death, glial activation and cell proliferation in the olfactory bulbs ipsilateral to the lesion (Veyrac *et al.,* 2005). Bauer *et al.,* (2003) demonstrated that administration of the α_2 antagonist, dexefaroxan for 7 days, resulted in increased neuronal survival in the olfactory bulbs of adult rats, which undergo constant apoptosis and neurogenesis. This

effect is thought to be a result of reducing the apoptotic fate of telencephalic stem cell progenies in the olfactory bulb. The α_2 antagonist, idazoxan, protects against incomplete forebrain ischemia-induced neuronal damage in rats, by increasing extracellular NA in the brain and activating survival-promoting and trophic processes (Gustafson *et al.,* 1990). In an *in vitro* model of excitotoxicity, the selective β_1 antagonist betaxolol increased survival of retinal neurons following glutamate-induced excitotoxic insult (Baptiste *et al.,* 2002). Treatment with clenbuterol, a β_2 adrenoceptor agonist, enhanced the regeneration of motor neuron axons in mice with motor neuron degeneration (Zeman *et al.,* 2004). Junker and colleagues (2002) examined the effects of both β_1 and β_2 adrenergic agonists clenbuterol and dobutamine in an *in vitro* model of excitotoxicity and an *in vivo* model of cerebral ischemia. Both clenbuterol and dobutamine protected against glutamate-induced cell death in mixed hippocampal cultures. These effects were blocked by the non-selective β-antagonist propranolol, and the β_2 selective antagonists ICI 118,551 and butoxamine. Pre-treatment with clenbuterol reduced infarct size, following focal cerebral ischemia. This protective effect was blocked by propranolol and butoxamine, but when administered in combination with the β_1 antagonist metaprolol, this effect was augmented. These results indicate that stimulation of $\beta_{1/2}$ receptors offers neuroprotection *in vitro*, whereas neuroprotection *in vivo* is mediated via the β_2 adrenoceptor.

5.3 NA confers neuroprotection through the promotion of pro-survival pathways

Administration of desipramine, a selective NA re-uptake inhibitor and tricyclic antidepressant, to hippocampus-derived adult neural stem cells inhibited LPS-induced apoptosis and resulted in an increase in the expression of the anti-apoptotic gene, Bcl-2 and nestin, a protein involved in axonal growth (Huang *et al.,* 2007). Treatment with NA dose-dependently protects cortical neurons against oxidative neuronal necrosis following exposure to Fe^{2+} or H_2O_2 (Noh *et al.,* 1999).

An investigation into the effects of α_2-adrenoceptors on apoptosis in the developing brain revealed that *in vivo* treatment with the α_2 antagonist yohimbine decreases the expression of the pro-apoptotic Bax gene in the cerebellum, increases the expression of the anti-apoptotic Bcl-xl gene in the cortex and hippocampus, and induces an increase in the ratio between Bax/Bcl-xl in the cerebellum, cortex and hippocampus, in 6 day old rat pups. This

effect was abolished following the administration of the α_2 agonist clonidine (Il'inykh *et al.*, 2008).

In rats exposed to unpredictable stress, a reduction in the expression of Bcl-2 throughout the brain was reported and administration of the selective NRI, RBX, resulted in an increase in Bcl-2 expression in the cortex and amygdala. RBX did not have any effect on Bcl-xl expression in the cortex, but significantly increased Bcl-xl expression in the hippocampus, specifically the CA1 region (Kosten *et al.*, 2008). Treatment with clenbuterol increases Bcl-2 and down-regulates Bax expression in the rat hippocampus and striatum, following transient forebrain ischemia. Clenbuterol also provokes an increase in Bcl-2 and Bax expression in the striatum and hippocampus of the non-ischemic brain (Zhu *et al.*, 1999).

As discussed previously, NA plays a significant role in the modulation of cytokine expression. Its potent anti-inflammatory properties suggest that NA could significantly affect the KP. Previous studies have demonstrated that stressors such as electric shock and restraint or immobilisation result in the simultaneous activation of brain catecholamine and indoleamine systems in addition to an increase in brain tryptophan levels (Dunn 1988a, 1988b, 1992). KYNA has been shown to partially inhibit the release of [^{3}H]NA from rat hippocampal slices (Barik and Wonnacott, 2006). Furthermore Popova (2002) suggested that KYNA decreases noradrenergic transmission in the rat brain by enhancing the conversion of tyrosine into dopamine, thus increasing its transport to nerve terminals. However, no work to date has determined the effect of modulating noradrenergic transmission within the CNS on inflammogen or excitotoxin-induced activation of the KP. Thus, in this series of experiments IDO expression is examined to determine what effects modulation of central NA may have on the induction of the KP in response to KA *in vivo*.

5.4 NA promotes the expression of protective neurotrophins

NA has the ability to influence the production of neurotrophins within the CNS. Numerous studies have reported that chronic antidepressant treatment (including selective NRIs) results in the upregulation of BDNF synthesis and signalling in the brain (Duman *et al.*, 2004; Coppell *et al.*, 2003; Castren, 2004). However, central depletion of NA using DSP-4 lesioning, and axonal knife-cut degeneration reportedly results in a significant increase in

BDNF and NGF expression in the hippocampus, suggestive of an inhibitory influence of NA on NGF and BDNF expression in the hippocampus (Hutter *et al.,* 1996).

In contrast, voluntary exercise increases levels of BDNF via the increased activation of monoaminergic neurotransmitter signalling, and several reports suggest that this effect is dependent on noradrenergic activation (Garcia *et al.,* 2003; Ivy *et al.,* 2003; Russo-Neustadt and Chen, 2005). This effect is mediated by the protein kinase A/ cAMP pathway which is activated by NA released during exercise, leading to the activation of the transcription factor CREB and enhanced BDNF synthesis. A similar process is also proposed to account for the upregulation of BDNF following chronic antidepressant treatment (Duman *et al.,* 2001; Russo-Neustadt and Chen, 2005). NA, when applied to primary hippocampal neurons, provokes a dose and time dependent increase in BDNF protein (Chen *et al.,* 2007). In attempts to elucidate which signalling pathways are involved in this NA-induced BDNF increase, a concomitant time-dependent increase in the expression of PI3K was observed and subsequent phosphorylation of its downstream signalling molecule, glycogen synthase kinase (GSK-3β) was reported following NA application. GSK-3β is involved in apoptosis in its dephosphorylated state (Hetman *et al.,* 2000; Crowder and Freeman, 2000), whereas the phosphorylated form is associated with inhibition of apoptosis and neuronal survival. Application of NA results in increases in the signalling molecules, phospho-ERK (extracellular receptor kinase) 1 and 2, which are both activated by the MAPK pathway and are involved in cell growth, differentiation and survival via transcriptional regulation. In addition, studies carried out by Chen and Russo-Neustadt (2007) reported that the BDNF-enhancing effects of NA are dependent on NO signalling, in primary hippocampal neurons. Increases in BDNF immunoreactivity were reportedly induced by both NA and by the NO donor, sodium nitroprusside, and the NA-induced increase in BDNF was prevented by compounds which removed NO from culture medium (haemoglobin), or inhibited the synthesis of NO within the cell (Nω-nitro-L-arginine methyl ester, L-NAME).

The aim of this series of experiments was to investigate the role of endogenous NA in the KA model of excitotoxicity. Pharmacological manipulations of NA and its receptors were utilised in these studies to examine if the reported anti-inflammatory, anti-apoptotic and neuroprotective effects of NA, as discussed earlier, can protect against KA-induced

hippocampal cell loss. The following pharmacological tools, outlined below, were employed to meet the objective of these experiments.

DMI is a tricyclic antidepressant and a NRI. It preferentially inhibits the NET and has also been shown to down-regulate central β-adrenergic receptors, following chronic administration to rodents (Bauer and Tejani-Butt, 1992; Hebert *et al.,* 2001; Mandela and Ordway, 2006; Argenti and D'Mello, 1994). It has a half life of 3-4 hours in the brain and 4-5 hours in the serum (Kozisek *et al.,* 2007). DMI can also inhibit the serotonin transporter, although to a much lesser extent than the NET (Leonard, 1997) and binds to a number of non-noradrenergic receptors such as histamine, muscarinic and NMDA receptors (Rang *et al.*, 1999). In addition, DMI can also elicit cardiotoxic effects in overdose by inhibition of sodium and calcium channels (Deffois and Carter, 1996).

DMI has been shown to decrease IFN-γ expression following LPS challenge in human blood cultures (Diamond *et al.,* 2006). Such anti-inflammatory effects of DMI *in vivo* have also been described by Kubera and colleagues (2000, 2001), where chronic DMI treatment to mice resulted in the suppression of con-A and LPS-stimulated IL-4 and IL-1, and increases in IL-10. Administration of DMI has been reported to almost completely ablate TNF-α in cultured neurons (Ignatowski *et al.,* 1997; Reynolds *et al.,* 2005). Furthermore, i.c.v infusion of TNF-α suppressed the ability of DMI to reduce immobility duration in the forced swim test, while administration of TNF-α antibody mimicked the therapeutic effect of DMI in rats during the forced swim test (Reynolds *et al.,* 2004).

In a model of chronic restraint, DMI attenuated the stress-related suppression of BDNF expression in the CA3 region of the hippocampus (Bravo *et al.,* 2009). Such effects have also been observed in cultured neuroblastoma cells treated long-term (48 hours) with DMI, which resulted in an increase in total BDNF mRNA levels (Donnici *et al.,* 2008). Central BDNF protein concentrations were reportedly increased in the frontal cortex, but not the hippocampus of rats, following chronic treatment with DMI (Balu *et al.,* 2008). *In vitro,* DMI has also been reported to protect against LPS-induced apoptosis in hippocampal-derived adult neural stem cells, in part by activating the expression of Bcl-2 (Huang *et al.,* 2007). After chronic treatment of mice, DMI induced a decrease in the potency of glycine to displace [^{3}H] 5, 7-dichlorokynurenic acid at the glycine recognition site of the NMDA receptor. This effect is attenuated by DSP-4-induced central NA depletion, suggesting that

these changes are dependent on a functionally intact NA system and a functional interaction between noradrenergic and glutamatergic signalling (Harkin *et al.,* 2000). Such changes to the NMDA receptor and to cytokine, BDNF and Bcl-2 expression support the rationale that DMI may confer neuroprotection in the rat brain in response to KA-induced hippocampal excitotoxicity.

RBX is a selective NRI that is also a weak inhibitor of the 5-HT transporter, but lacks activity at the dopamine transporter. RBX has no significant affinity for adrenergic, histaminergic or cholinergic receptors (Scates and Doraiswamy, 2000). It is rapidly absorbed and has an elimination half-life of 1-2 hours in rats (Dostert *et al* 1997). RBX elicits its effects by inhibiting NA reuptake, thus causing a sharp increase in the synaptic concentration of NA, followed by a down-regulation and desensitization of β and α_2 adrenoceptors, coupled with an increase in responsiveness of postsynaptic α_1 adrenoceptors (Scates and Doraiswamy, 2000). RBX was reported to be protective in cells derived from the substantia nigra on which the NET is present, where dopamine-dependent iron-induced cell death was reversed by pre-treatment with RBX (Paris *et al.,* 2005) Hashioka *et al.,* (2007) reported that RBX possesses novel anti-inflammatory properties, such as the dose-dependent inhibition of IFN-γ-induced pro-inflammatory mediators, IL-6 and NO, in murine microglial cells. RBX has also been reported to inhibit IFN-γ production in human blood following stimulation with Con-A (Diamond *et al.,* 2006). RBX has been shown to increase the expression of anti-apoptotic genes, Bcl-2 and Bcl-xl in the cortex and hippocampus, in rats exposed to chronic unpredictable stress (Kosten *et al.,* 2008). RBX in combination with voluntary physical exercise in the rat (wheel running paradigm) greatly enhances the production of BDNF in the hippocampus (Russo-Neustadt *et al.,* 2004). These observations suggest that RBX may possess neuroprotective properties. Also, as it is more selective for NA reuptake than DMI, RBX more clearly demonstrates the role of increased synaptic NA, in mediating NRI-induced neuroprotection, in models of neuronal degeneration.

Initial experiments to determine the putative neuroprotective properties of DMI and RBX in the KA model of hippocampal excitotoxicity showed that both compounds enhanced the KA-induced injury. To explore the role of NA in mediating KA-induced cell loss, antagonists and agonists at β adrenergic receptor were employed. Propranolol (PRP) is a non-selective β adrenergic antagonist. It is highly lipophilic and readily crosses the blood-

brain barrier. It has a half-life of 3-4 hours but its effects can last up to 12 hours due to the actions of the active metabolite, 4-hydroxypropranolol (Elghozi *et al.,* 1979), making it a suitable antagonist to use with the current model. PRP specifically competes with NA and related agonists at the β adrenergic receptor for available receptor sites and has been shown to decrease the production of pro-inflammatory cytokines, such as TNF-α and IL-6 in the cerebrospinal fluid of rats, following subarachnoid haemorrhage (Kato *et al.,* 2009). It has been shown to attenuate brain injury in the cortex and striatum of rats after focal transient cerebral ischemia (Goyagi *et al.,* 2006). Mikami *et al* (2008) demonstrated that PRP dose-dependently inhibits caspase 3-like activity in SH-SY5Y neuroblastoma cells, in response to staurosporine-induced apoptosis. In this study they also demonstrated that PRP inhibits staurosporine-induced release of cytochrome-c in isolated mitochondria, suggesting that PRP has anti-apoptotic properties. By contrast, PRP has been reported to inhibit both exercise-induced and antidepressant-induced BDNF expression, via its inhibition of NA signalling (Ivy *et al.,* 2003). PRP has previously been used in combination with RBX in behavioural studies and was shown to reverse the ability of RBX to attenuate stress-induced escape deficit in rats (Grappi *et al.,* 2003). These observations suggest that PRP is an appropriate β blocker to use in the current model, to investigate the mechanism mediating the ability of NA-uptake inhibitors to exacerbate the KA-induced hippocampal injury. Thus, in this series of experiments PRP is used in combination with RBX, in order to examine whether the ability of RBX to exacerbate the excitotoxic response to KA is mediated by β-adrenergic receptors.

β_1 and β_2 adrenoceptors are found throughout the CNS and are both coupled to the G_s protein, and stimulate the activation of adenylate cyclase. β_2 adrenoceptors are more highly expressed on microglia and cells of the immune system and produce greater amounts of cAMP upon stimulation than β_1 adrenoceptor (Mori *et al.,* 2002). Evidence in the literature has indicated a neuroprotective role for β_2 adrenoceptor stimulation in models of ischemic damage and brain injury (Junker *et al.,* 2002; Culmsee *et al.,* 1999b; Semkova *et al.,* 1996; Zhu *et al.,* 1998). Hence, the role of the β_2 adrenoceptor in the KA model of excitotoxicity was examined for putative neuroprotective properties.

Clenbuterol (CLN) is a selective β_2 adrenergic agonist. It is highly lipophilic and readily crosses the blood-brain barrier (Desaphy *et al.,* 2003). It has a long half-life in the rat, up to

30 hours in the plasma (Yamamoto *et al.,* 1985), which renders it useful in the current model. β adrenoceptor down-regulation in the rat cortex is induced following administration of CLN (Newman-Tancredi *et al.,* 1996). CLN has been shown to enhance the production of KYNA *in vitro* in cortical slices and glial cultures, and *in vivo* in the rat cortex, in a cAMP and PKA-dependent manner. As KYNA is able to block the glycine site of the NMDA receptor and to antagonise the α7 cholinergic-nicotinic receptor, such effects of CLN indicate that this β_2 agonist may possess neuroprotective properties in the KA model of excitotoxicity (Luchoskwa *et al.,* 2008, 2009).

Izeboud and colleagues (1999) reported that CLN has anti-inflammatory properties and is capable of potently suppressing LPS-induced IL-6 and TNF-α both *in vitro* in human monocyte-derived macrophages, and *in vivo* in rat plasma. CLN increases the production of neurotrophic factors such as NGF (Culmsee *et al.,* 1999a), which is thought to contribute to neuronal survival and neurogenesis. In this regard, CLN has been used in various rodent models of cerebral ischemia and has been shown to have neuroprotective properties (Junker *et al.,* 2002; Culmsee *et al.,* 1999b; Semkova *et al.,* 1996; Zhu *et al.,* 1998). In a model of glutamate-induced excitotoxicity in mixed neuronal/glial hippocampal cultures, CLN has been shown to be protective and reduces cell death by increasing the expression of NGF (Semkova *et al.,* 1996). It has been used in combination with the NMDA antagonist, memantine, in a model of permanent focal ischemic stroke in mice. CLN acted synergistically with memantine to reduce infarct volume (Culmsee *et al.,* 2004; Culmsee *et al.,* 2007). Furthermore, CLN has been shown to increase the expression of the anti-apoptotic gene, Bcl-2, and to decrease the expression of the pro-apoptotic gene, Bax, in the rat hippocampus and striatum, indicating that an increase in the ratio of Bcl-2: Bax may contribute to the anti-apoptotic effects of CLN (Zhu *et al.,* 1999). These observations suggest that CLN may be neuroprotective in the KA model of excitotoxicity. The aim of this study was to examine the effects of acute administration of CLN on KA-induced hippocampal cell loss and associated apoptosis, cytokine expression and BDNF expression and to elucidate the role of the β_2 adrenergic receptor in KA-induced hippocampal excitotoxicity.

The aim of the current series of experiments is to elucidate a role for NA in the regulation of KA-induced hippocampal excitotoxicity. As described above, NA confers notable anti-inflammatory and neuroprotective properties in both *in vitro* and *in vivo* models.

Pharmacological manipulation of NA and its receptors are utilised in the present experiments to gain insights into the mechanism of this neuroprotection.

The null hypothesis of the DMI and RBX study was that no protective effects would be seen following pre-treatment with these NRI's, and that no protection against hippocampal cell loss would be apparent. The null hypothesis of the PRP and RBX study was that PRP would further exacerbate the damage seen with RBX and KA. The null hypothesis of the CLN study was that no protection against KA-induced apoptosis, inflammation or alterations in neurotrophic factors, would be seen following pre-treatment with this drug.

Influence of the noradrenaline re-uptake inhibitors desipramine and reboxetine, on KA-induced inflammatory and degenerative changes in the hippocampus

5.5 Experimental protocol

Animals were randomly divided into treatment groups and housed individually for one week prior to KA administration.

Desipramine (15mg/ml) was prepared in 0.89% (w/v) saline and administered in an injection volume of 1ml/kg giving a dose of 15mg/kg. It was injected i.p. 1 hour prior to KA treatment (10mg/kg, s.c) in order for the animals to have the drug present in their systems before and during KA insult. Controls received saline in an equivalent volume.

RBX (15mg/kg) was prepared in 0.89% (w/v) saline and administered in an injection volume of 1 ml/kg giving a dose of 15 mg/kg. It was injected i.p. 1 hour prior to KA treatment (10mg/kg, s.c) in order for the animals to have the drug present in their systems before and during KA insult. Controls received saline in an equivalent volume.

In both experiments seizure behaviour was monitored for 3 hours following KA administration, as previously described and animals were euthanised 24 hours after KA administration.

5.6 Results

5.6.1 Pre-treatment with DMI does not alter KA-induced seizure behaviour

DMI (15mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. KA induced an increase in seizure behaviour according to the Racine scale. Wet dog shakes, forelimb clonus and forelimb clonus with rearing and falling was apparent in both groups. Pre-treatment with DMI did not significantly affect KA-induced seizures.

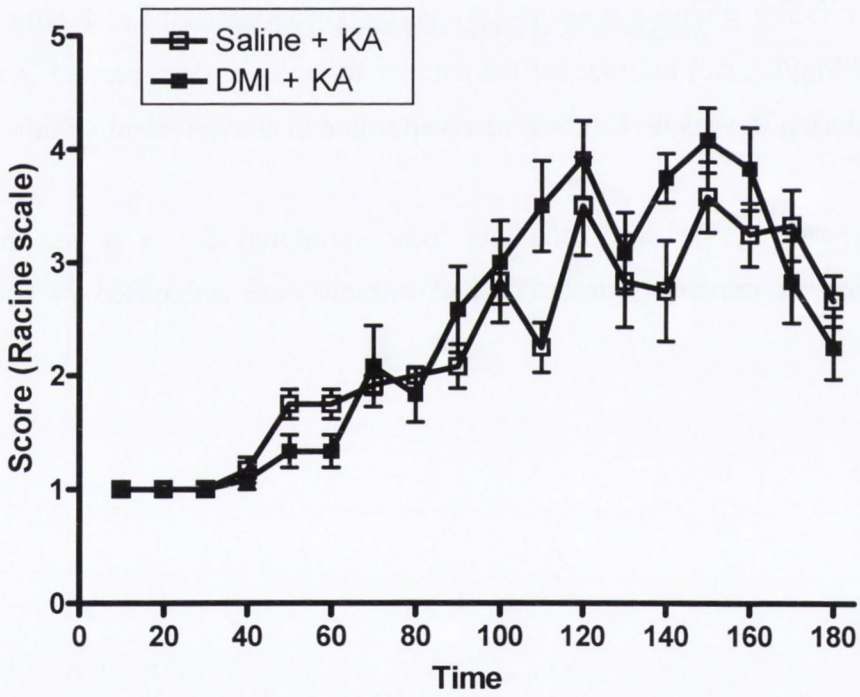
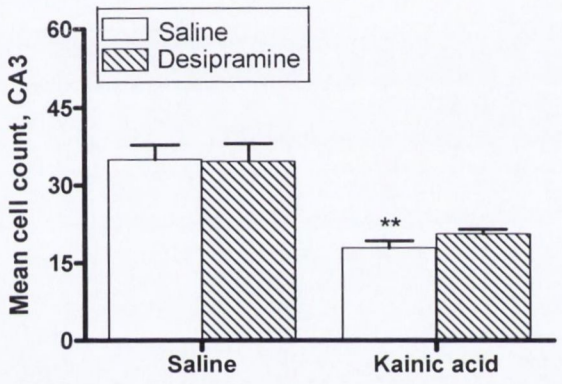


Figure 5.1 KA-induced seizure behaviour is not affected by pre-treatment with DMI
Animals were scored 1-5 according to what stage they were in, using the Racine scale for 1 minute every 10 minutes over a 3 hour observation period. A two-way repeated measures ANOVA revealed a significant time effect [$F(17, 175) = 24.9$, $P<0.001$] on seizure behaviour, but no interaction effect between time and DMI, or DMI alone was seen. Pre-treatment with DMI did not significantly alter KA-induced seizure behaviour. Data is expressed as mean ± SEM over time (n=12).

5.6.2 Pre-treatment with DMI does not influence KA-induced hippocampal cell loss

Animals received DMI (15mg/kg) 1 hour prior to KA (10mg/kg) and were euthanised 24 hours later. Counts of viable cells revealed a reduction in the number of viable cells in KA treated rats in the CA3 and CA1 regions of the hippocampus, when compared to saline treated controls. Prior treatment with DMI did not affect the KA-induced reduction in viable cells when compared to their KA treated counterparts.

(a)



(b)

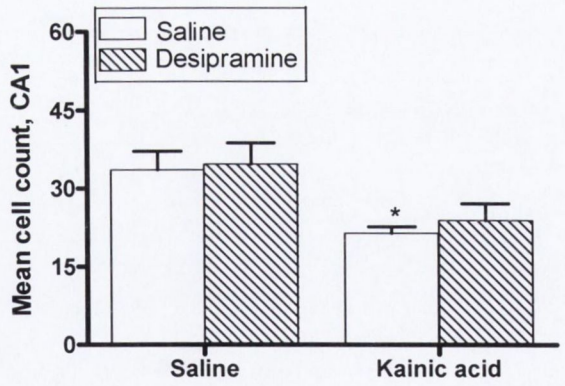


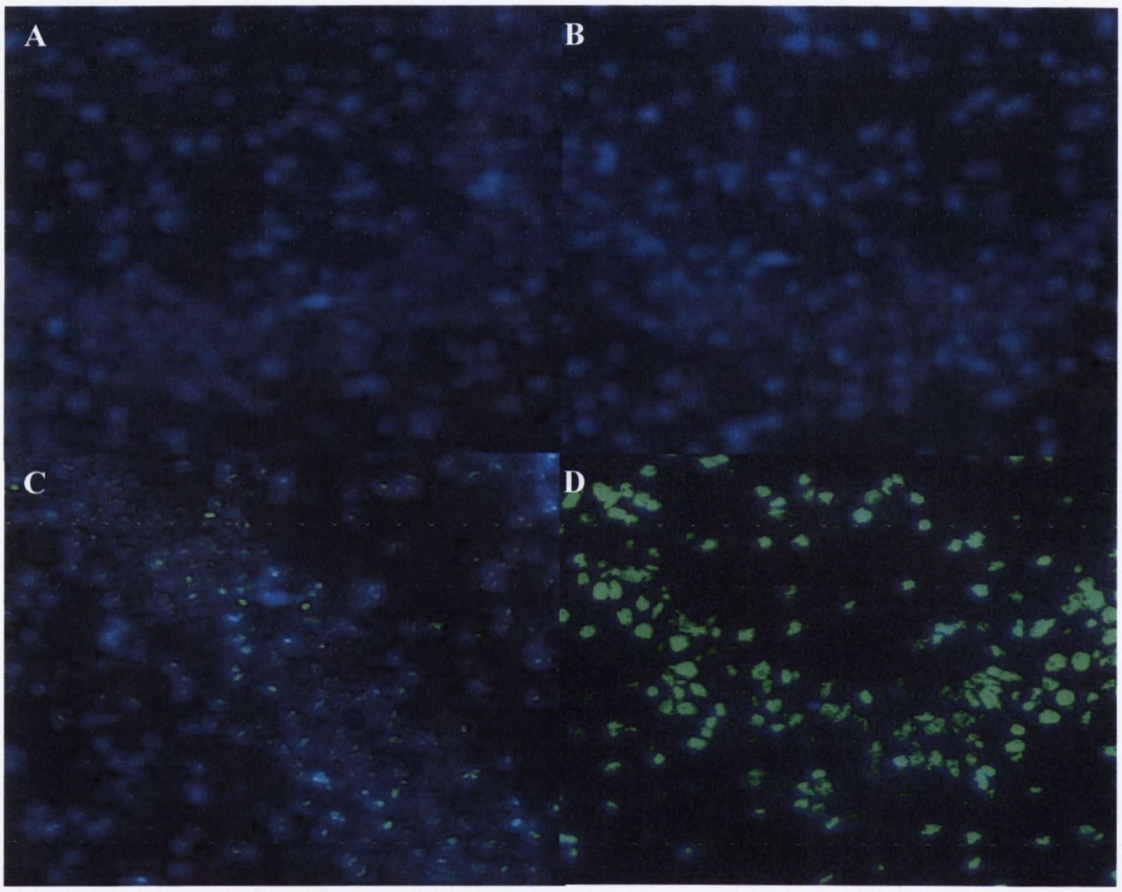
Figure 5.2 KA-induced hippocampal cell loss in the CA3 and CA1, is not attenuated by prior treatment with DMI

Two-way ANOVA showed an effect of KA on cell number in the (a) CA3 [$F(1, 27) = 35.25$, $P<0.001$] (b) CA1 [$F(1, 26) = 14.68$, $P<0.001$]. No effects of DMI were observed, either alone or in combination with KA. *Post hoc* comparisons revealed that KA provoked a significant reduction in cell number in the CA3 and CA1 when compared to saline treated controls. Data is expressed as mean±SEM (n=6-9). *P<0.05; **P<0.01 vs. saline counterparts (Neuman-Keuls *post hoc* test).

5.6.3 Pre-treatment with DMI influences KA-induced DNA fragmentation in the hippocampus

Animals received DMI (15mg/kg) 1 hour prior to KA (10mg/kg) and were euthanised 24 hours later. TUNEL staining of hippocampal slices following KA administration resulted in an increased number of TUNEL positive cells in the (i) CA3 and (ii) CA1 indicating an increase in DNA fragmentation. TUNEL staining was enhanced in the CA3 of KA treated animals which were pre-treated with DMI.

(i) CA3 of the hippocampus



(ii) CA1 of the hippocampus

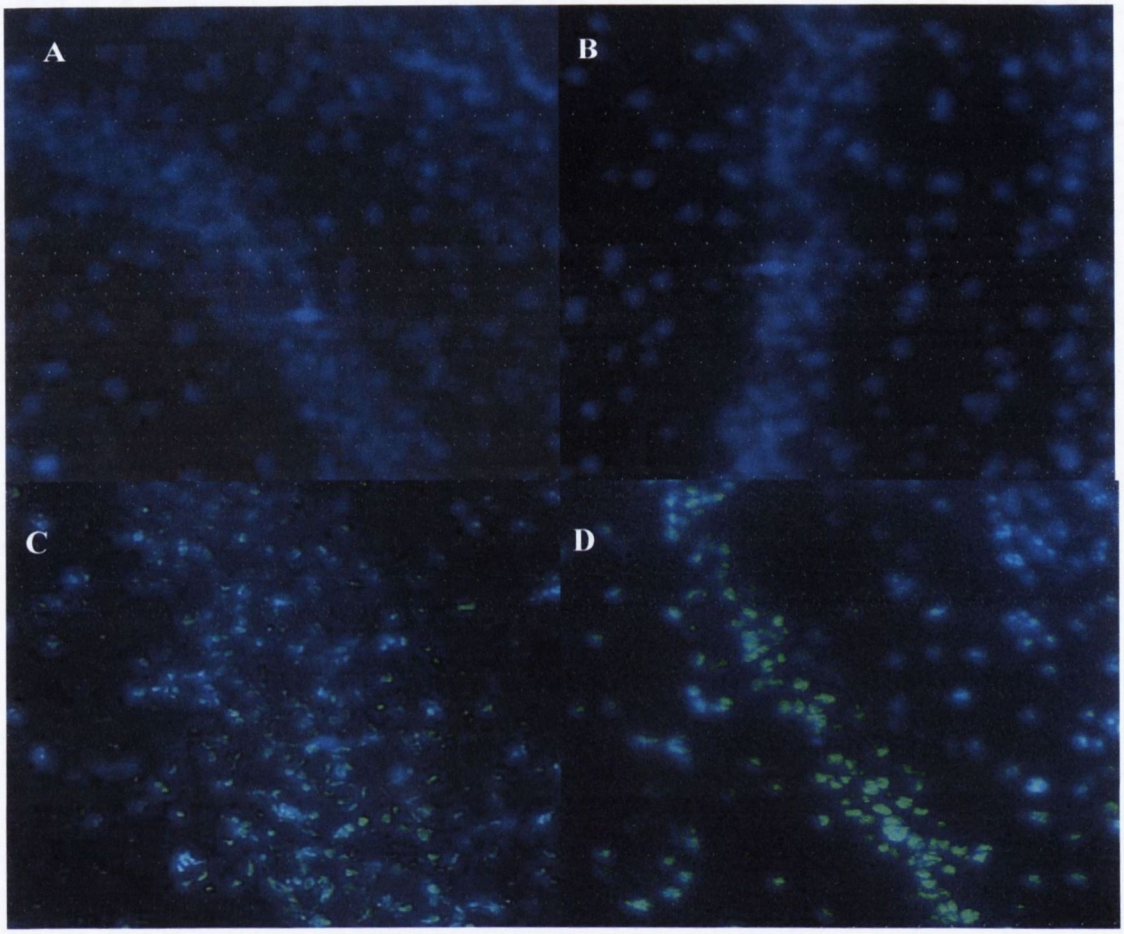
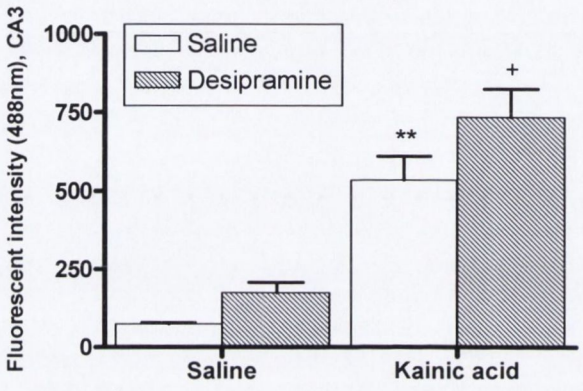


Figure 5.3 KA-induced DNA fragmentation in the CA3 and CA1, was augmented by pre-treatment with DMI

TUNEL staining of the (i) CA3 and (ii) CA1, where green indicates TUNEL positive staining and blue is a Hoechst dye to indicate co-localisation of DNA fragmentation with hippocampal cells. (A) Saline treated (B) DMI and saline (C) KA induces DNA fragmentation as indicated by TUNEL positive staining, which is (D) augmented following pre-treatment with DMI (20X magnification).

Quantification of TUNEL staining intensity in hippocampal slices shows an increase in staining intensity in the (a) CA3 and (b) CA1 following KA administration. The increase in TUNEL staining intensity in the CA3 following KA administration was enhanced by pre-treatment with DMI. A similar trend wasseen in the CA1 but this did not reach statistical significance.

(a)



(b)

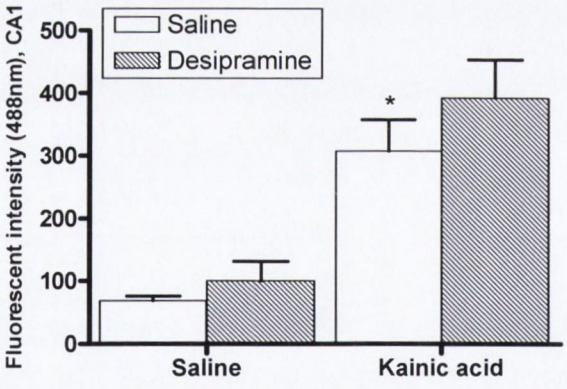


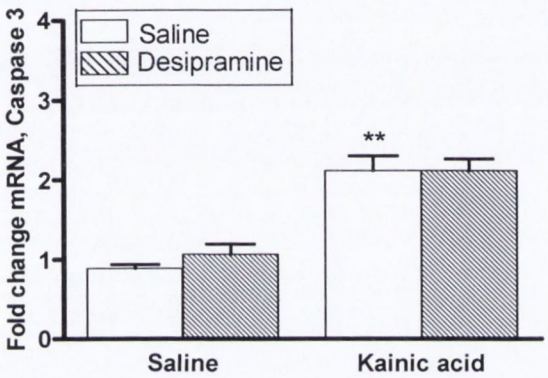
Figure 5.4 KA provokes an increase in TUNEL intensity in the (a) CA3 and (b) CA1 of the hippocampus, which is enhanced by prior treatment with DMI in the CA3.

Two-way ANOVA of mean TUNEL intensity showed an effect of KA and of DMI alone in the (a) CA3 [$F(1, 18) = 72.57$, $P<0.001$] and [$F(1, 18) = 6.24$, $P<0.05$] respectively, and (b) CA1 [$F(1, 18) = 17.06$, $P<0.001$]. No effects of DMI were observed, either alone or in combination with KA, in the CA1. *Post hoc* comparisons revealed that TUNEL intensity was increased in the CA3 and CA1 following KA administration, when compared to saline treated controls. Pre-treatment with DMI, enhanced KA-induced TUNEL intensity in the CA3, when compared to KA treated counterparts. Data is expressed as mean±SEM (n=4-8). *P< 0.05; **P<01 vs. saline counterparts. $^{+}$P<0.05 vs. KA treated counterparts (Neuman-Keuls *post hoc* test).

5.6.4 Pre-treatment with DMI does not influence KA-induced caspase-3 expression but affects caspase 3 activity, in the hippocampus

Animals received DMI (15mg/kg) 1 hour prior to KA (10mg/kg) and were euthanised 24 hours later. KA induced a 2-fold increase in hippocampal caspase-3 expression, which was not affected by prior treatment with DMI. Pre-treatment with DMI displayed a trend towards increasing caspase 3 activity, both alone and in combination with KA, however this result failed to achieve statistical significance.

(a)



(b)

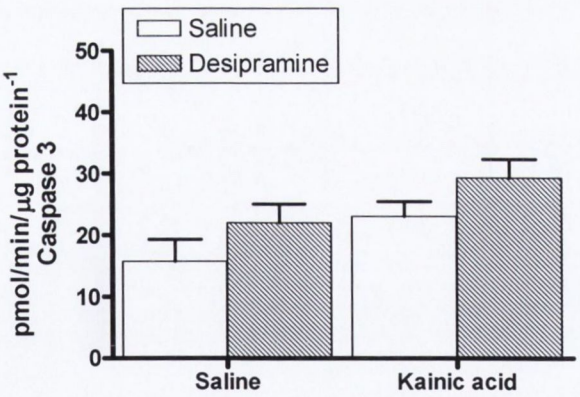


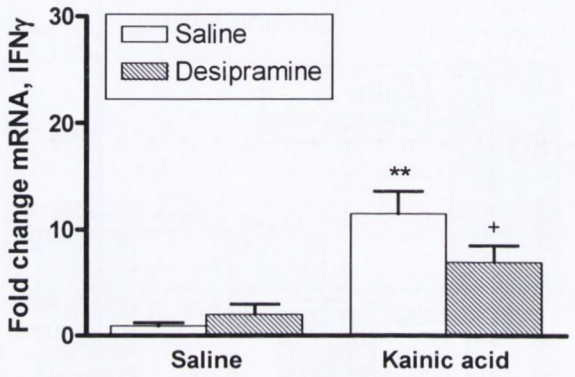
Figure 5.5 KA-induced hippocampal caspase-3 expression, and activity is not influenced, by pre-treatment with DMI

Two-way ANOVA of mRNA fold change showed an effect of KA only in (a) [$F(1, 18) = 46.9$, $P<0.001$] caspase 3 expression, and (b) [$F(1, 16) = 4.62$, $P<0.05$] caspase 3 activity. *Post hoc* comparisons revealed that KA increased caspase-3 expression when compared to saline treated controls. No effects of DMI were observed, either alone or in combination with KA. Pre-treatment with DMI shows a trend towards augmenting KA-induced caspase 3 activity, although this failed to achieve statistical significance. Data is expressed as mean ± SEM (n=4-8). **P<0.01 vs. saline counterparts (Neuman-Keuls *post hoc* test).

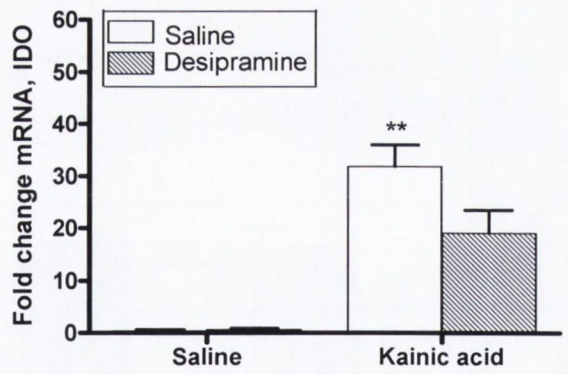
5.6.5 Pre-treatment with DMI attenuates KA-induced hippocampal IFN-γ, IDO and iNOS, but not CD-11b or GFAP expression.

Animals received DMI (15mg/kg) 1 hour prior to KA (10mg/kg) and were euthanised 24 hours later. KA induced a 10, 30, 80, 4 and 3-fold increase in hippocampal (a) IFN-γ, (b) IDO (c) iNOS (d) CD-11b and (e) GFAP expression respectively, when compared to saline counterparts. The increases in IFN-γ and iNOS were attenuated following pre-treatment with DMI.

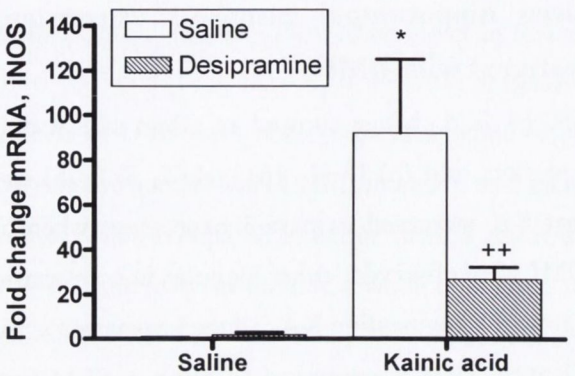
(a)



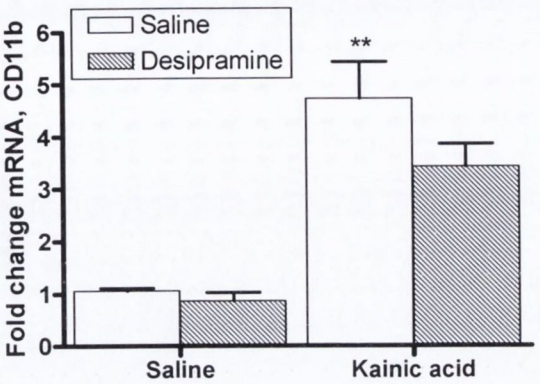
(b)



(c)



(d)



(e)

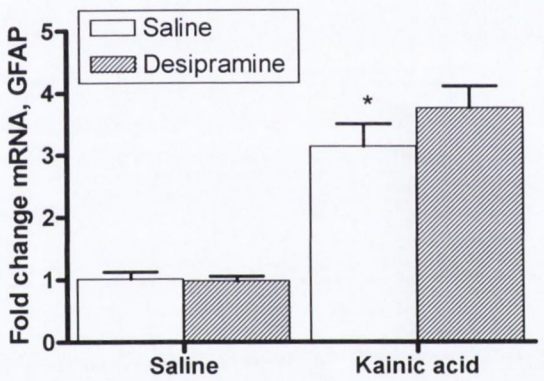


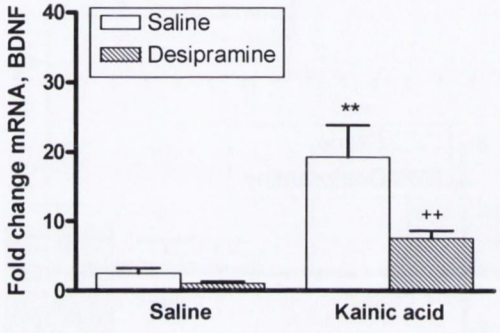
Figure 5.6 KA-induced hippocampal IFN-γ and iNOS expression is attenuated, but IDO, CD11b and GFAP expression is unchanged following pre-treatment with DMI

A two-way ANOVA of mRNA fold change revealed (a) a KA effect in IFN-γ expression [$F(1, 19) = 22.18$, $P<0.001$], and a trend towards an interaction effect [$F(1, 19) = 2.91$, $P=0.1$], (b) a KA effect only in IDO expression [$F(1, 17) = 37.4$, $P<0.001$], (c) a KA effect in iNOS expression [$F(1, 16) = 10.86$, $P<0.01$], and a trend towards an interaction effect [$F(1, 16) = 3.57$, $P=0.07$], (d) a KA effect only in CD11b expression [$F(1, 14) = 45.6$, $P<0.001$] and (e) a KA effect only in GFAP expression [$F(1, 18) = 46.9$, $P<0.001$]. No effects of DMI were observed, either alone or in combination with KA. *Post hoc* comparisons revealed that KA-induced increased IFN-γ, IDO, iNOS, CD11b and GFAP expression when compared to saline treated controls. DMI attenuated the KA-induced increase in IFN-γ and iNOS expression when compared to KA treatment alone. Data is expressed as mean ± SEM (n=4-8). *$P<0.05$; ** $P<0.01$ vs. saline counterparts, $^{+}P<0.05$; $^{++}P<0.01$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

5.6.6 Pre-treatment with DMI attenuates KA-induced BDNF mRNA expression without influencing the KA-induced increase in hippocampal BDNF concentrations

Animals received DMI (15mg/kg) 1 hour prior to KA (10mg/kg) and were euthanised 24 hours later. KA induced a 20-fold increase in BDNF mRNA expression when compared to saline controls. Pre-treatment with DMI significantly attenuated this response. Similarly, KA provoked an increase in BDNF protein when compared to saline controls. Pre-treatment with DMI did not affect this response.

