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.
MODELS OF NEURODEGENERATION IN THE HIPPOCAMPUS:
MOLECULAR AND BEHAVIOURAL FACTORS PROMOTING
NEUROPROTECTION AND NEUROPATHOLOGY

by

Oliviero L. Gobbo

A dissertation submitted for the degree of Doctor of Philosophy of the University of Dublin, Trinity College, Dublin 2, Ireland.
This research was conducted in the Department of Psychology, Trinity College Institute of Neuroscience.

October 2002
Declaration

I declare that this work has not been submitted previously as an exercise for a degree at this or any other university and that it is entirely my own work. The Trinity College Library may lend or copy this thesis without restriction.

Signed

Oliviero Gobbo
A Fosca

È per te che sono verdi gli alberi e rosa i fiocchi in maternità
è per te che il sole brucia a luglio è per te tutta questa città
è per te che sono bianchi i muri e la colomba vola
è per te il dieci dicembre è per te la campanella a scuola
è per te ogni cosa che c'è ..........

Lorenzo Cherubini
Acknowledgements

My eternal gratitude goes to:

Shane O’Mara for trusting me and to give me the chance to study in Trinity College, although I did not speak English. I thank him also for his enthusiasm and support over this thesis.

Marylene for her encouragements and for bringing up our daughter alone during this work.

I would like to thank my parents for their support, and the dream team (Michael Anderson, Sean Commins, David Delany, Richard Roche and Kendra Shaw) for their help, friendship, support and the parties throughout the years.

All my encouragements and thanks go to my new lab members: Jorge Brotons, Thelma Cowley, Maeve Mangaoang and Sinead Mullally.

I would like to thank all my Trinity friends and colleagues, particularly Deirdre Bonini, Martijn Goudbeek, Fiona Newell and Claire Walsh, for their friendship and support.

Finally, special thanks for their technical assistance go to Marina Lynch’s team (Aileen Lynch, Franck Maher and Darren Martin), Eddie Bolger and June Switzer.
Merci à mes amis et anciens collègues de France qui m’ont apportés leur aide et leur soutien, qu’ils trouvent ici le témoignage de ma gratitude.

Thanks for musical assistance go to Francesco Perilli and Vanessa Incontrada: Their nocturnal help was important during the writing of this thesis.

Gloria al Padre. San Leopoldo Mandic, prega per noi!
Summary

We investigated the consequences of brain insult after ischemia and kainic acid using different behavioural tasks, because both ischemia or KA model induce a similar neurodegenerative process to that resulting from traumatic brain injury, as well as some aspects of Alzheimer’s and Parkinson’s diseases. We have investigated some of the mechanisms which might be engaged by these pathologies; we have shown possible mechanisms which are involved in the process of recovery, focusing attention on prostaglandin production involved in the inflammation pathway generated from arachidonic acid via the action of the cyclooxygenases (COX), especially COX-2 (Cryer and Dubois, 1999). We also examined BDNF protein levels (one of most important trophic factors in the hippocampus, and which is augmented during brain injury); we examined if increases in BDNF protein levels could protect against cell death, following brain insult.
Abbreviations

4 VO: Four vessel occlusion
AA: Arachidonic acid
AMPA: Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
ANOVA: Analysis of variance
Anti-IgY: immunoglobulin Y antibody
AP5: 2-Amino-5-phosphonopentanoic acid
ATPases: Enzyme producing adenosine triphosphatase
BDNF: Brain-derived neurotrophic factor
BrdU: Bromodeoxyuridine
BSA: Bovine serum albumin
CA1: Cornu Ammonis 1 (sub-region of the hippocampus)
CA2: Cornu Ammonis 2 (sub-region of the hippocampus)
CA3: Cornu Ammonis 3 (sub-region of the hippocampus)
CB: Cerebellum
CNS: Central nervous system
CNTF: Ciliary neurotrophic factor
COX-1: Cyclooxygenase isoform 1
COX-2: Cyclooxygenase isoform 2
COX-3: Cyclooxygenase isoform 3
CPP: 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid
Cxlb: Celecoxib
DG: Dentate gyrus
DMSO: Dimethyl sulfoxide
DNA: Desoxyribonucleic acid
GABA: Gamma-aminobutyric acid
GluR5: glutamate receptor 5, subtype of kainate receptor
GluR6: glutamate receptor 6, subtype of kainate receptor
GluR7: glutamate receptor 7, subtype of kainate receptor
HRP: Horseradish peroxidase
IEG: Immediate-early gene
i.p.: Intraperitoneal
ITI: Intertrial interval
KA: Kainic acid
KA1r: Kainic acid receptor 1
KA2r: Kainic acid receptor 2
kDa: Kilo Dalton
LTP: Long-term potentiation
LV: lateral ventricle
mAb: Monoclonal antibody
MD, SM: the thalamic mediodorsal and submedius nuclei
mf: Mossy fibers
MPTP: 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
mRNA: messenger ribonucleic acid
n: Number
NADH: nicotinamide adenine dinucleotide H
NADPH: nicotinamide adenine dinucleotide phosphate H
NGF: Nerve growth factor
NMDA: N-methyl-D-aspartate
NOS: Reactive oxygen species
NSAID: Nonsteroidal anti-inflammatory drugs
NT-3, NT-4, NT-5: Neurotrophin 3, 4, 5
OB: Olfactory Bulb
p75
NGFR : 75 kDa molecular weight, low affinity neurotrophin receptor
pAb: Polyclonal antibody
PGD₂: Prostaglandin D₂
PGE₂: Prostaglandin E₂
PGF₂: Prostaglandin F₂
PGG₂: Prostaglandin G₂
PGH₂: Prostaglandin H₂
PGL₂: Prostaglandin I₂
PLA₂: Phospholypase A₂
PLC: Phospholypase C
pp: Perforant pathway
PTP megachannel: Permeability transition pore megachannel
RMS: Rostral migratory stream
sc: Schaffer collaterals
sem: Standard error mean
SUB and S: Subiculum
SVZ: Subventricular zone
SVZ-OB: Subventricular zone-olfactory Bulb
tPA : Tissue plasminogen activator
trk A, trk B or trk C: Tyrosine kinase-type A, B, C receptors
Chapter I: Literature review

Contents

1. Introduction ................................................................. 7
2. The hippocampus ........................................................... 12
3. The anatomy of the hippocampal formation .................... 15
4. Neurodegeneration and glutamate ............................... 17
5. Ischemia and kainic acid models in the rat ................. 19
6. Cyclooxygenases ......................................................... 27
7. Neurotrophins .............................................................. 33
8. Neurogenesis ............................................................... 42
9. Enriched environment and exercise .......................... 44
10. The Morris watermaze .................................................. 48
11. Olfactory memory task ............................................... 50
12. Object exploration task ............................................... 53
13. Objectives of this thesis ............................................. 55

Chapter II: Enriched-environment housing enhances performance on odour discrimination, object exploration and spatial learning tasks independently of transient global ischemia.

Contents

1. Introduction ................................................................. 60

2. Materials and Methods ............................................... 62
   2.1. Animals ................................................................. 62
   2.2. Housing conditions .................................................. 62
   2.3. Ischemia surgery ..................................................... 63
   2.4. Behavioural testing .................................................. 64
      2.4.1 Odour discrimination task ........................................ 64
      2.4.2 Watermaze ......................................................... 64
      2.4.3 Object exploration task .......................................... 65
   2.5 BDNF Elisa procedure ............................................... 66
   2.6 Quantitation of protein using the Bradford assay ........ 67
   2.7 Histology ............................................................... 68
2.8 Statistics ................................................................................................................................. 69

3. Results.................................................................................................................................. 71

3.1. Odour discrimination task ............................................................................................. 71

3.2 Watermaze task analysis .................................................................................................. 72

3.2.1 Velocity analysis ....................................................................................................... 72

3.2.2 Escape latency and total distance swam .................................................................. 73

3.2.3 Direct swim analysis .................................................................................................. 74

3.3 Object exploration task .................................................................................................... 76

3.4 Measurement of BDNF ..................................................................................................... 80

3.5 Histology ............................................................................................................................ 81

4. Discussion ............................................................................................................................. 86

Chapter III: Running and celecoxib can protect against spatial memory deficits following
kainic acid administration .......................................................................................................... 92

Contents .................................................................................................................................... 93

1. Introduction .......................................................................................................................... 96

2. Materials and Methods .......................................................................................................... 99

2.1 Animals .............................................................................................................................. 99

2.2 Housing conditions .......................................................................................................... 99