(a)



(b)

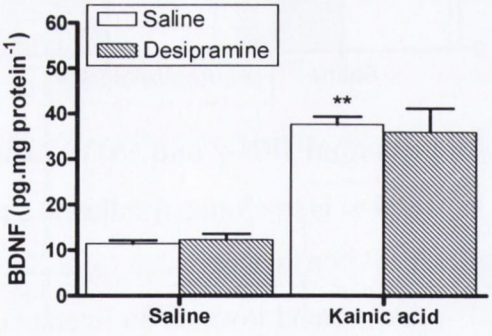


Figure 5.7 KA-induced hippocampal BDNF mRNA expression was attenuated, and BDNF protein was unchanged, following pre-treatment with DMI

A two-way ANOVA of fold change in (a) BDNF mRNA expression, showed a KA effect, an effect of DMI alone and a trend towards an interaction effect [$F(1, 17) = 16.65$, $P<0.001$], [$F(1, 17) = 5.35$, $P<0.05$], [$F(1, 17) = 3.2$, $P=0.089$], respectively. *Post hoc* comparisons revealed that KA increased BDNF mRNA expression when compared to saline treated controls, an effect which was attenuated by pre-treatment with DMI. A two-way ANOVA of (b) BDNF protein concentrations showed an effect of KA only [$F(1, 16) = 35.82$, $P<0.001$]. No effects of DMI were observed, either alone or in combination with KA. *Post hoc* comparisons revealed that KA increased BDNF protein when compared to saline treated controls. This response was not affected by pre-treatment with DMI. Data is expressed as mean ± SEM (n=3-7). **$P<0.01$ vs. saline counterparts, $^{++}P<0.01$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

5.6.7 Pre-treatment with RBX does not alter KA-induced seizure behaviour

RBX (15mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. KA induced an increase in seizure behaviour according to the Racine scale. Wet dog shakes, forelimb clonus, forelimb clonus with rearing and falling, was experienced in a similar manner by both groups. However, pre-treatment with RBX does not significantly alter KA-induced seizures.

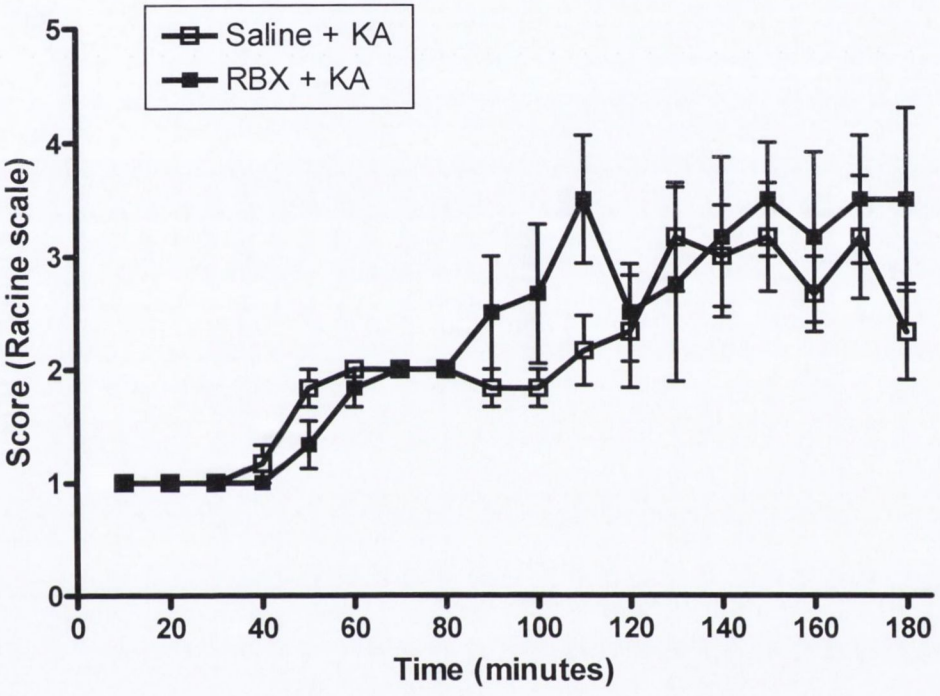
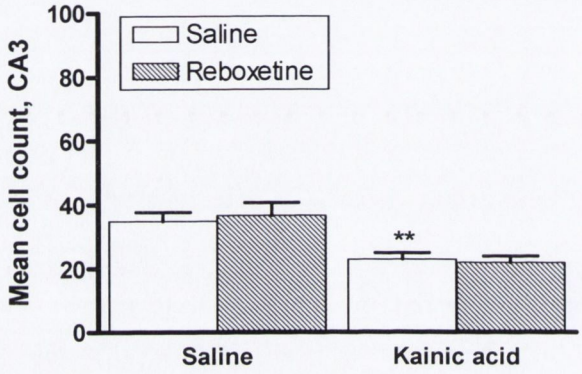


Figure 5.8 KA-induced seizure behaviour is not affected by pre-treatment with RBX
Animals were scored 1-5 according to what stage they were in, using the Racine scale for 1 minute every 10 minutes over a 3 hour observation period. A two-way repeated measures ANOVA revealed a significant effect of time [$F(17, 170) = 5.44$, $P<0.001$] and an interaction effect between time and RBX [$F(17, 170) = 1.98$, $P<0.05$] on seizure behaviour. Pre-treatment with RBX did not significantly alter KA-induced seizure behaviour. Data is expressed as mean ± SEM over time. (n=6).

5.6.8 Pre-treatment with RBX does not affect KA-induced hippocampal cell loss

RBX (15mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. Counts of viable cells revealed a reduction in the number of viable cells in KA treated rats, in the CA3 and CA1 of the hippocampus, when compared to saline treated controls. Prior treatment with RBX did not affect the KA-induced reduction in viable cells when compared to their KA treated counterparts.

(a)



(b)

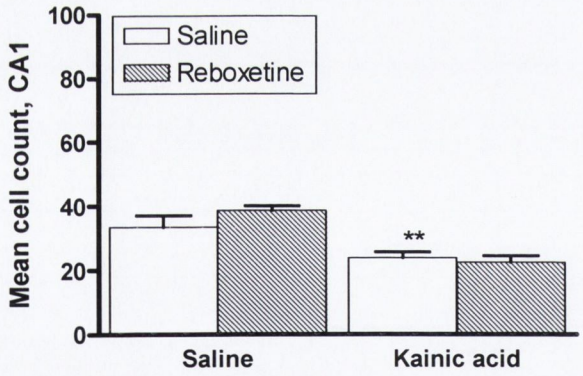


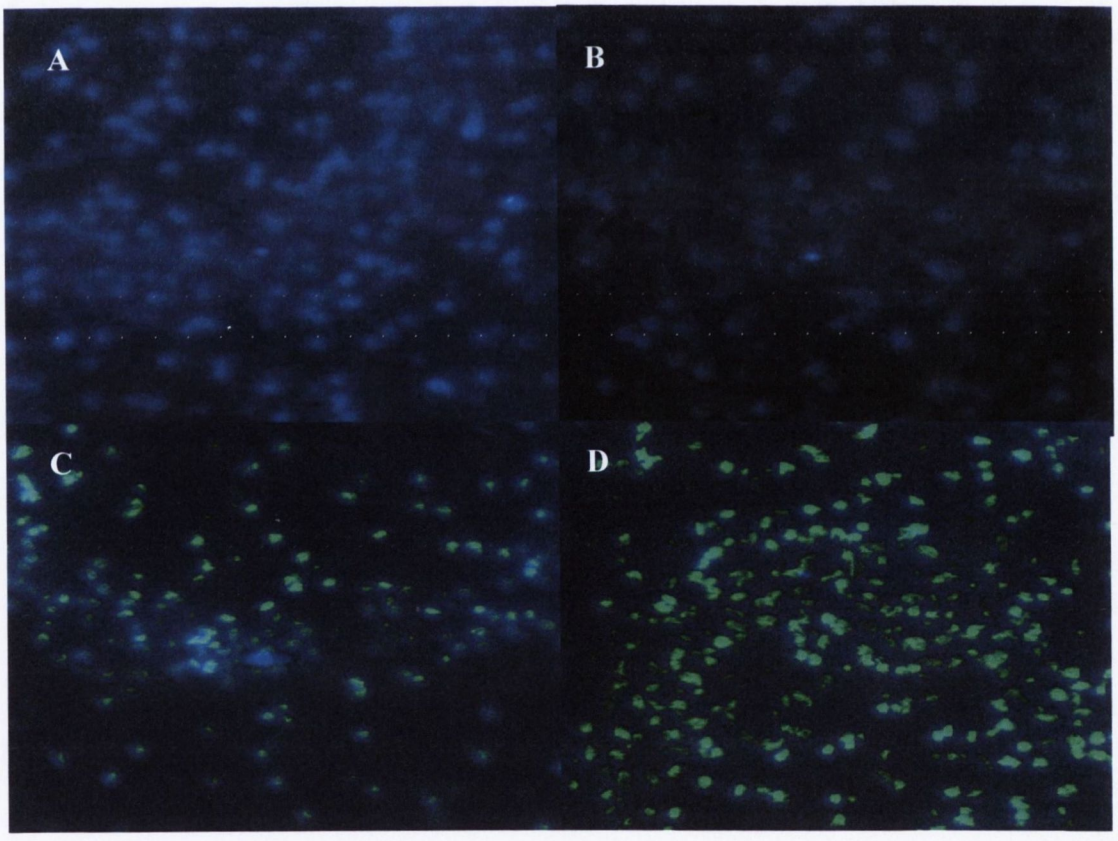
Figure 5.9 KA-induced hippocampal cell loss in the CA3 and CA1 is not attenuated by prior treatment with RBX

Two-way ANOVA showed an effect of KA on cell number in the (a) CA3 [$F(1, 17) = 24.88$, $P<0.001$] (b) CA1 [$F(1, 17) = 24.06$, $P<0.001$] following KA administration. No effects of RBX were observed, either alone or in combination with KA. *Post hoc* comparisons revealed that KA provoked a significant reduction in cell number in the CA3 and CA1 when compared to saline treated controls. Data is expressed as mean±SEM (n=4-6). **$P<0.01$ vs. saline counterparts (Neuman-Keuls *post hoc* test).

5.6.9 Pre-treatment with RBX does not significantly affect KA-induced DNA fragmentation in the hippocampus

RBX (15mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. TUNEL staining of hippocampal slices following KA administration resulted in an increased number of TUNEL positive cells in the (i) CA3 and (ii) CA1 indicating an increase in DNA fragmentation. TUNEL staining was enhanced in KA treated animals which were pre-treated with RBX, but this result failed to achieve statistical significance.

(i) CA3 of the hippocampus



(ii) CA1 of the hippocampus

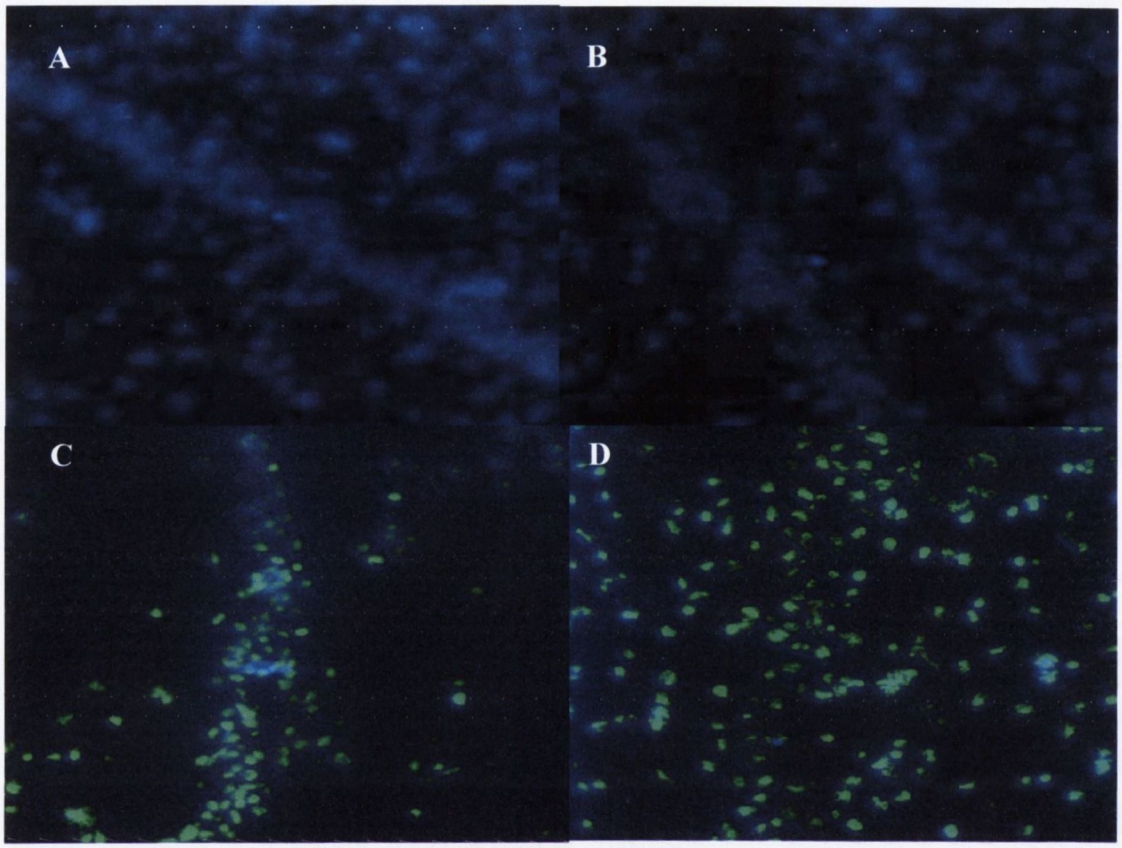
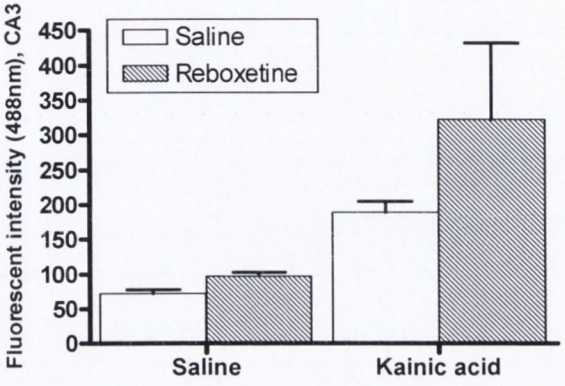


Figure 5.1010 KA-induced DNA fragmentation in the CA3 and CA1 is augmented by pre-treatment with RBX

TUNEL staining of the (i) CA3 and (ii) CA1 where green indicates TUNEL positive staining and blue is a Hoechst dye to indicate co-localisation of DNA fragmentation and hippocampal cells. (A) Saline treated (B) RBX and saline (C) KA induces DNA fragmentation as indicated by TUNEL positive staining, which appears to be (D) augmented following pre-treatment with RBX. (20X magnification)

Quantification of TUNEL staining intensity in hippocampal slices shows an increase in staining intensity in the (a) CA3 and (b) CA1 following KA administration. The increase in TUNEL staining following KA administration was enhanced by pre-treatment with RBX, albeit not significantly.

(a)



(b)

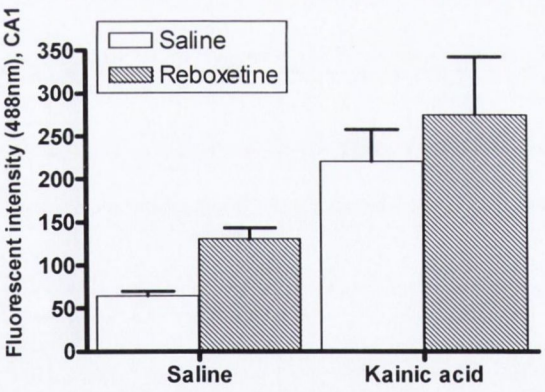


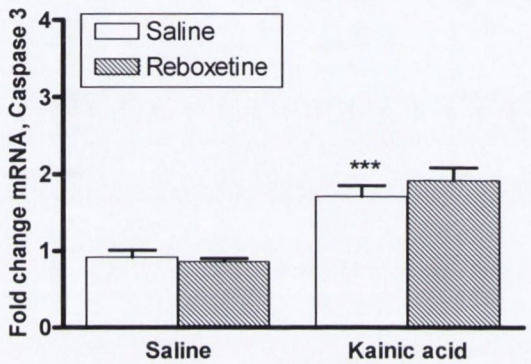
Figure 5.11 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus which is not significantly affected by RBX

A two-way ANOVA of mean TUNEL intensity showed an effect of KA only in the (a) CA3 [$F(1, 17) = 10.22$, $P<0.01$] and (b) CA1 [$F(1, 21) = 12.28$, $P<0.01$]. No effects of RBX were observed, either alone or in combination with KA. In the CA3 and CA1 it appears that RBX may enhance KA-induced DNA fragmentation, this effect however, failed to achieve significance. Data is expressed as mean±SEM (n=6). (Neuman-Keuls *post hoc* test).

5.6.10 Pre-treatment with RBX does not significantly influence KA-induced caspase-3 expression or caspase 3 activity, in the hippocampus

RBX (15mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later rats were euthanised. KA induced a 1.5-2 fold increase in hippocampal caspase 3 expression, which was not affected by prior treatment with RBX. KA induced caspase 3 activity, and pre-treatment with RBX augmented this, albeit not significantly.

(a)



(b)

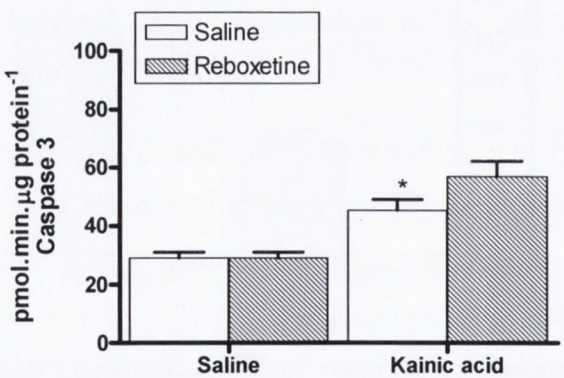


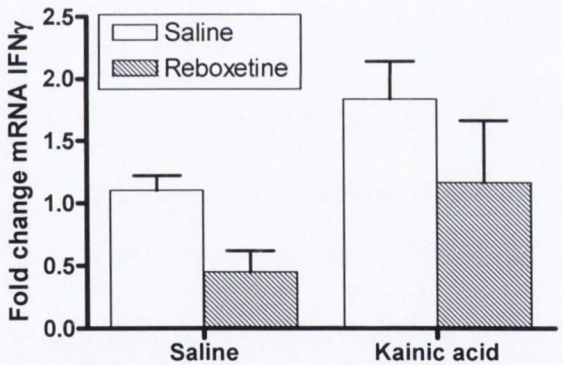
Figure 5.12 KA-induced hippocampal caspase-3 expression is unaltered, but caspase 3 activity is augmented, following pre-treatment with RBX

Two-way ANOVA showed an effect of KA only in (a) [$F(1, 13) = 48.85$, $P<0.001$] fold change in caspase 3 expression, and (b) [$F(1, 12) = 32.17$, $P<0.001$] caspase 3 activity. *Post hoc* comparisons revealed that KA increased caspase 3 expression when compared to saline treated controls, an effect which was not affected by pre-treatment with RBX. KA induced caspase 3 activity when compared to saline treated controls, an effect which was augmented following pre-treatment with RBX, albeit not significantly. No effects of RBX were observed either alone or in combination with KA. Data is expressed as mean ± SEM (n=6). *$P<0.05$; ***$P<0.001$ vs. saline counterparts, $^{+}P<0.05$ when compared to KA counterparts (Neuman-Keuls *post hoc* test).

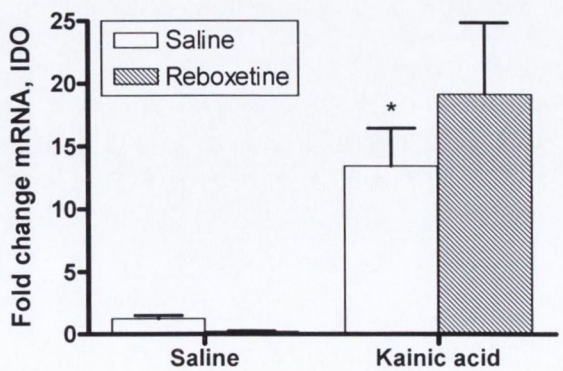
5.6.11 Pre-treatment with RBX does not influence KA-induced IFN-γ, IDO, CD-11b or GFAP expression but potentiates KA-induced iNOS expression in the hippocampus

RBX (15mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later, rats were euthanised. KA induced a 1.5, 12, 5, 4 and 1.5-fold increase in hippocampal (a) IFN-γ, (b) IDO (c) iNOS and (d) CD-11b respectively when compared to saline counterparts. KA failed to influence the expression of (e) GFAP. The increases in IFN-γ, IDO and CD-11b were not influenced following pre-treatment with RBX. By contrast pre-treatment with RBX potentiated the KA-induced increase in iNOS expression. RBX alone failed to influence the expression of each of the markers with the exception of GFAP where a reduction in expression was observed when compared to saline treated controls.

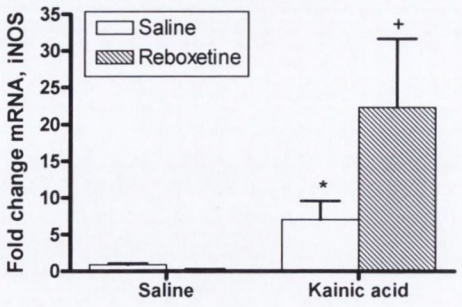
(a)



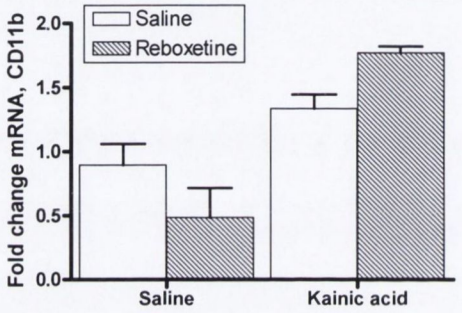
(b)



(c)



(d)



(e)

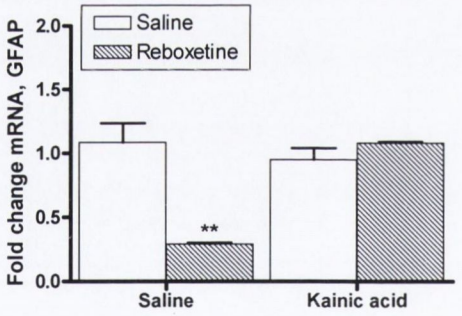


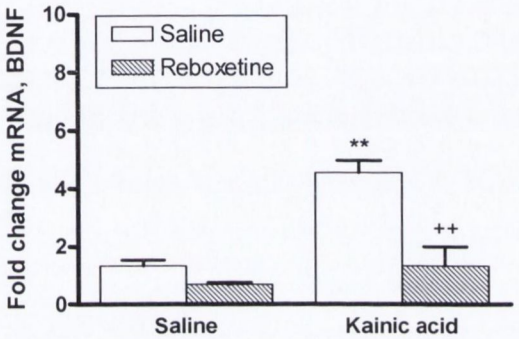
Figure 5.13 KA-induced hippocampal iNOS expression is augmented, but IFN-γ, IDO, CD-11b and GFAP expression is unchanged, following pre-treatment with RBX

Two-way ANOVA of fold change revealed (a) a KA effect [$F(1, 11) = 6.38$, $P<0.05$] and a RBX effect [$F(1, 11) = 5.41$, $P<0.05$] in IFN-γ expression (b) a KA effect only [$F(1, 10) = 21.03$, $P<0.001$] in IDO expression (c) a KA effect [$F(1, 11) = 11.82$, $P<0.01$] and a trend towards an interaction effect [$F(1, 11) = 3.82$, $P=0.07$] in iNOS expression (d) a KA effect [$F(1, 11) = 17.88$, $P<0.01$] in CD-11b expression and (e) a KA effect [$F(1, 10) = 9.43$, $P<0.05$], a RBX effect [$F(1, 10) = 9.65$, $P<0.05$], and an interaction effect [$F(1, 10) = 18.9$, $P<0.01$] in GFAP expression. *Post hoc* comparisons revealed that KA increased IDO and iNOS expression when compared to saline treated controls. RBX potentiated iNOS expression when compared to KA treated controls. RBX alone reduced GFAP expression when compared to saline treated controls. Data is expressed as mean ± SEM (n=3-5). *$P<0.05$; **$P<0.01$ vs. saline counterparts, $^{+}P<0.05$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

5.6.12 Pre-treatment with RBX attenuates KA-induced BDNF expression and BDNF concentrations

RBX (15mg/kg) was administered 1 hour prior to KA (10mg/kg) and 24 hours later, rats were euthanised. KA induced a 5-fold increase in BDNF expression when compared to saline controls. In a similar fashion KA provoked an increase in BDNF protein when compared to saline controls. Pre-treatment with RBX attenuated both of these responses.

(a)



(b)

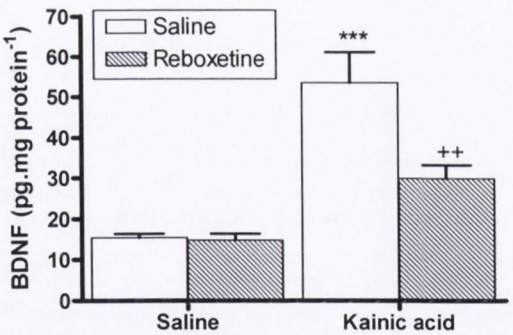


Figure 5.14 KA-induced hippocampal BDNF expression and BDNF protein is attenuated following pre-treatment

A two-way ANOVA of fold change in (a) BDNF expression showed an interaction between KA and RBX, a RBX alone effect and a KA effect [$F(1, 10) = 11.33$, $P<0.001$], [$F(1, 10) = 25.49$, $P<0.01$], [$F(1, 10) = 25.66$, $P<0.001$], respectively, and (b) BDNF concentrations showed an interaction between KA and RBX, a RBX alone effect and a KA effect [$F(1, 15) = 4.73$, $P<0.05$], [$F(1, 15) = 5.3$, $P<0.05$], [$F(1, 15) = 25.27$, $P<0.001$], respectively. *Post hoc* comparisons revealed that KA increased BDNF expression and concentration when compared to saline treated controls. RBX attenuated the KA-induced increase in BDNF expression and concentration, when compared to KA treated controls. Data is expressed as mean ± SEM (n=3-6). **P<0.0; ***P<0.001 vs. saline counterparts, $^{++}$P<0.01 vs. KA counterparts (Neuman-Keuls *post hoc* test).

Influence of the β adrenoceptor antagonist-propranolol alone and in combination with reboxetine on KA-induced inflammatory and degenerative changes in the hippocampus

5.7 Experimental protocol

PRP (10mg/ml) was prepared in 0.89% (w/v) saline and administered in an injection volume of 1ml/kg giving a dose of 10mg/kg. RBX was prepared as previously described. PRP (10 mg/kg) and/or RBX (15mg/kg) were administered (i.p) one hour prior to KA (10mg/kg, s.c.) administration.

Seizure behaviour was monitored for 3 hours following KA administration as previously described and animals were euthanised 24 hours after KA administration.

5.7.1 Pre-treatment with PRP and RBX in combination reduces KA-induced seizure behaviours

Animals received PRP (10mg/kg) and RBX (15mg/kg) 1 hour prior to KA administration and were euthanised 24 hours later. KA induced an increase in seizure behaviour according to the Racine scale, which was augmented following pre-treatment with RBX. This effect was ameliorated following co-administration of RBX and PRP. Those rats receiving RBX, PRP and KA experienced a much milder seizure pattern compared to KA controls, with very few experiencing stage 4 or 5 seizures.

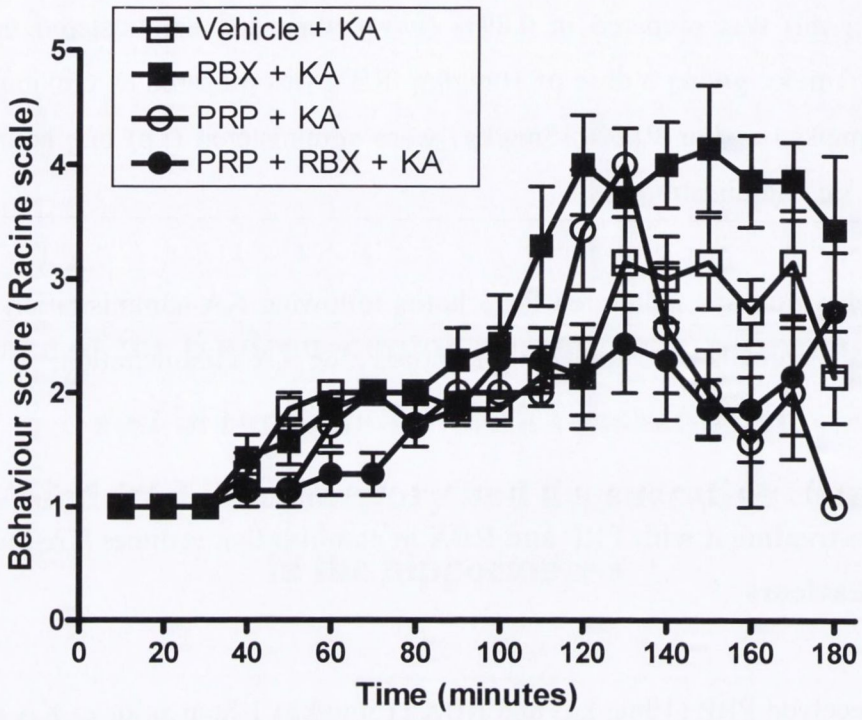


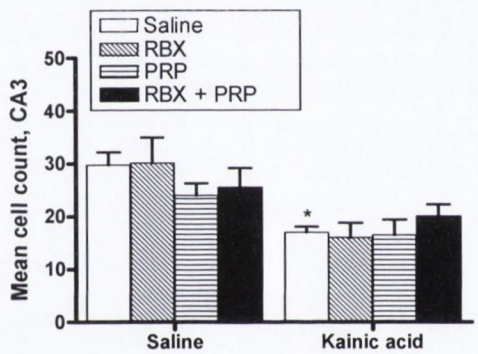
Figure 5.15 RBX augments KA induced seizure behaviours, an effect which is ameliorated by the co-administration of PRP and RBX

Animals were scored 1-5 according to what stage they were in, using the Racine scale for 1 minute, every 10 minutes, over a 3 hour observation period. A three-way repeated measures ANOVA of behavioural scores showed an effect of PRP [$F(1,22) = 14.45$, $P<0.001$], PRP x RBX [$F(1,22) = 6.11$, $P<0.05$], time [$F(17, 374) = 27.37$, $P<0.001$], PRP x time [$F(17,374)= 2.48$ $P<0.001$], and an interaction between RBX, PRP and time [$F(17, 374) = 2.38$, $P<0.01$]. Data is expressed as mean ± SEM over time. (n=7).

5.7.2 Pre-treatment with RBX or PRP treatment, alone or in combination, does not affect KA-induced hippocampal cell loss

Animals received PRP (10mg/kg) and RBX (15mg/kg) 1 hour prior to KA injection and were euthanised 24 hours later. Counts of viable cells in the CA3 and CA1 regions of the hippocampus showed that KA induced a decrease in cell number, when compared to saline controls. Pre-treatment with RBX and PRP had no effect on KA-induced cell loss in the CA3 and CA1 regions of the hippocampus when compared to their KA treated counterparts.

(a)



(b)

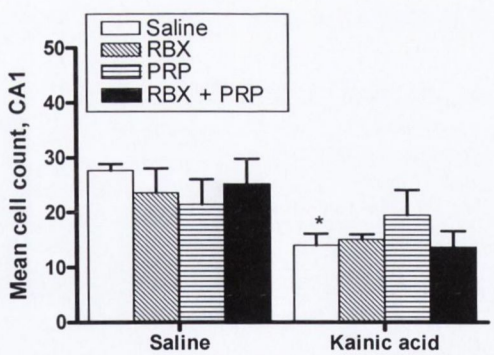


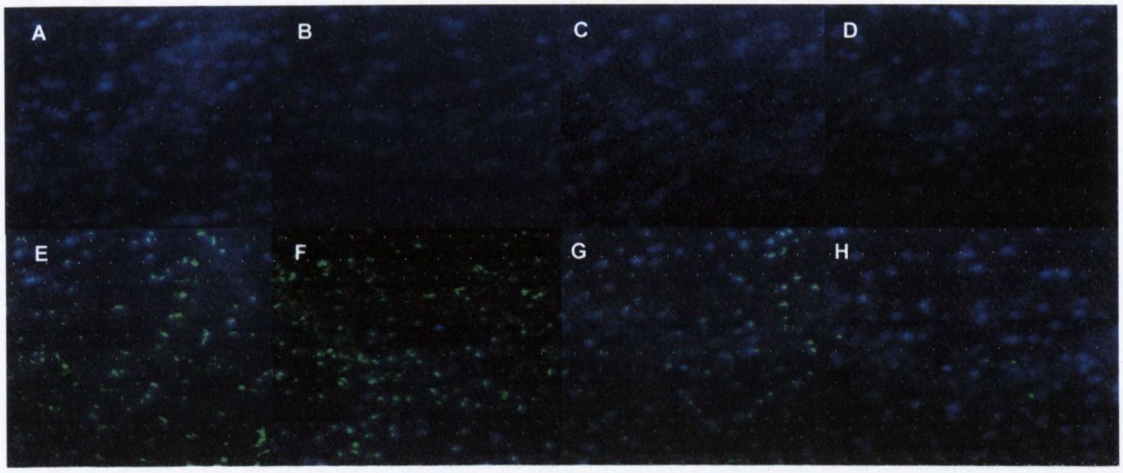
Figure 5.16 KA-induced hippocampal cell loss in the CA3 and CA1 is unchanged following pre-treatment with RBX, PRP or RBX and PRP in combination

A three-way ANOVA showed an effect of KA only on cell number in the (a) CA3 [$F(1, 44) = 18.28$, $P<0.001$] and (b) CA1 [$F(1, 17) = 5.91$, $P<0.05$] regions, following KA administration. No effects of RBX or PRP, alone or in combination, were observed either alone or in combination with KA *Post hoc* comparisons revealed that KA provoked a significant reduction in cell number in the CA3 and CA1 when compared to saline-treated controls. Data is expressed as mean ± SEM (n=4-9). * $P<0.05$ vs. saline counterparts (Neuman-Keuls *post hoc* test).

5.7.3 Pre-treatment with RBX and PRP influences KA-induced DNA fragmentation

Animals received PRP (10mg/kg) and RBX (15mg/kg) 1 hour prior to KA injection and were euthanised 24 hours later. TUNEL staining of hippocampal slices following KA administration resulted in an increased number of TUNEL positive cells in the (i) CA3 and (ii) CA1 regions of the hippocampus, indicating an increase in DNA fragmentation. Pre-treatment with RBX enhanced the KA-induced increase in TUNEL positive cells, an effect which was ameliorated by co-administration of PRP and RBX. These results failed to achieve statistical significance.

(i) CA3 of the hippocampus



(ii) CA1 of the hippocampus

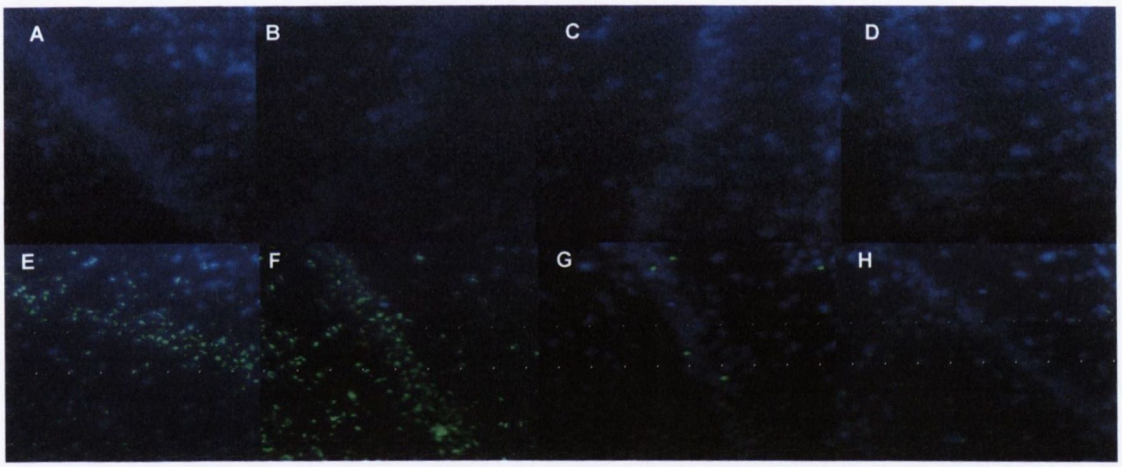
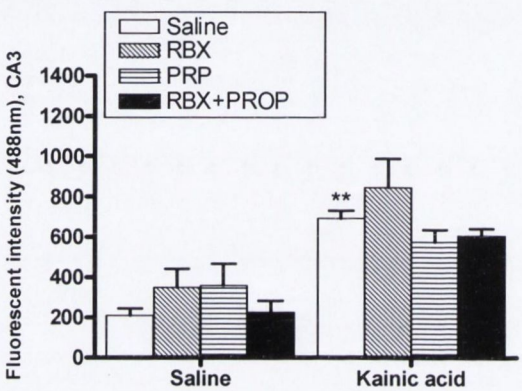


Figure 5.1717 KA-induced DNA fragmentation in the CA3 and CA1 is influenced by pre-treatment with PRP and RBX in combination

TUNEL staining of the (i) CA3 and (ii) CA1 where green indicates TUNEL positive staining and blue is a Hoechst dye to indicate co-localisation of DNA fragmentation and hippocampal cells. A) Saline treated (B) RBX and saline (C) PRP and saline (D) RBX and PRP and saline (E) KA induces DNA fragmentation as indicated by TUNEL positive staining, which is (F) enhanced following pre-treatment with RBX (G) KA-induced TUNEL staining is reduced following pre-treatment with PRP (H) co-administration of PRP with RBX attenuates the RBX induced augmentation of KA-induced DNA fragmentation. (20X magnification).

Quantification of TUNEL staining intensity in hippocampal slices shows an increase in staining intensity in the (a) CA3 and (b) CA1 following KA administration. Pre-treatment with RBX enhanced the KA-induced increase in TUNEL staining, although this did not achieve statistical significance. In contrast, pre-treatment with PRP, and RBX and PRP in combination, attenuates the RBX+KA-induced increase in TUNEL positive staining in the CA1.

(a)



(b)

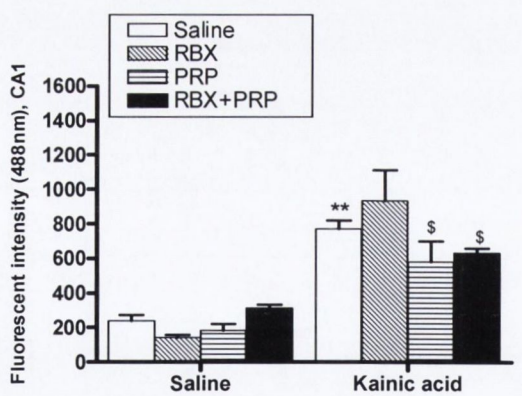


Figure 5.18 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus which is influenced by pre-treatment with RBX and PRP

Three-way ANOVA of mean TUNEL intensity showed an effect of KA in the (a) CA3 [$F(1, 40) = 44.22$, $P<0.001$] and (b) CA1 [$F(1, 23) = 97.81$, $P<0.001$] and an interaction effect between KA and PRP, in the CA1 [$F(1, 23) = 8.54$, $P<0.01$]. *Post hoc* comparisons revealed that TUNEL intensity was increased in the CA3 and CA1 following KA administration when compared to saline treated controls. Pre-treatment with PRP, and RBX and PRP in combination, attenuated RBX+KA-induced TUNEL intensity in the CA1, when compared to RBX+KA treated counterparts. Data is expressed as mean±SEM (n=4-9). **$P<0.01$ vs. saline counterparts; $^{\$}P<0.05$ vs. RBX and KA counterparts (Neuman-Keuls *post hoc* test).

5.7.4 Pre-treatment with PRP and RBX alone or in combination do not influence KA-induced caspase 3 expression in the hippocampus

Animals received PRP (10mg/kg) and RBX (15mg/kg) 1 hour prior to KA injection and were euthanised 24 hours later. KA induced a 2-fold increase in hippocampal caspase 3 expression, when compared to saline counterparts. This change was not influenced by prior treatment with PRP or RBX alone or in combination.

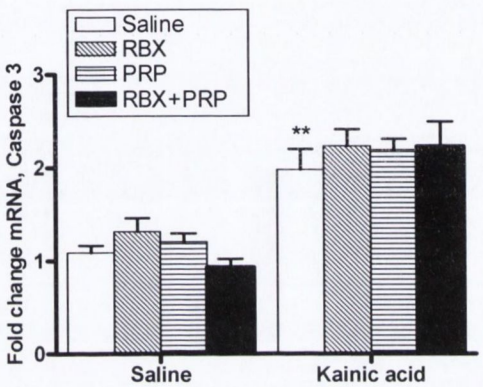


Figure 5.19 KA-induced hippocampal caspase 3 expression was not influenced by pre-treatment with PRP and RBX alone or in combination

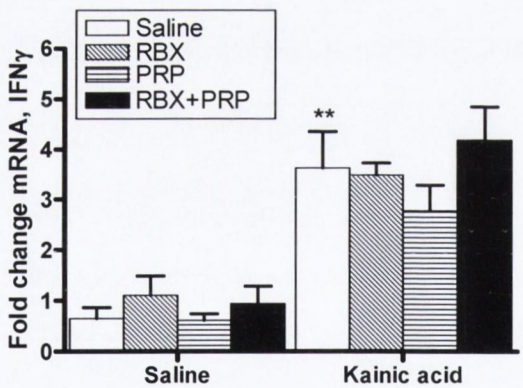
Three-way ANOVA of fold change in expression showed an effect of KA only [$F(1, 52) = 69.66$, $P<0.001$]. *Post hoc* comparisons revealed that KA increased caspase 3 expression when compared to saline treated controls. No effects of PRP or RBX alone or in combination were observed either alone or in combination with KA. Data is expressed as mean ± SEM (n=6). **P<0.01 vs. saline counterparts (Neuman-Keuls *post hoc* test).

It was not possible to accurately measure caspase 3 activity in these samples.

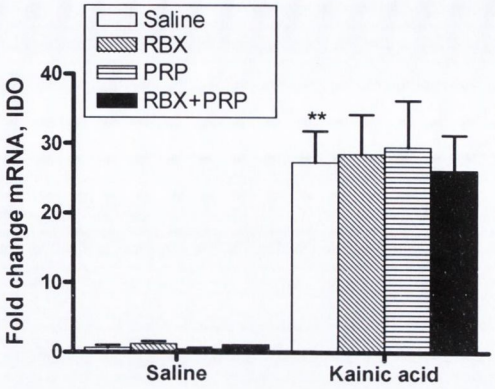
5.7.5 Effect of PRP or RBX alone and in combination on KA-induced IFN-γ, IDO, iNOS, CD-11b and GFAP expression in the hippocampus

Animals received PRP (10mg/kg) and RBX (15mg/kg) 1 hour prior to KA injection and were euthanised 24 hours later. KA induced a 4, 30, 30, 5 and 4-fold increase in hippocampal (a) IFN-γ, (b) IDO (c) iNOS (d) CD-11b and (e) GFAP respectively when compared to saline counterparts. The increases in IFN-γ, IDO and GFAP were not influenced following pre-treatment with PRP or RBX alone or in combination. Pre-treatment with RBX potentiated the KA-induced increase in iNOS expression, whereas PRP blocked KA-induced iNOS and the ability of RBX to augment KA-induced iNOS expression. Prior treatment with PRP alone and in combination with RBX attenuated KA-induced CD-11b expression.