2.3 Kainic acid injection ....................................................................................................... 100

2.4 Voluntary physical activity protocol ............................................................................. 102

2.5 COX inhibitors ............................................................................................................... 102

2.6 Voluntary physical activity protocol and COX inhibitor ............................................. 103

2.7 Behavioural testing ......................................................................................................... 105

2.7.1 Odour discrimination task ....................................................................................... 105

2.7.2 Watermaze ................................................................................................................. 105

2.7.3 Object exploration task ............................................................................................ 106

2.7.4 Open field testing ...................................................................................................... 107

2.8 BDNF Elisa procedure .................................................................................................... 108

2.9 Quantitation of protein using the Bradford assay ......................................................... 109

2.10 Histology .......................................................................................................................... 110

2.11 Statistics ............................................................................................................................ 110
3. Results ................................................................................................................................... 112

3.1 Odour discrimination task .................................................................................................. 112

3.2 Watermaze task analysis .................................................................................................... 113

3.2.1 Velocity analysis ............................................................................................................ 113

3.2.2 KA dose assessment – impairment of learning in the watermaze ............................... 114

3.2.3 Visible platform ............................................................................................................ 117

3.2.4 Enriched environment ................................................................................................. 120

3.2.5 Kainic acid + ibuprofen ............................................................................................... 121

3.2.6 Running before KA injection ....................................................................................... 123

3.2.7 Kainic acid + celecoxib 6 mg/kg .................................................................................. 126

3.2.8 Running + KA + celecoxib 6 mg/kg ............................................................................ 129

3.2.9 Running + KA + celecoxib 40 mg/kg ......................................................................... 132

3.3 Object exploration task .................................................................................................... 135

3.3.1 Run + KA and celecoxib + KA ................................................................................... 135

3.3.2 Running + KA + celecoxib 6 mg/kg ........................................................................... 137

3.3.3 Running + KA + celecoxib 40 mg/kg ......................................................................... 139

3.4 Spontaneous activity in the open field ............................................................................. 141

3.5 Measurement of BDNF ..................................................................................................... 142

3.5.1 BDNF 6 hours after injuries and after running for 5 nights ........................................ 142

3.5.2 Running + kainic acid group ....................................................................................... 143

3.5.3 Kainic acid + celecoxib 6 mg/kg and run + KA+ celecoxib groups ............................ 144

3.5.4 Celecoxib 40 mg/kg groups ....................................................................................... 145

3.6 Histology .......................................................................................................................... 147

4. Discussion ............................................................................................................................ 157

Chapter IV: Evaluation of celecoxib pre-administration in kainic acid-induced seizure 166

Contents .................................................................................................................................... 167

1. Introduction .......................................................................................................................... 168

2. Materials and Methods ......................................................................................................... 171

2.1 Animals ............................................................................................................................... 171

2.2 Kainic acid and celecoxib injections ............................................................................... 171

2.3 Behavioural testing .......................................................................................................... 172
2.3.1 Watermaze ................................................................. 172
2.3.2 Object exploration task .............................................. 172
2.3.3 Open field testing ....................................................... 174
2.4 BDNF Elisa procedure ................................................... 174
2.5 Quantitation of protein using the Bradford assay .......... 175
2.6 Histology ........................................................................ 176
2.7 Statistics ......................................................................... 176
3. Results .............................................................................. 178
3.1 Watermaze task analysis ................................................. 178
3.2 Object exploration task .................................................. 180
3.3 Spontaneous activity in the open field ......................... 183
3.4 Measurement of BDNF ................................................... 184
3.5 Histology ........................................................................ 185
4. Discussion ......................................................................... 187

Chapter V: General discussion ............................................. 191

Contents ............................................................................. 192

1. Summary of our results (table 1) ..................................... 193
2. The hippocampus ........................................................... 199
3. Enriched environment and running ................................ 200
4. Brain-derived neurotrophic factor (BDNF) ..................... 201
5. Cyclooxygenases ............................................................. 203
6. BDNF and cyclooxygenases .......................................... 207
7. Histology ........................................................................ 208
8. Concluding remarks ...................................................... 208

References .......................................................................... 210

Publications ......................................................................... 255
Chapter I: Literature review
### Contents

1. Introduction .......................................................... 7
2. The hippocampus .................................................... 12
3. The anatomy of the hippocampal formation ............... 15
4. Neurodegeneration and glutamate .......................... 17
5. Ischemia and kainic acid models in the rat .......... 19
6. Cyclooxygenases ...................................................... 27
7. Neurotrophins ........................................................ 33
8. Neurogenesis .......................................................... 42
9. Enriched environment and exercise ....................... 44
10. The Morris watermaze .......................................... 48
11. Olfactory memory task ......................................... 50
12. Object exploration task ....................................... 53
13. Objectives of this thesis ...................................... 55
1. Introduction

Every day we use the mnemonic properties of the brain, largely without conscious effort: recalling the various experiences of our personal life, learning new things, recognizing people, objects, acquiring skills and solving problems. In the 19th Century many differing theories of brain function were proposed, such as the phrenological theory of F. J. Gall, which suggested that each faculty of the mind is concerned with a particular skill and keeps its own memory. Closer to contemporary thinking were the suggestions of M. de Biran, who distinguished “representative” memory (recollection of ideas and events), “mechanical” memory (concerned with habits and skills) and “sensitive” memory (which concerns feelings). By the second half of the 20th Century, new hypotheses were proposed about the existence of different types of memory and the brain areas that might be involved these memory classes. Studies with amnesic patients, such as the famous patient H.M. described by Scoville and Milner (1957), have revealed much about the neuronal bases of memory. The case of H.M. is particularly instructive: he had been suffering from epilepsy that was refractory to anticonvulsant medication, and surgery was therefore the treatment option. The conducted surgery was a medial temporal-lobe resection removing the hippocampal formation and the amygdala bilaterally. After the operation, he had fewer seizures but he had a dramatic and sustained episodic memory impairment - memory for the events of everyday life. Other studies of brain damaged patients that have provided interesting hypotheses about the biological basis of memory include that of patient K.F., described by Warrington and Shallice (1969). K.F. suffered a motorcycle accident with a left parieto-occipital fracture, which produced impairments
such as poor language functions and deficits in oral and written spelling; furthermore, he was incapable of repeating verbal stimuli (e.g. letters, words or sentences). These cases led Zola-Morgan and Squire (see review by Squire, 1992) to propose a scheme concerning the varying types and overall organization of memory. Squire and Knowlton (1994) suggested additional hypotheses regarding the brain areas which might be involved in these memories (figure 1). These authors distinguish two major kinds of memory, “declarative” and “non-declarative” (or procedural memory). Declarative memory involves in the recollection of our own previous experiences, recognition of personal scenes, objects and what we learn at school or in the news - the events of everyday life. Non-declarative memory, by contrast, is concerned with skills, habits or basic associative learning (e.g. Pavlovian conditioning), however procedural memory also has declarative components and in general is a class of memory that is difficult to articulate. The information sequence involved in memory can be divided into three steps (Squire, 1987) as follows:

1)- information encoding, 2)- its storage and 3)- its recollection.

Other schemes for the division of memory have been presented: Eichenbaum et al. (1999), for example suggests that there are five major divisions of memory:

- Episodic memory is the explicit recollection of incidents that occurred at a particular time and place in one’s personal past.

- Semantic memory refers to the general knowledge of facts and concepts that is not linked to any particular time and place.

- The perceptual representation system plays an important role in the identification of words and objects on the basis of their form and structure.
Procedural memory refers to the acquisition of skills and habits, which are acquired gradually through repetitive practice.

Working memory (or short-term memory) is concerned with short-term retention, operating over periods of seconds. This is in contrast to previous definitions of long-term memory.

A tentative match between brain areas and particular types of memory can be made and this might explain why selective brain damage impairs one form of memory without affecting others. The overall picture that is emerging suggests that there is no specific area for memory, but rather a cohesive group of structures, which must act in concert to generate memories. Some areas are more involved in one form of memory than others: H.M.'s lesions (Corkin et al., 1997) consist of the amygdala, enthorinal cortex and hippocampus, suggesting that at least some of these structures play a major role in his memory deficit. At the cellular level, it is now widely assumed that information is stored in the brain in the form of changes in the efficacy of synaptic connections of those neurons which are activated during learning (Bliss and Collingridge, 1993; Laroche S, 1994). Donald Hebb (1949) made the original proposal for a mechanism for storing information in the brain, suggesting that the changes required for learning take place at the synapse and it is the changes in synaptic strength that constitute memory traces; in particular he proposed that when neurons fire together, that this will result in a preferential relationship between them, sustained by metabolic changes in the cells (Bliss and Collingridge, 1993). In an important discovery, Bliss and Lomo (1973) discovered that the electrical stimulation of an excitatory afferent pathway to the hippocampus resulted in an increase in the strength of the activated synapses, and that this change in
synaptic strength remained present for a long time. This effect has come to be called long-term potentiation (LTP) and has been suggested as a biological mechanism underlying the role of the hippocampus in memory.