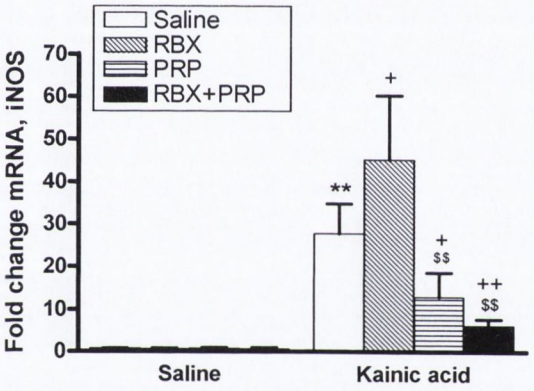
(a)



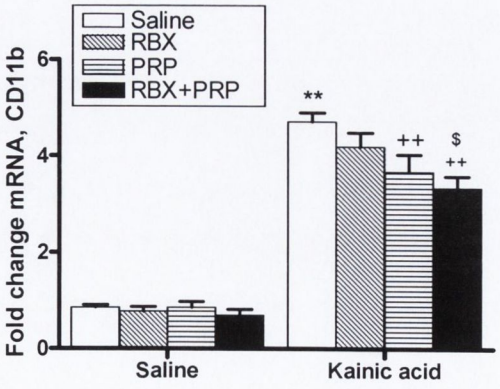
(b)


Saline
RBX
PRP
RBX+PRP
Fold change mRNA, IDO
40
30
20
10
0
**
Saline
Kainic acid

(c)


Saline
RBX
PRP
RBX+PRP
Fold change mRNA, iNOS
70
60
50
40
30
20
10
0
**
+
+
$$
++
$$
Saline
Kainic acid

(d)


Saline
RBX
PRP
RBX+PRP
Fold change mRNA, CD11b
6
4
2
0
**
++
$
++
Saline
Kainic acid

(e)

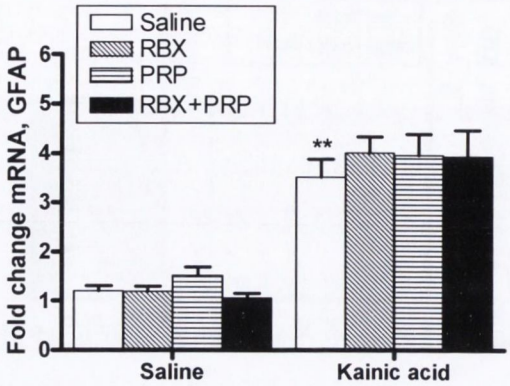


Figure 5.20 KA-induced hippocampal iNOS and CD-11b expression was attenuated, and IFN-γ, IDO and GFAP expression was unchanged following pre-treatment with RBX and PRP

Three-way ANOVA of fold change in expression showed (a) effects of KA only on IFN-γ expression [F (1, 42) = 61.2, P<0.001], (b) effects of KA only on IDO expression [F (1, 22) = 53.18, P<0.001] (c) an interaction between PRP and KA [F (1, 41) = 15.43, P<0.001], a PRP effect [F (1, 41) = 15.35, P<0.001], as well as a KA effect [F (1, 41) = 42.35, P<0.001] on iNOS expression, no effect of RBX alone or in combination with KA or PRP was observed, (d) an interaction between PRP and KA [F (1, 49) = 11.42, P<0.01], as well as a KA effect [F (1, 49) = 567.5, P<0.001], a RBX effect [F (1, 49) = 4.2, P<0.05], and a PRP effect [F (1, 49) = 13.63, P<0.001], on CD11b expression, and (e) an effect of KA only on GFAP expression [F (1, 49) = 137.5, P<0.001]. *Post hoc* comparisons revealed that KA increased IFN-γ, IDO, iNOS, CD11b and GFAP expression. Pre-treatment with RBX or PRP either alone or in combination had no effect on IFN-γ, IDO and GFAP expression. Pre-treatment with RBX, enhanced KA-induced expression of iNOS. However, pre-treatment with PRP and the combination of PRP and RBX ameliorated the RBX-enhanced KA-induced iNOS expression, and also decreased KA-induced CD11b expression. Data is expressed as mean±SEM over time (n=4-9). **P<0.01 vs. saline counterparts, $^{+}$P<0.05; $^{++}$P<0.01 vs. KA counterparts; $^{\$}$P<0.05; $^{\$\$}$P<0.01 vs. RBX+KA counterparts (Neuman-Keuls *post hoc* test).

5.7.6 Pre-treatment with RBX but not PRP attenuates KA-induced BDNF expression

Animals received PRP (10mg/kg) and RBX (15mg/kg) 1 hour prior to KA injection and were euthanised 24 hours later. KA induced a 6-fold increase in BDNF expression when compared to saline controls. KA-induced BDNF expression was not influenced by prior treatment with PRP. PRP however partially attenuated the ability of RBX to suppress KA-induced BDNF expression in the hippocampus, but this failed to achieve statistical significance.

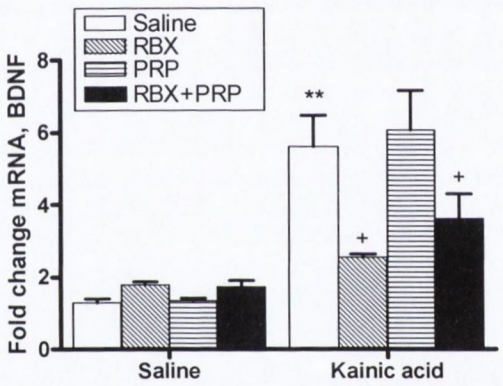


Figure 5.21 RBX attenuates KA-induced BDNF expression, an effect which is unaffected by co-administration of PRP and RBX

Three-way ANOVA of fold change of BDNF expression showed an interaction between KA and RBX [$F(1, 50) = 11.69$, $P<0.01$], a RBX effect [$F(1, 50) = 6.14$, $P<0.05$] and a KA effect [$F(1, 50) = 38.86$, $P<0.001$]. *Post hoc* comparisons revealed that KA increased BDNF expression when compared to saline treated controls. This response was attenuated by prior treatment with RBX but not PRP. RBX and PRP in combination also attenuated KA-induced BDNF expression. Data is expressed as mean ± SEM (n=4-9). **$P<0.001$ vs. saline counterparts, $^{+}P<0.05$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

It was not possible to measure BDNF protein concentrations in this study due to repeated technical difficulties.

Influence of the β_2 adrenoceptor agonist clenbuterol on KA-induced inflammation and degeneration in the hippocampus

5.8 Experimental protocol

CLN (0.5mg/ml) was prepared in 0.89% (w/v) saline and administered in an injection volume of 1 ml/kg giving a dose of 0.5 mg/kg. It was injected i.p. 1 hour prior to KA (10mg/kg, s.c). Seizure behaviour was monitored for 3 hours following KA administration as previously described and animals were euthanised 24 hours after KA administration.

5.8.1 Pre-treatment with CLN does not alter KA-induced seizure behaviour

Animals received CLN (0.5 mg/kg) 1 hour prior to KA and were euthanised 24 hours later. KA induced an increase in seizure behaviour according to the Racine scale which was not affected following pre-treatment with CLN. Wet dog shakes were observed in both groups, but a higher frequency was seen in the KA alone group. Forelimb clonus, with rearing and falling was observed in both groups.

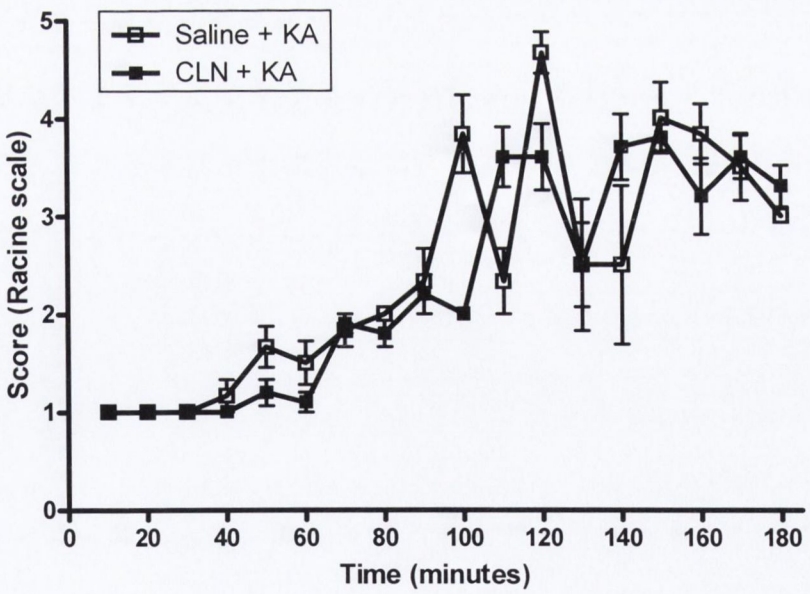


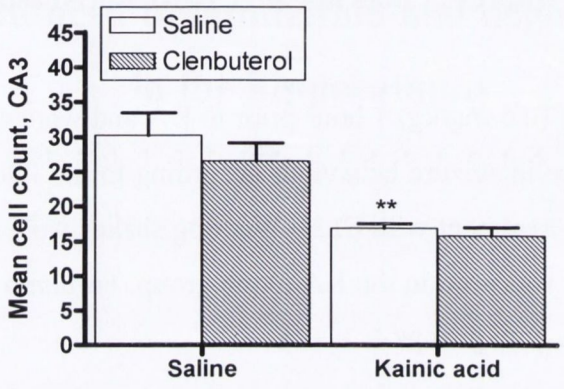
Figure 5.22 KA-induced seizure behaviour is affected by pre-treatment with CLN

Animals were scored 1-5 according to what stage they were in, using the Racine scale for 1 minute every 10 minutes over a 3 hour observation period. A two-way repeated measures ANOVA revealed a significant effect of time [$F(17, 221) = 30.43$, $P<0.001$] and an interaction between time and CLN [$F(17, 221) = 3.018$, $P<0.001$] on seizure behaviour. Pre-treatment with CLN significantly ameliorated KA-induced seizure behaviour at 100 minutes. Data is expressed as mean ± SEM over time. (n=9).

5.8.2 Pre-treatment with CLN does not influence KA-induced hippocampal cell loss

Animals received CLN (0.5 mg/kg) 1 hour prior to KA and were euthanised 24 hours later. Counts of viable cells revealed a reduction in the number of viable cells in KA treated rats in the CA3 and CA1 of the hippocampus, when compared to saline treated controls. Prior treatment with CLN did not affect the KA-induced reduction in viable cells when compared to their KA treated counterparts.

(a)



(b)

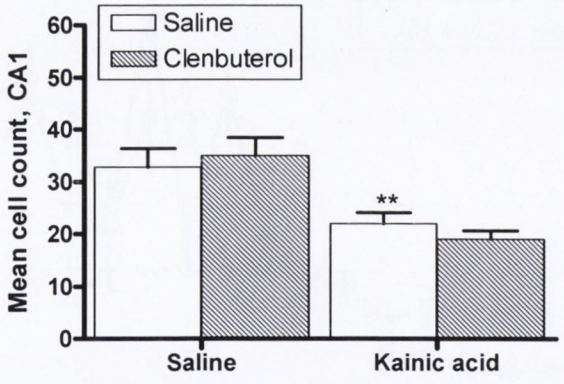


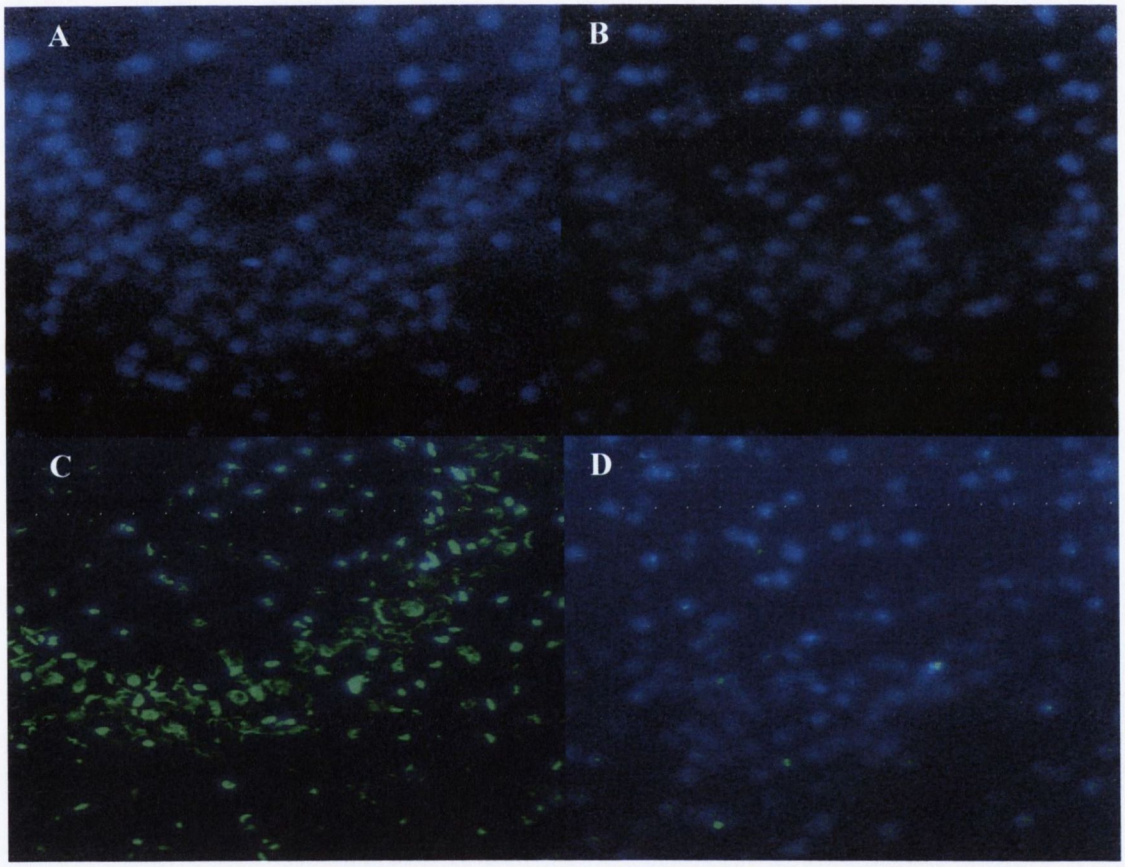
Figure 5.23 KA-induced hippocampal cell loss in the CA3 and CA1 is not attenuated by prior treatment with CLN

Two-way ANOVA showed an effect of KA only on cell number in the (a) CA3 [$F(1, 21) = 36.57$, $P<0.001$] (b) CA1 [$F(1, 21) = 24.39$, $P<0.001$]. No effects of CLN were observed either alone or in combination with KA. *Post hoc* comparisons revealed that KA provoked a significant reduction in cell number in the CA3 and CA1 when compared to saline treated controls. Data is expressed as mean±SEM (n=4-8). *P<0.05; **P<0.01 vs. saline counterparts (Neuman-Keuls *post hoc* test).

5.8.3 Pre-treatment with CLN attenuates KA-induced DNA fragmentation in the hippocampus

Animals received CLN (0.5 mg/kg) 1 hour prior to KA and were euthanised 24 hours later. TUNEL staining of hippocampal slices following KA administration resulted in an increased number of TUNEL positive cells in the (i) CA3 and (ii) CA1 indicating an increase in DNA fragmentation. KA-induced TUNEL staining was attenuated following pre-treatment with CLN.

(i) CA3 of the hippocampus



(ii) CA1 of the hippocampus

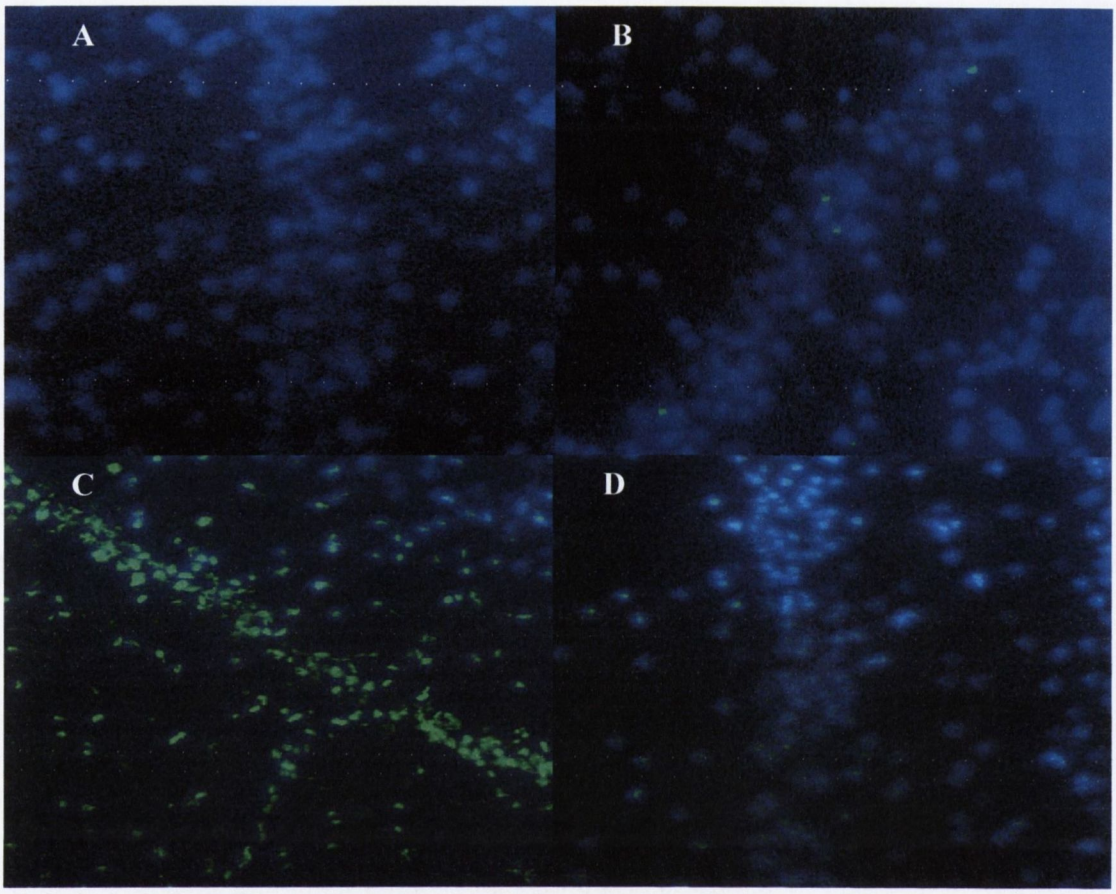
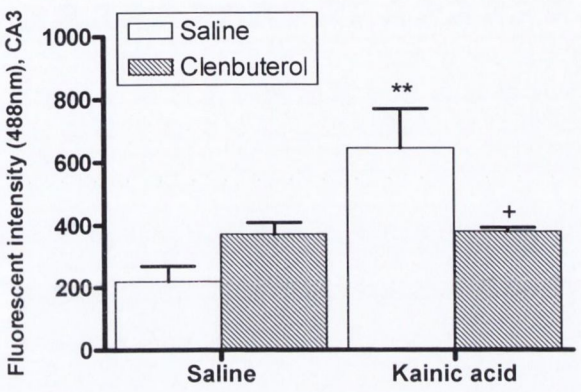


Figure 5.2424 KA-induced DNA fragmentation in the CA3 and CA1, which was attenuated by pre-treatment with CLN

TUNEL staining of the (i) CA3 and (ii) CA1 where green indicates TUNEL positive staining and blue is a Hoechst dye to indicate co-localisation of DNA fragmentation and hippocampal cells. (A) Saline treated (B) CLN and saline (C) KA induces DNA fragmentation as indicated by TUNEL positive staining, which is (D) ameliorated following pre-treatment with CLN. (20X magnification)

Quantification of TUNEL staining intensity in hippocampal slices shows an increase in staining in the (a) CA3 and (b) CA1 following KA administration. The increase in TUNEL staining following KA administration was attenuated by pre-treatment with CLN.

(a)



(b)

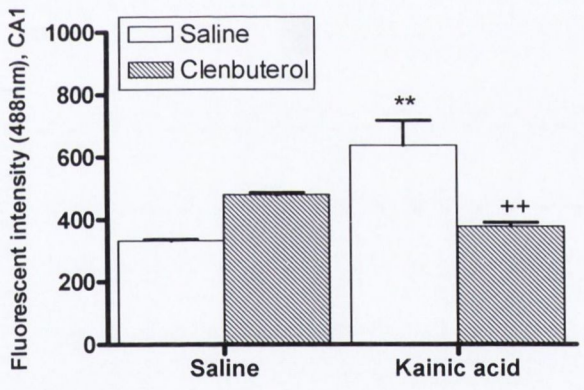


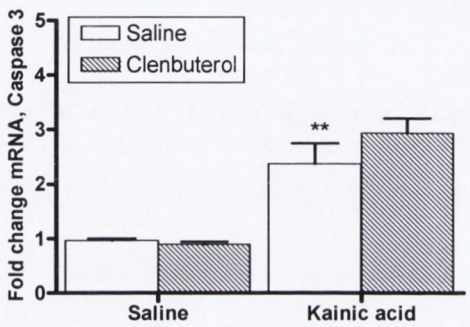
Figure 5.25 KA provokes an increase in DNA fragmentation in the (a) CA3 and (b) CA1 of the hippocampus which is attenuated by prior treatment with CLN

Two-way ANOVA of mean TUNEL intensity showed an interaction effect and an effect of KA in the (a) CA3 [$F(1, 18) = 7.58$, $P<0.05$], [$F(1, 18) = 8.2$, $P<0.05$] and (b) a KA effect [$F(1, 17) = 4.78$, $P<0.05$], an interaction effect [$F(1, 17) = 17.99$, $P<0.001$], in the CA1. No effect of CLN alone was seen in either the CA3 or CA1. *Post hoc* comparisons revealed that TUNEL intensity was increased in the CA3 and CA1 following KA administration when compared to saline treated controls, an effect which was attenuated by pre-treatment with CLN. Data is expressed as mean±SEM (n=6). *$P< 0.05$; ** $P < 0.01$ vs. saline counterparts. $^{+}P<0.05$; $^{++}P<0.01$ vs. KA treated counterparts (Neuman-Keuls *post hoc* test).

5.8.4 Pre-treatment with CLN does not influence KA-induced caspase-3 expression, but significantly attenuates KA-induced caspase 3 activity in the hippocampus

Animals received CLN (0.5 mg/kg) 1 hour prior to KA and were euthanised 24 hours later. KA induced a 2-3 fold increase in hippocampal caspase 3 expression, which was not affected by prior treatment with CLN. KA induced activated caspase 3, which was ameliorated following pre-treatment with CLN

(a)



(b)

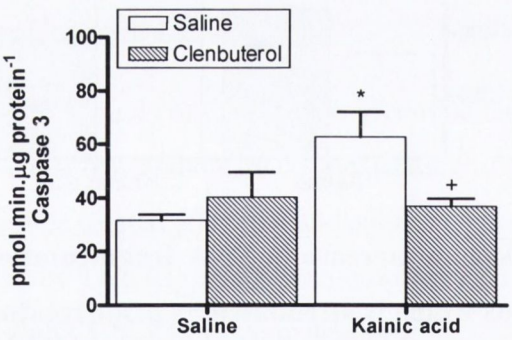


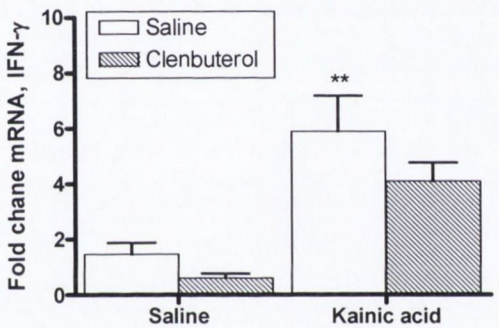
Figure 5.26 KA-induced hippocampal caspase-3 expression is not influenced, but caspase 3 activity is significantly ameliorated, by pre-treatment with CLN

Two-way ANOVA showed an effect of KA only in (a) [$F(1, 20) = 36.03$, $P<0.001$] caspase 3 expression and (b) a KA effect [$F(1, 21) = 4.75$, $P<0.05$], and an interaction effect between CLN and KA [$F(1, 21) = 7.15$, $P<0.05$] in caspase 3 activity. *Post hoc* comparisons revealed that KA increased caspase 3 expression when compared to saline treated controls, an effect which was not affected by pre-treatment with CLN. *Post hoc* comparisons revealed that KA increased caspase 3 activity when compared to saline treated controls, an effect which was ameliorated by pre-treatment with CLN. Data is expressed as mean ± SEM (n=4-9). **$P<0.01$ vs. saline counterparts, $^{+}P<0.05$ vs. KA treated counterparts (Neuman-Keuls *post hoc* test).

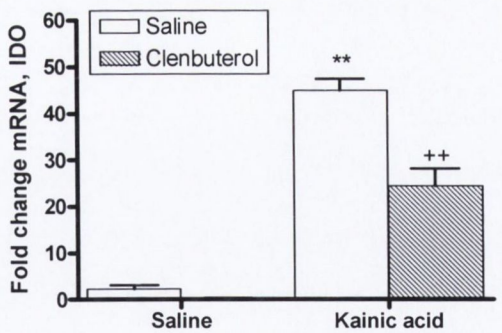
5.8.5 Pre-treatment with CLN affects KA-induced hippocampal IFN-γ, IDO, iNOS, CD11b and GFAP expression.

Animals received CLN (0.5 mg/kg) 1 hour prior to KA and were euthanised 24 hours later. KA induced a 6, 40, 40, 5 and 3-fold increase in hippocampal (a) IFN-γ, (b) IDO (c) iNOS (d) CD-11b and (e) GFAP expression respectively when compared to saline counterparts. The increases in IDO and iNOS were attenuated following pre-treatment with CLN. The increase in CD-11b was not affected by CLN. Pre-treatment with CLN augmented KA-induced GFAP expression, however this failed to achieve statistical significance.

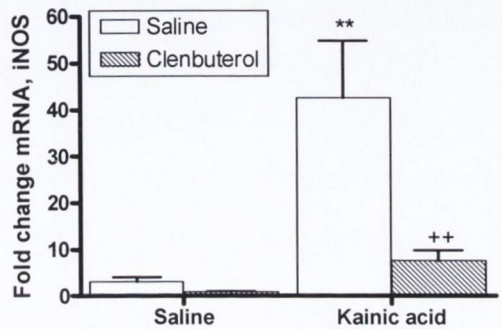
(a)



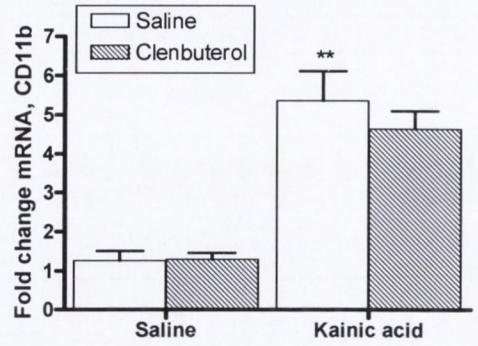
(b)



(c)



(d)



(e)

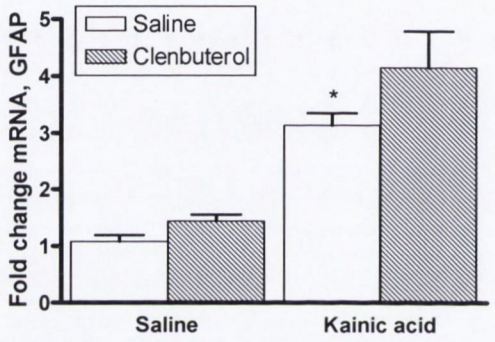


Figure 5.27 KA-induced IDO and iNOS expression is attenuated, GFAP expression is augmented, and CD11b and IFN-γ expression is unaffected, following pre-treatment with CLN

Two-way ANOVA of fold change showed a KA effect in (a) IFN-γ expression [$F(1, 20) = 20.44$, $P<0.001$] (b) IDO expression [$F(1, 17) = 70{,}69$, $P<0.001$], as well as a CLN effect [$F(1, 17) = 8.07$, $P<0.05$], and an interaction effect [$F(1, 17) = 5.42$, $P<0.05$] (c) iNOS expression [$F(1, 19) = 15.74$, $P<0.001$], as well as a CLN effect [$F(1, 19) = 10.09$, $P<0.01$], and an interaction effect [$F(1, 19) = 7.8$, $P<0.05$], (d) CD-11b expression [$F(1, 12) = 45.33$, $P<0.001$] and (e) GFAP expression [$F(1, 17) = 25.77$, $P<0.001$]. *Post hoc* comparisons revealed that KA increased IFN-γ, IDO, iNOS, CD-11b and GFAP expression when compared to saline treated controls. CLN attenuated the KA-induced increase in IDO and iNOS expression when compared to KA treated controls. CLN augmented KA-induced GFAP expression when compared to KA treatment alone, although this failed to achieve statistical significance. Data is expressed as mean ± SEM (n=4-9). *$P<0.05$; **$P<0.01$ vs. saline counterparts, $^{+}P<0.05$; $^{++}P<0.01$; $^{++}P<0.01$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

5.8.6 Pre-treatment with CLN augments NGF expression, KA-induced BDNF expression and BDNF concentrations in the hippocampus

Animals received CLN (0.5 mg/kg) 1 hour prior to KA and were euthanised 24 hours later. KA failed to induce NGF expression in the hippocampus, but pre-treatment with CLN, in combination with KA resulted in an increased expression of NGF mRNA.

KA induced a 3 fold increase in BDNF expression when compared to saline controls. Pre-treatment with CLN enhanced this response. In a similar fashion KA provoked an increase in BDNF protein when compared to saline controls. Pre-treatment with CLN also enhanced this response.

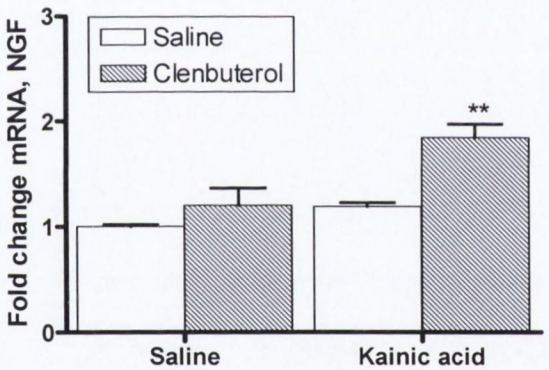
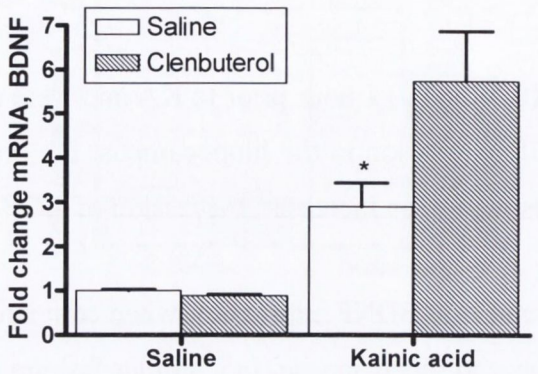


Figure 5.28 KA failed to induce hippocampal NGF expression alone, but pre-treatment with CLN resulted in an increase in NGF expression, following KA administration

Two-way ANOVA of fold change in NGF expression showed an effect of KA [$F(1, 20) = 9.2$, $P<0.01$] and an effect of CLN [$F(1, 20) = 9.75$, $P<0.01$]. *Post hoc* comparisons revealed that CLN in combination with KA, increased NGF expression when compared to saline or CLN counterparts. Data is expressed as mean ± SEM (n=4-9). **$P<0.01$ vs. saline counterparts, (Neuman-Keuls *post hoc* test).

(a)



(b)

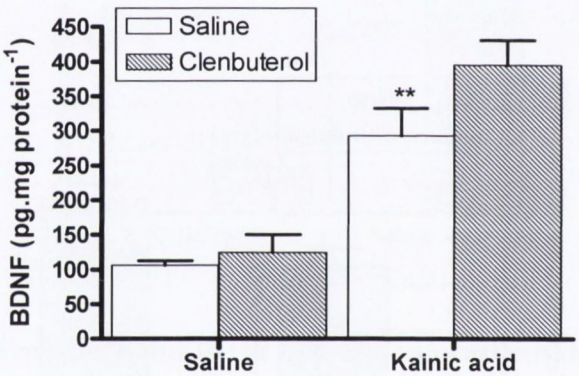


Figure 5.29 KA-induced hippocampal BDNF expression and BDNF protein shows a trend towards an increase following pre-treatment with CLN.

Two-way ANOVA of fold change in (a) BDNF expression showed an effect of KA only [$F(1, 20) = 12.35$, $P<0.01$]. *Post hoc* comparisons revealed that KA increased BDNF expression when compared to saline treated controls. CLN enhanced the KA-induced increase in BDNF expression when compared to KA treatment alone, but this did not achieve statistical significance. Two-way ANOVA of (b) BDNF concentrations showed an effect of KA only [$F(1, 19) = 38.37$, $P<0.001$. No effects of CLN were observed either alone or in combination with KA. *Post hoc* comparisons revealed that KA increased BDNF when compared to saline treated controls. This response was enhanced by prior treatment with CLN, although this did not achieve statistical significance. Data is expressed as mean ± SEM (n=4-9). * $P<0.05$; ** $P<0.01$ vs. saline counterparts, $^{+}P<0.05$ vs. KA counterparts (Neuman-Keuls *post hoc* test).

Table 5.1 Summary of DMI results

Target	DMI and Vehicle	KA and Vehicle	DMI and KA
Hippocampal cell loss CA3	–	↓	–
Hippocampal cell loss CA1	–	↓	–
DNA fragmentation CA3	–	↑	↑
DNA fragmentation CA1	–	↑	–
Caspase 3	–	↑	–
Caspase 3 activity	–	–	–
IFN-γ	–	↑	↓
IDO	–	↑	–
iNOS	–	↑	↓
CD11b	–	↑	–
GFAP	–	↑	–
BDNF mRNA	–	↑	↓
BDNF protein	–	↑	–

Table 5.2 Summary table of RBX results

Target	RBX and Vehicle	KA and Vehicle	RBX and KA
Hippocampal cell loss CA3	–	↓	–
Hippocampal cell loss CA1	–	↓	–
DNA fragmentation CA3	–	↑	–
DNA fragmentation CA1	–	↑	–
Caspase 3	–	↑	–
Caspase 3 activity	–	↑	–
IFN-γ	–	–	–
IDO	–	↑	–
iNOS	–	↑	↑
CD11b	–	–	–
GFAP	↓	–	–
BDNF mRNA	–	↑	↓
BDNF protein	–	↑	↓

Table 5.3 Summary table of RBX and PRP results

Target	KA and Vehicle	RBX and KA	PRP and KA	PRP+RBX+KA
Hippocampal cell loss CA3	↓	–	–	–
Hippocampal cell loss CA1	↓	–	–	–
DNA fragmentation CA3	↑	–	–	–
DNA fragmentation CA1	↑	–	↓	↓
Caspase 3	↑	–	–	–
IFN-γ	↑	–	–	–
IDO	↑	–	–	–
iNOS	↑	↑	↓	↓
CD11b	↑	–	↓	↓
GFAP	↓	–	–	–
BDNF mRNA	↑	↓	–	↓
BDNF protein	–	↑	↓	↓

Table 5.4 Summary of CLN results

Target	CLN and Vehicle	KA and Vehicle	CLN and KA
Hippocampal cell loss CA3	–	↓	–
Hippocampal cell loss CA1	–	↓	–
DNA fragmentation CA3	–	↑	↓
DNA fragmentation CA1	–	↑	↓
Caspase 3	–	↑	–
Caspase 3 activity	–	↑	↓
IFN-γ	–	↑	–
IDO	–	↑	↓
iNOS	–	↑	↓
CD11b	–	↑	–
GFAP	–	↑	–
NGF	–	–	↑
BDNF mRNA	–	↑	–
BDNF protein	–	↑	–

Summary of results where (–) indicates no significant change relative to either Vehicle or KA and Vehicle controls, where appropriate, and (↑/↓) indicates an increase/decrease relative to either Vehicle or KA and Vehicle controls, where appropriate.

5.9 Discussion

Firstly, the current study determined if pre-treatment with DMI or RBX could elicit neuroprotective effects in the KA model of hippocampal excitotoxicity. In a similar manner to the studies in the previous chapter, all animals receiving KA showed seizures and related stereotyped behaviours within 3 hours. Expression of IFN-γ, CD-11b and IDO in addition to iNOS and GFAP were increased in the hippocampus 24 hours post KA administration. The increase in these inflammatory markers was accompanied by an increase in expression of the apoptotic marker caspase 3, increase in TUNEL staining and a reduction in Cresyl violet staining of viable hippocampal cells. Pre-treatment with DMI, significantly attenuated the KA-induced increase in hippocampal IFN-γ, iNOS and BDNF expression and enhanced DNA fragmentation in the CA3, but failed to influence KA-induced seizures, hippocampal cell loss, caspase-3, CD-11b or GFAP expression or BDNF protein. RBX produced a similar response to DMI in respect of cellular degenerative markers, yet in contrast to DMI, failed to influence KA-induced IFN-γ and IDO expression, enhanced iNOS expression and attenuated both the KA-induced increase in BDNF expression and BDNF protein in the hippocampus.

Secondly, in order to determine if the exacerbating actions of the NRIs were mediated by β-adrenergic receptors, PRP was assessed alone and in combination with RBX, following KA administration. Pre-treatment with PRP attenuated KA-induced increase in hippocampal iNOS, CD-11b and DNA fragmentation, but failed to influence KA-induced hippocampal cell loss, IFN-γ, IDO, caspase-3, GFAP or BDNF expression. PRP was however able to attenuate the ability of RBX to enhance KA-induced DNA fragmentation and to attenuate KA-induced BDNF expression, although these results did not achieve statistical significance. Finally, pre-treatment with the β adrenergic receptor agonist CLN attenuated KA-induced DNA fragmentation, caspase 3 activity, IFN-γ, IDO and iNOS expression whilst demonstrating a trend towards enhancing KA-induced GFAP and BDNF expression in addition to BDNF concentrations in the hippocampus. Overall the results support a modulatory role for NA in the regulation of KA-induced excitotoxicity and suggest that the β-adrenoceptor is at least partially responsible for the neuroprotective properties attributed to the modulation of NA transmission centrally. Further consideration of these changes is considered in turn below.

5.9.1 DMI attenuates KA related inflammation yet augments KA-induced apoptosis in the hippocampus

DMI showed a trend towards influencing the behavioural response to KA, with animals in the DMI and KA group exhibiting few stage two behaviours over the first hour, compared to the corresponding KA control group. However, over the course of the second hour of observation, DMI showed a trend towards enhancing the behavioural response to KA, where animals were noted to show more stage 4 and 5 behaviours, compared to the KA control group. The response was very severe in some cases and resulted in death in 2/6 animals, on two separate occasions. There is a body of evidence which infers that NA has a protective role against seizure progression, in a variety of seizure models (for reviews see: Giorgi *et al.,* 2004; Weinshenker and Szot, 2002). However, a number of studies to date which have explored the influence of DMI in seizure progression have reported that DMI lowers the seizure threshold and markedly increases the incidence and intensity of seizures (Escorihuela *et al.,* 1989; Peterson *et al.,* 1985; Arai *et al.,* 2003). Coupled with its ability to block the NET, DMI also blocks α_1 adrenoceptors, an effect which has been shown to be pro-convulsant (Weinshenker *et al.,* 2001; Weinshenker and Szot, 2002) and which may account for the ability of DMI to enhance KA-induced seizures, as observed in the present investigation. Alternatively, it is not unreasonable to suggest that an increase in extracellular NA, subsequent to inhibition of the nerve terminal transporter, may lead to the activation of the inhibitory α_2 adrenoceptor, thus decreasing the overall availability of synaptic NA and contributing to an overall pro-convulsant action.

The effects of chronic administration of DMI was examined (results not presented) in the KA model of excitotoxicity. A dose regime of 7.5 mg/kg (i.p) everyday for 14 days, with a period of 24 hours between the last injection and the administration of KA, was employed. This does was chosen based on previous experiments carried out in our lab, where the animals did not tolerate a higher dose very well, and dramatic weight loss was seen both in that experiment and the one carried out here. It was expected that chronic administration of DMI would confer protective effects, similar to those reported in the literature, such as the upregulation of BDNF and anti-apoptotic factors such as Bcl-2. However, no changes in cell loss or cytokine, caspase 3 and BDNF expression was seen, when compared to KA counterparts, and it was not possible to determine DNA fragmentation. This may have

been due to a number of reasons, such as dose, duration and length of time between the last dose and KA treatment. As no significant changes were seen in this study, it was decided to focus on the acute modulation of NA in the KA model.

In the current study, acute pre-treatment with DMI did not afford protection against KA-induced hippocampal cell loss. Previously, DMI has been reported to be neurotoxic *in vitro* in cultured dorsal root ganglion cells, by reducing neuronal numbers when compared to controls (Haller *et al.,* 2007). By contrast, Haynes *et al.,* (2004) reported that DMI, when administered chronically to rats, affords protection against DEX-induced neuronal damage in the hippocampus and striatum, but no such protective actions were observed following either acute or chronic administration in the current study. Despite the fact that cell loss is evident 24 hours after KA administration, as indicated by cresyl violet staining, perhaps 24 hours is an insufficient time allowed to observe subsequent detrimental or protective effects.

Pre-treatment with DMI enhanced KA-induced DNA fragmentation in the CA3 of the hippocampus, as represented by increased TUNEL staining. These changes suggest that DMI may enhance KA-induced apoptosis in the hippocampus. Caspase 3 activity is not altered significantly, and this is probably due to the fact the tissue was snap frozen before being prepared in Krebs buffer. It is known that seizures are accompanied by an array of cellular and molecular disturbances, and that the seizure intensity correlates to the extent of cellular damage (Henshall and Simon, 2005; Fujikawa, 2005; Haut *et al.,* 2004; Shorvon, 2002). Thus, the added intensity of seizure behaviour observed following DMI treatment may correspond to an increase in apoptosis.

DMI has previously been reported to suppress pro-inflammatory cytokine production both *in vitro* and *in vivo* (Roumestan *et al.,* 2007; Diamond *et al.,* 2006; Reynolds *et al.,* 2005; Kubera, 2000; Ignatowski *et al.,* 1997; O'Sullivan *et al.,* 2009). In line with this, pre-treatment with DMI attenuated KA-induced IFN-γ, IDO and iNOS expression. As previously discussed, changes in IDO expression may be related to the corresponding suppression of IFN-γ, which is a potent inducer of IDO expression. The trend towards a suppression of IDO expression in turn suggests that pre-treatment with DMI inhibits activation of the KP. In addition to these markers, it was also of interest to determine if these changes were associated with a suppression of microglia, astrocytes or both. Whilst

KA-induced CD11b expression was, moderately but not significantly reduced, GFAP expression was not altered by pre-treatment with DMI. Thus it is possible that DMI exerts its anti-inflammatory effects via a suppression of microglia, as was demonstrated previously by O'Sullivan and colleagues (2009) *in vitro* in primary cortical glial cells stimulated by LPS. The notable anti-inflammatory activity of DMI might suggest that DMI confers protective properties. However, as observed following DEX administration, it is possible to suppress KA-induced inflammation without affording protective benefits. Overall, the results suggest that seizure intensity is most closely associated with the degree of cell loss and apoptosis observed, and that inflammation is playing a secondary role over the 24 hour period following KA administration when initial cell loss occurs.

KA induces the expression of BDNF in the brain, an effect thought to occur as a compensatory mechanism to protect the brain against the ensuing damage caused by KA (Dugich-Djordjevic *et al.,* 1992; Zafra *et al.,* 1990; Thoenen *et al.,* 1991). Consistent with the literature, KA induced BDNF expression and increased protein concentration in the hippocampus. Pre-treatment with DMI attenuated KA-induced BDNF expression, whilst no changes were observed with regard to the KA-induced increase in hippocampal BDNF concentrations. Other investigators have reported that BDNF expression is unaltered following chronic treatment with DMI in rats (Coppell and Zetterstrom, 2003), whilst some have reported a reduction in the expression of BDNF in rats, following chronic and acute treatment with DMI (Khundakar and Zetterstrom, 2006; Donnici *et al.,* 2008). The results of the current study are in line with these findings. It is possible that the DMI-related reduction in BDNF, is associated with the increase in apoptotic damage and DNA fragmentation, where signalling and expression pathways regulating growth factor expression and cellular apoptosis synergise to enhance KA-related damage.