Although many experiments concerning memory are conducted in humans, it is easier to investigate certain processes (especially biological ones) using animals. Even if it is difficult to adequately compare both on a psychological level, animal experimentation can provide insights into the underlying neuronal mechanisms of memory. An analogue of declarative memory is one of most studied forms in animals. In non-human primates, the “delayed non-matching-to-sample” task has been used, where the animal must choose the difference between familiar and unfamiliar objects after a delay of few seconds to few minutes, usually after frontal brain damage. In contrast, rodents are more useful for revealing aspects of cognitive processing accomplished by the hippocampus.
Figure 1: Multiple memory systems and their associated brain structures. Modified from Squire and Knowlton (1994).
2. The hippocampus

After their initial description of H.M., Scoville and Milner tested other cases with post-operative hippocampal formation lesions. In the 1950’s, there were about 300 lobotomies conducted on schizophrenic patients in Canadian hospitals; they selected 10 of these cases (Scoville and Milner, 1957) for further study, testing their intelligence on the Wechsler-Bellevue Intelligence Scale, and their memory on the Wechsler Memory Scale. The results showed that for patients with hippocampal lesions, general intelligence was unimpaired but that they suffered memory loss, especially memory for recent events. Memory for older experiences before surgery was well preserved, but it was difficult or impossible for them to remember new or recent facts and events. Scoville and Milner (1957) concluded that the hippocampal formation (figure 2) plays a critical role in the retention of current experience.

In a then revolutionary proposal, O’Keefe and Nadel (1978) proposed that the hippocampus mediates “cognitive mapping”, i.e. there is a neuronal representation of the physical environment in the hippocampal formation. Thus, a hippocampal lesion should impair spatial learning on a spatial learning task such as the Morris watermaze. Many hundreds of papers have since shown an essential involvement of the hippocampal formation in a variety of so-called “allocentric” spatial tasks, which require an animal or human to locate itself relative to the environment (for reviews see Kessels et al., 2001; Warburton et al., 2001; Burgess et al., 2001; Gaffan et al., 2000). Furthermore, many studies have shown that hippocampal neurons are activated when an animal occupies a particular position in space - “place” cells (O’Keefe, 1979; O’Mara, 1995). Furthermore,
the hippocampal formation is activated during spatial navigation in virtual reality mazes (Maguire et al., 1998) by humans.

Does the hippocampal formation also represent non-spatial information?

Dusek and Eichenbaum, exploiting the excellent olfactory memory capacities of the rat (1997) showed also that the hippocampal formation is involved in non-spatial memory. In this study, rats initially learnt different sets of odour-pairs that shared common elements e.g. A-B, B-C, C-D and were then tested for their capacity to infer indirect relationships between items not explicitly paired (A-C or B-D). Normal rats, as well as rats with selective hippocampal lesions, readily acquired the paired associates, and normal subjects demonstrated the associative inference between indirectly related items. However, rats with hippocampal lesions showed no inferential capacity, implicating the hippocampus in mediating representations that link indirectly associated elements in memory and in the expression of these associations through inferential judgments (Burton, 2000). Dusek and Eichenbaum’s results (1997) extend the properties of cognitive mapping and their mediation by hippocampal mechanisms to non-spatial dimensions of memory organization in animals, indicating that the role of the hippocampal region in declarative memory expression may be as general in rats as it is in humans.
Figure 2: The hippocampal formation
3. The anatomy of the hippocampal formation