Chronic antidepressant treatment has been shown to increase the expression of BDNF (Duman *et al.,* 1997; Russo-Neustadt *et al.,* 2000; Balu *et al.,* 2008). Balu and colleagues (2008) reported that chronic treatment (21days) with DMI (10 mg/kg) resulted in an increase of BDNF protein levels in the frontal cortex, but not the hippocampus. Conversely, chronic treatment (10 mg/kg for 21 days) with DMI has been shown to increase BDNF mRNA in the dentate gyrus (Jacobsen and Mork, 2004). In the chronic DMI study carried out in tandem with this thesis (results not presented), no change in BDNF mRNA was seen in the hippocampus. This may be explained by dose or timing

issues, where 7.5mg/kg was not a high enough dose, or perhaps, 14 days was not long enough to elicit an increase in BDNF expression. Further examination of this is required to examine if chronic administration of DMI can confer neuroprotection in the KA-model of excitotoxicity.

As there is accumulating evidence in support of a neuroprotective role for NA both *in vivo* and *in vitro,* the results obtained in this study appear to work against this. To my knowledge the results of this study represent the first appraisal of DMI in the KA model of hippocampal excitotoxicity *in vivo*. A number of factors can be put forward to account for the effects observed. Firstly, many studies reporting the neuroprotective effects of DMI involve chronic administration (Jeannotte *et al.,* 2009; Bravo *et al.,* 2009; Lapiz *et al.,* 2007; Bondi *et al.,* 2007; Vinet *et al.,* 2004; Yalcin *et al.,* 2005; Canciani *et al.,* 2006; Connor *et al.,* 2000), thus acute administration may not be sufficient to confer protection in this model. Although a chronic study with DMI was carried out, the parameters of that study may not have been sufficient to appropriately address the role of chronic administration of DMI in the KA model, and further examination of chronic DMI at different dose and duration regimes may provide a valuable insight into its role in this excitotoxic model. Secondly, DMI may act to enhance intracellular concentrations of NA to promote cellular toxicity. In support of this Nakai and colleagues (1999) demonstrated in an *in vitro* model of ischemia, using rat spinal cord slices, that large amounts of NA is released in response to ischemia, and concentrations of NA were potentiated and sustained by application of DMI. Furthermore, as NA has been shown in some studies to lower the seizure threshold (Escorihuela *et al.,* 1989; Peterson *et al.,* 1985; Arai *et al.,* 2003), it is possible that the actions of DMI may synergise with KA to intensify seizures, culminating in increased hippocampal damage. A further possibility may be attributed to the ability of DMI to bind to adrenergic, histminergic, and cholinergic receptors, which influence its properties independently of endogenous NA. In order to take account of such off-target effects, the experiment was attempted with a highly selective inhibitor of the NET, RBX.

5.9.2 RBX augments KA-induced apoptosis associated with an inhibition of BDNF expression in the hippocampus

As discussed previously RBX is more selective for inhibition of the NET than DMI, and has no significant affinity for dopaminergic, adrenergic, histaminergic or cholinergic

receptors (Scates and Doraiswamy, 2000). It has been shown to possess anti-inflammatory, anti-apoptotic properties and promote pro-survival pathways (Hashioka *et al.,* 2007; Kosten *et al.,* 2008; Russo-Neustadt *et al.,* 2005). Thus RBX represents a selective pharmacological tool whereby inhibition of NA transport may be assessed in the KA-model of hippocampal excitotoxicity. RBX did not influence KA-induced seizures in the first hour of observation following KA administration. However, over the course of the second hour it was noted that animals that had received RBX and KA displayed a trend towards an augmented seizure response, with more frequent stage 4 and 5 seizures (resulting in 2/6 deaths on two separate occasions), when compared to those animals treated with KA alone. RBX was reported to lower the seizure threshold in NET +/+ wild type mice (Ahern *et al.,* 2006), an effect which could be responsible for the increased seizure activity in the current investigation.

RBX had no effect on KA-induced hippocampal cell loss. Pre-treatment with RBX however showed a trend towards enhancing KA-induced DNA fragmentation. This effect was observed in both the CA3 and CA1, but these results failed to achieve statistical significance due to the variable nature of intensity values. In a similar manner to that seen for DMI, this enhancement of TUNEL staining was accompanied by a trend towards an increase in caspase 3 activity. These results echo those found following pre-treatment with DMI and support the possibility that increased seizure severity correlates with an exacerbated hippocampal injury following KA administration. However, chronic administration of RBX has been shown to influence the expression of apoptotic markers. RBX was reported to increase the expression of Bcl-xl but not Bcl-2 in the hippocampus (Kosten *et al.,* 2008), therefore it is likely that chronic administration of RBX may afford protection against KA-induced apoptosis in the hippocampus.

It has been reported in the literature that RBX has anti-inflammatory properties, suppressing pro-inflammatory cytokines such as IFN-γ, IL-6 and NO *in vitro* in LPS-stimulated human blood cultures, and in IFN-γ-stimulated murine microglial cells (Diamond *et al.,* 2006; Hashioka *et al.,* 2007). In the current study KA-induced IFN-γ expression was not significantly altered by pre-treatment with RBX, however, a trend towards attenuation was observed. By contrast KA-induced IDO expression was not influenced by RBX, which is possibly related to the lack of a robust increase in IFN-γ. Thus pre-treatment with RBX, by contrast to DMI, did not silence the induction of IDO.

Activation of the KP leads to increased production of QUIN, which may contribute to, and exacerbate hippocampal excitotoxicity. Moreover as pre-treatment with RBX produced a robust enhancement of KA-induced iNOS expression, subsequent production of NO and potent nitrosylating agents may lead to the deamination of DNA. NO readily reacts with the superanion free radical O_2^- to form the peroxynitrite anion $ONOO^-$, which further contributes to oxidative stress and cellular damage (Beckman and Koppenol, 1996). Thus changes to inflammatory markers in response to RBX treatment are conducive to provoking cellular toxicity and degeneration. In order to determine if these changes in proinflammatory mediators are associated with microglia or astrocytes, the expression of CD11b and GFAP was also determined. KA-induced CD11b expression showed a trend towards being enhanced albeit not significantly following RBX treatment. RBX alone induced a decrease in GFAP expression, however in this experiment KA failed to induce GFAP expression, a response unaltered by RBX. Overall the results suggest that pre-treatment with RBX results in an increase in activated microglia which may account for the observed changes in IFN-γ and iNOS.

Overall the effects of DMI and RBX were similar with respect to changes in seizure behaviour and cellular apoptosis. However RBX was not as effective as DMI in suppressing KA-induced inflammatory markers, and produced an opposite effect of DMI in relation to iNOS expression. Such differences between the two NET inhibitors have not been previously reported and further experiments will be required to clarify the mechanisms mediating the differential effects of both agents. Perhaps the ability of DMI to influence the NMDA receptor and its affinity for cholinergic, histamatergic and adrenergic receptors contribute to its anti-inflammatory properties. It is possible that such effects are not seen following pre-treatment with RBX due to its specificity for the NET. With regard to the trend towards enhancement of KA-induced seizure behaviours and apoptosis common to both RBX and DMI, such effects are likely to be related to the enhancement of extracellular availability of NA, which contributes to a lowering of the seizure threshold and excitotoxicity in the model.

BDNF protein and expression were found to be increased following KA administration. Pre-treatment with RBX attenuated this response. There is a correlation between the KA-induced increase in apoptosis and the reduction in expression and availability of the neurotrophin, BDNF (Han *et al.*, 2000; Perez-Navarro *et al.*, 2005). Changes to BDNF

may be considered a reaction to seizure activity, apoptosis and inflammation as discussed above. In other paradigms too, RBX has been reported to influence changes in central BDNF. Donnici *et al.,* (2008) demonstrated that treatment with RBX causes a decrease in total BDNF mRNA in human neuroblastoma cells.

Previous reports have demonstrated that RBX has novel anti-inflammatory, antiapoptotic and pro-survival properties (Diamond *et al.,* 2006; Kosten *et al.,* 2008; Duman *et al.,* 2001; Russo-Neustadt and Chen, 2005). Such reports are in contrast to the results of the present investigation. Many of the reported protective effects of RBX to date were obtained following chronic administration. Acute effects of RBX are less well characterised and it is possible that RBX fails to confer protection against acute and rapid degeneration such as that which occurs in response to KA administration. Acutely RBX provokes an elevation of synaptic NA (Scates and Doraiswamy, 2000) which may work in concert with extracellular glutamate to exacerbate KA induced hippocampal cell damage. Alternatively it is possible that an increase in extracellular NA leads to the activation of the inhibitory α_2 adrenoceptor, thus decreasing extracellular NA. Such a mechanism would be consistent with the protective effects of chronic RBX administration, which is associated with the down-regulation of α_2 adrenoceptors. Moreover it has been previously reported that elevated synaptic levels of NA seen in NET knock-out mice, are more resistant to KA-induced seizures (Kaminski *et al.,* 2005). Thus enhanced availability of NA may confer protective, rather than mediate degenerative effects in concert with glutamate. It is also possible that down-regulation of the β adrenergic receptor following chronic RBX administration (Scates and Doraiswamy, 2000) contributes to the reported protective effects of RBX *in vivo*. Furthermore β_1 adrenoceptors have been implicated in promoting seizure activity (Pericic *et al.,* 2000; Louis *et al.,* 1982). Thus in order to further investigate the role of β adrenoceptors in mediating KA-induced hippocampal apoptosis the effects of pre-treatment with the non-selective β blocker, PRP was examined alone and in combination with RBX. The effects of PRP are long-lasting and it has previously been used to elucidate the mechanisms of action of RBX with success by other investigators (Grappi *et al.,* 2003).

5.9.3 PRP attenuates KA-induced inflammatory and degenerative changes and counteracts the RBX-induced augmentation of apoptosis in the hippocampus

KA-induced seizures showed a trend towards an increase following pre-treatment with RBX and this action was attenuated following co-administration with PRP. Those rats receiving RBX, PRP and KA experienced a milder seizure pattern compared to KA controls, with very few experiencing stage 4 or 5 seizures. RBX, as before, induced death in 2/7 of the rats receiving both RBX and KA. Animals receiving PRP and KA do experience stage 4 and 5 seizures, but for a very short time period (between 110 and 140 minutes). In this regard PRP has been previously reported to have anticonvulsant properties in various seizure models (For review see: Fischer, 2002) consistent with the observations of the current study. For example, PRP has additive anticonvulsant effects in combination with the barbiturate phenobarbital (Fischer and Muller, 1988) and reduces seizures to stage 3 seizures (unilateral forelimb seizures) in fully kindled rats (Loscher and Schmidt, 1988). However, in the pentylenetetrazole (PTZ) seizure model, PRP does not have anticonvulsant properties (Fischer, 2002). It is postulated that PRP's anticonvulsant properties are not related to its β adrenoceptor blockade (Lasek *et al.,* 1983; Jaeger *et al.,* 1979) but rather due to its membrane stabilizing properties (Fischer *et al.,* 1985; Khanna *et al.,* 1989; Madan and Barar, 1974). Overall, in line with reports of the anti-convulsant properties of PRP, PRP shows anticonvulsant activity the KA model of excitotoxicity and attenuates the RBX induced augmentation of KA-induced seizures suggesting such effects are produced via β adrenergic receptor activation.

KA-induced hippocampal cell loss is not affected by pre-treatment with RBX, PRP or the combination of RBX and PRP, in either the CA3 or CA1. However KA-induced DNA fragmentation showed a trend towards being enhanced following pre-treatment with RBX, albeit not significantly in the CA3. Co-administration of PRP with RBX attenuated this effect in the CA1. As observed in the previous experiments, no changes were found in response to KA-induced caspase 3 expression following PRP or RBX treatments alone or in combination. Overall the effects of PRP observed in the current investigation are consistent with anti-apoptotic actions of the drug reported by others. PRP has been reported to inhibit caspase 3-like activity in a dose dependent manner, to directly inhibit caspase 9 activity and to reduce staurosporine-induced release of cytochrome c from the

mitochondria. These effects in turn reduce DNA fragmentation observed by a concomitant reduction in TUNEL positive staining in an *in vitro* model of apoptosis (Mikami *et al.,* 2008). PRP was also reported to inhibit cytochrome c, released from rat brain mitochondria stimulated with recombinant Bax, and caspase 8-cleaved Bid or Bcl-2 homology 3 protein, suggesting that PRP inhibits Bax-induced permeability changes by a direct interaction with the lipid membrane (Polster *et al.,* 2003).

No significant changes are seen in KA-induced IFN-γ or IDO expression, following pre-treatment with PRP, RBX alone or in combination consistent with the results obtained from the previous study with RBX, where no significant changes were observed in KA-induced IFN-γ or IDO expression. However, there is evidence within the literature to suggest an anti-inflammatory role of PRP and RBX individually (Kato *et al.,* 2009; Hashioka *et al.,* 2007; Diamond *et al.,* 2006). By contrast KA-induced inflammation may not be susceptible to change by either RBX or PRP. In the previous study, a robust increase in KA-induced iNOS expression was obtained following pre-treatment with RBX, an effect which is mirrored in this study. This effect is amenable to attenuation following co-administration of PRP, in fact administration of PRP alone attenuates KA-induced iNOS expression, suggesting that this pro-inflammatory mediator is susceptible to regulation by β adrenoceptors following KA administration. To implicate microglia in this response, KA-induced CD11b expression was significantly attenuated by PRP alone, and in response to the combination of RBX and PRP, suggesting that PRP may exert its anti-inflammatory effects via an inhibition of activated microglia. By contrast expression of the astrocytic marker GFAP was not altered following pre-treatment with RBX, PRP or the combination of both.

KA-induced BDNF expression is attenuated by pre-treatment with RBX, an effect also seen in the previous study. This response was not affected by prior treatment with PRP alone. Co-administration of PRP and RBX displayed a similar effect to RBX alone. Administration of PRP has been shown to inhibit exercise-induced BDNF expression in the hippocampus (Ivy *et al.,* 2003), but such an effect was not observed in the KA model. These results suggest that the mechanism whereby RBX attenuates KA-induced BDNF expression may not involve β-adrenoceptor activation.

A number of studies have reported a role for β adrenoceptors in mechanisms underlying neurodegenerative processes. In mice lacking the β_2 adrenoceptor, a marked attenuation of post-ischemic damage was reported, an effect that was also obtained in mice treated with the selective β_2 adrenergic antagonist, ICI 118,551 (Han *et al.,* 2009). A range of β antagonists including PRP, metaprolol and butoxamine were reported to reduce levels of the pro-inflammatory cytokine IL-6 in the cerebrospinal fluid, following subarachnoid haemorrhage in rats (Kato *et al.,* 2009). Hypoxia-induced retinal ganglion cell death was reduced following pre-treatment with β antagonists, betaxolol, timolol and nipradilol (Chen *et al.,* 2007). Using a series of beta blockers, it was demonstrated that neuroprotection is preferentially mediated via the β_2 adrenoceptor *in vivo*, whereas neuroprotection is achieved *in vitro* via stimulation of both $\beta_{1/2}$ adrenoceptors (Junker *et al.,* 2002). Stimulation of β_2 receptors is also protective in various models of stroke (Rami *et al.,* 2003; Culmsee *et al.,* 2007; Semkova *et al.,* 1996).

In addition to the range of studies reporting that blockade of the β adrenoceptors confers neuroprotection (Chen *et al.,* 2007; Yu *et al.,* 2007; Chan *et al.,* 2007; Kato *et al.,* 2009; Goyagi *et al.,* 2006; Mikami *et al* 2008), there is a body of literature that supports the contrary (Wang *et al.,* 2009; Feinstein *et al.,* 1993; Nakamura *et al.,* 1998; Mori *et al.,* 2002; Dello Russo *et al.,* 2004; Zeman *et al.,* 2004; Zhu *et al.,* 1999). In this study PRP blocks the RBX-induced enhancement of iNOS expression and shows a trend towards ameliorating RBX-induced enhancement of DNA fragmentation. These results indicate that in the current model of excitotoxicity, RBX augments KA-induced hippocampal apoptosis, an effect which is susceptible to regulation by β-adrenergic receptors. However, as PRP is a non-selective β adrenergic antagonist, it is not clear if such effects are attributable to the β_1 or the β_2 adrenoceptor. Reports have demonstrated a neuroprotective role for the β_2 adrenoceptor with few effects associated with β_1 adrenoceptor activation. For example, activation of β_2 adrenoceptors has been associated with the prevention of Aβ evoked inhibition of LTP in the dentate gyrus of adult rats, an effect not seen following activation of β_1 adrenoceptors (Wang *et al.,* 2009). Administration of the selective β_1 antagonist, metaprolol with CLN, reduced infarct volume following global ischemia, an effect which was abolished following the administration of the selective β_2 antagonist, butoxamine (Junker *et al.,* 2002). Numerous reports illustrate the protective role of β_2 adrenoceptor stimulation, by increasing protective neurotrophins and decreasing DNA

fragmentation and apoptosis seen following brain injury *in vitro* and *in vivo*, and reducing infarct volume following stroke (Luchoskwa *et al.*, 2008; Izeboud and colleagues 1999; Junker *et al.*, 2002; Culmsee *et al.*, 1999a, 1999b; Semkova *et al.*, 1996; Zhu *et al.*, 1998). In addition to the experiments carried out thus far, such reports provided a strong rationale for an investigation of the effects of β_2 adrenoceptor activation in the KA-model of hippocampal excitotoxicity.

5.9.4 CLN has neuroprotective properties in the KA model of hippocampal excitotoxicity

CLN has demonstrated a number of neuroprotective properties in a wide range of models of neurodegeneration. Specifically, *in vitro* and *in vivo* models of excitotoxicity, CLN has been reported to afford neuroprotection by reducing inflammation and apoptosis, and promoting the production of pro-survival molecules such as NGF and BDNF (Rami *et al.*, 2003; Junker *et al.*, 2002; Culmsee *et al.*, 1999b; Semkova *et al.*, 1996; Zhu *et al.*, 1998; Semkova *et al.*, 1999). It is postulated that CLN mediates its protective effects via its influence on neurotrophin production (Rami *et al.*, 2003; Semkova *et al.*, 1999; Culmsee *et al.*, 1999a, 1999b; Colangelo *et al.*, 1998). The results of the present investigation are consistent with these reports to date and suggest that activation of the β_2 adrenoceptor produces protection in the KA model of hippocampal excitotoxicity.

KA-induced seizure behaviour showed a trend towards being reduced following pre-treatment with CLN over the first 100 minutes. Following this, there was no discernable difference between the KA-treated groups. CLN has been shown to exert anticonvulsant effects in various seizure models, and in the PTZ model of myoclonic seizures where pre-treatment with low-dose CLN resulted in reduced tonic convulsions, but had no effect on generalised clonic seizures (Fischer *et al.*, 2001). These findings are in line with the results obtained in the present investigation.

KA-induced hippocampal cell loss was not altered by pre-treatment with CLN, in the CA3 or CA1. In other reports, CLN has been shown to protect against glutamate-induced excitotoxicity in mixed hippocampal cultures, an effect which was blocked by the non-selective β antagonist, PRP, and the selective β_2 antagonists, ICI 118,551 and butoxamine

(Junker *et al.,* 2002). Protective effects were also reported by Semkova and colleagues (1996), who demonstrated that CLN protected hippocampal neurons, in an *in vivo* model of transient forebrain ischemia.

KA-induced DNA fragmentation and caspase-3 activity were attenuated following pre-treatment with CLN, in both the CA3 and CA1 of the hippocampus. Others have reported similar actions of CLN. Following transient global ischemia, treatment with CLN was reported to attenuate DNA fragmentation and DNA laddering, in the rat hippocampus (Rami *et al.,* 2003). When administered 3 hours prior to transient global ischemia, CLN attenuated DNA fragmentation in the striatum and hippocampus (Zhu *et al.,* 1998). CLN has previously been reported to regulate apoptotic pathways. Zhu *et al.,* (1999) reported that CLN provokes an increase in Bcl-2 in both non-ischemic and ischemic hippocampus and striatum.

Pre-treatment with CLN attenuates KA-induced IFN-γ, IDO and iNOS expression showing anti-inflammatory properties of CLN in the KA model. Similar results have previously been reported both *in vitro* and *in vivo*. Izeboud and colleagues (1999) reported that CLN suppresses the LPS-induced pro-inflammatory cytokines-IL-6 and TNF-α in the human U-937 cell line, and in the plasma of adult male wistar rats. CLN has been shown to suppress the release of LPS-induced IL-1β and TNF-α in peripheral blood mononuclear cells, an effect which was shown to be mediated by its ability to increase intracellular cAMP levels (Yoshimura *et al.,* 1997). In order to determine if the anti-inflammatory effects of CLN obtained in the current study were mediated by microglia or astrocytes, CD11b and GFAP expression were determined respectively. No significant changes were observed in KA-induced CD11b expression following pre-treatment with CLN. However, a trend towards an increase in KA-induced GFAP expression was obtained following pre-treatment with CLN. This result correlates with reports from Junker and colleagues (2002) who demonstrated that treatment with CLN induced profound morphological changes in cultured astrocytes, which transformed into activated astroglia, an effect which was blocked by butoxamine and propranolol.

KA-induced BDNF expression and protein is augmented following pre-treatment with CLN. Neurotrophin regulation has been shown to be affected by CLN in numerous studies (McCauslin *et al.,* 2006; Colangelo *et al.,* 2004; Rami *et al.,* 2003; Samina and Tomlinson,

2000; Culmsee *et al.,* 1999). The main focus of these studies was NGF regulation, and despite NGF expression not being induced by KA, the combination of CLN and KA was sufficient to induce the expression of NGF. Culmsee and colleagues (1999) demonstrated that increased NGF expression in response to CLN contributes to its neuroprotective effects both *in vitro* and *in vivo*. Using NGF antisense oligonucleotide, they reported that the protective effects of CLN against glutamate-mediated excitotoxicity were abolished following administration of the NGF antisense. Infarct volume induced by glutamate was found to be reduced by CLN, an effect that was reversed following administration of NGF antisense oligonucleotide. It has been postulated that an increase in NGF inhibits DNA degradation after global ischemia, suggesting that the ability of CLN to increase NGF has knock-on anti-apoptotic effects (Zhu *et al.,* 1998). Such effects are consistent with the protective properties of CLN in the current study, where KA-induced BDNF, is enhanced, with the net result of attenuating KA-induced apoptosis.

Overall the results of this study indicate that CLN is anti-inflammatory, anti-apoptotic and promotes pro-survival pathways in the KA model of excitotoxicity. The results presented correlate with the findings of previous studies, which also demonstrate a clear neuroprotective role for CLN in various degenerative models. It is possible that its neuroprotective effects are not reflected in KA-induced hippocampal cell loss, as 24 hours post KA may not be enough time for the molecular changes to transfer into pathological changes. The increase in GFAP expression may account for the anti-inflammatory effects induced by CLN. It has been reported that CLN induces the release of KYNA (Luchowska *et al.,* 2009), an effect which is mediated by astrocytes. It is possible that this phenomenon is occurring in this study too, and would be of interest to examine hippocampal KYNA levels in future studies. As astrocytes are important for the sequestration of extracellular glutamate, astrocytic activation by CLN may also help control glutamate concentrations in the synapse, thus alleviating the KA-induced damage. An additional mechanism by which CLN may exert protective effects is via its attenuation of IDO expression, indicating a decrease in the KP, thus decreasing the amount of QUIN produced. Moreover stimulation of the β_2 adrenoceptor results in the upregulation of cAMP, activating CREB and its subsequent downstream signalling molecules including BDNF. Thus CLN may afford protective effects via multiple mechanisms working alone or in concert to rescue cells from excitotoxin-induced degeneration.

6.0 General discussion

Overall, direct inhibition of inflammation does not prevent KA-induced hippocampal cell loss and apoptosis, whereas direct inhibition of the KP protects against hippocampal cell loss and apoptosis, but not inflammation. Hence, modulation of intracellular NA can be both degenerative and protective in the KA model of excitotoxicity.

The KA model is used to represent a number of disease states, namely temporal lobe epilepsy, Huntington's chorea, excitotoxicity and acute neurodegeneration (Sharma *et al.*, 2008; Coyle and Schwarcz, 1976; Wang *et al.*, 2005). In this thesis KA-induced excitotoxicity was utilised to represent acute neurodegeneration. As many of the features of acute neurodegeneration are also seen in chronic neurodegenerative conditions, this model may give some indications as to the clinical relevance of the drugs tested in this thesis. Excitotoxicity has been shown to play a key role in neurodegenerative disorders such as AD, PD and HD (Rothstein, 1996; Wong *et al.*, 2002; Hynd *et al*, 2004; Tannenberg *et al*, 2004; Fujikawa, 2005; Yi and Hazell, 2006) and the KA model may provide some insights into the pathogenesis of these conditions. Acute neurodegeneration refers to conditions in which neurons are rapidly damaged and cell death occurs within a short period of time. However, the consequences of this cell death and rapid brain damage can cause long-term degeneration and lead to chronic conditions. It is for this reason that the results of this thesis are not only relevant to acute neurodegeneration, but also have implications for chronic neurodegeneration.

The hippocampus is a central structure within the brain that is commonly affected in neurodegenerative disorders. The KA model of hippocampal excitotoxicity provides a useful insight into the pathogenesis of these diseases. The protective strategies illustrated by the work carried out in this thesis, demonstrating that stimulation of the β_2 adrenoceptor prevents acute hippocampal degeneration, may be clinically relevant in chronic neurodegenerative diseases involving excitotoxic damage within the hippocampus.

Pro- and anti-inflammatory cytokines are synthesized by microglia and astrocytes in the rodent brain, within 30 minutes of seizure onset (Minami *et al.*, 1991; Vezzani *et al.*, 1999, 2000; De Simoni *et al.*, 2000). Seizure activity results in an increase in cytokine levels in the hippocampus, where they influence the ongoing epileptic activity (Minami *et al.*, 1990,

1991; Eriksson *et al.,* 1999; De Simoni *et al.,* 2000; Vezzani *et al.,* 1999, 2000). Cytokine expression is greater in intensity and distribution when seizures are associated with neuronal damage, suggesting that neuronal injury and cytokine over-expression are interconnected. Viviani and colleagues (2003) found that IL-1β induces Src kinase family-mediated tyrosine phosphorylation of the NR2A/B subunit of the NMDA receptor. This was shown to mediate upregulation of NMDA activity by increasing its channel gating properties (Ali and Salter, 2001). Thus, induction of IL-1β during seizures may contribute to the occurrence of neuronal damage by inducing posttranslational modifications in NR2 subunit (Rizzi *et al.,* 2003). Peripheral administration of dizocilpine and 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (R-(CPP)), a non-competitive and a competitive NMDA-receptor antagonist, respectively, blocked in a dose-dependent manner the expression of IL-1β in the rat brain, suggesting the involvement of this group of glutamate receptors in the initiation of cytokine synthesis in the brain (Eriksson *et al.,* 2000). It was reported that microglial cells have functional glutamate receptors of the AMPA-type, suggesting that the induction of IL-1β by KA is mainly indirect, through release of endogenous glutamate (Noda *et al.,* 2000; Kohler *et al.,* 1979). IL-1β and TNF-α impair astrocytic glutamate uptake both in primary cultures from rat postnatal hippocampus, and in human cortical astrocytes, producing a dose-dependent inhibition of GluT mRNA expression (Ye and Sontheimer, 1996; Hu *et al*, 2004). It is possible that the observed pro-convulsant effect of pro-inflammatory cytokines, is the result of an increase in glutamate available for the activation of NMDA- and non-NMDA-receptors, due to the inhibition of its uptake by astrocytes.

As KA represents a model of acute neurodegeneration, it was initially postulated that inflammation may be a key process in driving hippocampal cell loss and related molecular alterations. However, the findings of this thesis indicate that the inflammatory process does not appear to mediate the cellular and apoptotic damage elicited by KA administration. Robust anti-inflammatory effects were observed following DEX and DMI, but these failed to affect DNA fragmentation, apoptosis or cell loss. Other anti-inflammatory strategies, such as the use of COX-2 inhibitors have been successful in conferring neuroprotective properties. The expression of COX-2 is reportedly increased in neurodegenerative disorders such as stroke, PD, HD and AD (Boyd *et al.,* 2007; Huntjens *et al.,* 2009; Breder *et al.,* 1995; Saldana *et al.,* 2008). It has been reported that there is a relationship between excitotoxicity, oxidative stress and COX-2 in disease conditions (Breder *et al.,* 1995; Gendron *et al.,* 2005). Neuroprotective effects of various COX inhibitors have been

documented against colchicines, 3-nitropropionic acid and QUIN- induced neurotoxicities (Kumar *et al.,* 2006; Kalonia *et al.,* 2009; Salzberg-Brenhouse *et al.,* 2003). Recent reports have demonstrated that an array of COX-2 inhibitors have been shown to be neuroprotective *in vivo* against QUIN-induced striatal damage in rats, by improving impaired motor coordination, oxidative damage (in the form of increased nitrite concentration, depleted superoxide dismutase and catalase levels) and alterations in mitochondrial enzyme complex activity (Kalonia *et al.,* 2009).

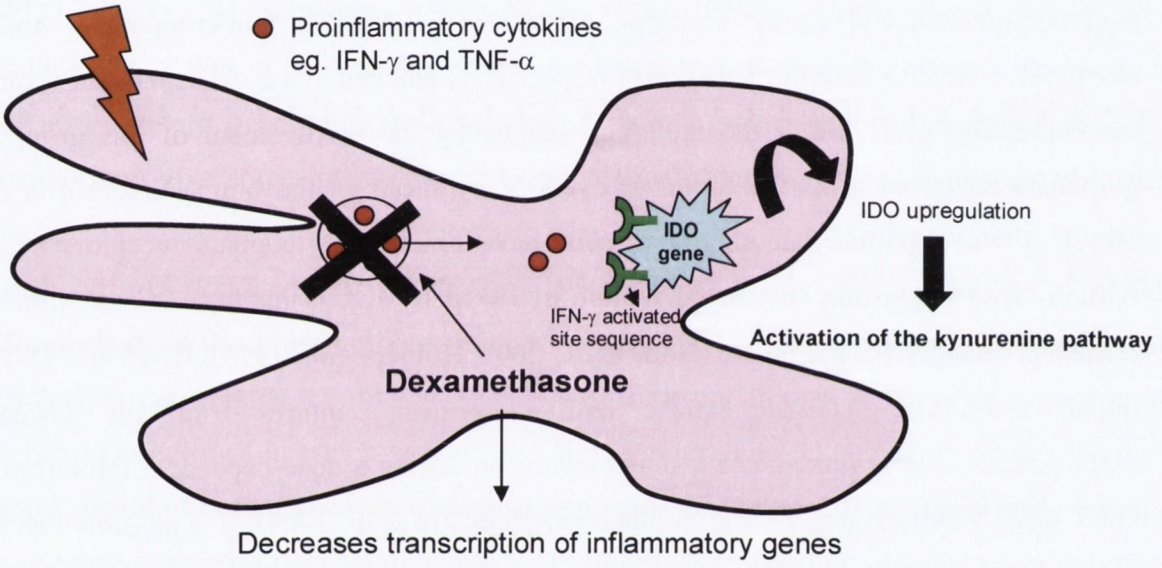


Figure 6.1 Mechanism of action of DEX

It is clear that anti-inflammatory strategies do provide neuroprotection in various models of neurodegeneration (Lim *et al.,* 2009; Memo *et al.,* 2002; Kalonia *et al.,* 2009), so why do the anti-inflammatory effects of DEX and DMI not confer neuroprotection in the KA model? Perhaps 24 hours post intervention is not enough time to see translational changes in key genes such as caspase 3, and hippocampal cell loss. Kalonia and colleagues (2009) demonstrated neuroprotective changes 21 days following initial COX-2 inhibitor treatment and QUIN insult. It would be of interest to examine changes in apoptotic markers and cell loss at a later time point post KA, to test the hypothesis that anti-inflammatory changes may confer neuroprotection. Also, the examination of a wider array of markers, such as oxidative stress and mitochondrial dysfunction, may provide a greater insight into the biochemical changes occurring post DEX and DMI intervention.

CLN was the only drug examined in this thesis to possess both anti-inflammatory and anti-apoptotic effects. CLN was also the only drug to increase GFAP expression, whereas the other drugs primarily affected CD11b expression, indicating that CLN predominantly affects astrocytic activation, whereas other drugs affect microglial activation. Under normal conditions, astrocytes function to sequester glutamate from the synapse, thus controlling the build up of excess glutamate. In neuroinflammatory conditions astrocytes are capable of releasing glutamate by reacting to synaptically released neurotransmitters, coupled with intracellular calcium elevations, which result in the release of glutamate via regulated exocytosis. This process is affected by pro-inflammatory cytokines such as TNF-α, and prostaglandins (Vesce *et al.,* 2007). It is therefore not unreasonable to suggest that the ability of CLN to suppress inflammatory mediators, and to increase the activation of astrocytes, causes these astrocytes to sequester glutamate from the synapse via GluT, and thus contribute to the neuroprotective properties of CLN. In the brain, astrocytes were identified as the dominant cell type expressing β_1 and β_2 adrenoceptors (Hosli and Hosli, 1993; Mantyh *et al.,* 1995). A pronounced increase in β_2 adrenoceptor expression was seen predominantly in glial cells following brain injury (Hodges-Savola *et al.,* 1996). Astrocytes are the main source for growth factors in the brain under both physiological and degenerative conditions, indicating that astrocyte activation may be an endogenous mechanism of neuroprotection (Oderfeld-Nowak and Bacia, 1994; Schwartz *et al.,* 1993). Activation of astrocytes has been associated with increased expression of β_2 adrenoceptors (Hodges-Savola *et al.,* 1996), and CLN has been shown to enhance astrocytic activation in ischemic brain tissue (Culmsee *et al.,* 1999a). CLN also induced NGF synthesis in cultured astrocytes, suggesting a contribution of these glial cells to the neuroprotective effects of CLN in mixed hippocampal cultures and in rats (Culmsee *et al.,* 1999b).

The KP has previously been examined in the KA model, and inhibition of kynureninase and KMO using mNBA was successful in inhibiting QUIN production, thus protecting against KA-mediated damage (Behan and Stone, 2000). The results presented in this thesis demonstrate a role of the KP in the KA model, but it remains unclear what the significance of this is. In each experiment, it was observed that IDO expression mirrored the expression IFN-γ, in line with what is reported in the literature where pro-inflammatory cytokines, namely IFN-γ and TNF-α, induce the expression of IDO (Schroecksnadel *et al.,* 2003; Takikawa *et al.,* 1999; Popov *et al.,* 2006). Further investigation of the expression of enzymes of the KP showed that a suppression of IDO expression is coupled with no

change in L-KYN and 3-HK levels, following DEX administration. In contrast, administration of 1 MT, which increased IDO expression, was also not accompanied by changes in the levels of KP enzymes such as L-KYN, 3-HK or KYNA. These results indicate that IDO expression is not a useful indication of KP activation and alterations in KP metabolites, and future investigations should focus on IDO protein using mass spectrometry or western blotting.

As previously discussed, a wide range of studies have reported that NA has innate anti-inflammatory, anti-apoptotic and pro-survival neuroprotective properties (Feinstein *et al.*, 1993; Nakamura *et al.*, 1998; Mori *et al.*, 2002; Dello Russo *et al.*, 2004: Il'inykh *et al.*, 2008; Kosten *et al.*, 2008; Chen *et al.*, 2007; Chen and Russo-Neustadt, 2007). It was thus postulated that NA would have protective effects in the KA model of excitotoxicity. As was demonstrated by pre-treatment with the NRI's DMI and RBX, increasing synaptic availability of NA augmented KA-induced hippocampal damage. Although their effects on inflammatory mediators differed, both drugs provoked a similar exacerbatory effect on KA-induced apoptosis and DNA fragmentation, suggesting that the specificity for the NET displayed by both NRI's is what accounts for this exacerbatory action.

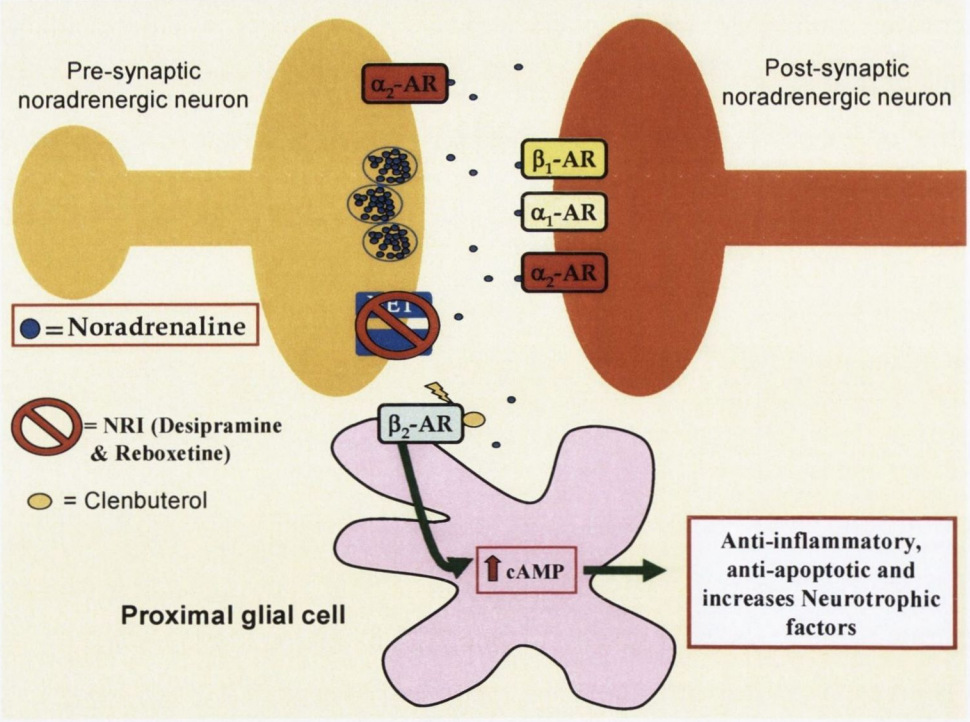


Figure 6.2 Mechanism of action of DMI, RBX and CLN

In the studies presented here an increase in synaptic availability of NA through the use of NRI's DMI and RBX, was shown to augment KA-induced damage. However, stimulation of the β_2 adrenoceptor using the highly lipophilic β_2 agonist, CLN, was shown to be anti-

inflammatory, anti-apoptotic and to increase the production of protective neurotrophins. This protective mechanism is more than likely due to an increase in cAMP which also leads to an increase in BDNF production, thus protecting against neuronal death.

In the hippocampus, glutamate, NA and 5-HT systems are known to interact (Millan, 2004). In particular, NA and 5-HT can inhibit glutamate release by acting at presynaptic α_2-adrenergic and 5-HT_{1D} receptors (Kamisaki *et al.,* 1992). Conversely, glutamate has been shown to enhance NA and 5-HT release, by acting at presynaptic NMDA and AMPA receptors (Fink *et al.,* 1990; Pittaluga and Raiteri, 1990; Raiteri *et al.,* 1992; Wang *et al.,* 1992; Pittaluga *et al.,* 1997, 2007). Thus NA could provide neuroprotective properties through its inhibition of glutamate release. However, Nakai and colleagues (1999) demonstrated in an *in vitro* model of ischemia that large amounts of NA were released in response to ischemic damage, indicating that during ischemic conditions, glutamate enhances the release of NA. Furthermore, as NA has been shown in some studies to lower the seizure threshold (Escorihuela *et al.,* 1989; Peterson *et al.,* 1985; Arai *et al.,* 2003), NA may be responsible for the increase in seizure intensity seen following pre-treatment with NRI's, which is coupled with an increase in DNA fragmentation and apoptotic damage.

It is well established that chronic treatment with NRI's downregulate β adrenoceptor expression in the CNS (Harkin *et al.,* 2000; Lafaille *et al.,* 1991) and it was originally believed that this effect was necessary in order to achieve antidepressant effects (Sulser, 1978). However, a range of clinically efficacious antidepressants have no effect on β adrenoceptor expression (Sartorius *et al.*, 2007; Leonard, 1991). Investigation of β adrenoceptor density in the rat cortex and cerebellum revealed β_1 adrenoceptor density was decreased in cortical tissue following chronic DMI treatment, but no effects on β_2 adrenoceptor density were evident. Despite a higher density of β_2 adrenoceptors in the cerebellum, DMI had no effects on β_2 adrenoceptor density in this region (Paetsch and Greenshaw, 1993). This down-regulation is accompanied by a reduction in central β adrenoceptor sensitivity, demonstrated by Handley and Singh (1986) who reported that forty-eight hours after withdrawal from chronic, but not acute pre-treatment with DMI, the potentiating effects of dobutamine, prenalterol and salbutamol on the head-twitch response to 5-hydroxytryptophan, were significantly decreased. This is consistent with a reduction in β adrenoceptor density (For review see: Manji and Brown, 1987). The results of this thesis indicate a degenerative role for the β_1 adrenoceptor following acute administration of

the NRI's DMI and RBX. Perhaps this functional down-regulation of the β adrenoceptor, namely the β_1 adrenoceptor, may play a protective role following chronic administration of NRI's. Chronic administration of DMI did not convey any protective/degenerative changes in the KA model, but it would be of interest to investigate this further, PRP in combination with chronic RBX treatment may provide a more useful insight into this hypothesis.

Chronic treatment with NRI's is the manner in which these drugs are used clinically for the treatment of depression. The issues raised in this thesis regarding the exacerbated KA-induced hippocampal damage seen following pre-treatment with NRI's may have important implications for patients who suffer from post-stroke depression. Further investigation into the mechanisms behind this exacerbated response, and extensive examination of the role of chronic NRI administration in the KA model, are warranted.

As was demonstrated in this thesis, PRP ameliorated the pro-degenerative effects of RBX, when it was used in combination with KA, indicating that the exacerbated damage seen following pre-treatment with NRI's is mediated by β adrenoceptors. Also, stimulation of the β_2 adrenoceptor confers neuroprotective properties, as was demonstrated in this study, as well as others (Luchoskwa *et al.,* 2008, 2009; Junker *et al.,* 2002; Culmsee *et al.,* 1999b; Semkova *et al.,* 1996; Zhu *et al.,* 1998). Hence, perhaps NRI's elicit their pro-degenerative effects via the β_1 receptor, which is downregulated following chronic administration. This hypothesis may explain the protective effects observed by other investigators following chronic RBX (Paris *et al.,* 2005; Hashioka *et al*, 2007; Kosten *et al.,* 2008; Russo-Neustadt *et al.,* 2004). It would be of interest to examine the effects of chronic administration of these drugs in the KA model.

In the current investigation both RBX and DMI were found to attenuate KA-induced BDNF expression following their acute administration. BDNF has been shown to regulate noradrenergic transmission, drug-related neuronal changes and maintains NA innervation in the aged brain (Juric *et al.,* 2008). Loss of BDNF in this model of acute degeneration may account for the concurrent increase in apoptosis and DNA fragmentation observed in these studies. It has been shown previously that i.c.v injection of BDNF prior to hypoxic-ischemic injury prevents the induction of caspase 3 *in vivo* (Han *et al.,* 2000), indicating that a loss of BDNF may promote caspase 3 induction following ischemic injury. Moreover, central application of BDNF protects against KA-induced striatal damage and

blocks caspase 3 activation in adult male Fischer rats (Perez-Navarro *et al.,* 2005). Other neurotrophic factors also possess neuroprotective properties. As was demonstrated by Culmsee and colleagues (1999), an increase in NGF induced by β_2 adrenoceptor stimulation was responsible for the corresponding decrease in glutamate-induced neuronal cell death *in vitro*, and the reduction in infarct volume following permanent focal cerebral ischemia *in vivo*. BDNF phosphorylates Bad protein, which prevents the association of the mitochondrially localized Bcl-xl with Bcl-2, and therefore inhibits apoptosis. It is possible that the attenuated BDNF levels in these studies affects this downstream signalling, thus augmenting pro-apoptotic pathways. The mechanism involved in the NRI-induced attenuation of KA-induced BDNF warrants further investigation.