The hippocampal formation comprises of the hippocampus (areas CA1 and CA3 (CA=Cornu Ammonis)), the dentate gyrus and subiculum. The entorhinal cortex, closely associated with the hippocampal formation, is the posterior part of the pyriform cortex (Amaral & Witter, 1995). Hippocampal pyramidal cells have extensive apical and basal dendrites and axons that bifurcate, sending projections running both anterior and posterior in the alveus. Hippocampal area CA1 pyramidal cells mainly project to the septal nuclei, the subiculum (Amaral et al., 1991), and also to other structures. The subiculum receives afferents from the hippocampus proper, mainly from area CA1 (O'Mara et al., 2001a) and from entorhinal cortex (Witter et al., 2000). Subicular cells project to entorhinal cortex, deep layers of perirhinal cortex, and also to subcortical structures such as the mammillary bodies, hypothalamus, amygdala and nucleus accumbens (O'Mara et al., 2001a). The mammillary body projections to the pons may provide an important link between the hippocampus and the cerebellum, permitting hippocampal influences on motor behaviour. The major afferents to the hippocampal formation (the dentate gyrus and Ammon's horn) come from the entorhinal cortex via the perforant path, which in turn receives input from the entire neocortex (Witter et al., 2000). Finally, there are commissural afferents from the pyramidal cells of contralateral hippocampus and from contralateral entorhinal cortex (see schematic diagram figure 3a and 3b).
Figure 3a: The intrinsic connections of the hippocampal formation, reprinted from O’Mara et al. (2001a).
Figure 3b: Diagram shows a coronal section of hippocampus, indicating the various sub-regions. CA1 and CA3: Ammons horn region 1 and 3; DG: dentate gyrus; S: subiculum; mf: Mossy fibers; pp: perforant pathway; sc: Schaffer collaterals

4. Neurodegeneration and glutamate

The overstimulation of excitatory amino acid receptors by glutamate (the principal excitatory neurotransmitter in the brain) has been suggested as a major cause of injury or death of neurons (Meldrum, 2002); and has been implicated in brain insults such as stroke, hypoglycemia, trauma (Piot-Grosjean et al., 2001), and epilepsy, as well as chronic neurodegenerative states such as Huntington's disease, amyotrophic lateral sclerosis, and perhaps Alzheimer's disease (Meldrum and Garthwaite, 1990; Choi, 1988b). Excitatory neurotransmitters are also important in the developmental plasticity of synaptic connections in the nervous system. However, in pathologic conditions, excessive
activation of glutamate receptors may produce neuronal injury or death. This excitotoxicity may constitute a final common pathway for neuronal injury due to diseases with diverse pathophysiologic processes (Lipton and Rosenberg, 1994). There are 2 main sub-types of glutamate receptors:

- ionotropic glutamate receptors (receptors that are coupled directly to membrane ion channels)
- metabotropic glutamate receptors (receptors that are coupled to G proteins and modulate intracellular second messengers such as inositol trisphosphate, calcium, and cyclic nucleotides)

Ionotropic receptors are probably more implicated in excitotoxicity than other receptor types. They can be divided in 3 main types based on their selective agonists (see figure 4):

- N-methyl-D-aspartate (NMDA)
- \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)
- kainic acid

The activation of glutamate receptors causes an influx of sodium and calcium ions. High intracellular levels of calcium then initiate signalling cascades within susceptible neurons that cause neuronal death through as yet undefined sequences of molecular events (Choi, 1988a).
5. Ischemia and kainic acid models in the rat

There are several different models currently used to induce neurodegeneration. We will focus here on two models which involve excitotoxicity: a major one of these is the ischemia model induced in the rat by the four-vessel occlusion method (4VO) (Pulsinelli and Brierley, 1979), which reproduces many of the features of ischemic stroke, which commonly occurs due to occlusion of brain blood vessels by blood clots originating from the heart or atherosclerotic arterial plaques in humans. Major disability can result, with the loss, for example, of the ability to communicate, coordinate or reason; one of the most
celebrated cases is that of patient R.B., who developed anterograde amnesia following an ischemic episode, and which also caused extensive bilateral neuronal loss in area CA1 of his hippocampal formation.

The neuronal damage inflicted by 4VO is often assessed by measuring pyramidal cell loss in the CA1 hippocampal field, which is perhaps the most sensitive area to ischemia in the brain. However, the duration of induced global ischemia depends critically on the duration of arterial occlusion: 15 minutes occlusion induces selective damage in the CA1 sub-field; 30 minutes produces larger cell loss within the area CA1 and more variably in the areas CA2, CA3, striatum and cortex (Nunn et al., 1994).