It is of interest to note the review by Zhang and Kimelberg (2005), reporting that high concentrations of NA may elicit neuronal damage by inducing an imbalance between cerebral oxygen supply and demand, thus increasing the sensitivity of pyramidal neurons to glutamate. They also provide a wide range of evidence indicating a protective role of α_2 agonists in animal models of ischemia (Globus *et al.,* 1989; Hoffman *et al.,* 1991a, 1991b; Kapinya *et al.,* 2002; Maier *et al.,* 1993). Specifically, the α_2 agonist, dexemedotomidine suppressed KA-induced seizures and prevented hippocampal cell death in rats (Halonen *et al.,* 1995). Pre-treatment with the α_2 antagonists prazosin, yohimbine, idazoxan and clonidine, or idazoxan alone resulted in a higher mortality rate following hypoxic-ischemic brain injury in neonatal rats (Yuan *et al.,* 2001; Antier *et al.,* 1999). These investigations demonstrate the role of the α_2 adrenoceptor in excitotoxic/ischemic conditions, which compliment the findings presented here, demonstrating a degenerative role of NA when introduced into an excitotoxic environment, and a protective role of suppressing NA in this model. Further investigation of the role α_2 antagonists, and the α_1 receptor in the KA model would help to confirm conclusively the role of endogenous NA within this model.

Stimulation of the β_2 adrenoceptor has been reported in numerous studies to confer neuroprotection in several models of degeneration (Luchoskwa *et al.,* 2008; Izeboud and colleagues 1999; Junker *et al.,* 2002; Culmsee *et al.,* 1999a, 1999b; Semkova *et al.,* 1996; Zhu *et al.,* 1998; Culmsee *et al.,* 2004). Here the first description of CLN's neuroprotective effects in the KA model are presented. As was previously demonstrated, CLN increased GFAP expression, a marker of astrocytic activation, and as astrocytes express KAT-II, activation of astrocytes may in turn lead to the production of KYNA (Luchoskwa *et al.,*

2008). KYNA is an NMDA antagonist and has been shown to be neuroprotective in previous studies (Sas *et al.,* 2008; Rejdak *et al*, 2007). Astrocytes are a known source of BDNF, and NA is involved in the release of astrocytic BDNF via $\beta_{1/2}$ and α_1 adrenoceptors (Juric *et al.,* 2007). Perhaps the increase in astrocytic activation contributes to the augmented levels of BDNF seen in this study. Further investigation of this theory would involve the use of an inhibitor of astrocyte activation, such as arundic acid, to see if astrocytes are directly responsible for the increased BDNF in this model.

Stimulation of the β_2 adrenoceptor results in the upregulation of cAMP, activating CREB and PKA, and subsequent downstream signalling molecules, such as BDNF. Although activation of the β_2 adrenoceptor can be attributed to the G_s protein, β_2 adrenoceptors have been shown to couple to G_i proteins, resulting in the activation of the ERK and p38 MAPK (Daaka *et al.,* 1997). The ERK cascade functions in cellular proliferation and differentiation, whereas the p38 pathway is involved in gene transcription and is related to cytokine production and apoptosis (Johnson and Lapadat, 2002). Perhaps modulation of these pathways can contribute to the neuroprotective effects of CLN. The upregulation of BDNF leads to the activation of CREB and ERK signalling pathways, which inhibit the pro-apoptotic factor Bad, and promotes Bcl-2 production, among other anti-apoptotic proteins (Chen *et al.,* 1999; Coyle and Duman, 2003). This mechanism could account for the concurrent suppression of DNA fragmentation and apoptosis seen following pre-treatment with CLN.

Overall, the results of this thesis indicate that inflammation is not a key mediator of KA-induced hippocampal cell loss and apoptosis, and that acute alterations of central noradrenergic tone can indeed exacerbate KA-induced hippocampal damage and seizure behaviour. However, stimulation of the β_2 adrenoceptor proved to be neuroprotective in this acute model of neurodegeneration.

This outcome could represent a clinically feasible neuroprotective strategy, as CLN has been shown previously to be neuroprotective in animal models of stroke and ALS (Culmsee *et al.,* 1999, 2004; Zhu *et al.,* 1998; Teng *et al*, 2006) and is safe for human consumption (Yamamoto *et al.,* 1985; Aboul-Enein and Serignese, 1999; Yuen *et al.,* 2005). However, as CLN has powerful anabolic and lipolytic effects, long-term usage has been shown to cause alterations in cardiac muscle structure and function (Duncan *et al.,*

2000), which poses a problem for use as a neuroprotectant in chronic diseases such as AD, PD and HD. However, use in the treatment of acute conditions such as stroke or traumatic brain injury remain clinically feasible.

7.0 Future Directions

The research presented in this thesis has yielded a number of important leads for future research as outlined below.

1. Using the D-isoform of 1 MT, an investigation of IDO protein levels, free tryptophan and quantification of QUIN levels in the brain, in a time course manner, would provide useful insights into the role of the KP in the KA model.

2. The investigation of chronic administration of DMI and RBX, alone and in combination with PRP in future studies would demonstrate if the protective effects of these drugs seen currently in the literature could be applied to KA-induced hippocampal cell loss, and would demonstrate if protective/degenerative effects of chronic administration of NRI's is mediated by β adrenoceptors.

3. Further investigation of the role of the β adrenoceptor following acute administration of RBX is required. As the β adrenoceptor has been identified as a mediator of the exacerbated damage induced by RBX and KA, the use of the selective β_1 antagonist, metaprolol and the selective β_2 antagonist, ICI 118,551 in combination with RBX, would identify which receptor this effect is mediated by, in the KA model.

4. It is possible that the large increase in extracellular NA following pre-treatment with NRI's leads to the activation of the inhibitory α_2 adrenoceptor, thus decreasing the overall amount of NA. In order to test this theory co-administration of RBX and the α_2 antagonist idazoxan would demonstrate the involvement of the α_2 adrenoceptor in this study.

5. Future research could focus on the role of the α adrenoceptor in the KA model of excitotoxicty. Recent reports have implicated α_2 adrenoceptors in modulating neuroprotection. Examination of α_1 adrenoceptor agonism and antagonism in the KA model might provide further insights into the role of NA in this model.

6. In order to confirm the mechanism by which CLN exerts its protective effects, using it in combination with a selective β_2 antagonist, such as ICI-118551, would demonstrate if stimulation of the β_2 adrenoceptor is indeed the mechanism by which this drug operates. Also using CLN in combination with an inhibitor of cAMP would demonstrate if the protective effects are mediated by increasing cAMP signalling.

7. The mechanism by which CLN affords neuroprotection in the KA model could be further explored. Reports have implicated CLN-induced increases in NGF as a possible mechanism of neuroprotection. In order to explore the theory that increased BDNF following pre-treatment with CLN is responsible for neuroprotection in the KA model, central application of anti-BDNF following pre-treatment with CLN would provide useful insights into the mechanism underlying the neuroprotective properties of CLN. Also the investigation of CLN at a later time point to investigate if any changes in hippocampal cell loss are evident beyond 24 hours.

8. Further investigation of the role of astrocytes in the KA model would be of interest in future studies. Here it was postulated that the increase in astrocytic activity, seen following CLN administration, was responsible for the neuroprotective properties of this drug. Administration of the inhibitor of astrocytic activity, arundic acid, with CLN, would illustrate the role of astrocytes in CLN-mediated neuroprotection in the KA model of hippocampal excitotoxicity.

VIII Bibliography

Aboul-Enein HY & Serignese V (1999). Quantitative determination of clenbuterol enantiomers in human plasma by high-performance liquid chromatography using the macrocyclic antibiotic chiral stationary phase teicoplanin. *Biomed Chromatogr* **13,** 520-524.

Abrahamson EE, Ikonomovic MD, Ciallella JR, Hope CE, Paljug WR, Isanski BA, Flood DG, Clark RS & DeKosky ST (2006). Caspase inhibition therapy abolishes brain trauma-induced increases in Abeta peptide: implications for clinical outcome. *Exp Neurol* **197,** 437-450.

Acarin L, Gonzalez B & Castellano B (2000). STAT3 and NFkappaB activation precedes glial reactivity in the excitotoxically injured young cortex but not in the corresponding distal thalamic nuclei. *J Neuropathol Exp Neurol* **59,** 151-163.

Acsady L, Kamondi A, Sik A, Freund T & Buzsaki G (1998). GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J Neurosci* **18,** 3386-3403.

Aggarwal BB (2003). Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* **3,** 745-756.

Ahern TH, Javors MA, Eagles DA, Martillotti J, Mitchell HA, Liles LC & Weinshenker D (2006). The effects of chronic norepinephrine transporter inactivation on seizure susceptibility in mice. *Neuropsychopharmacology* **31,** 730-738.

Ahlquist RP (1948). A study of the adrenotropic receptors. *Am J Physiol* **153,** 586-600.

Akama KT & Van Eldik LJ (2000). Beta-amyloid stimulation of inducible nitric-oxide synthase in astrocytes is interleukin-1beta- and tumor necrosis factor-alpha (TNFalpha)-dependent, and involves a TNFalpha receptor-associated factor- and NFkappaB-inducing kinase-dependent signaling mechanism. *J Biol Chem* **275,** 7918-7924.

Alblas J, van Corven EJ, Hordijk PL, Milligan G & Moolenaar WH (1993). Gi-mediated activation of the p21ras-mitogen-activated protein kinase pathway by alpha 2-adrenergic receptors expressed in fibroblasts. *J Biol Chem* **268,** 22235-22238.

Ali DW & Salter MW (2001). NMDA receptor regulation by Src kinase signalling in excitatory synaptic transmission and plasticity. *Curr Opin Neurobiol* **11,** 336-342.

Alkondon M, Pereira EF, Yu P, Arruda EZ, Almeida LE, Guidetti P, Fawcett WP, Sapko MT, Randall WR, Schwarcz R, Tagle DA & Albuquerque EX (2004). Targeted deletion of the kynurenine aminotransferase ii gene reveals a critical role of endogenous kynurenic acid in the regulation of synaptic transmission via alpha7 nicotinic receptors in the hippocampus. *J Neurosci* **24,** 4635-4648.

Allan SM, Parker LC, Collins B, Davies R, Luheshi GN & Rothwell NJ (2000). Cortical cell death induced by IL-1 is mediated via actions in the hypothalamus of the rat. *Proc Natl Acad Sci U S A* **97,** 5580-5585.

Allan SM & Rothwell NJ (2000). Cortical death caused by striatal administration of AMPA and interleukin-1 is mediated by activation of cortical NMDA receptors. *J Cereb Blood Flow Metab* **20,** 1409-1413.

Allan SM & Rothwell NJ (2001). Cytokines and acute neurodegeneration. *Nat Rev Neurosci* **2,** 734-744.

Allan SM & Rothwell NJ (2003). Inflammation in central nervous system injury. *Philos Trans R Soc Lond B Biol Sci* **358,** 1669-1677.

Allen JW, Eldadah BA, Huang X, Knoblach SM & Faden AI (2001). Multiple caspases are involved in beta-amyloid-induced neuronal apoptosis. *J Neurosci Res* **65,** 45-53.

Amadoro G, Ciotti MT, Costanzi M, Cestari V, Calissano P & Canu N (2006). NMDA receptor mediates tau-induced neurotoxicity by calpain and ERK/MAPK activation. *Proc Natl Acad Sci U S A* **103,** 2892-2897.

Amaral DG & Witter MP (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* **31,** 571-591.

Antier D, Franconi F & Sannajust F (1999). Idazoxan does not prevent but worsens focal hypoxic-ischemic brain damage in neonatal Wistar rats. *J Neurosci Res* **58,** 690-696.

Arai S, Morita K, Kitayama S, Kumagai K, Kumagai M, Kihira K & Dohi T (2003). Chronic inhibition of the norepinephrine transporter in the brain participates in seizure sensitization to cocaine and local anesthetics. *Brain Res* **964,** 83-90.

Argenti D & D'Mello AP (1994). Design of a desipramine dosing regimen for the rapid induction and maintenance of maximal cortical beta-adrenoceptor downregulation. *Neuropharmacology* **33,** 1117-1124.

Arundine M & Tymianski M (2003). Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calcium* **34,** 325-337.

Aschner M, Allen JW, Kimelberg HK, LoPachin RM & Streit WJ (1999). Glial cells in neurotoxicity development. *Annu Rev Pharmacol Toxicol* **39,** 151-173.

Asensio VC & Campbell IL (2001). Chemokines and viral diseases of the central nervous system. *Adv Virus Res* **56,** 127-173.

Awasthi A, Matsunaga Y & Yamada T (2005). Amyloid-beta causes apoptosis of neuronal cells via caspase cascade, which can be prevented by amyloid-beta-derived short peptides. *Exp Neurol* **196,** 282-289.

Baker AF, Briehl MM, Dorr R & Powis G (1996). Decreased antioxidant defence and increased oxidant stress during dexamethasone-induced apoptosis: bcl-2 prevents the loss of antioxidant enzyme activity. *Cell Death Differ* **3,** 207-213.

Balakumar P & Singh M (2006). Anti-tumour necrosis factor-alpha therapy in heart failure: future directions. *Basic Clin Pharmacol Toxicol* **99,** 391-397.

Ballestas ME & Benveniste EN (1997). Elevation of cyclic AMP levels in astrocytes antagonizes cytokine-induced adhesion molecule expression. *J Neurochem* **69,** 1438-1448.

Balu DT, Hoshaw BA, Malberg JE, Rosenzweig-Lipson S, Schechter LE & Lucki I (2008). Differential regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. *Brain Res* **1211,** 37-43.

Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG & Miller FD (1998). The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *J Cell Biol* **140,** 911-923.

Baptiste DC, Hartwick AT, Jollimore CA, Baldridge WH, Chauhan BC, Tremblay F & Kelly ME (2002). Comparison of the neuroprotective effects of adrenoceptor drugs in retinal cell culture and intact retina. *Invest Ophthalmol Vis Sci* **43,** 2666-2676.

Barbacid M (1994). The Trk family of neurotrophin receptors. *J Neurobiol* **25,** 1386-1403.

Barde YA, Edgar D & Thoenen H (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO J* **1,** 549-553.

Barik J & Wonnacott S (2006). Indirect modulation by alpha7 nicotinic acetylcholine receptors of noradrenaline release in rat hippocampal slices: interaction with glutamate and GABA systems and effect of nicotine withdrawal. *Mol Pharmacol* **69,** 618-628.

Bariohay B, Lebrun B, Moyse E & Jean A (2005). Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. *Endocrinology* **146,** 5612-5620.

Barone FC, Arvin B, White RF, Miller A, Webb CL, Willette RN, Lysko PG & Feuerstein GZ (1997). Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury. *Stroke* **28,** 1233-1244.

Barone FC & Feuerstein GZ (1999). Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* **19,** 819-834.

Bauer ME & Tejani-Butt SM (1992). Effects of repeated administration of desipramine or electroconvulsive shock on norepinephrine uptake sites measured by [3H]nisoxetine autoradiography. *Brain Res* **582,** 208-214.

Bauer S, Moyse E, Jourdan F, Colpaert F, Martel JC & Marien M (2003). Effects of the alpha 2-adrenoreceptor antagonist dexefaroxan on neurogenesis in the olfactory bulb of the adult rat in vivo: selective protection against neuronal death. *Neuroscience* **117,** 281-291.

Beal MF (1998). Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. *Ann Neurol* **44,** S110-114.

Beatty WL, Belanger TA, Desai AA, Morrison RP & Byrne GI (1994). Role of tryptophan in gamma interferon-mediated chlamydial persistence. *Ann N Y Acad Sci* **730,** 304-306.

Beckman JS & Koppenol WH (1996). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* **271,** C1424-1437.

Behan WM & Stone TW (2000). Role of kynurenines in the neurotoxic actions of kainic acid. *Br J Pharmacol* **129,** 1764-1770.

Ben-Ari Y (1985). Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14,** 375-403.

Ben-Ari Y (2001). Cell death and synaptic reorganizations produced by seizures. *Epilepsia* **42 Suppl 3,** 5-7.

Ben-Ari Y, Tremblay E, Ottersen OP & Meldrum BS (1980). The role of epileptic activity in hippocampal and "remote" cerebral lesions induced by kainic acid. *Brain Res* **191,** 79-97.

Bender DA & McCreanor GM (1982). The preferred route of kynurenine metabolism in the rat. *Biochim Biophys Acta* **717,** 56-60.

Benkovic SA, O'Callaghan JP & Miller DB (2004). Sensitive indicators of injury reveal hippocampal damage in C57BL/6J mice treated with kainic acid in the absence of tonic-clonic seizures. *Brain Res* **1024,** 59-76.

Bennett SA, Stevenson B, Staines WA & Roberts DC (1995). Periodic acid-Schiff (PAS)-positive deposits in brain following kainic acid-induced seizures: relationships to fos induction, neuronal necrosis, reactive gliosis, and blood-brain barrier breakdown. *Acta Neuropathol* **89,** 126-138.

Benveniste EN, Huneycutt BS, Shrikant P & Ballestas ME (1995). Second messenger systems in the regulation of cytokines and adhesion molecules in the central nervous system. *Brain Behav Immun* **9,** 304-314.

Berridge MJ, Lipp P & Bootman MD (2000). The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* **1,** 11-21.

Berthelsen S & Pettinger WA (1977). A functional basis for classification of alpha-adrenergic receptors. *Life Sci* **21,** 595-606.

Betz AL, Yang GY & Davidson BL (1995). Attenuation of stroke size in rats using an adenoviral vector to induce overexpression of interleukin-1 receptor antagonist in brain. *J Cereb Blood Flow Metab* **15,** 547-551.

Bhardwaj A & Aggarwal BB (2003). Receptor-mediated choreography of life and death. *J Clin Immunol* **23,** 317-332.

Billiau A, Heremans H, Vandekerckhove F, Dijkmans R, Sobis H, Meulepas E & Carton H (1988). Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN-gamma. *J Immunol* **140,** 1506-1510.

Binder DK (2007). Neurotrophins in the dentate gyrus. *Prog Brain Res* **163,** 371-397.

Binder DK, Routbort MJ, Ryan TE, Yancopoulos GD & McNamara JO (1999). Selective inhibition of kindling development by intraventricular administration of TrkB receptor body. *J Neurosci* **19,** 1424-1436.

Binder DK & Scharfman HE (2004). Brain-derived neurotrophic factor. *Growth Factors* **22,** 123-131.

Biscoe TJ, Evans RH, Headley PM, Martin MR & Watkins JC (1976). Structure-activity relations of excitatory amino acids on frog and rat spinal neurones. *Br J Pharmacol* **58,** 373-382.

Blasko I, Veerhuis R, Stampfer-Kountchev M, Saurwein-Teissl M, Eikelenboom P & Grubeck-Loebenstein B (2000). Costimulatory effects of interferon-gamma and interleukin-1beta or tumor necrosis factor alpha on the synthesis of Abeta1-40 and Abeta1-42 by human astrocytes. *Neurobiol Dis* **7,** 682-689.

Blochl A & Thoenen H (1996). Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons. *Mol Cell Neurosci* **7,** 173-190.

Block ML, Zecca L & Hong JS (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* **8,** 57-69.

Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen IM, Ricci JE, Edris WA, Sutherlin DP, Green DR & Salvesen GS (2003). A unified model for apical caspase activation. *Mol Cell* **11,** 529-541.

Bondi CO, Barrera G, Lapiz MD, Bedard T, Mahan A & Morilak DA (2007). Noradrenergic facilitation of shock-probe defensive burying in lateral septum of rats, and modulation by chronic treatment with desipramine. *Prog Neuropsychopharmacol Biol Psychiatry* **31,** 482-495.

Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y & Rothwell NJ (2001). Role of IL-1alpha and IL-1beta in ischemic brain damage. *J Neurosci* **21,** 5528-5534.

Boyd JD, Jang H, Shepherd KR, Faherty C, Slack S, Jiao Y & Smeyne RJ (2007). Response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) differs in mouse strains and reveals a divergence in JNK signaling and COX-2 induction prior to loss of neurons in the substantia nigra pars compacta. *Brain Res* **1175,** 107-116.

Boyd JG & Gordon T (2002). A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. *Eur J Neurosci* **15,** 613-626.

Braak H & Braak E (1997). Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol Aging* **18,** 351-357.

Bravo JA, Diaz-Veliz G, Mora S, Ulloa JL, Berthoud VM, Morales P, Arancibia S & Fiedler JL (2009). Desipramine prevents stress-induced changes in depressive-like behavior and hippocampal markers of neuroprotection. *Behav Pharmacol* **20,** 273-285.

Breder CD, Dewitt D & Kraig RP (1995). Characterization of inducible cyclooxygenase in rat brain. *J Comp Neurol* **355,** 296-315.

Bregola G, Frigati L, Zucchini S & Simonato M (2000). Different patterns of induction of fibroblast growth factor-2 and brain-derived neurotrophic factor messenger RNAs during kindling epileptogenesis, and development of a herpes simplex vector for fibroblast growth factor-2 gene transfer in vivo. *Epilepsia* **41 Suppl 6,** S122-126.

Brenner T, Yamin A, Abramsky O & Gallily R (1993). Stimulation of tumor necrosis factor-alpha production by mycoplasmas and inhibition by dexamethasone in cultured astrocytes. *Brain Res* **608,** 273-279.

Brenner T, Yamin A & Gallily R (1994). Mycoplasma triggering of nitric oxide production by central nervous system glial cells and its inhibition by glucocorticoids. *Brain Res* **641,** 51-56.

Brown AW & Brierley JB (1972). Anoxic-ischaemic cell change in rat brain light microscopic and fine-structural observations. *J Neurol Sci* **16,** 59-84.

Brown GC & Bal-Price A (2003). Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. *Mol Neurobiol* **27,** 325-355.

Buchmeier NA & Schreiber RD (1985). Requirement of endogenous interferon-gamma production for resolution of Listeria monocytogenes infection. *Proc Natl Acad Sci U S A* **82,** 7404-7408.

Buisson A, Lesne S, Docagne F, Ali C, Nicole O, MacKenzie ET & Vivien D (2003). Transforming growth factor-beta and ischemic brain injury. *Cell Mol Neurobiol* **23,** 539-550.

Buntinx M, Moreels M, Vandenabeele F, Lambrichts I, Raus J, Steels P, Stinissen P & Ameloot M (2004). Cytokine-induced cell death in human oligodendroglial cell lines: I. Synergistic effects of IFN-gamma and TNF-alpha on apoptosis. *J Neurosci Res* **76,** 834-845.

Burda J, Danielisova V, Nemethova M, Gottlieb M, Kravcukova P, Domorakova I, Mechirova E & Burda R (2009). Postconditioning and anticonditioning: possibilities to interfere to evoked apoptosis. *Cell Mol Neurobiol* **29,** 821-825.

Buss H, Dorrie A, Schmitz ML, Hoffmann E, Resch K & Kracht M (2004). Constitutive and interleukin-1-inducible phosphorylation of p65 NF-{kappa}B at serine 536 is mediated by multiple protein kinases including I{kappa}B kinase (IKK)-{alpha}, IKK{beta}, IKK{epsilon}, TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein-associated factor II31-mediated interleukin-8 transcription. *J Biol Chem* **279,** 55633-55643.

Buttini M, Sauter A & Boddeke HW (1994). Induction of interleukin-1 beta mRNA after focal cerebral ischaemia in the rat. *Brain Res Mol Brain Res* **23,** 126-134.

Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR, Jr. & Trendelenburg U (1994). International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol Rev* **46,** 121-136.

Cahoon-Metzger SM, Wang G & Scott SA (2001). Contribution of BDNF-mediated inhibition in patterning avian skin innervation. *Dev Biol* **232,** 246-254.

Campbell IL, Abraham CR, Masliah E, Kemper P, Inglis JD, Oldstone MB & Mucke L (1993). Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proc Natl Acad Sci U S A* **90,** 10061-10065.

Canciani L, Giaroni C, Zanetti E, Giuliani D, Pisani R, Moro E, Trinchera M, Crema F, Lecchini S & Frigo G (2006). Functional interaction between alpha2-adrenoceptors, mu- and kappa-opioid receptors in the guinea pig myenteric plexus: effect of chronic desipramine treatment. *Eur J Pharmacol* **553,** 269-279.

Candelario-Jalil E, Ajamieh HH, Sam S, Martinez G & Leon Fernandez OS (2000). Nimesulide limits kainate-induced oxidative damage in the rat hippocampus. *Eur J Pharmacol* **390,** 295-298.

Canova C, Baudet C, Chevalier G, Brachet P & Wion D (1997). Noradrenaline inhibits the programmed cell death induced by 1,25-dihydroxyvitamin D3 in glioma. *Eur J Pharmacol* **319,** 365-368.

Cardenas A, Moro MA, Hurtado O, Leza JC, Lorenzo P, Castrillo A, Bodelon OG, Bosca L & Lizasoain I (2000). Implication of glutamate in the expression of inducible nitric oxide synthase after oxygen and glucose deprivation in rat forebrain slices. *J Neurochem* **74,** 2041-2048.

Carlin JM, Borden EC & Byrne GI (1989). Interferon-induced indoleamine 2,3-dioxygenase activity inhibits Chlamydia psittaci replication in human macrophages. *J Interferon Res* **9,** 329-337.

Carmignoto G, Pizzorusso T, Tia S & Vicini S (1997). Brain-derived neurotrophic factor and nerve growth factor potentiate excitatory synaptic transmission in the rat visual cortex. *J Physiol* **498 (Pt 1),** 153-164.

Carpenedo R, Chiarugi A, Russi P, Lombardi G, Carla V, Pellicciari R, Mattoli L & Moroni F (1994). Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. *Neuroscience* **61,** 237-243.

Carpenedo R, Pittaluga A, Cozzi A, Attucci S, Galli A, Raiteri M & Moroni F (2001). Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *Eur J Neurosci* **13,** 2141-2147.

Carter AR, Berry EM & Segal RA (2003). Regional expression of p75NTR contributes to neurotrophin regulation of cerebellar patterning. *Mol Cell Neurosci* **22,** 1-13.

Castillo CG, Mendoza S, Freed WJ & Giordano M (2006). Intranigral transplants of immortalized GABAergic cells decrease the expression of kainic acid-induced seizures in the rat. *Behav Brain Res* **171,** 109-115.

Castren E (2004). Neurotrophic effects of antidepressant drugs. *Curr Opin Pharmacol* **4,** 58-64.

Castren E, Pitkanen M, Sirvio J, Parsadanian A, Lindholm D, Thoenen H & Riekkinen PJ (1993). The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. *Neuroreport* **4,** 895-898.

Ceresoli-Borroni G & Schwarcz R (2001). Neonatal asphyxia in rats: acute effects on cerebral kynurenine metabolism. *Pediatr Res* **50,** 231-235.

Chan AS, Ng LW, Poon LS, Chan WW & Wong YH (2007). Dopaminergic and adrenergic toxicities on SK-N-MC human neuroblastoma cells are mediated through G protein signaling and oxidative stress. *Apoptosis* **12,** 167-179.

Chang CK, Llanes S & Schumer W (1997). Effect of dexamethasone on NF-kB activation, tumor necrosis factor formation, and glucose dyshomeostasis in septic rats. *J Surg Res* **72,** 141-145.

Chao MV & Bothwell M (2002). Neurotrophins: to cleave or not to cleave. *Neuron* **33,** 9-12.

Chaouloff F, Elghozi JL, Guezennec Y & Laude D (1985). Effects of conditioned running on plasma, liver and brain tryptophan and on brain 5-hydroxytryptamine metabolism of the rat. *Br J Pharmacol* **86,** 33-41.

Chaouloff F, Kennett GA, Serrurrier B, Merino D & Curzon G (1986). Amino acid analysis demonstrates that increased plasma free tryptophan causes the increase of brain tryptophan during exercise in the rat. *J Neurochem* **46,** 1647-1650.

Chelmicka-Schorr E, Kwasniewski MN, Thomas BE & Arnason BG (1989). The beta-adrenergic agonist isoproterenol suppresses experimental allergic encephalomyelitis in Lewis rats. *J Neuroimmunol* **25,** 203-207.

Chen B, Wang JF, Hill BC & Young LT (1999). Lithium and valproate differentially regulate brain regional expression of phosphorylated CREB and c-Fos. *Brain Res Mol Brain Res* **70,** 45-53.

Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, Bian J, Guo L, Farrell LA, Hersch SM, Hobbs W, Vonsattel JP, Cha JH & Friedlander RM (2000). Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* **6,** 797-801.

Chen MJ, Nguyen TV, Pike CJ & Russo-Neustadt AA (2007). Norepinephrine induces BDNF and activates the PI-3K and MAPK cascades in embryonic hippocampal neurons. *Cell Signal* **19,** 114-128.

Chen MJ & Russo-Neustadt AA (2007). Nitric oxide signaling participates in norepinephrine-induced activity of neuronal intracellular survival pathways. *Life Sci* **81,** 1280-1290.

Chen PW, Mellon JK, Mayhew E, Wang S, He YG, Hogan N & Niederkorn JY (2007). Uveal melanoma expression of indoleamine 2,3-deoxygenase: establishment of an immune privileged environment by tryptophan depletion. *Exp Eye Res* **85,** 617-625.

Chen X, Bao G, Hua Y, Li Y, Wang Z & Zhang X (2009). The effects of topiramate on caspase-3 expression in hippocampus of basolateral amygdala (BLA) electrical kindled epilepsy rat. *J Mol Neurosci* **38,** 201-206.

Chen YN, Yamada H, Mao W, Matsuyama S, Aihara M & Araie M (2007). Hypoxia-induced retinal ganglion cell death and the neuroprotective effects of beta-adrenergic antagonists. *Brain Res* **1148,** 28-37.

Chin MH, Qian WJ, Wang H, Petyuk VA, Bloom JS, Sforza DM, Lacan G, Liu D, Khan AH, Cantor RM, Bigelow DJ, Melega WP, Camp DG, 2nd, Smith RD & Smith DJ (2008). Mitochondrial dysfunction, oxidative stress, and apoptosis revealed by proteomic and transcriptomic analyses of the striata in two mouse models of Parkinson's disease. *J Proteome Res* **7,** 666-677.

Cho IH, Hong J, Suh EC, Kim JH, Lee H, Lee JE, Lee S, Kim CH, Kim DW, Jo EK, Lee KE, Karin M & Lee SJ (2008). Role of microglial IKKbeta in kainic acid-induced hippocampal neuronal cell death. *Brain* **131,** 3019-3033.

Choi DW (1987). Ionic dependence of glutamate neurotoxicity. *J Neurosci* **7,** 369-379.

Choi DW (1992). Excitotoxic cell death. *J Neurobiol* **23,** 1261-1276.

Choi DW, Maulucci-Gedde M & Kriegstein AR (1987). Glutamate neurotoxicity in cortical cell culture. *J Neurosci* **7,** 357-368.

Choi-Lundberg DL & Bohn MC (1995). Ontogeny and distribution of glial cell line-derived neurotrophic factor (GDNF) mRNA in rat. *Brain Res Dev Brain Res* **85,** 80-88.

Chon SY, Hassanain HH, Pine R & Gupta SL (1995). Involvement of two regulatory elements in interferon-gamma-regulated expression of human indoleamine 2,3-dioxygenase gene. *J Interferon Cytokine Res* **15,** 517-526.

Cohen GM (1997). Caspases: the executioners of apoptosis. *Biochem J* **326 (Pt 1),** 1-16.

Colangelo AM, Johnson PF & Mocchetti I (1998). beta-adrenergic receptor-induced activation of nerve growth factor gene transcription in rat cerebral cortex involves CCAAT/enhancer-binding protein delta. *Proc Natl Acad Sci U S A* **95,** 10920-10925.

Colangelo AM, Mallei A, Johnson PF & Mocchetti I (2004). Synergistic effect of dexamethasone and beta-adrenergic receptor agonists on the nerve growth factor gene transcription. *Brain Res Mol Brain Res* **124,** 97-104.

Colbourne F, Sutherland GR & Auer RN (1999). Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia. *J Neurosci* **19,** 4200-4210.

Collino M, Thiemermann C, Mastrocola R, Gallicchio M, Benetti E, Miglio G, Castiglia S, Danni O, Murch O, Dianzani C, Aragno M & Fantozzi R (2008). Treatment with the glycogen synthase kinase-3beta inhibitor, TDZD-8, affects transient cerebral ischemia/reperfusion injury in the rat hippocampus. *Shock* **30,** 299-307.

Conner JM, Lauterborn JC, Yan Q, Gall CM & Varon S (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J Neurosci* **17,** 2295-2313.

Connor TJ, Harkin A, Kelly JP & Leonard BE (2000). Olfactory bulbectomy provokes a suppression of interleukin-1beta and tumour necrosis factor-alpha production in response to an in vivo challenge with lipopolysaccharide: effect of chronic desipramine treatment. *Neuroimmunomodulation* **7,** 27-35.

Coppell AL, Pei Q & Zetterstrom TS (2003). Bi-phasic change in BDNF gene expression following antidepressant drug treatment. *Neuropharmacology* **44,** 903-910.

Cosgaya JM, Chan JR & Shooter EM (2002). The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science* **298,** 1245-1248.

Cotecchia S, Kobilka BK, Daniel KW, Nolan RD, Lapetina EY, Caron MG, Lefkowitz RJ & Regan JW (1990). Multiple second messenger pathways of alpha-adrenergic receptor subtypes expressed in eukaryotic cells. *J Biol Chem* **265,** 63-69.

Coyle JT (1983). Neurotoxic action of kainic acid. *J Neurochem* **41,** 1-11.

Coyle JT (1987). Kainic acid: insights into excitatory mechanisms causing selective neuronal degeneration. *Ciba Found Symp* **126,** 186-203.

Coyle JT & Duman RS (2003). Finding the intracellular signaling pathways affected by mood disorder treatments. *Neuron* **38,** 157-160.

Coyle JT & Schwarcz R (1976). Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. *Nature* **263,** 244-246.

Crespel A, Coubes P, Rousset MC, Brana C, Rougier A, Rondouin G, Bockaert J, Baldy-Moulinier M & Lerner-Natoli M (2002). Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. *Brain Res* **952,** 159-169.

Crowder RJ & Freeman RS (2000). Glycogen synthase kinase-3 beta activity is critical for neuronal death caused by inhibiting phosphatidylinositol 3-kinase or Akt but not for death caused by nerve growth factor withdrawal. *J Biol Chem* **275,** 34266-34271.

Culmsee C, Junker V, Kremers W, Thal S, Plesnila N & Krieglstein J (2004). Combination therapy in ischemic stroke: synergistic neuroprotective effects of memantine and clenbuterol. *Stroke* **35,** 1197-1202.

Culmsee C, Junker V, Thal S, Kremers W, Maier S, Schneider HJ, Plesnila N & Krieglstein J (2007). Enantio-selective effects of clenbuterol in cultured neurons and astrocytes, and in a mouse model of cerebral ischemia. *Eur J Pharmacol* **575,** 57-65.

Culmsee C, Semkova I & Krieglstein J (1999). NGF mediates the neuroprotective effect of the beta2-adrenoceptor agonist clenbuterol in vitro and in vivo: evidence from an NGF-antisense study. *Neurochem Int* **35,** 47-57.

Culmsee C, Stumm RK, Schafer MK, Weihe E & Krieglstein J (1999). Clenbuterol induces growth factor mRNA, activates astrocytes, and protects rat brain tissue against ischemic damage. *Eur J Pharmacol* **379,** 33-45.

Currier AR, Ziegler MH, Riley MM, Babcock TA, Telbis VP & Carlin JM (2000). Tumor necrosis factor-alpha and lipopolysaccharide enhance interferon-induced antichlamydial indoleamine dioxygenase activity independently. *J Interferon Cytokine Res* **20,** 369-376.

Czeh B, Simon M, Schmelting B, Hiemke C & Fuchs E (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology* **31,** 1616-1626.

Daaka Y, Luttrell LM & Lefkowitz RJ (1997). Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* **390,** 88-91.

Daikhin Y & Yudkoff M (2000). Compartmentation of brain glutamate metabolism in neurons and glia. *J Nutr* **130,** 1026S-1031S.

Dammann O & Leviton A (2004). Inflammatory brain damage in preterm newborns--dry numbers, wet lab, and causal inferences. *Early Hum Dev* **79,** 1-15.

Das S & Basu A (2008). Inflammation: a new candidate in modulating adult neurogenesis. *J Neurosci Res* **86,** 1199-1208.

De Groot CJ, Langeveld CH, Jongenelen CA, Montagne L, Van Der Valk P & Dijkstra CD (1997). Establishment of human adult astrocyte cultures derived from postmortem multiple sclerosis and control brain and spinal cord regions: immunophenotypical and functional characterization. *J Neurosci Res* **49,** 342-354.

De Keyser J, Zeinstra E & Wilczak N (2004). Astrocytic beta2-adrenergic receptors and multiple sclerosis. *Neurobiol Dis* **15,** 331-339.

de Montigny C & Tardif D (1981). Differential excitatory effects of kainic acid on CA3 and CA1 hippocampal pyramidal neurons: further evidence for the excitotoxic hypothesis and for a receptor-mediated action. *Life Sci* **29,** 2103-2111.

De Simoni MG, Perego C, Ravizza T, Moneta D, Conti M, Marchesi F, De Luigi A, Garattini S & Vezzani A (2000). Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. *Eur J Neurosci* **12,** 2623-2633.

Debillon T, Gras-Leguen C, Leroy S, Caillon J, Roze JC & Gressens P (2003). Patterns of cerebral inflammatory response in a rabbit model of intrauterine infection-mediated brain lesion. *Brain Res Dev Brain Res* **145,** 39-48.

Dechant G & Barde YA (2002). The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat Neurosci* **5,** 1131-1136.

Deffois A, Fage D & Carter C (1996). Inhibition of synaptosomal veratridine-induced sodium influx by antidepressants and neuroleptics used in chronic pain. *Neurosci Lett* **220,** 117-120.

del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X & Feuerstein GZ (2000). Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain Pathol* **10,** 95-112.

Dello Russo C, Boullerne AI, Gavrilyuk V & Feinstein DL (2004). Inhibition of microglial inflammatory responses by norepinephrine: effects on nitric oxide and interleukin-1beta production. *J Neuroinflammation* **1,** 9.

Desaphy JF, Pierno S, De Luca A, Didonna P & Camerino DC (2003). Different ability of clenbuterol and salbutamol to block sodium channels predicts their therapeutic use in muscle excitability disorders. *Mol Pharmacol* **63,** 659-670.

Dheen ST, Kaur C & Ling EA (2007). Microglial activation and its implications in the brain diseases. *Curr Med Chem* **14,** 1189-1197.

Diamond M, Kelly JP & Connor TJ (2006). Antidepressants suppress production of the Th1 cytokine interferon-gamma, independent of monoamine transporter blockade. *Eur Neuropsychopharmacol* **16,** 481-490.

Diguet E, Gross CE, Tison F & Bezard E (2004). Rise and fall of minocycline in neuroprotection: need to promote publication of negative results. *Exp Neurol* **189,** 1-4.

Dingledine R, Borges K, Bowie D & Traynelis SF (1999). The glutamate receptor ion channels. *Pharmacol Rev* **51,** 7-61.

Distelhorst CW (2002). Recent insights into the mechanism of glucocorticosteroid-induced apoptosis. *Cell Death Differ* **9,** 6-19.

Doble A (1999). The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacol Ther* **81,** 163-221.

Dommergues MA, Patkai J, Renauld JC, Evrard P & Gressens P (2000). Proinflammatory cytokines and interleukin-9 exacerbate excitotoxic lesions of the newborn murine neopallium. *Ann Neurol* **47,** 54-63.

Dong H, Csernansky CA, Goico B & Csernansky JG (2003). Hippocampal neurogenesis follows kainic acid-induced apoptosis in neonatal rats. *J Neurosci* **23,** 1742-1749.

Donnelly S, Loscher CE, Lynch MA & Mills KH (2001). Whole-cell but not acellular pertussis vaccines induce convulsive activity in mice: evidence of a role for toxin-induced interleukin-1beta in a new murine model for analysis of neuronal side effects of vaccination. *Infect Immun* **69,** 4217-4223.

Donnici L, Tiraboschi E, Tardito D, Musazzi L, Racagni G & Popoli M (2008). Time-dependent biphasic modulation of human BDNF by antidepressants in neuroblastoma cells. *BMC Neurosci* **9,** 61.

Dostert P, Benedetti MS & Poggesi I (1997). Review of the pharmacokinetics and metabolism of reboxetine, a selective noradrenaline reuptake inhibitor. *Eur Neuropsychopharmacol* **7 Suppl 1,** S23-35; discussion S71-23.

Doyle KP, Simon RP & Stenzel-Poore MP (2008). Mechanisms of ischemic brain damage. *Neuropharmacology* **55,** 310-318.

D'Sa C & Duman RS (2002). Antidepressants and neuroplasticity. *Bipolar Disord* **4,** 183-194.

Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP & Paul SM (2001). Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proc Natl Acad Sci U S A* **98,** 14669-14674.

Dudek FE, Obenaus A, Schweitzer JS & Wuarin JP (1994). Functional significance of hippocampal plasticity in epileptic brain: electrophysiological changes of the dentate granule cells associated with mossy fiber sprouting. *Hippocampus* **4,** 259-265.

Dugan LL SS, Canzoniero LMT, Handran SD, Rothman SM, Lin TS, Goldberg MP, Elger CE, Helmstaedter C, Kurthen M. (1995). Chronic epilepsy and cognition. *Lancet Neurology* **3,** 663-672.

Dugich-Djordjevic MM, Tocco G, Willoughby DA, Najm I, Pasinetti G, Thompson RF, Baudry M, Lapchak PA & Hefti F (1992). BDNF mRNA expression in the developing rat brain following kainic acid-induced seizure activity. *Neuron* **8,** 1127-1138.

Duman RS (2004). Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular Med* **5,** 11-25.

Duman RS, Heninger GR & Nestler EJ (1997). A molecular and cellular theory of depression. *Arch Gen Psychiatry* **54,** 597-606.

Duman RS, Malberg J & Nakagawa S (2001). Regulation of adult neurogenesis by psychotropic drugs and stress. *J Pharmacol Exp Ther* **299,** 401-407.

Duncan ND, Williams DA & Lynch GS (2000). Deleterious effects of chronic clenbuterol treatment on endurance and sprint exercise performance in rats. *Clin Sci (Lond)* **98,** 339-347.

Dunn AJ (1988). Changes in plasma and brain tryptophan and brain serotonin and 5-hydroxyindoleacetic acid after footshock stress. *Life Sci* **42,** 1847-1853.

Dunn AJ (1988). Stress-related activation of cerebral dopaminergic systems. *Ann N Y Acad Sci* **537,** 188-205.

Dunn AJ & Chuluyan HE (1992). The role of cyclo-oxygenase and lipoxygenase in the interleukin-1-induced activation of the HPA axis: dependence on the route of injection. *Life Sci* **51,** 219-225.

Duong TT, Finkelman FD, Singh B & Strejan GH (1994). Effect of anti-interferon-gamma monoclonal antibody treatment on the development of experimental allergic encephalomyelitis in resistant mouse strains. *J Neuroimmunol* **53,** 101-107.

Duong TT, St Louis J, Gilbert JJ, Finkelman FD & Strejan GH (1992). Effect of anti-interferon-gamma and anti-interleukin-2 monoclonal antibody treatment on the development of actively and passively induced experimental allergic encephalomyelitis in the SJL/J mouse. *J Neuroimmunol* **36,** 105-115.

Dykens JA (1994). Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated CA2+ and Na+: implications for neurodegeneration. *J Neurochem* **63,** 584-591.

Eisenhofer G (2001). The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol Ther* **91,** 35-62.

Ekegren T, Grundstrom E, Lindholm D & Aquilonius SM (1999). Upregulation of Bax protein and increased DNA degradation in ALS spinal cord motor neurons. *Acta Neurol Scand* **100,** 317-321.

Elghozi JL, Bianchetti G, Morselli PL & Meyer P (1979). Brain distribution of propranolol in the rat. *Eur J Pharmacol* **55,** 319-322.

Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A & Nagata S (1998). A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* **391,** 43-50.

Endoh M, Maiese K, Pulsinelli WA & Wagner JA (1993). Reactive astrocytes express NADPH diaphorase in vivo after transient ischemia. *Neurosci Lett* **154,** 125-128.

Erickson JB, Flanagan EM, Russo S & Reinhard JF, Jr. (1992). A radiometric assay for kynurenine 3-hydroxylase based on the release of 3H2O during hydroxylation of L-[3,5-3H]kynurenine. *Anal Biochem* **205,** 257-262.

Eriksson C, Tehranian R, Iverfeldt K, Winblad B & Schultzberg M (2000). Increased expression of mRNA encoding interleukin-1beta and caspase-1, and the secreted isoform of interleukin-1 receptor antagonist in the rat brain following systemic kainic acid administration. *J Neurosci Res* **60,** 266-279.

Eriksson C, Van Dam AM, Lucassen PJ, Bol JG, Winblad B & Schultzberg M (1999). Immunohistochemical localization of interleukin-1beta, interleukin-1 receptor antagonist and interleukin-1beta converting enzyme/caspase-1 in the rat brain after peripheral administration of kainic acid. *Neuroscience* **93,** 915-930.

Eriksson C, Zou LP, Ahlenius S, Winblad B & Schultzberg M (2000). Inhibition of kainic acid induced expression of interleukin-1 beta and interleukin-1 receptor antagonist mRNA in the rat brain by NMDA receptor antagonists. *Brain Res Mol Brain Res* **85,** 103-113.

Ernfors P, Bengzon J, Kokaia Z, Persson H & Lindvall O (1991). Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. *Neuron* **7,** 165-176.

Escorihuela RM, Boix F, Corda MG, Tobena A & Fernandez Teruel A (1989). Chronic but not acute antidepressant treatment increases pentetrazol-induced convulsions in mice. *J Pharm Pharmacol* **41,** 143-144.

Espey MG, Chernyshev ON, Reinhard JF, Jr., Namboodiri MA & Colton CA (1997). Activated human microglia produce the excitotoxin quinolinic acid. *Neuroreport* **8,** 431-434.

Estrada Sanchez AM, Mejia-Toiber J & Massieu L (2008). Excitotoxic neuronal death and the pathogenesis of Huntington's disease. *Arch Med Res* **39,** 265-276.

Evans MC, Griffiths T & Meldrum BS (1984). Kainic acid seizures and the reversibility of calcium loading in vulnerable neurons in the hippocampus. *Neuropathol Appl Neurobiol* **10,** 285-302.

Faherty CJ, Xanthoudakis S & Smeyne RJ (1999). Caspase-3-dependent neuronal death in the hippocampus following kainic acid treatment. *Brain Res Mol Brain Res* **70,** 159-163.

Fallarino F, Vacca C, Orabona C, Belladonna ML, Bianchi R, Marshall B, Keskin DB, Mellor AL, Fioretti MC, Grohmann U & Puccetti P (2002). Functional expression of indoleamine 2,3-dioxygenase by murine CD8 alpha(+) dendritic cells. *Int Immunol* **14,** 65-68.

Fan LW, Lin S, Pang Y, Rhodes PG & Cai Z (2006). Minocycline attenuates hypoxia-ischemia-induced neurological dysfunction and brain injury in the juvenile rat. *Eur J Neurosci* **24,** 341-350.

Favaron M, Manev H, Siman R, Bertolino M, Szekely AM, DeErausquin G, Guidotti A & Costa E (1990). Down-regulation of protein kinase C protects cerebellar granule neurons in primary culture from glutamate-induced neuronal death. *Proc Natl Acad Sci U S A* **87,** 1983-1987.

Favrais G, Couvineau A, Laburthe M, Gressens P & Lelievre V (2007). Involvement of VIP and PACAP in neonatal brain lesions generated by a combined excitotoxic/inflammatory challenge. *Peptides* **28,** 1727-1737.

Fawcett JP, Bamji SX, Causing CG, Aloyz R, Ase AR, Reader TA, McLean JH & Miller FD (1998). Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. *J Neurosci* **18,** 2808-2821.

Fazekas I, Szakacs R, Mihaly A, Zador Z, Krisztin-Peva B, Juhasz A & Janka Z (2006). Alterations of seizure-induced c-fos immunolabelling and gene expression in the rat cerebral cortex following dexamethasone treatment. *Acta Histochem* **108,** 463-473.

Feinstein DL (1998). Suppression of astroglial nitric oxide synthase expression by norepinephrine results from decreased NOS-2 promoter activity. *J Neurochem* **70,** 1484-1496.

Feinstein DL, Galea E & Reis DJ (1993). Norepinephrine suppresses inducible nitric oxide synthase activity in rat astroglial cultures. *J Neurochem* **60,** 1945-1948.

Feinstein DL, Heneka MT, Gavrilyuk V, Dello Russo C, Weinberg G & Galea E (2002). Noradrenergic regulation of inflammatory gene expression in brain. *Neurochem Int* **41,** 357-365.

Feldman RS MJ, Quenzer LF (1997). *Principles of Neuropsychopharmacology*. Sinauer Associates Inc., Massachusetts.

Felszeghy K, Banisadr G, Rostene W, Nyakas C & Haour F (2004). Dexamethasone downregulates chemokine receptor CXCR4 and exerts neuroprotection against hypoxia/ischemia-induced brain injury in neonatal rats. *Neuroimmunomodulation* **11,** 404-413.

Fernandes-Alnemri T, Litwack G & Alnemri ES (1994). CPP32, a novel human apoptotic protein with homology to Caenorhabditis elegans cell death protein Ced-3 and mammalian interleukin-1 beta-converting enzyme. *J Biol Chem* **269,** 30761-30764.

Ferrer I, Blanco R & Carmona M (2001). Differential expression of active, phosphorylation-dependent MAP kinases, MAPK/ERK, SAPK/JNK and p38, and specific transcription factor substrates following quinolinic acid excitotoxicity in the rat. *Brain Res Mol Brain Res* **94,** 48-58.

Ferrer I, Marin C, Rey MJ, Ribalta T, Goutan E, Blanco R, Tolosa E & Marti E (1999). BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J Neuropathol Exp Neurol* **58,** 729-739.

Filipowski R, Hetman, M., Kaminska, B. and Kaczmarek L., (1994). DNA fragmentation in rat brain after intraperitoneal administration of kainate. *Neuroreport* **5,** 1538-1540.

Fink K, Bonisch H & Gothert M (1990). Presynaptic NMDA receptors stimulate noradrenaline release in the cerebral cortex. *Eur J Pharmacol* **185,** 115-117.

Fischer W (2002). Anticonvulsant profile and mechanism of action of propranolol and its two enantiomers. *Seizure* **11,** 285-302.

Fischer W, Kittner H, Regenthal R, Malinowska B & Schlicker E (2001). Anticonvulsant and sodium channel blocking activity of higher doses of clenbuterol. *Naunyn Schmiedebergs Arch Pharmacol* **363,** 182-192.

Fischer W, Lasek R & Muller M (1985). Anticonvulsant effects of propranolol and their pharmacological modulation. *Pol J Pharmacol Pharm* **37,** 883-896.

Fischer W & Muller M (1988). Pharmacological modulation of central monoaminergic systems and influence on the anticonvulsant effectiveness of standard antiepileptics in maximal electroshock seizure. *Biomed Biochim Acta* **47,** 631-645.

Fitzgerald KA & O'Neill LA (2000). The role of the interleukin-1/Toll-like receptor superfamily in inflammation and host defence. *Microbes Infect* **2,** 933-943.

Flanders KC, Ren RF & Lippa CF (1998). Transforming growth factor-betas in neurodegenerative disease. *Prog Neurobiol* **54,** 71-85.

Forman MS, Trojanowski JQ & Lee VM (2004). Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. *Nat Med* **10,** 1055-1063.

Forrest CM, Youd P, Kennedy A, Gould SR, Darlington LG & Stone TW (2002). Purine, kynurenine, neopterin and lipid peroxidation levels in inflammatory bowel disease. *J Biomed Sci* **9,** 436-442.

Foster AC, Miller LP, Oldendorf WH & Schwarcz R (1984). Studies on the disposition of quinolinic acid after intracerebral or systemic administration in the rat. *Exp Neurol* **84,** 428-440.

Foster GA & Roberts PJ (1981). Kainic acid stimulation of cerebellar cyclic GMP levels: potentiation by glutamate and related amino acids. *Neurosci Lett* **23,** 67-70.

Frohman EM, Vayuvegula B, van den Noort S & Gupta S (1988). Norepinephrine inhibits gamma-interferon-induced MHC class II (Ia) antigen expression on cultured brain astrocytes. *J Neuroimmunol* **17,** 89-101.

Fujikawa DG (2005). Prolonged seizures and cellular injury: understanding the connection. *Epilepsy Behav* **7 Suppl 3,** S3-11.

Fujikawa DG, Shinmei SS & Cai B (1999). Lithium-pilocarpine-induced status epilepticus produces necrotic neurons with internucleosomal DNA fragmentation in adult rats. *Eur J Neurosci* **11,** 1605-1614.

Fujikawa DG, Shinmei SS & Cai B (2000). Kainic acid-induced seizures produce necrotic, not apoptotic, neurons with internucleosomal DNA cleavage: implications for programmed cell death mechanisms. *Neuroscience* **98,** 41-53.

Fujikawa DG, Shinmei SS & Cai B (2000). Seizure-induced neuronal necrosis: implications for programmed cell death mechanisms. *Epilepsia* **41 Suppl 6,** S9-13.

Gagliardi RJ (2000). Neuroprotection, excitotoxicity and NMDA antagonists. *Arq Neuropsiquiatr* **58,** 583-588.

Galea E & Feinstein DL (1999). Regulation of the expression of the inflammatory nitric oxide synthase (NOS2) by cyclic AMP. *FASEB J* **13,** 2125-2137.

Galea E, Feinstein DL & Reis DJ (1992). Induction of calcium-independent nitric oxide synthase activity in primary rat glial cultures. *Proc Natl Acad Sci U S A* **89,** 10945-10949.

Galea E, Heneka MT, Dello Russo C & Feinstein DL (2003). Intrinsic regulation of brain inflammatory responses. *Cell Mol Neurobiol* **23,** 625-635.

Gall C, Brecha N, Karten HJ & Chang KJ (1981). Localization of enkephalin-like immunoreactivity to identified axonal and neuronal populations of the rat hippocampus. *J Comp Neurol* **198,** 335-350.

Gall C & Lauterborn J (1992). The dentate gyrus: a model system for studies of neurotrophin regulation. *Epilepsy Res Suppl* **7,** 171-185.

Gall C, Lauterborn J, Bundman M, Murray K & Isackson P (1991). Seizures and the regulation of neurotrophic factor and neuropeptide gene expression in brain. *Epilepsy Res Suppl* **4,** 225-245.

Gall CM (1993). Seizure-induced changes in neurotrophin expression: implications for epilepsy. *Exp Neurol* **124,** 150-166.

Ganguli R, Yang Z, Shurin G, Chengappa KN, Brar JS, Gubbi AV & Rabin BS (1994). Serum interleukin-6 concentration in schizophrenia: elevation associated with duration of illness. *Psychiatry Res* **51,** 1-10.

Garcia C, Chen MJ, Garza AA, Cotman CW & Russo-Neustadt A (2003). The influence of specific noradrenergic and serotonergic lesions on the expression of hippocampal brain-derived neurotrophic factor transcripts following voluntary physical activity. *Neuroscience* **119,** 721-732.

Garrigue-Antar L, Munoz-Antonia T, Antonia SJ, Gesmonde J, Vellucci VF & Reiss M (1995). Missense mutations of the transforming growth factor beta type II receptor in human head and neck squamous carcinoma cells. *Cancer Res* **55,** 3982-3987.

Gary DS, Getchell TV, Getchell ML & Mattson MP (2002). Olfactory bulbectomy protects hippocampal pyramidal neurons against excitotoxic death. *Exp Neurol* **176,** 266-268.

Gass JT & Olive MF (2008). Glutamatergic substrates of drug addiction and alcoholism. *Biochem Pharmacol* **75,** 218-265.

Gavrilyuk V, Dello Russo C, Heneka MT, Pelligrino D, Weinberg G & Feinstein DL (2002). Norepinephrine increases I kappa B alpha expression in astrocytes. *J Biol Chem* **277,** 29662-29668.

Gehler S, Gallo G, Veien E & Letourneau PC (2004). p75 neurotrophin receptor signaling regulates growth cone filopodial dynamics through modulating RhoA activity. *J Neurosci* **24,** 4363-4372.

Gendron TF, Brunette E, Tauskela JS & Morley P (2005). The dual role of prostaglandin E(2) in excitotoxicity and preconditioning-induced neuroprotection. *Eur J Pharmacol* **517,** 17-27.

Gillardon F, Bottiger B, Schmitz B, Zimmermann M & Hossmann KA (1997). Activation of CPP-32 protease in hippocampal neurons following ischemia and epilepsy. *Brain Res Mol Brain Res* **50,** 16-22.

Ginsberg MD (2008). Neuroprotection for ischemic stroke: past, present and future. *Neuropharmacology* **55,** 363-389.

Giorgi FS, Pizzanelli C, Biagioni F, Murri L & Fornai F (2004). The role of norepinephrine in epilepsy: from the bench to the bedside. *Neurosci Biobehav Rev* **28,** 507-524.

Girnun GD, Domann FE, Moore SA & Robbins ME (2002). Identification of a functional peroxisome proliferator-activated receptor response element in the rat catalase promoter. *Mol Endocrinol* **16,** 2793-2801.

Globus MY, Busto R, Dietrich WD, Martinez E, Valdes I & Ginsberg MD (1989). Direct evidence for acute and massive norepinephrine release in the hippocampus during transient ischemia. *J Cereb Blood Flow Metab* **9,** 892-896.

Good PF, Hsu A, Werner P, Perl DP & Olanow CW (1998). Protein nitration in Parkinson's disease. *J Neuropathol Exp Neurol* **57,** 338-342.

Goyagi T, Kimura T, Nishikawa T, Tobe Y & Masaki Y (2006). Beta-adrenoreceptor antagonists attenuate brain injury after transient focal ischemia in rats. *Anesth Analg* **103,** 658-663.

Grappi S, Nanni G, Leggio B, Rauggi R, Scheggi S, Masi F & Gambarana C (2003). The efficacy of reboxetine in preventing and reverting a condition of escape deficit in rats. *Biol Psychiatry* **53,** 890-898.

Graveland GA, Williams RS & DiFiglia M (1985). Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science* **227,** 770-773.

Griffin WS, Sheng JG, Gentleman SM, Graham DI, Mrak RE & Roberts GW (1994). Microglial interleukin-1 alpha expression in human head injury: correlations with neuronal and neuritic beta-amyloid precursor protein expression. *Neurosci Lett* **176,** 133-136.

Griffith OW & Mulcahy RT (1999). The enzymes of glutathione synthesis: gamma-glutamylcysteine synthetase. *Adv Enzymol Relat Areas Mol Biol* **73,** 209-267, xii.

Gross CG (1998). *Brain, vision, memory. Tales in the history of Neuroscience*. MIT press, Cambridge.

Guidetti P, Bates GP, Graham RK, Hayden MR, Leavitt BR, MacDonald ME, Slow EJ, Wheeler VC, Woodman B & Schwarcz R (2006). Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington disease mice. *Neurobiol Dis* **23,** 190-197.

Guillemin GJ & Brew BJ (2002). Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease. *Redox Rep* **7,** 199-206.

Guillemin GJ, Kerr SJ, Smythe GA, Smith DG, Kapoor V, Armati PJ, Croitoru J & Brew BJ (2001). Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. *J Neurochem* **78,** 842-853.

Gustafson I, Miyauchi Y & Wieloch TW (1989). Postischemic administration of idazoxan, an alpha-2 adrenergic receptor antagonist, decreases neuronal damage in the rat brain. *J Cereb Blood Flow Metab* **9,** 171-174.

Gustafson I, Westerberg E & Wieloch T (1990). Protection against ischemia-induced neuronal damage by the alpha 2-adrenoceptor antagonist idazoxan: influence of time of administration and possible mechanisms of action. *J Cereb Blood Flow Metab* **10,** 885-894.

Gutierrez R (2003). The GABAergic phenotype of the "glutamatergic" granule cells of the dentate gyrus. *Prog Neurobiol* **71,** 337-358.

Haenisch B, Bilkei-Gorzo A, Caron MG & Bonisch H (2009). Knockout of the norepinephrine transporter and pharmacologically diverse antidepressants prevent behavioral and brain neurotrophin alterations in two chronic stress models of depression. *J Neurochem* **111,** 403-416.

Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH & Kern SE (1996). DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* **271,** 350-353.

Hailer NP (2008). Immunosuppression after traumatic or ischemic CNS damage: it is neuroprotective and illuminates the role of microglial cells. *Prog Neurobiol* **84,** 211-233.

Haller I, Lirk P, Keller C, Wang GK, Gerner P & Klimaschewski L (2007). Differential neurotoxicity of tricyclic antidepressants and novel derivatives in vitro in a dorsal root ganglion cell culture model. *Eur J Anaesthesiol* **24,** 702-708.

Halonen T, Kotti T, Tuunanen J, Toppinen A, Miettinen R & Riekkinen PJ (1995). Alpha 2-adrenoceptor agonist, dexmedetomidine, protects against kainic acid-induced convulsions and neuronal damage. *Brain Res* **693,** 217-224.

Han BH, D'Costa A, Back SA, Parsadanian M, Patel S, Shah AR, Gidday JM, Srinivasan A, Deshmukh M & Holtzman DM (2000). BDNF blocks caspase-3 activation in neonatal hypoxia-ischemia. *Neurobiol Dis* **7,** 38-53.

Han RQ, Ouyang YB, Xu L, Agrawal R, Patterson AJ & Giffard RG (2009). Postischemic brain injury is attenuated in mice lacking the beta2-adrenergic receptor. *Anesth Analg* **108,** 280-287.

Handley SL & Singh L (1986). Chronic antidepressant treatment reduces central beta-adrenoceptor sensitivity in a behavioural test. *Eur J Pharmacol* **127,** 97-103.

Hansen AM, Driussi C, Turner V, Takikawa O & Hunt NH (2000). Tissue distribution of indoleamine 2,3-dioxygenase in normal and malaria-infected tissue. *Redox Rep* **5,** 112-115.

Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J & Moskowitz MA (1997). Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. *Proc Natl Acad Sci U S A* **94,** 2007-2012.

Harkany T, Penke B & Luiten PG (2000). beta-Amyloid excitotoxicity in rat magnocellular nucleus basalis. Effect of cortical deafferentation on cerebral blood flow regulation and implications for Alzheimer's disease. *Ann N Y Acad Sci* **903,** 374-386.

Harkin A, Nowak G & Paul IA (2000). Noradrenergic lesion antagonizes desipramine-induced adaptation of NMDA receptors. *Eur J Pharmacol* **389,** 187-192.

Hashioka S, Klegeris A, Monji A, Kato T, Sawada M, McGeer PL & Kanba S (2007). Antidepressants inhibit interferon-gamma-induced microglial production of IL-6 and nitric oxide. *Exp Neurol* **206,** 33-42.

Hassanain HH, Chon SY & Gupta SL (1993). Differential regulation of human indoleamine 2,3-dioxygenase gene expression by interferons-gamma and -alpha. Analysis of the regulatory region of the gene and identification of an interferon-gamma-inducible DNA-binding factor. *J Biol Chem* **268,** 5077-5084.

Haut SR, Veliskova J & Moshe SL (2004). Susceptibility of immature and adult brains to seizure effects. *Lancet Neurol* **3,** 608-617.

Hayashi Y, Jikihara I, Yagi T, Fukumura M, Ohashi Y, Ohta Y, Takagi H & Maeda M (2001). Immunohistochemical investigation of caspase-1 and effect of caspase-1 inhibitor in delayed neuronal death after transient cerebral ischemia. *Brain Res* **893,** 113-120.

Haynes LE, Barber D & Mitchell IJ (2004). Chronic antidepressant medication attenuates dexamethasone-induced neuronal death and sublethal neuronal damage in the hippocampus and striatum. *Brain Res* **1026,** 157-167.

Hebert C, Habimana A, Elie R & Reader TA (2001). Effects of chronic antidepressant treatments on 5-HT and NA transporters in rat brain: an autoradiographic study. *Neurochem Int* **38,** 63-74.

Heida JG & Pittman QJ (2005). Causal links between brain cytokines and experimental febrile convulsions in the rat. *Epilepsia* **46,** 1906-1913.

Heneka MT, Galea E, Gavriluyk V, Dumitrescu-Ozimek L, Daeschner J, O'Banion MK, Weinberg G, Klockgether T & Feinstein DL (2002). Noradrenergic depletion potentiates beta -amyloid-induced cortical inflammation: implications for Alzheimer's disease. *J Neurosci* **22,** 2434-2442.

Heneka MT, Gavrilyuk V, Landreth GE, O'Banion MK, Weinberg G & Feinstein DL (2003). Noradrenergic depletion increases inflammatory responses in brain: effects on IkappaB and HSP70 expression. *J Neurochem* **85,** 387-398.

Heneka MT, Klockgether T & Feinstein DL (2000). Peroxisome proliferator-activated receptor-gamma ligands reduce neuronal inducible nitric oxide synthase expression and cell death in vivo. *J Neurosci* **20,** 6862-6867.

Henrich-Noack P, Prehn JH & Krieglstein J (1996). TGF-beta 1 protects hippocampal neurons against degeneration caused by transient global ischemia. Dose-response relationship and potential neuroprotective mechanisms. *Stroke* **27,** 1609-1614; discussion 1615.

Henshall DC, Chen J & Simon RP (2000). Involvement of caspase-3-like protease in the mechanism of cell death following focally evoked limbic seizures. *J Neurochem* **74,** 1215-1223.

Henshall DC & Simon RP (2005). Epilepsy and apoptosis pathways. *J Cereb Blood Flow Metab* **25,** 1557-1572.

Herber DL, Mercer M, Roth LM, Symmonds K, Maloney J, Wilson N, Freeman MJ, Morgan D & Gordon MN (2007). Microglial activation is required for Abeta clearance after intracranial injection of lipopolysaccharide in APP transgenic mice. *J Neuroimmune Pharmacol* **2,** 222-231.

Hertz L, Chen Y, Gibbs ME, Zang P & Peng L (2004). Astrocytic adrenoceptors: a major drug target in neurological and psychiatric disorders? *Curr Drug Targets CNS Neurol Disord* **3,** 239-267.

Herve C, Beyne P, Jamault H & Delacoux E (1996). Determination of tryptophan and its kynurenine pathway metabolites in human serum by high-performance liquid chromatography with simultaneous ultraviolet and fluorimetric detection. *J Chromatogr B Biomed Appl* **675,** 157-161.

Hetman M & Xia Z (2000). Signaling pathways mediating anti-apoptotic action of neurotrophins. *Acta Neurobiol Exp (Wars)* **60,** 531-545.

Hewett SJ, Csernansky CA & Choi DW (1994). Selective potentiation of NMDA-induced neuronal injury following induction of astrocytic iNOS. *Neuron* **13,** 487-494.

Hodges-Savola C, Rogers SD, Ghilardi JR, Timm DR & Mantyh PW (1996). Beta-adrenergic receptors regulate astrogliosis and cell proliferation in the central nervous system in vivo. *Glia* **17,** 52-62.

Hoffman WE, Cheng MA, Thomas C, Baughman VL & Albrecht RF (1991). Clonidine decreases plasma catecholamines and improves outcome from incomplete ischemia in the rat. *Anesth Analg* **73,** 460-464.

Hoffman WE, Kochs E, Werner C, Thomas C & Albrecht RF (1991). Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the alpha 2-adrenergic antagonist atipamezole. *Anesthesiology* **75,** 328-332.

Hosli E & Hosli L (1993). Receptors for neurotransmitters on astrocytes in the mammalian central nervous system. *Prog Neurobiol* **40,** 477-506.

Howells DW, Porritt MJ, Wong JY, Batchelor PE, Kalnins R, Hughes AJ & Donnan GA (2000). Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp Neurol* **166,** 127-135.

Hsu YT, Wolter KG & Youle RJ (1997). Cytosol-to-membrane redistribution of Bax and Bcl-X(L) during apoptosis. *Proc Natl Acad Sci U S A* **94,** 3668-3672.

Hu RQ, Koh S, Torgerson T & Cole AJ (1998). Neuronal stress and injury in C57/BL mice after systemic kainic acid administration. *Brain Res* **810,** 229-240.

Hu S, Sheng WS, Ehrlich LC, Peterson PK & Chao CC (2000). Cytokine effects on glutamate uptake by human astrocytes. *Neuroimmunomodulation* **7,** 153-159.

Huang EJ & Reichardt LF (2001). Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* **24,** 677-736.

Huang YY, Peng CH, Yang YP, Wu CC, Hsu WM, Wang HJ, Chan KH, Chou YP, Chen SJ & Chang YL (2007). Desipramine activated Bcl-2 expression and inhibited lipopolysaccharide-induced apoptosis in hippocampus-derived adult neural stem cells. *J Pharmacol Sci* **104,** 61-72.

Hunot S & Hirsch EC (2003). Neuroinflammatory processes in Parkinson's disease. *Ann Neurol* **53 Suppl 3,** S49-58; discussion S58-60.

Hunter CL, Quintero EM, Gilstrap L, Bhat NR & Granholm AC (2004). Minocycline protects basal forebrain cholinergic neurons from mu p75-saporin immunotoxic lesioning. *Eur J Neurosci* **19,** 3305-3316.

Huntjens DR, Spalding DJ, Danhof M & Della Pasqua OE (2009). Differences in the sensitivity of behavioural measures of pain to the selectivity of cyclo-oxygenase inhibitors. *Eur J Pain* **13,** 448-457.

Hutter P, Johansson M, Saria A & Humpel C (1996). Acute and chronic noradrenergic regulation of neurotrophin messenger RNA expression in rat hippocampus: evidence from lesions and organotypic cultures. *Neuroscience* **70,** 15-29.

Hynd MR, Scott HL & Dodd PR (2004). Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochem Int* **45,** 583-595.

Iadecola C, Zhang F, Casey R, Nagayama M & Ross ME (1997). Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. *J Neurosci* **17,** 9157-9164.

Ignarro LJ, Cirino G, Casini A & Napoli C (1999). Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol* **34,** 879-886.

Ignatowski TA, Noble BK, Wright JR, Gorfien JL, Heffner RR & Spengler RN (1997). Neuronal-associated tumor necrosis factor (TNF alpha): its role in noradrenergic functioning and modification of its expression following antidepressant drug administration. *J Neuroimmunol* **79,** 84-90.

Ikeda T, Koo H, Xia YX, Ikenoue T & Choi BH (2002). Bimodal upregulation of glial cell line-derived neurotrophic factor (GDNF) in the neonatal rat brain following ischemic/hypoxic injury. *Int J Dev Neurosci* **20,** 555-562.

Il'inykh FA, Bannova AV, Kalinina TS & Dygalo NN (2008). [Effects of ligands of alpha2-adrenoceptors on mRNA level of apoptotic proteins in the developing rat brain]. *Izv Akad Nauk Ser Biol*, 104-109.

Ilzecka J, Kocki T, Stelmasiak Z & Turski WA (2003). Endogenous protectant kynurenic acid in amyotrophic lateral sclerosis. *Acta Neurol Scand* **107,** 412-418.

Imamura N, Hida H, Aihara N, Ishida K, Kanda Y, Nishino H & Yamada K (2003). Neurodegeneration of substantia nigra accompanied with macrophage/microglia infiltration after intrastriatal hemorrhage. *Neurosci Res* **46,** 289-298.

Inoue I, Goto S, Matsunaga T, Nakajima T, Awata T, Hokari S, Komoda T & Katayama S (2001). The ligands/activators for peroxisome proliferator-activated receptor alpha (PPARalpha) and PPARgamma increase Cu2+,Zn2+-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells. *Metabolism* **50,** 3-11.

Isackson PJ, Huntsman MM, Murray KD & Gall CM (1991). BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. *Neuron* **6,** 937-948.

Isobe T & Lillehoj HS (1993). Dexamethasone suppresses T cell-mediated immunity and enhances disease susceptibility to Eimeria mivati infection. *Vet Immunol Immunopathol* **39,** 431-446.

Iversen LL (1974). Uptake mechanisms for neurotransmitter amines. *Biochem Pharmacol* **23,** 1927-1935.

Ivy AS, Rodriguez FG, Garcia C, Chen MJ & Russo-Neustadt AA (2003). Noradrenergic and serotonergic blockade inhibits BDNF mRNA activation following exercise and antidepressant. *Pharmacol Biochem Behav* **75,** 81-88.

Iwashita A, Muramatsu Y, Yamazaki T, Muramoto M, Kita Y, Yamazaki S, Mihara K, Moriguchi A & Matsuoka N (2007). Neuroprotective efficacy of the peroxisome proliferator-activated receptor delta-selective agonists in vitro and in vivo. *J Pharmacol Exp Ther* **320,** 1087-1096.

Izeboud CA, Monshouwer M, van Miert AS & Witkamp RF (1999). The beta-adrenoceptor agonist clenbuterol is a potent inhibitor of the LPS-induced production of TNF-alpha and IL-6 in vitro and in vivo. *Inflamm Res* **48,** 497-502.

Jacobsen JP & Mork A (2004). The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. *Brain Res* **1024,** 183-192.

Jaeger V, Esplin B & Capek R (1979). The anticonvulsant effects of propranolol and beta-adrenergic blockade. *Experientia* **35,** 80-81.

Jander S, Schroeter M & Stoll G (2000). Role of NMDA receptor signaling in the regulation of inflammatory gene expression after focal brain ischemia. *J Neuroimmunol* **109,** 181-187.

Jankowsky JL & Patterson PH (2001). The role of cytokines and growth factors in seizures and their sequelae. *Prog Neurobiol* **63,** 125-149.

Javelaud D & Mauviel A (2005). Crosstalk mechanisms between the mitogen-activated protein kinase pathways and Smad signaling downstream of TGF-beta: implications for carcinogenesis. *Oncogene* **24,** 5742-5750.

Jeannotte AM, McCarthy JG, Redei EE & Sidhu A (2009). Desipramine modulation of alpha-, gamma-synuclein, and the norepinephrine transporter in an animal model of depression. *Neuropsychopharmacology* **34,** 987-998.

Jia L, Schweikart K, Tomaszewski J, Page JG, Noker PE, Buhrow SA, Reid JM, Ames MM & Munn DH (2008). Toxicology and pharmacokinetics of 1-methyl-d-tryptophan: absence of toxicity due to saturating absorption. *Food Chem Toxicol* **46,** 203-211.

Jiang X & Wang X (2000). Cytochrome c promotes caspase-9 activation by inducing nucleotide binding to Apaf-1. *J Biol Chem* **275,** 31199-31203.

Johnson GL & Lapadat R (2002). Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* **298,** 1911-1912.

Johnston GA, Curtis DR, Davies J & McCulloch RM (1974). Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid. *Nature* **248,** 804-805.

Jorgensen MB, Finsen BR, Jensen MB, Castellano B, Diemer NH & Zimmer J (1993). Microglial and astroglial reactions to ischemic and kainic acid-induced lesions of the adult rat hippocampus. *Exp Neurol* **120,** 70-88.

Jourdain P, Nikonenko I, Alberi S & Muller D (2002). Remodeling of hippocampal synaptic networks by a brief anoxia-hypoglycemia. *J Neurosci* **22,** 3108-3116.

Junker V, Becker A, Huhne R, Zembatov M, Ravati A, Culmsee C & Krieglstein J (2002). Stimulation of beta-adrenoceptors activates astrocytes and provides neuroprotection. *Eur J Pharmacol* **446,** 25-36.

Juric DM, Loncar D & Carman-Krzan M (2008). Noradrenergic stimulation of BDNF synthesis in astrocytes: mediation via alpha1- and beta1/beta2-adrenergic receptors. *Neurochem Int* **52,** 297-306.

Kalinin S, Gavrilyuk V, Polak PE, Vasser R, Zhao J, Heneka MT & Feinstein DL (2007). Noradrenaline deficiency in brain increases beta-amyloid plaque burden in an animal model of Alzheimer's disease. *Neurobiol Aging* **28,** 1206-1214.

Kalonia H, Kumar P, Kumar A & Nehru B (2009). Effects of caffeic acid, rofecoxib, and their combination against quinolinic acid-induced behavioral alterations and disruption in glutathione redox status. *Neurosci Bull* **25,** 343-352.

Kaminski RM, Shippenberg TS, Witkin JM & Rocha BA (2005). Genetic deletion of the norepinephrine transporter decreases vulnerability to seizures. *Neurosci Lett* **382,** 51-55.

Kamisaki Y, Hamahashi T, Hamada T, Maeda K & Itoh T (1992). Presynaptic inhibition by clonidine of neurotransmitter amino acid release in various brain regions. *Eur J Pharmacol* **217,** 57-63.

Kamiya H (2002). Kainate receptor-dependent presynaptic modulation and plasticity. *Neurosci Res* **42,** 1-6.

Kamiya H & Ozawa S (1998). Kainate receptor-mediated inhibition of presynaptic Ca2+ influx and EPSP in area CA1 of the rat hippocampus. *J Physiol* **509 (Pt 3),** 833-845.

Kang H & Suzuki Y (2001). Requirement of non-T cells that produce gamma interferon for prevention of reactivation of Toxoplasma gondii infection in the brain. *Infect Immun* **69,** 2920-2927.

Kang KH, Lee KH, Kim MY & Choi KH (2001). Caspase-3-mediated cleavage of the NF-kappa B subunit p65 at the NH2 terminus potentiates naphthoquinone analog-induced apoptosis. *J Biol Chem* **276,** 24638-24644.

Kapinya KJ, Lowl D, Futterer C, Maurer M, Waschke KF, Isaev NK & Dirnagl U (2002). Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. *Stroke* **33,** 1889-1898.

Kariko K, Weissman D & Welsh FA (2004). Inhibition of toll-like receptor and cytokine signaling--a unifying theme in ischemic tolerance. *J Cereb Blood Flow Metab* **24,** 1288-1304.

Karin M (1999). The beginning of the end: IkappaB kinase (IKK) and NF-kappaB activation. *J Biol Chem* **274,** 27339-27342.

Kato H, Kawaguchi M, Inoue S, Hirai K & Furuya H (2009). The effects of beta-adrenoceptor antagonists on proinflammatory cytokine concentrations after subarachnoid hemorrhage in rats. *Anesth Analg* **108,** 288-295.

Kermer P, Liman J, Weishaupt JH & Bahr M (2004). Neuronal apoptosis in neurodegenerative diseases: from basic research to clinical application. *Neurodegener Dis* **1,** 9-19.

Kesner RP, Lee I & Gilbert P (2004). A behavioral assessment of hippocampal function based on a subregional analysis. *Rev Neurosci* **15,** 333-351.

Khanna N, Ray A, Alkondon M & Sen P (1989). Effect of beta-adrenoceptor antagonists and some related drugs on maximal electroshock seizures in mice. *Indian J Exp Biol* **27,** 128-130.

Khundakar AA & Zetterstrom TS (2006). Biphasic change in BDNF gene expression following antidepressant drug treatment explained by differential transcript regulation. *Brain Res* **1106,** 12-20.

Kim BJ, Kim MJ, Park JM, Lee SH, Kim YJ, Ryu S, Kim YH & Yoon BW (2009). Reduced neurogenesis after suppressed inflammation by minocycline in transient cerebral ischemia in rat. *J Neurol Sci* **279,** 70-75.

Kitazawa M, Oddo S, Yamasaki TR, Green KN & LaFerla FM (2005). Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-

dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease. *J Neurosci* **25,** 8843-8853.

Klegeris A, McGeer EG & McGeer PL (2007). Therapeutic approaches to inflammation in neurodegenerative disease. *Curr Opin Neurol* **20,** 351-357.

Kleinert H, Pautz A, Linker K & Schwarz PM (2004). Regulation of the expression of inducible nitric oxide synthase. *Eur J Pharmacol* **500,** 255-266.

Klotz L, Sastre M, Kreutz A, Gavrilyuk V, Klockgether T, Feinstein DL & Heneka MT (2003). Noradrenaline induces expression of peroxisome proliferator activated receptor gamma (PPARgamma) in murine primary astrocytes and neurons. *J Neurochem* **86,** 907-916.

Knipper M, da Penha Berzaghi M, Blochl A, Breer H, Thoenen H & Lindholm D (1994). Positive feedback between acetylcholine and the neurotrophins nerve growth factor and brain-derived neurotrophic factor in the rat hippocampus. *Eur J Neurosci* **6,** 668-671.

Knipper M, Leung LS, Zhao D & Rylett RJ (1994). Short-term modulation of glutamatergic synapses in adult rat hippocampus by NGF. *Neuroreport* **5,** 2433-2436.

Koch RA & Barish ME (1994). Perturbation of intracellular calcium and hydrogen ion regulation in cultured mouse hippocampal neurons by reduction of the sodium ion concentration gradient. *J Neurosci* **14,** 2585-2593.

Kohler C, Schwarcz R & Fuxe K (1979). Intrahippocampal injections of ibotenic acid provide histological evidence for a neurotoxic mechanism different from kainic acid. *Neurosci Lett* **15,** 223-228.

Kokaia M, Ernfors P, Kokaia Z, Elmer E, Jaenisch R & Lindvall O (1995). Suppressed epileptogenesis in BDNF mutant mice. *Exp Neurol* **133,** 215-224.

Konan KV & Taylor MW (1996). Importance of the two interferon-stimulated response element (ISRE) sequences in the regulation of the human indoleamine 2,3-dioxygenase gene. *J Biol Chem* **271,** 19140-19145.

Kondratyev A & Gale K (2000). Intracerebral injection of caspase-3 inhibitor prevents neuronal apoptosis after kainic acid-evoked status epilepticus. *Brain Res Mol Brain Res* **75,** 216-224.

Kosten TA, Galloway MP, Duman RS, Russell DS & D'Sa C (2008). Repeated unpredictable stress and antidepressants differentially regulate expression of the bcl-2 family of apoptotic genes in rat cortical, hippocampal, and limbic brain structures. *Neuropsychopharmacology* **33,** 1545-1558.

Kovalenko T, Osadchenko I, Nikonenko A, Lushnikova I, Voronin K, Nikonenko I, Muller D & Skibo G (2006). Ischemia-induced modifications in hippocampal CA1 stratum radiatum excitatory synapses. *Hippocampus* **16,** 814-825.

Kozisek ME, Deupree JD, Burke WJ & Bylund DB (2007). Appropriate dosing regimens for treating juvenile rats with desipramine for neuropharmacological and behavioral studies. *J Neurosci Methods* **163,** 83-91.

Krief S, Lonnqvist F, Raimbault S, Baude B, Van Spronsen A, Arner P, Strosberg AD, Ricquier D & Emorine LJ (1993). Tissue distribution of beta 3-adrenergic receptor mRNA in man. *J Clin Invest* **91,** 344-349.

Kronfol Z & Remick DG (2000). Cytokines and the brain: implications for clinical psychiatry. *Am J Psychiatry* **157,** 683-694.

Krueger BK (1990). Kinetics and block of dopamine uptake in synaptosomes from rat caudate nucleus. *J Neurochem* **55,** 260-267.

Krupinski J, Kumar P, Kumar S & Kaluza J (1996). Increased expression of TGF-beta 1 in brain tissue after ischemic stroke in humans. *Stroke* **27,** 852-857.

Kubera M, Holan V, Mathison R & Maes M (2000). The effect of repeated amitriptyline and desipramine administration on cytokine release in C57BL/6 mice. *Psychoneuroendocrinology* **25,** 785-797.

Kubera M, Maes M, Holan V, Basta-Kaim A, Roman A & Shani J (2001). Prolonged desipramine treatment increases the production of interleukin-10, an anti-inflammatory cytokine, in C57BL/6 mice subjected to the chronic mild stress model of depression. *J Affect Disord* **63,** 171-178.

Kuhar MJ CP, Lambert PD, ed (1999). *Basic Neurochemistry*. Lippincott, Williams and Wilkins, Philadelphia.

Kuida K, Zheng TS, Na S, Kuan C, Yang D, Karasuyama H, Rakic P & Flavell RA (1996). Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* **384,** 368-372.

Kumar S (1999). Mechanisms mediating caspase activation in cell death. *Cell Death Differ* **6,** 1060-1066.

Kumar VL, Roy S, Sehgal R & Padhy BM (2006). A comparative study on the efficacy of rofecoxib in monoarticular arthritis induced by latex of Calotropis procera and Freund's complete adjuvant. *Inflammopharmacology* **14,** 17-21.

Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H & Morikawa K (2000). Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* **407,** 971-977.

Kuno R, Yoshida Y, Nitta A, Nabeshima T, Wang J, Sonobe Y, Kawanokuchi J, Takeuchi H, Mizuno T & Suzumura A (2006). The role of TNF-alpha and its receptors in the production of NGF and GDNF by astrocytes. *Brain Res* **1116,** 12-18.

Kwidzinski E, Bunse J, Aktas O, Richter D, Mutlu L, Zipp F, Nitsch R & Bechmann I (2005). Indolamine 2,3-dioxygenase is expressed in the CNS and down-regulates autoimmune inflammation. *FASEB J* **19,** 1347-1349.

Laane E, Panaretakis T, Pokrovskaja K, Buentke E, Corcoran M, Soderhall S, Heyman M, Mazur J, Zhivotovsky B, Porwit A & Grander D (2007). Dexamethasone-induced apoptosis in acute lymphoblastic leukemia involves differential regulation of Bcl-2 family members. *Haematologica* **92,** 1460-1469.

Lafaille F, Welner SA & Suranyi-Cadotte BE (1991). Regulation of serotonin type 2 (5-HT2) and beta-adrenergic receptors in rat cerebral cortex following novel and classical antidepressant treatment. *J Psychiatry Neurosci* **16,** 209-214.

Laflamme N, Lacroix S & Rivest S (1999). An essential role of interleukin-1beta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. *J Neurosci* **19,** 10923-10930.

Lafon-Cazal M, Culcasi M, Gaven F, Pietri S & Bockaert J (1993). Nitric oxide, superoxide and peroxynitrite: putative mediators of NMDA-induced cell death in cerebellar granule cells. *Neuropharmacology* **32,** 1259-1266.

Lands AM, Arnold A, McAuliff JP, Luduena FP & Brown TG, Jr. (1967). Differentiation of receptor systems activated by sympathomimetic amines. *Nature* **214,** 597-598.

Langer SZ (1974). Presynaptic regulation of catecholamine release. *Biochem Pharmacol* **23,** 1793-1800.

Lapiz MD, Bondi CO & Morilak DA (2007). Chronic treatment with desipramine improves cognitive performance of rats in an attentional set-shifting test. *Neuropsychopharmacology* **32,** 1000-1010.

Lasek R, Fischer W & Muller M (1983). [Anticonvulsant activity of beta-adrenolytics in electroshock]. *Pharmazie* **38,** 202-203.

Lassmann H (1991). Inflammation and the blood-brain barrier. *Brain Pathol* **1,** 88.

Lau LT & Yu AC (2001). Astrocytes produce and release interleukin-1, interleukin-6, tumor necrosis factor alpha and interferon-gamma following traumatic and metabolic injury. *J Neurotrauma* **18,** 351-359.

Lauterborn JC, Rivera S, Stinis CT, Hayes VY, Isackson PJ & Gall CM (1996). Differential effects of protein synthesis inhibition on the activity-dependent expression of BDNF transcripts: evidence for immediate-early gene responses from specific promoters. *J Neurosci* **16,** 7428-7436.

Lavrik IN, Golks A & Krammer PH (2005). Caspases: pharmacological manipulation of cell death. *J Clin Invest* **115,** 2665-2672.

Lawrence CB, Allan SM & Rothwell NJ (1998). Interleukin-1beta and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rat. *Eur J Neurosci* **10,** 1188-1195.

Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier GG & Earnshaw WC (1994). Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature* **371,** 346-347.

Lee PH, Grimes L & Hong JS (1989). Glucocorticoids potentiate kainic acid-induced seizures and wet dog shakes. *Brain Res* **480,** 322-325.

Leonard BE (1991). Antidepressants. Current concepts of mode of action. *Encephale* **17 Spec No 1,** 127-131.

Leonard BE (1997). Neurotransmitters in depression: noradrenaline and serotonin and their interactions. *J Clin Psychopharmacol* **17 Suppl 1,** 1S.

Leonard BE & Myint A (2009). The psychoneuroimmunology of depression. *Hum Psychopharmacol* **24,** 165-175.

Lerma J, Paternain AV, Rodriguez-Moreno A & Lopez-Garcia JC (2001). Molecular physiology of kainate receptors. *Physiol Rev* **81,** 971-998.

Lessmann V & Brigadski T (2009). Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. *Neurosci Res* **65,** 11-22.

Lessmann V, Gottmann K & Malcangio M (2003). Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol* **69,** 341-374.

Levi-Montalcini R & Angeletti PU (1968). Nerve growth factor. *Physiol Rev* **48,** 534-569.

Levy LM, Attwell D, Hoover F, Ash JF, Bjoras M & Danbolt NC (1998). Inducible expression of the GLT-1 glutamate transporter in a CHO cell line selected for low endogenous glutamate uptake. *FEBS Lett* **422,** 339-342.

Li M, Wang X, Meintzer MK, Laessig T, Birnbaum MJ & Heidenreich KA (2000). Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta. *Mol Cell Biol* **20,** 9356-9363.

Liem RK & Messing A (2009). Dysfunctions of neuronal and glial intermediate filaments in disease. *J Clin Invest* **119,** 1814-1824.

Lim CM, Kim SW, Park JY, Kim C, Yoon SH & Lee JK (2009). Fluoxetine affords robust neuroprotection in the postischemic brain via its anti-inflammatory effect. *J Neurosci Res* **87,** 1037-1045.

Lin LF, Doherty DH, Lile JD, Bektesh S & Collins F (1993). GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* **260,** 1130-1132.

Linvall OaB, A., ed (1983). *Chemical neuroanatomy.* Raven press, New York.

Liochev SI & Fridovich I (2001). The oxidation of 3-hydroxyanthranilic acid by Cu,Zn superoxide dismutase: mechanism and possible consequences. *Arch Biochem Biophys* **388,** 281-284.

Lipton SA & Rosenberg PA (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* **330,** 613-622.

Liu H, Cao Y, Basbaum AI, Mazarati AM, Sankar R & Wasterlain CG (1999). Resistance to excitotoxin-induced seizures and neuronal death in mice lacking the preprotachykinin A gene. *Proc Natl Acad Sci U S A* **96,** 12096-12101.

Liu J, Zhao ML, Brosnan CF & Lee SC (1996). Expression of type II nitric oxide synthase in primary human astrocytes and microglia: role of IL-1beta and IL-1 receptor antagonist. *J Immunol* **157,** 3569-3576.

Liu T, Clark RK, McDonnell PC, Young PR, White RF, Barone FC & Feuerstein GZ (1994). Tumor necrosis factor-alpha expression in ischemic neurons. *Stroke* **25,** 1481-1488.

Liu X, Zou H, Slaughter C & Wang X (1997). DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* **89,** 175-184.

Liu YF (1998). Expression of polyglutamine-expanded Huntingtin activates the SEK1-JNK pathway and induces apoptosis in a hippocampal neuronal cell line. *J Biol Chem* **273,** 28873-28877.

Liu Z & Martin LJ (2001). Motor neurons rapidly accumulate DNA single-strand breaks after in vitro exposure to nitric oxide and peroxynitrite and in vivo axotomy. *J Comp Neurol* **432,** 35-60.

Lob S, Konigsrainer A, Schafer R, Rammensee HG, Opelz G & Terness P (2008). Levo- but not dextro-1-methyl tryptophan abrogates the IDO activity of human dendritic cells. *Blood* **111,** 2152-2154.

Loddick SA, Turnbull AV & Rothwell NJ (1998). Cerebral interleukin-6 is neuroprotective during permanent focal cerebral ischemia in the rat. *J Cereb Blood Flow Metab* **18,** 176-179.

Loddick SA, Wong ML, Bongiorno PB, Gold PW, Licinio J & Rothwell NJ (1997). Endogenous interleukin-1 receptor antagonist is neuroprotective. *Biochem Biophys Res Commun* **234,** 211-215.

Lodish AB, S. Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell (2000). *Molecular cell biology.* W.H Freeman and Co.

London ED & Coyle JT (1979). Specific binding of [3H]kainic acid to receptor sites in rat brain. *Mol Pharmacol* **15,** 492-505.

Lopez E, Pozas E, Rivera R & Ferrer I (1999). Bcl-2, Bax and Bcl-x expression following kainic acid administration at convulsant doses in the rat. *Neuroscience* **91,** 1461-1470.

Loscher W & Schmidt D (1988). Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res* **2,** 145-181.

Lothman EW & Collins RC (1981). Kainic acid induced limbic seizures: metabolic, behavioral, electroencephalographic and neuropathological correlates. *Brain Res* **218,** 299-318.

Louis WJ, Papanicolaou J, Summers RJ & Vajda FJ (1982). Role of central beta-adrenoceptors in the control of pentylenetetrazol-induced convulsions in rats. *Br J Pharmacol* **75,** 441-446.

Luchowska E, Kloc R, Olajossy B, Wnuk S, Wielosz M, Owe-Larsson B & Urbanska EM (2009). beta-adrenergic enhancement of brain kynurenic acid production mediated via cAMP-related protein kinase A signaling. *Prog Neuropsychopharmacol Biol Psychiatry* **33,** 519-529.

Luchowska E, Kloc R, Wnuk S, Olajossy B, Wielosz M & Urbanska EM (2008). Clenbuterol enhances the production of kynurenic acid in brain cortical slices and glial cultures. *Pharmacol Rep* **60,** 574-577.

Luo X, Tang A, Pi L, Xiao L, Ao X, Pu Y & Wang R (2008). Determination of kynurenine in serum by high-performance liquid chromatography with on-column fluorescence derivatization. *Clin Chim Acta* **389,** 186-188.

MacDonald RL & Barker JL (1979). Enhancement of GABA-mediated postsynaptic inhibition in cultured mammalian spinal cord neurons: a common mode of anticonvulsant action. *Brain Res* **167,** 323-336.

Mackay GM, Forrest CM, Stoy N, Christofides J, Egerton M, Stone TW & Darlington LG (2006). Tryptophan metabolism and oxidative stress in patients with chronic brain injury. *Eur J Neurol* **13,** 30-42.

MacKenzie CR, Hadding U & Daubener W (1998). Interferon-gamma-induced activation of indoleamine 2,3-dioxygenase in cord blood monocyte-derived macrophages inhibits the growth of group B streptococci. *J Infect Dis* **178,** 875-878.

MacMicking J, Xie QW & Nathan C (1997). Nitric oxide and macrophage function. *Annu Rev Immunol* **15,** 323-350.

Madan BR & Barar FS (1974). Anticonvulsant activity of some beta-adrenoceptor blocking agents in mice. *Eur J Pharmacol* **29,** 1-4.

Madrigal JL, Feinstein DL & Dello Russo C (2005). Norepinephrine protects cortical neurons against microglial-induced cell death. *J Neurosci Res* **81,** 390-396.

Madrigal JL, Kalinin S, Richardson JC & Feinstein DL (2007). Neuroprotective actions of noradrenaline: effects on glutathione synthesis and activation of peroxisome proliferator activated receptor delta. *J Neurochem* **103,** 2092-2101.

Madrigal JL, Leza JC, Polak P, Kalinin S & Feinstein DL (2009). Astrocyte-derived MCP-1 mediates neuroprotective effects of noradrenaline. *J Neurosci* **29,** 263-267.

Maes M, Jacobs MP, Suy E, Minner B, Leclercq C, Christiaens F & Raus J (1990). Suppressant effects of dexamethasone on the availability of plasma L-tryptophan and tyrosine in healthy controls and in depressed patients. *Acta Psychiatr Scand* **81,** 19-23.

Maier C, Steinberg GK, Sun GH, Zhi GT & Maze M (1993). Neuroprotection by the alpha 2-adrenoreceptor agonist dexmedetomidine in a focal model of cerebral ischemia. *Anesthesiology* **79,** 306-312.

Mandela P & Ordway GA (2006). The norepinephrine transporter and its regulation. *J Neurochem* **97,** 310-333.

Manji H & Brown JH (1987). The antidepressant effect of beta-adrenoreceptor subsensitivity: a brief review and clinical implications. *Can J Psychiatry* **32,** 788-797.

Mantyh PW, Rogers SD, Allen CJ, Catton MD, Ghilardi JR, Levin LA, Maggio JE & Vigna SR (1995). Beta 2-adrenergic receptors are expressed by glia in vivo in the normal and injured central nervous system in the rat, rabbit, and human. *J Neurosci* **15,** 152-164.

Marcyniuk B, Mann DM & Yates PO (1986). Loss of nerve cells from locus coeruleus in Alzheimer's disease is topographically arranged. *Neurosci Lett* **64,** 247-252.

Marien MR, Colpaert FC & Rosenquist AC (2004). Noradrenergic mechanisms in neurodegenerative diseases: a theory. *Brain Res Brain Res Rev* **45,** 38-78.

Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B & et al. (1995). Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* **268,** 1336-1338.

Martel JC, Chopin P, Colpaert F & Marien M (2000). Drug treatments to reduce excitotoxicity in vivo: a potential for alpha2-adrenoceptor antagonists? *Amino Acids* **19,** 239-252.

Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE & Portera-Cailliau C (1998). Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: A perspective on the contributions of apoptosis and necrosis. *Brain Res Bull* **46,** 281-309.

Martin LJ & Liu Z (2002). DNA damage profiling in motor neurons: a single-cell analysis by comet assay. *Neurochem Res* **27,** 1093-1104.

Martin LJ & Liu Z (2002). Injury-induced spinal motor neuron apoptosis is preceded by DNA single-strand breaks and is p53- and Bax-dependent. *J Neurobiol* **50,** 181-197.

Martin LJ, Liu Z, Chen K, Price AC, Pan Y, Swaby JA & Golden WC (2007). Motor neuron degeneration in amyotrophic lateral sclerosis mutant superoxide dismutase-1 transgenic mice: mechanisms of mitochondriopathy and cell death. *J Comp Neurol* **500,** 20-46.

Martin LJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, Price DL & Lee MK (2006). Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *J Neurosci* **26,** 41-50.

Massague J, Blain SW & Lo RS (2000). TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* **103,** 295-309.

Massague J & Chen YG (2000). Controlling TGF-beta signaling. *Genes Dev* **14,** 627-644.

Mastrangelo MA, Sudol KL, Narrow WC & Bowers WJ (2009). Interferon-{gamma} differentially affects Alzheimer's disease pathologies and induces neurogenesis in triple transgenic-AD mice. *Am J Pathol* **175,** 2076-2088.

Matsuoka Y, Kitamura Y, Okazaki M, Terai K & Taniguchi T (1999). Kainic acid-induced activation of nuclear factor-kappaB in rat hippocampus. *Exp Brain Res* **124,** 215-222.

Mattson MP, Partin J & Begley JG (1998). Amyloid beta-peptide induces apoptosis-related events in synapses and dendrites. *Brain Res* **807,** 167-176.

Mayne M, Ni W, Yan HJ, Xue M, Johnston JB, Del Bigio MR, Peeling J & Power C (2001). Antisense oligodeoxynucleotide inhibition of tumor necrosis factor-alpha expression is neuroprotective after intracerebral hemorrhage. *Stroke* **32,** 240-248.

McCauslin CS, Heath V, Colangelo AM, Malik R, Lee S, Mallei A, Mocchetti I & Johnson PF (2006). CAAT/enhancer-binding protein delta and cAMP-response element-binding protein mediate inducible expression of the nerve growth factor gene in the central nervous system. *J Biol Chem* **281,** 17681-17688.

McColl BW, Rothwell NJ & Allan SM (2007). Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via interleukin-1- and neutrophil-dependent mechanisms. *J Neurosci* **27,** 4403-4412.

McDonald ER, 3rd & El-Deiry WS (2003). Yeast two-hybrid screening as a means of deciphering tumor suppressor pathways. *Methods Mol Biol* **223,** 173-185.

McGeer EG, Innanen VT & McGeer PL (1976). Evidence on the cellular localization of adenyl cyclase in the neostriatum. *Brain Res* **118,** 356-358.

McGinty JF, Henriksen SJ, Goldstein A, Terenius L & Bloom FE (1983). Dynorphin is contained within hippocampal mossy fibers: immunochemical alterations after kainic acid administration and colchicine-induced neurotoxicity. *Proc Natl Acad Sci U S A* **80,** 589-593.

McKay LI & Cidlowski JA (1999). Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* **20,** 435-459.

McLin JP & Steward O (2006). Comparison of seizure phenotype and neurodegeneration induced by systemic kainic acid in inbred, outbred, and hybrid mouse strains. *Eur J Neurosci* **24,** 2191-2202.

McNamee EN, Ryan KM, Griffin EW, Gonzalez-Reyes RE, Ryan KJ, Harkin A & Connor TJ Noradrenaline acting at central beta-adrenoceptors induces interleukin-10 and

suppressor of cytokine signaling-3 expression in rat brain: Implications for neurodegeneration. *Brain Behav Immun* **24,** 660-671.

McNamee EN, Ryan KM, Kilroy D & Connor TJ (2009). Noradrenaline induces IL-1ra and IL-1 type II receptor expression in primary glial cells and protects against IL-1beta-induced neurotoxicity. *Eur J Pharmacol.*

Meda L, Cassatella MA, Szendrei GI, Otvos L, Jr., Baron P, Villalba M, Ferrari D & Rossi F (1995). Activation of microglial cells by beta-amyloid protein and interferon-gamma. *Nature* **374,** 647-650.

Medana IM, Day NP, Salahifar-Sabet H, Stocker R, Smythe G, Bwanaisa L, Njobvu A, Kayira K, Turner GD, Taylor TE & Hunt NH (2003). Metabolites of the kynurenine pathway of tryptophan metabolism in the cerebrospinal fluid of Malawian children with malaria. *J Infect Dis* **188,** 844-849.

Melchior B, Nerriere-Daguin V, Laplaud DA, Remy S, Wiertlewski S, Neveu I, Naveilhan P, Meakin SO & Brachet P (2003). Ectopic expression of the TrkA receptor in adult dopaminergic mesencephalic neurons promotes retrograde axonal NGF transport and NGF-dependent neuroprotection. *Exp Neurol* **183,** 367-378.

Meldrum BS (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr* **130,** 1007S-1015S.

Meldrum BS, Akbar MT & Chapman AG (1999). Glutamate receptors and transporters in genetic and acquired models of epilepsy. *Epilepsy Res* **36,** 189-204.

Mellor A (2005). Indoleamine 2,3 dioxygenase and regulation of T cell immunity. *Biochem Biophys Res Commun* **338,** 20-24.

Memo M, Blandini F, Nappi G & Spano P (2002). Neuroprotection: promise and pitfalls in anti-inflammatory treatment of Alzheimer's disease. *Funct Neurol* **17,** 175-176.

Merry DE & Korsmeyer SJ (1997). Bcl-2 gene family in the nervous system. *Annu Rev Neurosci* **20,** 245-267.

Metsis M, Timmusk T, Arenas E & Persson H (1993). Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. *Proc Natl Acad Sci U S A* **90,** 8802-8806.

Metz LM, Zhang Y, Yeung M, Patry DG, Bell RB, Stoian CA, Yong VW, Patten SB, Duquette P, Antel JP & Mitchell JR (2004). Minocycline reduces gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. *Ann Neurol* **55,** 756.

Metz R, Duhadaway JB, Kamasani U, Laury-Kleintop L, Muller AJ & Prendergast GC (2007). Novel tryptophan catabolic enzyme IDO2 is the preferred biochemical target of the antitumor indoleamine 2,3-dioxygenase inhibitory compound D-1-methyl-tryptophan. *Cancer Res* **67,** 7082-7087.

Mikami M, Goubaeva F, Song JH, Lee HT & Yang J (2008). beta-Adrenoceptor blockers protect against staurosporine-induced apoptosis in SH-SY5Y neuroblastoma cells. *Eur J Pharmacol* **589,** 14-21.

Mikova O, Yakimova R, Bosmans E, Kenis G & Maes M (2001). Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. *Eur Neuropsychopharmacol* **11,** 203-208.

Milatovic D, Gupta RC & Dettbarn WD (2002). Involvement of nitric oxide in kainic acid-induced excitotoxicity in rat brain. *Brain Res* **957,** 330-337.

Millan MJ (2004). The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. *Eur J Pharmacol* **500,** 371-384.

Miller AH, Maletic V & Raison CL (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* **65,** 732-741.

Miller DB & O'Callaghan JP (2005). Aging, stress and the hippocampus. *Ageing Res Rev* **4,** 123-140.

Miller RH (2006). Building bridges with astrocytes for spinal cord repair. *J Biol* **5,** 6.

Miltiadous P, Stamatakis A & Stylianopoulou F (2009). Neuroprotective Effects of IGF-I Following Kainic Acid-Induced Hippocampal Degeneration in the Rat. *Cell Mol Neurobiol.*

Minami M, Kuraishi Y & Satoh M (1991). Effects of kainic acid on messenger RNA levels of IL-1 beta, IL-6, TNF alpha and LIF in the rat brain. *Biochem Biophys Res Commun* **176,** 593-598.

Minami M, Kuraishi Y, Yamaguchi T, Nakai S, Hirai Y & Satoh M (1990). Convulsants induce interleukin-1 beta messenger RNA in rat brain. *Biochem Biophys Res Commun* **171,** 832-837.

Minc-Golomb D, Yadid G, Tsarfaty I, Resau JH & Schwartz JP (1996). In vivo expression of inducible nitric oxide synthase in cerebellar neurons. *J Neurochem* **66,** 1504-1509.

Minneman KP, Hegstrand LR & Molinoff PB (1979). The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung in vitro. *Mol Pharmacol* **16,** 21-33.

Mitchell JJ, Paiva M, Walker DW & Heaton MB (1999). BDNF and NGF afford in vitro neuroprotection against ethanol combined with acute ischemia and chronic hypoglycemia. *Dev Neurosci* **21,** 68-75.

Miyashita Y (2004). Cognitive memory: cellular and network machineries and their top-down control. *Science* **306,** 435-440.

Mody I & MacDonald JF (1995). NMDA receptor-dependent excitotoxicity: the role of intracellular Ca2+ release. *Trends Pharmacol Sci* **16,** 356-359.

Mogi M, Kondo T, Mizuno Y & Nagatsu T (2007). p53 protein, interferon-gamma, and NF-kappaB levels are elevated in the parkinsonian brain. *Neurosci Lett* **414,** 94-97.

Mori K, Ozaki E, Zhang B, Yang L, Yokoyama A, Takeda I, Maeda N, Sakanaka M & Tanaka J (2002). Effects of norepinephrine on rat cultured microglial cells that express alpha1, alpha2, beta1 and beta2 adrenergic receptors. *Neuropharmacology* **43,** 1026-1034.

Moriyama N, Taniguchi M, Miyano K, Miyoshi M & Watanabe T (2006). ANP inhibits LPS-induced stimulation of rat microglial cells by suppressing NF-kappaB and AP-1 activations. *Biochem Biophys Res Commun* **350,** 322-328.

Morrison RS, Wenzel HJ, Kinoshita Y, Robbins CA, Donehower LA & Schwartzkroin PA (1996). Loss of the p53 tumor suppressor gene protects neurons from kainate-induced cell death. *J Neurosci* **16,** 1337-1345.

Mourlevat S, Troadec JD, Ruberg M & Michel PP (2003). Prevention of dopaminergic neuronal death by cyclic AMP in mixed neuronal/glial mesencephalic cultures requires the repression of presumptive astrocytes. *Mol Pharmacol* **64,** 578-586.

Moustakas A & Heldin CH (2005). Non-Smad TGF-beta signals. *J Cell Sci* **118,** 3573-3584.

Mrak RE & Griffin WS (2005). Potential inflammatory biomarkers in Alzheimer's disease. *J Alzheimers Dis* **8,** 369-375.

Mu X, He J, Anderson DW, Trojanowski JQ & Springer JE (1996). Altered expression of bcl-2 and bax mRNA in amyotrophic lateral sclerosis spinal cord motor neurons. *Ann Neurol* **40,** 379-386.

Mudo G, Trovato-Salinaro A, Caniglia G, Cheng Q & Condorelli DF (2007). Cellular localization of mGluR3 and mGluR5 mRNAs in normal and injured rat brain. *Brain Res* **1149,** 1-13.

Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slingluff CL, Jr. & Mellor AL (2002). Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* **297,** 1867-1870.

Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H & Leonard B (2007). Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J Affect Disord* **98,** 143-151.

Nadeau S & Rivest S (2003). Glucocorticoids play a fundamental role in protecting the brain during innate immune response. *J Neurosci* **23,** 5536-5544.

Nadler JV (1981). Role of excitatory pathways in the hippocampal damage produced by kainic acid. *Adv Biochem Psychopharmacol* **27,** 395-402.

Nadler JV & Cuthbertson GJ (1980). Kainic acid neurotoxicity toward hippocampal formation: dependence on specific excitatory pathways. *Brain Res* **195,** 47-56.

Nadler JV, Perry BW, Gentry C & Cotman CW (1980). Degeneration of hippocampal CA3 pyramidal cells induced by intraventricular kainic acid. *J Comp Neurol* **192,** 333-359.

Nadler JV, Shelton DL, Perry BW & Cotman CW (1980). Regional distribution of [3H]kainic acid after intraventricular injection. *Life Sci* **26,** 133-138.

Nakagawa Y, Asai H, Kitoh J, Mori H & Nakano K (1995). Increase in the level of mRNA for 3-hydroxyanthranilate 3,4-dioxygenase in brain of epilepsy-prone El mice. *Biosci Biotechnol Biochem* **59,** 2191-2192.

Nakagawa Y, Asai H, Miura T, Kitoh J, Mori H & Nakano K (1998). Increased expression of 3-hydroxyanthranilate 3,4-dioxygenase gene in brain of epilepsy-prone El mice. *Brain Res Mol Brain Res* **58,** 132-137.

Nakai T, Milusheva E, Baranyi M, Uchihashi Y, Satoh T & Vizi ES (1999). Excessive release of [3H]noradrenaline and glutamate in response to simulation of ischemic conditions in rat spinal cord slice preparation: effect of NMDA and AMPA receptor antagonists. *Eur J Pharmacol* **366,** 143-150.

Nakamura A, Johns EJ, Imaizumi A, Abe T & Kohsaka T (1998). Regulation of tumour necrosis factor and interleukin-6 gene transcription by beta2-adrenoceptor in the rat astrocytes. *J Neuroimmunol* **88,** 144-153.

Nakane A, Minagawa T, Kohanawa M, Chen Y, Sato H, Moriyama M & Tsuruoka N (1989). Interactions between endogenous gamma interferon and tumor necrosis factor in host resistance against primary and secondary Listeria monocytogenes infections. *Infect Immun* **57,** 3331-3337.

Nakano K, Asai H & Kitoh J (1992). Abnormally high activity of 3-hydroxyanthranilate 3,4-dioxygenase in brain of epilepsy-prone El mice. *Brain Res* **572,** 1-4.

Narkilahti S, Nissinen J & Pitkanen A (2003). Administration of caspase 3 inhibitor during and after status epilepticus in rat: effect on neuronal damage and epileptogenesis. *Neuropharmacology* **44,** 1068-1088.

Nathan C (1992). Nitric oxide as a secretory product of mammalian cells. *FASEB J* **6,** 3051-3064.

Nawashiro H, Martin D & Hallenbeck JM (1997). Inhibition of tumor necrosis factor and amelioration of brain infarction in mice. *J Cereb Blood Flow Metab* **17,** 229-232.

Nelson KB, Dambrosia JM, Grether JK & Phillips TM (1998). Neonatal cytokines and coagulation factors in children with cerebral palsy. *Ann Neurol* **44,** 665-675.

Nemeth H, Robotka H, Kis Z, Rozsa E, Janaky T, Somlai C, Marosi M, Farkas T, Toldi J & Vecsei L (2004). Kynurenine administered together with probenecid markedly inhibits pentylenetetrazol-induced seizures. An electrophysiological and behavioural study. *Neuropharmacology* **47,** 916-925.

Newman-Tancredi A, Verriele L, Chaput C & Millan MJ (1996). Down-regulation of rat beta-adrenoceptors by clenbuterol or desipramine does not require chronic treatment: [3H] CGP-12177 binding reveals rapid (24 hour) modulation. *Brain Res Bull* **41,** 93-96.

Nguyen MD, Julien JP & Rivest S (2002). Innate immunity: the missing link in neuroprotection and neurodegeneration? *Nat Rev Neurosci* **3,** 216-227.

Nibuya M, Morinobu S & Duman RS (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* **15,** 7539-7547.

Nicholson DW (1996). ICE/CED3-like proteases as therapeutic targets for the control of inappropriate apoptosis. *Nat Biotechnol* **14,** 297-301.

Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, Gareau Y, Griffin PR, Labelle M, Lazebnik YA & et al. (1995). Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* **376,** 37-43.

Niizuma K, Endo H & Chan PH (2009). Oxidative stress and mitochondrial dysfunction as determinants of ischemic neuronal death and survival. *J Neurochem* **109 Suppl 1,** 133-138.

Nikonenko A, Schmidt S, Skibo G, Bruckner G & Schachner M (2003). Tenascin-R-deficient mice show structural alterations of symmetric perisomatic synapses in the CA1 region of the hippocampus. *J Comp Neurol* **456,** 338-349.

Nikonenko AG, Radenovic L, Andjus PR & Skibo GG (2009). Structural features of ischemic damage in the hippocampus. *Anat Rec (Hoboken)* **292,** 1914-1921.

Nishiyama K, Kwak S, Takekoshi S, Watanabe K & Kanazawa I (1996). In situ nick end-labeling detects necrosis of hippocampal pyramidal cells induced by kainic acid. *Neurosci Lett* **212,** 139-142.

Noda M, Nakanishi H, Nabekura J & Akaike N (2000). AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. *J Neurosci* **20,** 251-258.

Noh HS, Kim YS, Lee HP, Chung KM, Kim DW, Kang SS, Cho GJ & Choi WS (2003). The protective effect of a ketogenic diet on kainic acid-induced hippocampal cell death in the male ICR mice. *Epilepsy Res* **53,** 119-128.

Noh JS, Kim EY, Kang JS, Kim HR, Oh YJ & Gwag BJ (1999). Neurotoxic and neuroprotective actions of catecholamines in cortical neurons. *Exp Neurol* **159,** 217-224.

Novak L BP, Ascher P, Herbert A, and Prochiantz A (1984). Magnesium gates glutamate-activated channels in mouse central neurons. *Nature* **307,** 462-465.

Nunez G, Benedict MA, Hu Y & Inohara N (1998). Caspases: the proteases of the apoptotic pathway. *Oncogene* **17,** 3237-3245.

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL & Petersen CM (2004). Sortilin is essential for proNGF-induced neuronal cell death. *Nature* **427,** 843-848.

O'Connor JC, Andre C, Wang Y, Lawson MA, Szegedi SS, Lestage J, Castanon N, Kelley KW & Dantzer R (2009). Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. *J Neurosci* **29,** 4200-4209.

O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, Kelley KW & Dantzer R (2009). Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry* **14,** 511-522.

Oderfeld-Nowak B & Bacia A (1994). Expression of astroglial nerve growth factor in damaged brain. *Acta Neurobiol Exp (Wars)* **54,** 73-80.

Oh-hashi K, Ito M, Tanaka T, Hirata Y & Kiuchi K (2009). Biosynthesis, processing, and secretion of glial cell line-derived neurotrophic factor in astroglial cells. *Mol Cell Biochem* **323,** 1-7.

Ohtori S, Takahashi K, Moriya H & Myers RR (2004). TNF-alpha and TNF-alpha receptor type 1 upregulation in glia and neurons after peripheral nerve injury: studies in murine DRG and spinal cord. *Spine (Phila Pa 1976)* **29,** 1082-1088.

Okuda S, Nishiyama N, Saito H & Katsuki H (1998). 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. *J Neurochem* **70,** 299-307.

Olney JW (1969). Glutaate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. *J Neuropathol Exp Neurol* **28,** 455-474.

Olney JW, Adamo NJ & Ratner A (1971). Monosodium glutamate effects. *Science* **172,** 294.

Olney JW & de Gubareff T (1978). Glutamate neurotoxicity and Huntington's chorea. *Nature* **271,** 557-559.

Oprica M, Eriksson C & Schultzberg M (2003). Inflammatory mechanisms associated with brain damage induced by kainic acid with special reference to the interleukin-1 system. *J Cell Mol Med* **7,** 127-140.

Ordway GA, O'Donnell JM & Frazer A (1987). Effects of clenbuterol on central beta-1 and beta-2 adrenergic receptors of the rat. *J Pharmacol Exp Ther* **241,** 187-195.

Orrenius S, McConkey DJ, Bellomo G & Nicotera P (1989). Role of Ca2+ in toxic cell killing. *Trends Pharmacol Sci* **10,** 281-285.

Orth K, Chinnaiyan AM, Garg M, Froelich CJ & Dixit VM (1996). The CED-3/ICE-like protease Mch2 is activated during apoptosis and cleaves the death substrate lamin A. *J Biol Chem* **271,** 16443-16446.

O'Sullivan JB, Ryan KM, Curtin NM, Harkin A & Connor TJ (2009). Noradrenaline reuptake inhibitors limit neuroinflammation in rat cortex following a systemic

inflammatory challenge: implications for depression and neurodegeneration. *Int J Neuropsychopharmacol* **12,** 687-699.

Paetsch PR & Greenshaw AJ (1993). Effects of chronic antidepressant treatment on beta-adrenoceptor subtype binding in the rat cerebral cortex and cerebellum. *Mol Chem Neuropathol* **20,** 21-31.

Pannu R & Singh I (2006). Pharmacological strategies for the regulation of inducible nitric oxide synthase: neurodegenerative versus neuroprotective mechanisms. *Neurochem Int* **49,** 170-182.

Paris I, Martinez-Alvarado P, Perez-Pastene C, Vieira MN, Olea-Azar C, Raisman-Vozari R, Cardenas S, Graumann R, Caviedes P & Segura-Aguilar J (2005). Monoamine transporter inhibitors and norepinephrine reduce dopamine-dependent iron toxicity in cells derived from the substantia nigra. *J Neurochem* **92,** 1021-1032.

Parsons CG, Danysz W & Quack G (1999). Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist--a review of preclinical data. *Neuropharmacology* **38,** 735-767.

Patapoutian A & Reichardt LF (2001). Trk receptors: mediators of neurotrophin action. *Curr Opin Neurobiol* **11,** 272-280.

Patterson SL, Grover LM, Schwartzkroin PA & Bothwell M (1992). Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. *Neuron* **9,** 1081-1088.

Pearson VL, Rothwell NJ & Toulmond S (1999). Excitotoxic brain damage in the rat induces interleukin-1beta protein in microglia and astrocytes: correlation with the progression of cell death. *Glia* **25,** 311-323.

Pemberton LA, Kerr SJ, Smythe G & Brew BJ (1997). Quinolinic acid production by macrophages stimulated with IFN-gamma, TNF-alpha, and IFN-alpha. *J Interferon Cytokine Res* **17,** 589-595.

Penfield W & Milner B (1958). Memory deficit produced by bilateral lesions in the hippocampal zone. *AMA Arch Neurol Psychiatry* **79,** 475-497.

Perez-Navarro E, Gavalda N, Gratacos E & Alberch J (2005). Brain-derived neurotrophic factor prevents changes in Bcl-2 family members and caspase-3 activation induced by excitotoxicity in the striatum. *J Neurochem* **92,** 678-691.

Pericic D, Jazvinscak M, Svob D & Mirkovic K (2000). Beta-1 adrenoceptor antagonists potentiate the anticonvulsive effect of swim stress in mice. *Pharmacol Biochem Behav* **67,** 507-510.

Perkins MN & Stone TW (1982). An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res* **247,** 184-187.

Pesant M, Sueur S, Dutartre P, Tallandier M, Grimaldi PA, Rochette L & Connat JL (2006). Peroxisome proliferator-activated receptor delta (PPARdelta) activation protects H9c2 cardiomyoblasts from oxidative stress-induced apoptosis. *Cardiovasc Res* **69,** 440-449.

Peterson AC (1999). Evaluation of functionalized tryptophan derivatives and related compounds as competitive inhibitors of indoleamine 2,3–dioxygenase. *Med. Chem. Res.* **3,** 531-544.

Peterson SL, Trzeciakowski JP & St Mary JS (1985). Chronic but not acute treatment with antidepressants enhances the electroconvulsive seizure response in rats. *Neuropharmacology* **24,** 941-946.

Petruska JC & Mendell LM (2004). The many functions of nerve growth factor: multiple actions on nociceptors. *Neurosci Lett* **361,** 168-171.

Phillips HS, Hains JM, Armanini M, Laramee GR, Johnson SA & Winslow JW (1991). BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron* **7,** 695-702.

Pi LG, Tang AG, Mo XM, Luo XB & Ao X (2009). More rapid and sensitive method for simultaneous determination of tryptophan and kynurenic acid by HPLC. *Clin Biochem* **42,** 420-425.

Pinteaux E, Rothwell NJ & Boutin H (2006). Neuroprotective actions of endogenous interleukin-1 receptor antagonist (IL-1ra) are mediated by glia. *Glia* **53,** 551-556.

Pittaluga A, Bonfanti A & Raiteri M (1997). Differential desensitization of ionotropic non-NMDA receptors having distinct neuronal location and function. *Naunyn Schmiedebergs Arch Pharmacol* **356,** 29-38.

Pittaluga A, Raiteri L, Longordo F, Luccini E, Barbiero VS, Racagni G, Popoli M & Raiteri M (2007). Antidepressant treatments and function of glutamate ionotropic receptors mediating amine release in hippocampus. *Neuropharmacology* **53,** 27-36.

Pittaluga A & Raiteri M (1990). Release-enhancing glycine-dependent presynaptic NMDA receptors exist on noradrenergic terminals of hippocampus. *Eur J Pharmacol* **191,** 231-234.

Platt SR (2007). The role of glutamate in central nervous system health and disease--a review. *Vet J* **173,** 278-286.

Poeggeler B, Rassoulpour A, Guidetti P, Wu HQ & Schwarcz R (1998). Dopaminergic control of kynurenate levels and N-methyl-D-aspartate toxicity in the developing rat striatum. *Dev Neurosci* **20,** 146-153.

Pollard H, Charriaut-Marlangue C, Cantagrel S, Represa A, Robain O, Moreau J & Ben-Ari Y (1994). Kainate-induced apoptotic cell death in hippocampal neurons. *Neuroscience* **63,** 7-18.

Polster BM, Basanez G, Young M, Suzuki M & Fiskum G (2003). Inhibition of Bax-induced cytochrome c release from neural cell and brain mitochondria by dibucaine and propranolol. *J Neurosci* **23,** 2735-2743.

Pop C & Salvesen GS (2009). Human caspases: activation, specificity, and regulation. *J Biol Chem* **284,** 21777-21781.

Popov A, Abdullah Z, Wickenhauser C, Saric T, Driesen J, Hanisch FG, Domann E, Raven EL, Dehus O, Hermann C, Eggle D, Debey S, Chakraborty T, Kronke M, Utermohlen O & Schultze JL (2006). Indoleamine 2,3-dioxygenase-expressing dendritic cells form suppurative granulomas following Listeria monocytogenes infection. *J Clin Invest* **116,** 3160-3170.

Popova LD (2002). [Effect of kynurenic acid on catecholamine content in the brain of rats with different susceptibility to seizures]. *Fiziol Zh* **48,** 41-45.

Popp R, Fleming I & Busse R (1998). Pulsatile stretch in coronary arteries elicits release of endothelium-derived hyperpolarizing factor: a modulator of arterial compliance. *Circ Res* **82,** 696-703.

Portera-Cailliau C, Hedreen JC, Price DL & Koliatsos VE (1995). Evidence for apoptotic cell death in Huntington disease and excitotoxic animal models. *J Neurosci* **15,** 3775-3787.

Portera-Cailliau C, Price DL & Martin LJ (1997). Excitotoxic neuronal death in the immature brain is an apoptosis-necrosis morphological continuum. *J Comp Neurol* **378,** 70-87.

Poulsen KT, Armanini MP, Klein RD, Hynes MA, Phillips HS & Rosenthal A (1994). TGF beta 2 and TGF beta 3 are potent survival factors for midbrain dopaminergic neurons. *Neuron* **13,** 1245-1252.

Pralong E & Jones RS (1993). Interactions of dopamine with glutamate- and GABA-mediated synaptic transmission in the rat entorhinal cortex in vitro. *Eur J Neurosci* **5,** 760-767.

Pralong E, Magistretti P & Stoop R (2002). Cellular perspectives on the glutamate-monoamine interactions in limbic lobe structures and their relevance for some psychiatric disorders. *Prog Neurobiol* **67,** 173-202.

Pralong E & Magistretti PJ (1995). Noradrenaline increases K-conductance and reduces glutamatergic transmission in the mouse entorhinal cortex by activation of alpha 2-adrenoreceptors. *Eur J Neurosci* **7,** 2370-2378.

Prehn JH, Backhauss C & Krieglstein J (1993). Transforming growth factor-beta 1 prevents glutamate neurotoxicity in rat neocortical cultures and protects mouse neocortex from ischemic injury in vivo. *J Cereb Blood Flow Metab* **13,** 521-525.

Puig B & Ferrer I (2002). Caspase-3-associated apoptotic cell death in excitotoxic necrosis of the entorhinal cortex following intraperitoneal injection of kainic acid in the rat. *Neurosci Lett* **321,** 182-186.

Racay P, Tatarkova Z, Chomova M, Hatok J, Kaplan P & Dobrota D (2009). Mitochondrial calcium transport and mitochondrial dysfunction after global brain ischemia in rat hippocampus. *Neurochem Res* **34,** 1469-1478.

Racine RJ (1972). Modification of seizure activity by electrical stimulation. I. After-discharge threshold. *Electroencephalogr Clin Neurophysiol* **32,** 269-279.

Racine RJ (1972). Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* **32,** 281-294.

Racine RJ, Gartner JG & Burnham WM (1972). Epileptiform activity and neural plasticity in limbic structures. *Brain Res* **47,** 262-268.

Rahimi RA & Leof EB (2007). TGF-beta signaling: a tale of two responses. *J Cell Biochem* **102,** 593-608.

Rainbow TC & Biegon A (1983). Quantitative autoradiography of [3H]prazosin binding sites in rat forebrain. *Neurosci Lett* **40,** 221-226.

Raison CL, Capuron L & Miller AH (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* **27,** 24-31.

Raitala A, Pertovaara M, Karjalainen J, Oja SS & Hurme M (2005). Association of interferon-gamma +874(T/A) single nucleotide polymorphism with the rate of tryptophan catabolism in healthy individuals. *Scand J Immunol* **61,** 387-390.

Raiteri M, Garrone B & Pittaluga A (1992). N-methyl-D-aspartic acid (NMDA) and non-NMDA receptors regulating hippocampal norepinephrine release. II. Evidence for functional cooperation and for coexistence on the same axon terminal. *J Pharmacol Exp Ther* **260,** 238-242.

Rami A, Volkmann T, Agarwal R, Schoninger S, Nurnberger F, Saido TC & Winckler J (2003). beta2-Adrenergic receptor responsiveness of the calpain-calpastatin system and attenuation of neuronal death in rat hippocampus after transient global ischemia. *Neurosci Res* **47,** 373-382.

Rang HP DM, Ritter JM (1999). *Pharmacology*. Churchill Livingston, London.

Rasley A, Bost KL, Olson JK, Miller SD & Marriott I (2002). Expression of functional NK-1 receptors in murine microglia. *Glia* **37,** 258-267.

Reed JC (2000). Mechanisms of apoptosis. *Am J Pathol* **157,** 1415-1430.

Reimann-Philipp U, Ovase R, Weigel PH & Grammas P (2001). Mechanisms of cell death in primary cortical neurons and PC12 cells. *J Neurosci Res* **64,** 654-660.

Reiss JI, Dishman RK, Boyd HE, Robinson JK & Holmes PV (2009). Chronic activity wheel running reduces the severity of kainic acid-induced seizures in the rat: possible role of galanin. *Brain Res* **1266,** 54-63.

Rejdak K, Petzold A, Kocki T, Kurzepa J, Grieb P, Turski WA & Stelmasiak Z (2007). Astrocytic activation in relation to inflammatory markers during clinical exacerbation of relapsing-remitting multiple sclerosis. *J Neural Transm* **114,** 1011-1015.

Relton JK & Rothwell NJ (1992). Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. *Brain Res Bull* **29,** 243-246.

Reynolds JL, Ignatowski TA, Sud R & Spengler RN (2004). Brain-derived tumor necrosis factor-alpha and its involvement in noradrenergic neuron functioning involved in the mechanism of action of an antidepressant. *J Pharmacol Exp Ther* **310,** 1216-1225.

Reynolds JL, Ignatowski TA, Sud R & Spengler RN (2005). An antidepressant mechanism of desipramine is to decrease tumor necrosis factor-alpha production culminating in increases in noradrenergic neurotransmission. *Neuroscience* **133,** 519-531.

Riccio A, Ahn S, Davenport CM, Blendy JA & Ginty DD (1999). Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. *Science* **286,** 2358-2361.

Riedl SJ, Fuentes-Prior P, Renatus M, Kairies N, Krapp S, Huber R, Salvesen GS & Bode W (2001). Structural basis for the activation of human procaspase-7. *Proc Natl Acad Sci U S A* **98,** 14790-14795.

Rijkers K, Majoie HJ, Hoogland G, Kenis G, De Baets M & Vles JS (2009). The role of interleukin-1 in seizures and epilepsy: a critical review. *Exp Neurol* **216,** 258-271.

Rios C & Santamaria A (1991). Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. *Neurochem Res* **16,** 1139-1143.

Rizzi M, Perego C, Aliprandi M, Richichi C, Ravizza T, Colella D, Veliskova J, Moshe SL, De Simoni MG & Vezzani A (2003). Glia activation and cytokine increase in rat hippocampus by kainic acid-induced status epilepticus during postnatal development. *Neurobiol Dis* **14,** 494-503.

Roberts AB & Sporn MB (1993). Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta). *Growth Factors* **8,** 1-9.

Rodriguez-Martinez E, Camacho A, Maldonado PD, Pedraza-Chaverri J, Santamaria D, Galvan-Arzate S & Santamaria A (2000). Effect of quinolinic acid on endogenous antioxidants in rat corpus striatum. *Brain Res* **858,** 436-439.

Rodriquez-Moreno A ST (2007). Kainate receptors with a metabotropic modus operandi. *Trends Neurosci* **30,** 630-637.

Rogawski MA, ed (1985). *Norepinephrine*. Plenum press, New york.

Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell CJ, Roger V, Rumsfeld J, Sorlie P, Steinberger J, Thom T, Wasserthiel-Smoller S & Hong Y (2007). Heart disease and stroke statistics--2007 update: a report from the

American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* **115,** e69-171.

Rossi D, Brambilla L, Valori CF, Crugnola A, Giaccone G, Capobianco R, Mangieri M, Kingston AE, Bloc A, Bezzi P & Volterra A (2005). Defective tumor necrosis factor-alpha-dependent control of astrocyte glutamate release in a transgenic mouse model of Alzheimer disease. *J Biol Chem* **280,** 42088-42096.

Rossi DJ, Oshima T & Attwell D (2000). Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature* **403,** 316-321.

Rothman DL, Behar KL, Hetherington HP, den Hollander JA, Bendall MR, Petroff OA & Shulman RG (1985). 1H-Observe/13C-decouple spectroscopic measurements of lactate and glutamate in the rat brain in vivo. *Proc Natl Acad Sci U S A* **82,** 1633-1637.

Rothstein JD (1996). Excitotoxicity hypothesis. *Neurology* **47,** S19-25; discussion S26.

Rothwell NJ & Luheshi GN (2000). Interleukin 1 in the brain: biology, pathology and therapeutic target. *Trends Neurosci* **23,** 618-625.

Rotonda J, Nicholson DW, Fazil KM, Gallant M, Gareau Y, Labelle M, Peterson EP, Rasper DM, Ruel R, Vaillancourt JP, Thornberry NA & Becker JW (1996). The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nat Struct Biol* **3,** 619-625.

Roumestan C, Michel A, Bichon F, Portet K, Detoc M, Henriquet C, Jaffuel D & Mathieu M (2007). Anti-inflammatory properties of desipramine and fluoxetine. *Respir Res* **8,** 35.

Rousset CI, Kassem J, Olivier P, Chalon S, Gressens P & Saliba E (2008). Antenatal bacterial endotoxin sensitizes the immature rat brain to postnatal excitotoxic injury. *J Neuropathol Exp Neurol* **67,** 994-1000.

Rudoy CA VBE (2005). Cocaine Effects on Norepinephrine in the Amygdala: Cocaine Withdrawal-Related Anxiety and Stress-Related Relapse. *Cellscience* **2.**

Ruit KG, Osborne PA, Schmidt RE, Johnson EM, Jr. & Snider WD (1990). Nerve growth factor regulates sympathetic ganglion cell morphology and survival in the adult mouse. *J Neurosci* **10,** 2412-2419.

Russo-Neustadt AA, Beard RC, Huang YM & Cotman CW (2000). Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. *Neuroscience* **101,** 305-312.

Russo-Neustadt AA & Chen MJ (2005). Brain-derived neurotrophic factor and antidepressant activity. *Curr Pharm Des* **11,** 1495-1510.

Saarma M & Sariola H (1999). Other neurotrophic factors: glial cell line-derived neurotrophic factor (GDNF). *Microsc Res Tech* **45,** 292-302.

Saito K, Markey SP & Heyes MP (1994). 6-Chloro-D,L-tryptophan, 4-chloro-3-hydroxyanthranilate and dexamethasone attenuate quinolinic acid accumulation in brain and blood following systemic immune activation. *Neurosci Lett* **178,** 211-215.

Saldana M, Pujols L, Roca-Ferrer J, Cardozo A, Aguilar E, Bonastre M & Marin C (2008). Relevance of COX-2 gene expression in dementia with lewy bodies associated with Alzheimer pathology. *Mov Disord* **23,** 804-810.

Salzberg-Brenhouse HC, Chen EY, Emerich DF, Baldwin S, Hogeland K, Ranelli S, Lafreniere D, Perdomo B, Novak L, Kladis T, Fu K, Basile AS, Kordower JH & Bartus RT (2003). Inhibitors of cyclooxygenase-2, but not cyclooxygenase-1 provide structural and functional protection against quinolinic acid-induced neurodegeneration. *J Pharmacol Exp Ther* **306,** 218-228.

Samina Riaz S & Tomlinson DR (2000). Pharmacological modulation of nerve growth factor synthesis: a mechanistic comparison of vitamin D receptor and beta(2)-adrenoceptor agonists. *Brain Res Mol Brain Res* **85,** 179-188.

Sanni LA, Thomas SR, Tattam BN, Moore DE, Chaudhri G, Stocker R & Hunt NH (1998). Dramatic changes in oxidative tryptophan metabolism along the kynurenine pathway in experimental cerebral and noncerebral malaria. *Am J Pathol* **152,** 611-619.

Santamaria A, Galvan-Arzate S, Lisy V, Ali SF, Duhart HM, Osorio-Rico L, Rios C & St'astny F (2001). Quinolinic acid induces oxidative stress in rat brain synaptosomes. *Neuroreport* **12,** 871-874.

Santamaria A, Santamaria D, Diaz-Munoz M, Espinoza-Gonzalez V & Rios C (1997). Effects of N omega-nitro-L-arginine and L-arginine on quinolinic acid-induced lipid peroxidation. *Toxicol Lett* **93,** 117-124.

Sarac S, Afzal S, Broholm H, Madsen FF, Ploug T & Laursen H (2009). Excitatory amino acid transporters EAAT-1 and EAAT-2 in temporal lobe and hippocampus in intractable temporal lobe epilepsy. *APMIS* **117,** 291-301.

Sartorius N, Baghai TC, Baldwin DS, Barrett B, Brand U, Fleischhacker W, Goodwin G, Grunze H, Knapp M, Leonard BE, Lieberman J, Nakane Y, Pinder RM, Schatzberg AF, Svestka J, Baumann P, Ghalib K, Markowitz JC, Padberg F, Fink M, Furukawa T, Fountoulakis KN, Jensen P, Kanba S & Riecher-Rossler A (2007). Antidepressant medications and other treatments of depressive disorders: a CINP Task Force report based on a review of evidence. *Int J Neuropsychopharmacol* **10 Suppl 1,** S1-207.

Sas K, Robotka H, Rozsa E, Agoston M, Szenasi G, Gigler G, Marosi M, Kis Z, Farkas T, Vecsei L & Toldi J (2008). Kynurenine diminishes the ischemia-induced histological and electrophysiological deficits in the rat hippocampus. *Neurobiol Dis* **32,** 302-308.

Sas K, Robotka H, Toldi J & Vecsei L (2007). Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J Neurol Sci* **257,** 221-239.

Satake K, Matsuyama Y, Kamiya M, Kawakami H, Iwata H, Adachi K & Kiuchi K (2000). Up-regulation of glial cell line-derived neurotrophic factor (GDNF) following traumatic spinal cord injury. *Neuroreport* **11,** 3877-3881.

Sathasivam S, Ince PG & Shaw PJ (2001). Apoptosis in amyotrophic lateral sclerosis: a review of the evidence. *Neuropathol Appl Neurobiol* **27,** 257-274.

Scates AC & Doraiswamy PM (2000). Reboxetine: a selective norepinephrine reuptake inhibitor for the treatment of depression. *Ann Pharmacother* **34,** 1302-1312.

Scharfman H (2002). Does BDNF Contribute to Temporal Lobe Epilepsy? *Epilepsy Curr* **2,** 92-94.

Schielke GP, Yang GY, Shivers BD & Betz AL (1998). Reduced ischemic brain injury in interleukin-1 beta converting enzyme-deficient mice. *J Cereb Blood Flow Metab* **18,** 180-185.

Schmidt-Kastner R, Bedard A & Hakim A (1997). Transient expression of GAP-43 within the hippocampus after global brain ischemia in rat. *Cell Tissue Res* **288,** 225-238.

Schmidt-Kastner R & Freund TF (1991). Selective vulnerability of the hippocampus in brain ischemia. *Neuroscience* **40,** 599-636.

Schmidt-Kastner R, Wetmore C & Olson L (1996). Comparative study of brain-derived neurotrophic factor messenger RNA and protein at the cellular level suggests multiple roles in hippocampus, striatum and cortex. *Neuroscience* **74,** 161-183.

Schmierer B & Hill CS (2007). TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol* **8,** 970-982.

Schroecksnadel K, Kaser S, Ledochowski M, Neurauter G, Mur E, Herold M & Fuchs D (2003). Increased degradation of tryptophan in blood of patients with rheumatoid arthritis. *J Rheumatol* **30,** 1935-1939.

Schwarcz R & Pellicciari R (2002). Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. *J Pharmacol Exp Ther* **303,** 1-10.

Schwarcz R, Scholz D & Coyle JT (1978). Structure-activity relations for the neurotoxicity of kainic acid derivatives and glutamate analogues. *Neuropharmacology* **17,** 145-151.

Schwarcz R, Whetsell WO, Jr. & Mangano RM (1983). Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* **219,** 316-318.

Schwartz D, Engelhard D, Gallily R, Matoth I & Brenner T (1998). Glial cells production of inflammatory mediators induced by Streptococcus pneumoniae: inhibition by pentoxifylline, low-molecular-weight heparin and dexamethasone. *J Neurol Sci* **155,** 13-22.

Schwartz JP, Sheng JG, Mitsuo K, Shirabe S & Nishiyama N (1993). Trophic factor production by reactive astrocytes in injured brain. *Ann N Y Acad Sci* **679,** 226-234.

Schwarzer C, Williamson JM, Lothman EW, Vezzani A & Sperk G (1995). Somatostatin, neuropeptide Y, neurokinin B and cholecystokinin immunoreactivity in two chronic models of temporal lobe epilepsy. *Neuroscience* **69,** 831-845.

Schweitzer JB (1989). Nerve growth factor receptor-mediated transport from CSF labels cholinergic neurons: direct demonstration by a double-labeling study. *Brain Res* **490,** 390-396.

Schwinn DA, Page SO, Middleton JP, Lorenz W, Liggett SB, Yamamoto K, Lapetina EG, Caron MG, Lefkowitz RJ & Cotecchia S (1991). The alpha 1C-adrenergic receptor: characterization of signal transduction pathways and mammalian tissue heterogeneity. *Mol Pharmacol* **40,** 619-626.

Sciacca FL, Ferri C, Licastro F, Veglia F, Biunno I, Gavazzi A, Calabrese E, Martinelli Boneschi F, Sorbi S, Mariani C, Franceschi M & Grimaldi LM (2003). Interleukin-1B polymorphism is associated with age at onset of Alzheimer's disease. *Neurobiol Aging* **24,** 927-931.

Scoville WB & Milner B (1957). Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* **20,** 11-21.

Segal RA (2003). Selectivity in neurotrophin signaling: theme and variations. *Annu Rev Neurosci* **26,** 299-330.

Semba J, Miyoshi R & Kito S (1996). Nicotine protects against the dexamethasone potentiation of kainic acid-induced neurotoxicity in cultured hippocampal neurons. *Brain Res* **735,** 335-338.

Semkova I, Schilling M, Henrich-Noack P, Rami A & Krieglstein J (1996). Clenbuterol protects mouse cerebral cortex and rat hippocampus from ischemic damage and attenuates glutamate neurotoxicity in cultured hippocampal neurons by induction of NGF. *Brain Res* **717,** 44-54.

Seo SK, Choi JH, Kim YH, Kang WJ, Park HY, Suh JH, Choi BK, Vinay DS & Kwon BS (2004). 4-1BB-mediated immunotherapy of rheumatoid arthritis. *Nat Med* **10,** 1088-1094.

Sharma AK, Jordan WH, Reams RY, Hall DG & Snyder PW (2008). Temporal profile of clinical signs and histopathologic changes in an F-344 rat model of kainic acid-induced mesial temporal lobe epilepsy. *Toxicol Pathol* **36,** 932-943.

Sharma S & Kaur G (2005). Neuroprotective potential of dietary restriction against kainate-induced excitotoxicity in adult male Wistar rats. *Brain Res Bull* **67,** 482-491.

Shetty AK & Turner DA (2000). Fetal hippocampal grafts containing CA3 cells restore host hippocampal glutamate decarboxylase-positive interneuron numbers in a rat model of temporal lobe epilepsy. *J Neurosci* **20,** 8788-8801.

Shinozaki H & Konishi S (1970). Actions of several anthelmintics and insecticides on rat cortical neurones. *Brain Res* **24,** 368-371.

Shirayama Y, Chen AC, Nakagawa S, Russell DS & Duman RS (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* **22,** 3251-3261.

Shorvon S (2002). Does convulsive status epilepticus (SE) result in cerebral damage or affect the course of epilepsy--the epidemiological and clinical evidence? *Prog Brain Res* **135,** 85-93.

Siegel PM & Massague J (2003). Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat Rev Cancer* **3,** 807-821.

Sieradzan KA & Mann DM (2001). The selective vulnerability of nerve cells in Huntington's disease. *Neuropathol Appl Neurobiol* **27,** 1-21.

Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS & Bulloch K (2008). Steroid hormone receptor expression and function in microglia. *Glia* **56,** 659-674.

Siesjo BK, Katsura K, Zhao Q, Folbergrova J, Pahlmark K, Siesjo P & Smith ML (1995). Mechanisms of secondary brain damage in global and focal ischemia: a speculative synthesis. *J Neurotrauma* **12,** 943-956.

Silva NM, Rodrigues CV, Santoro MM, Reis LF, Alvarez-Leite JI & Gazzinelli RT (2002). Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with Toxoplasma gondii: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. *Infect Immun* **70,** 859-868.

Siman R, Noszek JC & Kegerise C (1989). Calpain I activation is specifically related to excitatory amino acid induction of hippocampal damage. *J Neurosci* **9,** 1579-1590.

Simi A, Tsakiri N, Wang P & Rothwell NJ (2007). Interleukin-1 and inflammatory neurodegeneration. *Biochem Soc Trans* **35,** 1122-1126.

Simmons ML & Murphy S (1992). Induction of nitric oxide synthase in glial cells. *J Neurochem* **59,** 897-905.

Simmons ML & Murphy S (1993). Cytokines regulate L-arginine-dependent cyclic GMP production in rat glial cells. *Eur J Neurosci* **5,** 825-831.

Simonato M, Bregola G, Armellin M, Del Piccolo P, Rodi D, Zucchini S & Tongiorgi E (2002). Dendritic targeting of mRNAs for plasticity genes in experimental models of temporal lobe epilepsy. *Epilepsia* **43 Suppl 5,** 153-158.

Simonian NA, Getz RL, Leveque JC, Konradi C & Coyle JT (1996). Kainic acid induces apoptosis in neurons. *Neuroscience* **75,** 1047-1055.

Siuciak JA, Lewis DR, Wiegand SJ & Lindsay RM (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol Biochem Behav* **56,** 131-137.

Skoog I (2000). Detection of preclinical Alzheimer's disease. *N Engl J Med* **343,** 502-503.

Sloviter RS, Dean E, Sollas AL & Goodman JH (1996). Apoptosis and necrosis induced in different hippocampal neuron populations by repetitive perforant path stimulation in the rat. *J Comp Neurol* **366,** 516-533.

Sly LM, Krzesicki RF, Brashler JR, Buhl AE, McKinley DD, Carter DB & Chin JE (2001). Endogenous brain cytokine mRNA and inflammatory responses to lipopolysaccharide are elevated in the Tg2576 transgenic mouse model of Alzheimer's disease. *Brain Res Bull* **56,** 581-588.

Smith SA, Monteith GR, Robinson JA, Venkata NG, May FJ & Roberts-Thomson SJ (2004). Effect of the peroxisome proliferator-activated receptor beta activator GW0742 in rat cultured cerebellar granule neurons. *J Neurosci Res* **77,** 240-249.

Sofroniew MV, Howe CL & Mobley WC (2001). Nerve growth factor signaling, neuroprotection, and neural repair. *Annu Rev Neurosci* **24,** 1217-1281.

Sossin WS & Barker PA (2007). Something old, something new: BDNF-induced neuron survival requires TRPC channel function. *Nat Neurosci* **10,** 537-538.

Spera PA, Ellison JA, Feuerstein GZ & Barone FC (1998). IL-10 reduces rat brain injury following focal stroke. *Neurosci Lett* **251,** 189-192.

Sperk G, Lassmann H, Baran H, Seitelberger F & Hornykiewicz O (1985). Kainic acid-induced seizures: dose-relationship of behavioural, neurochemical and histopathological changes. *Brain Res* **338,** 289-295.

Sriram K & O'Callaghan JP (2007). Divergent roles for tumor necrosis factor-alpha in the brain. *J Neuroimmune Pharmacol* **2,** 140-153.

Stamler JS (1994). Redox signaling: nitrosylation and related target interactions of nitric oxide. *Cell* **78,** 931-936.

Starke K, Gothert M & Kilbinger H (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol Rev* **69,** 864-989.

Stenqvist A, Agerman K, Marmigere F, Minichiello L & Ernfors P (2005). Genetic evidence for selective neurotrophin 3 signalling through TrkC but not TrkB in vivo. *EMBO Rep* **6,** 973-978.

Stevens B (2003). Glia: much more than the neuron's side-kick. *Curr Biol* **13,** R469-472.

Stone TW (1993). Neuropharmacology of quinolinic and kynurenic acids. *Pharmacol Rev* **45,** 309-379.

Stone TW (2001). Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. *Prog Neurobiol* **64,** 185-218.

Stone TW & Addae JI (2002). The pharmacological manipulation of glutamate receptors and neuroprotection. *Eur J Pharmacol* **447,** 285-296.

Stone TW & Darlington LG (2002). Endogenous kynurenines as targets for drug discovery and development. *Nat Rev Drug Discov* **1,** 609-620.

Stone TW, Forrest CM, Mackay GM, Stoy N & Darlington LG (2007). Tryptophan, adenosine, neurodegeneration and neuroprotection. *Metab Brain Dis* **22,** 337-352.

Storm-Mathisen J, Danbolt NC, Rothe F, Torp R, Zhang N, Aas JE, Kanner BI, Langmoen I & Ottersen OP (1992). Ultrastructural immunocytochemical observations on the localization, metabolism and transport of glutamate in normal and ischemic brain tissue. *Prog Brain Res* **94,** 225-241.

Stroemer RP & Rothwell NJ (1998). Exacerbation of ischemic brain damage by localized striatal injection of interleukin-1beta in the rat. *J Cereb Blood Flow Metab* **18,** 833-839.

Sulser F, Vetulani J & Mobley PL (1978). Mode of action of antidepressant drugs. *Biochem Pharmacol* **27,** 257-261.

Summers RJ & McMartin LR (1993). Adrenoceptors and their second messenger systems. *J Neurochem* **60,** 10-23.

Summers RJ, Papaioannou M, Harris S & Evans BA (1995). Expression of beta 3-adrenoceptor mRNA in rat brain. *Br J Pharmacol* **116,** 2547-2548.

Suzuki Y, Claflin J, Wang X, Lengi A & Kikuchi T (2005). Microglia and macrophages as innate producers of interferon-gamma in the brain following infection with Toxoplasma gondii. *Int J Parasitol* **35,** 83-90.

Swartz KJ, Matson WR, MacGarvey U, Ryan EA & Beal MF (1990). Measurement of kynurenic acid in mammalian brain extracts and cerebrospinal fluid by high-performance liquid chromatography with fluorometric and coulometric electrode array detection. *Anal Biochem* **185,** 363-376.

Szanto S, Koreny T, Mikecz K, Glant TT, Szekanecz Z & Varga J (2007). Inhibition of indoleamine 2,3-dioxygenase-mediated tryptophan catabolism accelerates collagen-induced arthritis in mice. *Arthritis Res Ther* **9,** R50.

Tabakman R, Jiang H, Shahar I, Arien-Zakay H, Levine RA & Lazarovici P (2005). Neuroprotection by NGF in the PC12 in vitro OGD model: involvement of mitogen-activated protein kinases and gene expression. *Ann N Y Acad Sci* **1053,** 84-96.

Takahashi A, Alnemri ES, Lazebnik YA, Fernandes-Alnemri T, Litwack G, Moir RD, Goldman RD, Poirier GG, Kaufmann SH & Earnshaw WC (1996). Cleavage of lamin A by Mch2 alpha but not CPP32: multiple interleukin 1 beta-converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. *Proc Natl Acad Sci U S A* **93,** 8395-8400.

Takemoto, ed (1978). *Isolation and structural identification of naturally occuring excitatory amino acids*, vol. 1-10. Raven Press, New York.

Takikawa O, Tagawa Y, Iwakura Y, Yoshida R & Truscott RJ (1999). Interferon-gamma-dependent/independent expression of indoleamine 2,3-dioxygenase. Studies with interferon-gamma-knockout mice. *Adv Exp Med Biol* **467,** 553-557.

Tamura (1954). Pharmacological studies on the principles of *Dignea simplex. Ag. J Pharm. soc Jpn* **74,** 1290-1293.

Tannenberg RK, Scott HL, Westphalen RI & Dodd PR (2004). The identification and characterization of excitotoxic nerve-endings in Alzheimer disease. *Curr Alzheimer Res* **1,** 11-25.

Tao L, Liu HR, Gao E, Teng ZP, Lopez BL, Christopher TA, Ma XL, Batinic-Haberle I, Willette RN, Ohlstein EH & Yue TL (2003). Antioxidative, antinitrative, and vasculoprotective effects of a peroxisome proliferator-activated receptor-gamma agonist in hypercholesterolemia. *Circulation* **108,** 2805-2811.

Tao X, West AE, Chen WG, Corfas G & Greenberg ME (2002). A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. *Neuron* **33,** 383-395.

Tatton WG, Chalmers-Redman R, Brown D & Tatton N (2003). Apoptosis in Parkinson's disease: signals for neuronal degradation. *Ann Neurol* **53 Suppl 3,** S61-70; discussion S70-62.

Tavares RG, Tasca CI, Santos CE, Alves LB, Porciuncula LO, Emanuelli T & Souza DO (2002). Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochem Int* **40,** 621-627.

Tazik S, Johnson S, Lu D, Johnson C, Youdim MB, Stockmeier CA & Ou XM (2009). Comparative neuroprotective effects of rasagiline and aminoindan with selegiline on dexamethasone-induced brain cell apoptosis. *Neurotox Res* **15,** 284-290.

Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen ZY, Lee FS, Kraemer RT, Nykjaer A & Hempstead BL (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neurosci* **25,** 5455-5463.

Teng YD, Choi H, Huang W, Onario RC, Frontera WR, Snyder EY & Sabharwal S (2006). Therapeutic effects of clenbuterol in a murine model of amyotrophic lateral sclerosis. *Neurosci Lett* **397,** 155-158.

Teunissen CE, Lutjohann D, von Bergmann K, Verhey F, Vreeling F, Wauters A, Bosmans E, Bosma H, van Boxtel MP, Maes M, Delanghe J, Blom HJ, Verbeek MM, Rieckmann P, De Bruijn C, Steinbusch HW & de Vente J (2003). Combination of serum markers related to several mechanisms in Alzheimer's disease. *Neurobiol Aging* **24,** 893-902.

Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS & Dixit VM (1995). Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* **81,** 801-809.

Thoenen H, Zafra F, Hengerer B & Lindholm D (1991). The synthesis of nerve growth factor and brain-derived neurotrophic factor in hippocampal and cortical neurons is regulated by specific transmitter systems. *Ann N Y Acad Sci* **640,** 86-90.

Thomas K & Davies A (2005). Neurotrophins: a ticket to ride for BDNF. *Curr Biol* **15,** R262-264.

Thompson CB (1995). Apoptosis in the pathogenesis and treatment of disease. *Science* **267,** 1456-1462.

Thornberry NA (1998). Caspases: key mediators of apoptosis. *Chem Biol* **5,** R97-103.

Thornberry NA & Lazebnik Y (1998). Caspases: enemies within. *Science* **281,** 1312-1316.

Tikka TM & Koistinaho JE (2001). Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* **166,** 7527-7533.

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M & Persson H (1993). Multiple promoters direct tissue-specific expression of the rat BDNF gene. *Neuron* **10,** 475-489.

Tokuhara D, Sakuma S, Hattori H, Matsuoka O & Yamano T (2007). Kainic acid dose affects delayed cell death mechanism after status epilepticus. *Brain Dev* **29,** 2-8.

Tonder N, Kragh J, Finsen BR, Bolwig TG & Zimmer J (1994). Kindling induces transient changes in neuronal expression of somatostatin, neuropeptide Y, and calbindin in adult rat hippocampus and fascia dentata. *Epilepsia* **35,** 1299-1308.

Torregrossa MM, Folk JE, Rice KC, Watson SJ & Woods JH (2005). Chronic administration of the delta opioid receptor agonist (+)BW373U86 and antidepressants on behavior in the forced swim test and BDNF mRNA expression in rats. *Psychopharmacology (Berl)* **183,** 31-40.

Toulmond S, Vige X, Fage D & Benavides J (1992). Local infusion of interleukin-6 attenuates the neurotoxic effects of NMDA on rat striatal cholinergic neurons. *Neurosci Lett* **144,** 49-52.

Traver S, Salthun-Lassalle B, Marien M, Hirsch EC, Colpaert F & Michel PP (2005). The neurotransmitter noradrenaline rescues septal cholinergic neurons in culture from degeneration caused by low-level oxidative stress. *Mol Pharmacol* **67,** 1882-1891.

Troadec JD, Marien M, Darios F, Hartmann A, Ruberg M, Colpaert F & Michel PP (2001). Noradrenaline provides long-term protection to dopaminergic neurons by reducing oxidative stress. *J Neurochem* **79,** 200-210.

Troadec JD, Marien M, Mourlevat S, Debeir T, Ruberg M, Colpaert F & Michel PP (2002). Activation of the mitogen-activated protein kinase (ERK(1/2)) signaling pathway by cyclic AMP potentiates the neuroprotective effect of the neurotransmitter noradrenaline on dopaminergic neurons. *Mol Pharmacol* **62,** 1043-1052.

Troost D, Aten J, Morsink F & de Jong JM (1995). Apoptosis in amyotrophic lateral sclerosis is not restricted to motor neurons. Bcl-2 expression is increased in unaffected post-central gyrus. *Neuropathol Appl Neurobiol* **21,** 498-504.

Troy CM, Friedman JE & Friedman WJ (2002). Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *J Biol Chem* **277,** 34295-34302.

Tsujimoto G, Tsujimoto A, Suzuki E & Hashimoto K (1989). Glycogen phosphorylase activation by two different alpha 1-adrenergic receptor subtypes: methoxamine selectively stimulates a putative alpha 1-adrenergic receptor subtype (alpha 1a) that couples with Ca2+ influx. *Mol Pharmacol* **36,** 166-176.

Tsunashima K, Schwarzer C, Kirchmair E, Sieghart W & Sperk G (1997). GABA(A) receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy. *Neuroscience* **80,** 1019-1032.

Tsutsui N & Kamiyama T (1998). Suppression of in vitro IFN-gamma production by spleen cells of Plasmodium chabaudi-infected C57BL/10 mice exposed to dexamethasone at a low dose. *Int J Immunopharmacol* **20,** 141-152.

Ueno A, Cho S, Cheng L, Wang J, Hou S, Nakano H, Santamaria P & Yang Y (2007). Transient upregulation of indoleamine 2,3-dioxygenase in dendritic cells by human chorionic gonadotropin downregulates autoimmune diabetes. *Diabetes* **56,** 1686-1693.

Unsain N, Nunez N, Anastasia A & Masco DH (2008). Status epilepticus induces a TrkB to p75 neurotrophin receptor switch and increases brain-derived neurotrophic factor interaction with p75 neurotrophin receptor: an initial event in neuronal injury induction. *Neuroscience* **154,** 978-993.

Vanoye-Carlo A, Morales T, Ramos E, Mendoza-Rodriguez A & Cerbon M (2008). Neuroprotective effects of lactation against kainic acid treatment in the dorsal hippocampus of the rat. *Horm Behav* **53,** 112-123.

Vaudry D, Gonzalez BJ, Basille M, Pamantung TF, Fontaine M, Fournier A & Vaudry H (2000). The neuroprotective effect of pituitary adenylate cyclase-activating polypeptide on cerebellar granule cells is mediated through inhibition of the CED3-related cysteine protease caspase-3/CPP32. *Proc Natl Acad Sci U S A* **97,** 13390-13395.

Vecsei L, Miller J, MacGarvey U & Beal MF (1992). Kynurenine and probenecid inhibit pentylenetetrazol- and NMDLA-induced seizures and increase kynurenic acid concentrations in the brain. *Brain Res Bull* **28,** 233-238.

Vesce S, Rossi D, Brambilla L & Volterra A (2007). Glutamate release from astrocytes in physiological conditions and in neurodegenerative disorders characterized by neuroinflammation. *Int Rev Neurobiol* **82,** 57-71.

Veyrac A, Didier A, Colpaert F, Jourdan F & Marien M (2005). Activation of noradrenergic transmission by alpha2-adrenoceptor antagonists counteracts deafferentation-induced neuronal death and cell proliferation in the adult mouse olfactory bulb. *Exp Neurol* **194,** 444-456.

Vezzani A, Conti M, De Luigi A, Ravizza T, Moneta D, Marchesi F & De Simoni MG (1999). Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures. *J Neurosci* **19,** 5054-5065.

Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, De Simoni MG, Sperk G, Andell-Jonsson S, Lundkvist J, Iverfeldt K & Bartfai T (2000). Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. *Proc Natl Acad Sci U S A* **97,** 11534-11539.

Vignau J, Jacquemont MC, Lefort A, Imbenotte M & Lhermitte M (2004). Simultaneous determination of tryptophan and kynurenine in serum by HPLC with UV and fluorescence detection. *Biomed Chromatogr* **18,** 872-874.

Vincent P & Mulle C (2009). Kainate receptors in epilepsy and excitotoxicity. *Neuroscience* **158,** 309-323.

Vinet J, Carra S, Blom JM, Brunello N, Barden N & Tascedda F (2004). Chronic treatment with desipramine and fluoxetine modulate BDNF, CaMKKalpha and CaMKKbeta mRNA levels in the hippocampus of transgenic mice expressing antisense RNA against the glucocorticoid receptor. *Neuropharmacology* **47,** 1062-1069.

Viswanath V, Wu Z, Fonck C, Wei Q, Boonplueang R & Andersen JK (2000). Transgenic mice neuronally expressing baculoviral p35 are resistant to diverse types of induced apoptosis, including seizure-associated neurodegeneration. *Proc Natl Acad Sci U S A* **97,** 2270-2275.

Vitkovic L, Konsman JP, Bockaert J, Dantzer R, Homburger V & Jacque C (2000). Cytokine signals propagate through the brain. *Mol Psychiatry* **5,** 604-615.

Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL & Marinovich M (2003). Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci* **23,** 8692-8700.

Vivien D & Ali C (2006). Transforming growth factor-beta signalling in brain disorders. *Cytokine Growth Factor Rev* **17,** 121-128.

Vodovotz Y, Lucia MS, Flanders KC, Chesler L, Xie QW, Smith TW, Weidner J, Mumford R, Webber R, Nathan C, Roberts AB, Lippa CF & Sporn MB (1996). Inducible nitric oxide synthase in tangle-bearing neurons of patients with Alzheimer's disease. *J Exp Med* **184,** 1425-1433.

von Lubitz DK & Diemer NH (1983). Cerebral ischemia in the rat: ultrastructural and morphometric analysis of synapses in stratum radiatum of the hippocampal CA-1 region. *Acta Neuropathol* **61,** 52-60.

Walser M & Hill SB (1993). Free and protein-bound tryptophan in serum of untreated patients with chronic renal failure. *Kidney Int* **44,** 1366-1371.

Wang D, Kanuma T, Mizunuma H, Takama F, Ibuki Y, Wake N, Mogi A, Shitara Y & Takenoshita S (2000). Analysis of specific gene mutations in the transforming growth factor-beta signal transduction pathway in human ovarian cancer. *Cancer Res* **60,** 4507-4512.

Wang H, Wu YB & Du XH (2005). Effect of dexamethasone on nitric oxide synthase and Caspase-3 gene expressions in endotoxemia in neonate rat brain. *Biomed Environ Sci* **18,** 181-186.

Wang J, Wei Q, Wang CY, Hill WD, Hess DC & Dong Z (2004). Minocycline up-regulates Bcl-2 and protects against cell death in mitochondria. *J Biol Chem* **279,** 19948-19954.

Wang JK, Andrews H & Thukral V (1992). Presynaptic glutamate receptors regulate noradrenaline release from isolated nerve terminals. *J Neurochem* **58,** 204-211.

Wang QW, Rowan MJ & Anwyl R (2009). Inhibition of LTP by beta-amyloid is prevented by activation of beta2 adrenoceptors and stimulation of the cAMP/PKA signalling pathway. *Neurobiol Aging* **30,** 1608-1613.

Wang X, Michie SA, Xu B & Suzuki Y (2007). Importance of IFN-gamma-mediated expression of endothelial VCAM-1 on recruitment of CD8+ T cells into the brain during chronic infection with Toxoplasma gondii. *J Interferon Cytokine Res* **27,** 329-338.

Wang X, Yue TL, Barone FC, White RF, Gagnon RC & Feuerstein GZ (1994). Concomitant cortical expression of TNF-alpha and IL-1 beta mRNAs follows early response gene expression in transient focal ischemia. *Mol Chem Neuropathol* **23,** 103-114.

Weinshenker D & Szot P (2002). The role of catecholamines in seizure susceptibility: new results using genetically engineered mice. *Pharmacol Ther* **94,** 213-233.

Weinshenker D, Szot P, Miller NS & Palmiter RD (2001). Alpha(1) and beta(2) adrenoreceptor agonists inhibit pentylenetetrazole-induced seizures in mice lacking norepinephrine. *J Pharmacol Exp Ther* **298,** 1042-1048.

Weise J, Engelhorn T, Dorfler A, Aker S, Bahr M & Hufnagel A (2005). Expression time course and spatial distribution of activated caspase-3 after experimental status epilepticus: contribution of delayed neuronal cell death to seizure-induced neuronal injury. *Neurobiol Dis* **18,** 582-590.

Wejksza K, Rzeski W, Okuno E, Kandefer-Szerszen M, Albrecht J & Turski WA (2005). Demonstration of kynurenine aminotransferases I and II and characterization of kynurenic acid synthesis in oligodendrocyte cell line (OLN-93). *Neurochem Res* **30,** 963-968.

Wells JE, Hurlbert RJ, Fehlings MG & Yong VW (2003). Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. *Brain* **126,** 1628-1637.

Wenzel HJ, Cole TB, Born DE, Schwartzkroin PA & Palmiter RD (1997). Ultrastructural localization of zinc transporter-3 (ZnT-3) to synaptic vesicle membranes within mossy fiber boutons in the hippocampus of mouse and monkey. *Proc Natl Acad Sci U S A* **94,** 12676-12681.

WHO (2002). Active ageing: a policy framework. Second United Nations world assembly on aging, Madrid, Spain.

Wiegmann K, Muthyala S, Kim DH, Arnason BG & Chelmicka-Schorr E (1995). Beta-adrenergic agonists suppress chronic/relapsing experimental allergic encephalomyelitis (CREAE) in Lewis rats. *J Neuroimmunol* **56,** 201-206.

Wilcockson DC, Campbell SJ, Anthony DC & Perry VH (2002). The systemic and local acute phase response following acute brain injury. *J Cereb Blood Flow Metab* **22,** 318-326.

Willenborg DO, Fordham S, Bernard CC, Cowden WB & Ramshaw IA (1996). IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J Immunol* **157,** 3223-3227.

Wong PC, Cai H, Borchelt DR & Price DL (2002). Genetically engineered mouse models of neurodegenerative diseases. *Nat Neurosci* **5,** 633-639.

Wu HQ, Guidetti P, Goodman JH, Varasi M, Ceresoli-Borroni G, Speciale C, Scharfman HE & Schwarcz R (2000). Kynurenergic manipulations influence excitatory synaptic function and excitotoxic vulnerability in the rat hippocampus in vivo. *Neuroscience* **97,** 243-251.

Xiang Z, Haroutunian V, Ho L, Purohit D & Pasinetti GM (2006). Microglia activation in the brain as inflammatory biomarker of Alzheimer's disease neuropathology and clinical dementia. *Dis Markers* **22,** 95-102.

Xiao LD, Luo XB, Pi LG & Tang AG (2008). Simultaneous determination of kynurenine and kynurenic acid concentrations in human serum by HPLC with dual wavelengths fluorescence detection. *Clin Chim Acta* **395,** 178-180.

Xie QW, Kashiwabara Y & Nathan C (1994). Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. *J Biol Chem* **269,** 4705-4708.

Xie QW, Whisnant R & Nathan C (1993). Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon gamma and bacterial lipopolysaccharide. *J Exp Med* **177,** 1779-1784.

Xiong H, McCabe L, Costello J, Anderson E, Weber G & Ikezu T (2004). Activation of NR1a/NR2B receptors by soluble factors from APP-stimulated monocyte-derived macrophages: implications for the pathogenesis of Alzheimer's disease. *Neurobiol Aging* **25,** 905-911.

Xu H, Steven Richardson J & Li XM (2003). Dose-related effects of chronic antidepressants on neuroprotective proteins BDNF, Bcl-2 and Cu/Zn-SOD in rat hippocampus. *Neuropsychopharmacology* **28,** 53-62.

Xu XP, Sun RP & Jin RF (2006). Effect of ketogenic diet on hippocampus mossy fiber sprouting and GluR5 expression in kainic acid induced rat model. *Chin Med J (Engl)* **119,** 1925-1929.

Yalcin I, Aksu F & Belzung C (2005). Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. *Eur J Pharmacol* **514,** 165-174.

Yamamoto I, Iwata K & Nakashima M (1985). Pharmacokinetics of plasma and urine clenbuterol in man, rat, and rabbit. *J Pharmacobiodyn* **8,** 385-391.

Yamasaki Y, Matsuura N, Shozuhara H, Onodera H, Itoyama Y & Kogure K (1995). Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke* **26,** 676-680; discussion 681.

Yamazaki T, Akiba H, Iwai H, Matsuda H, Aoki M, Tanno Y, Shin T, Tsuchiya H, Pardoll DM, Okumura K, Azuma M & Yagita H (2002). Expression of programmed death 1 ligands by murine T cells and APC. *J Immunol* **169,** 5538-5545.

Yan H, Frost P, Shi Y, Hoang B, Sharma S, Fisher M, Gera J & Lichtenstein A (2006). Mechanism by which mammalian target of rapamycin inhibitors sensitize multiple myeloma cells to dexamethasone-induced apoptosis. *Cancer Res* **66,** 2305-2313.

Yang DS, Kumar A, Stavrides P, Peterson J, Peterhoff CM, Pawlik M, Levy E, Cataldo AM & Nixon RA (2008). Neuronal apoptosis and autophagy cross talk in aging PS/APP mice, a model of Alzheimer's disease. *Am J Pathol* **173,** 665-681.

Yang J, Kawamura I & Mitsuyama M (1997). Requirement of the initial production of gamma interferon in the generation of protective immunity of mice against Listeria monocytogenes. *Infect Immun* **65,** 72-77.

Ye ZC & Sontheimer H (1996). Cytokine modulation of glial glutamate uptake: a possible involvement of nitric oxide. *Neuroreport* **7,** 2181-2185.

Yen GC & Hsieh CL (1997). Antioxidant effects of dopamine and related compounds. *Biosci Biotechnol Biochem* **61,** 1646-1649.

Yenari MA, Xu L, Tang XN, Qiao Y & Giffard RG (2006). Microglia potentiate damage to blood-brain barrier constituents: improvement by minocycline in vivo and in vitro. *Stroke* **37,** 1087-1093.

Yi JH & Hazell AS (2006). Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. *Neurochem Int* **48,** 394-403.

Yong VW, Moumdjian R, Yong FP, Ruijs TC, Freedman MS, Cashman N & Antel JP (1991). Gamma-interferon promotes proliferation of adult human astrocytes in vitro and reactive gliosis in the adult mouse brain in vivo. *Proc Natl Acad Sci U S A* **88,** 7016-7020.

Yong VW, Wells J, Giuliani F, Casha S, Power C & Metz LM (2004). The promise of minocycline in neurology. *Lancet Neurol* **3,** 744-751.

Yoo YM, Lee CJ, Lee U & Kim YJ (2006). Neuroprotection of adenoviral-vector-mediated GDNF expression against kainic-acid-induced excitotoxicity in the rat hippocampus. *Exp Neurol* **200,** 407-417.

Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH & Kim IO (1997). Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol* **177,** 19-26.

Yoshimura T, Kurita C, Nagao T, Usami E, Nakao T, Watanabe S, Kobayashi J, Yamazaki F, Tanaka H, Inagaki N & Nagai H (1997). Inhibition of tumor necrosis factor-alpha and interleukin-1-beta production by beta-adrenoceptor agonists from lipopolysaccharide-stimulated human peripheral blood mononuclear cells. *Pharmacology* **54,** 144-152.

Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH & Koistinaho J (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A* **96,** 13496-13500.

Yu ZK, Chen YN, Aihara M, Mao W, Uchida S & Araie M (2007). Effects of beta-adrenergic receptor antagonists on oxidative stress in purified rat retinal ganglion cells. *Mol Vis* **13,** 833-839.

Yuan J & Horvitz HR (1992). The Caenorhabditis elegans cell death gene ced-4 encodes a novel protein and is expressed during the period of extensive programmed cell death. *Development* **116,** 309-320.

Yuan J, Shaham S, Ledoux S, Ellis HM & Horvitz HR (1993). The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* **75,** 641-652.

Yuan SZ, Runold M, Hagberg H, Bona E & Lagercrantz H (2001). Hypoxic-ischaemic brain damage in immature rats: effects of adrenoceptor modulation. *Eur J Paediatr Neurol* **5,** 29-35.

Yuen AH, Yacoub MH & Smolenski RT (2005). Determination of clenbuterol concentration in human blood using liquid chromatography with electrospray/ion-trap tandem mass spectrometry. *Rapid Commun Mass Spectrom* **19,** 3603-3606.

Yun HY, Dawson VL & Dawson TM (1997). Nitric oxide in health and disease of the nervous system. *Mol Psychiatry* **2,** 300-310.

Zaczek R, Simonton S & Coyle JT (1980). Local and distant neuronal degeneration following intrastriatal injection of kainic acid. *J Neuropathol Exp Neurol* **39,** 245-264.

Zafra F, Hengerer B, Leibrock J, Thoenen H & Lindholm D (1990). Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO J* **9,** 3545-3550.

Zeman RJ, Peng H & Etlinger JD (2004). Clenbuterol retards loss of motor function in motor neuron degeneration mice. *Exp Neurol* **187,** 460-467.

Zhang Y & Kimelberg HK (2005). Neuroprotection by alpha 2-adrenergic agonists in cerebral ischemia. *Curr Neuropharmacol* **3,** 317-323.

Zhang Y, McCluskey K, Fujii K & Wahl LM (1998). Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-alpha, granulocyte-macrophage CSF, and IL-1 beta through prostaglandin-dependent and -independent mechanisms. *J Immunol* **161,** 3071-3076.

Zhao X, Bausano B, Pike BR, Newcomb-Fernandez JK, Wang KK, Shohami E, Ringger NC, DeFord SM, Anderson DK & Hayes RL (2001). TNF-alpha stimulates caspase-3 activation and apoptotic cell death in primary septo-hippocampal cultures. *J Neurosci Res* **64,** 121-131.

Zhao Z, Zhang HT, Bootzin E, Millan MJ & O'Donnell JM (2009). Association of changes in norepinephrine and serotonin transporter expression with the long-term behavioral effects of antidepressant drugs. *Neuropsychopharmacology* **34,** 1467-1481.

Zheng H, Zhu W, Zhao H, Wang X, Wang W & Li Z Kainic acid-activated microglia mediate increased excitability of rat hippocampal neurons in vitro and in vivo: crucial role of interleukin-1beta. *Neuroimmunomodulation* **17,** 31-38.

Zhu Y, Culmsee C, Semkova I & Krieglstein J (1998). Stimulation of beta2-adrenoceptors inhibits apoptosis in rat brain after transient forebrain ischemia. *J Cereb Blood Flow Metab* **18,** 1032-1039.

Zhu Y, Prehn JH, Culmsee C & Krieglstein J (1999). The beta2-adrenoceptor agonist clenbuterol modulates Bcl-2, Bcl-xl and Bax protein expression following transient forebrain ischemia. *Neuroscience* **90,** 1255-1263.

Zipp F & Aktas O (2006). The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. *Trends Neurosci* **29,** 518-527.

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S & Cattaneo E (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* **293,** 493-498.

IX Publications

Gleeson LC, Harkin A. (2008) Dexamethasone suppresses inflammatory and apoptotic responses but not seizure or neuronal cell loss in response to kainic acid. British Association of Psychopharmacology, Annual meeting, Harrogate, UK. *Abstract published in Journal of Psychopharmacology, Vol 22. No 5 (July 2008).*

Gleeson LC, Harkin A. (2009) Inhibitors of noradrenaline transport exacerbate excitotoxin-induced hippocampal apoptosis. British Neuroscience Association, Annual meeting, Liverpool, UK. *Abstract published in British Neuroscience Association abstracts*

Gleeson LC, Connor TJ, Harkin A. (2009) Noradrenaline re-uptake inhibition influences neuroinflammatory and degenerative changes associated with the excitotoxin kainic acid. Psychoneuroimmunology annual conference, Denver, Colorado, USA. *Abstract published in Brain, Behavior, and Immunity, Volume 23, Supplement 2, Pages S25-S64 (July 2009).*

Forthcoming Publications

Gleeson LC, Ryan KJ, Griffin EW, Connor TJ, Harkin A. (2010) The β_2-adrenoceptor agonist clenbuterol elicits neuroprotective, anti-inflammatory and neurotrophic actions in the kainic acid model of excitotoxicity. Submitted to *Brain Behavior and Immunity*