Ischemia induces energy deprivation, removing the homeostatic equilibrium, which is directed towards maintaining and monitoring the ionic gradients related to synaptic transmission and the generation of action potentials. Excitatory neurotransmission comprises much of the brain’s energy demand, and glutamate is the major excitatory neurotransmitter in brain (see above). Excessive activation of central excitatory amino acid receptors is toxic even in the presence of normal glucose and oxygen (Choi, 1990). Such excitotoxicity induces large increases in intracellular calcium, activates $\text{Ca}^{2+}$-dependent proteases, kinases, phospholipases and endonucleases, thereby promoting neuronal and glial cell death (figure 6). Free radicals are produced by damaged mitochondria and by the reaction of the molecular oxygen with iron released from protein binding sites by proteases, acidoses and oxygenases. Superoxide, the primary oxygen radical, is produced in tissue via a variety of enzymatic reactions or by auto-oxidation of tissue components (Kontos, 2001, figure 5a). However, there are multiple sources of oxygen radicals and they cause many pathological cellular effects such as lipid
peroxidation, protein denaturation and DNA damage. It seems that activation of AMPA receptors may also be an effective mechanism for increasing the production of oxygen radicals in cerebral ischemia (Kontos, 2001). Oxygen radicals are one of the molecules involved in the initiation of inflammatory pathways, because they damage the cell membrane and cause the release of phospholipids. These are hydrolysed by phospholipase A$_2$ (PLA$_2$) to generate arachidonic acid and lysophospholipids (Arai et al., 2001) (figure 5b). One of 3 forms of PLA$_2$ is Ca$^{2+}$-dependent (Dennis, 1997) and thus it is probably strongly stimulated during excitotoxicity.

Autoxidation of small molecules
Autoxidation of hemoglobin and myoglobin
Autoxidation of mitochondrial components
Oxidative enzymes (xanthine oxidase, NADH oxidases, NOS, cyclooxygenase, NADPH oxidase in phagocytic cells)
Oxidation of unsaturated fatty acids

Figure 5a: Cellular sources of superoxide (Kontos, 2001)
The second popular model of neurodegeneration is induced by the administration of kainic acid (figure 7), which is an agonist of the AMPA and kainate class of glutamate receptors. It was isolated fifty years ago from the seaweed *Digenea simplex* and has been extensively used in post-war Japan to eradicate *ascarasis*, a worm, which infects the human and animal intestine (Ben-Ari, 1985). Limbic seizures in the rat, induced by intracerebral or systemic injection of kainic acid, represent a valuable animal model for temporal lobe neurodegeneration, closely reflecting the clinical and neuropathological
symptoms of the disorder in humans (Ben-Ari, 1985). The potent excitotoxic and epileptogenic effects of kainate are thought to be due to the existence of specific receptors for kainate. This hypothesis was supported by the demonstration of high affinity binding sites for $[^{3}H]$kainate to rat brain (London and Coyle, 1979) and the demonstration that kainate produces distinct depolarising and desensitizing responses in dorsal root ganglia (Huettner, 1990). Canzoniero et al. (1996) have found in neurons derived from P19 embryonal carcinoma cells that the elevation in calcium produced by kainate involved the activation of voltage-gated calcium channels as a consequence of membrane depolarisation. However, channels gated by kainate displayed low permeability to calcium compared to those of NMDA receptors.

There are two different kinds of kainate receptor subunits, KA1r and KA2r, which show high affinity binding for $[^{3}H]$kainate (Werner et al., 1991) and 3 other subtypes with a low affinity for $[^{3}H]$kainate, GluR5, GluR6 and GluR7 (Chittajallu et al., 1999). They are widely distributed in the mammalian central nervous system, including the cerebellum, hippocampus and spinal cord (Wisden and Seeburg, 1993). However, the subtypes are not all found in the same brain areas: GluR5 mRNA is limited mainly to the subiculum, hippocampal area CA1 and the Purkinje cell layer of the cerebellum; GluR6 mRNA is found the dentate gyrus and CA3 region of the hippocampus and granule cells of cerebellum; KA1r mRNA is highest in the amygdala, hippocampal formation and entorhinal cortex. KA2r is widely distributed in the central nervous system, mainly in cerebral cortex, caudate putamen, hippocampus, entorhinal cortex and the granule cell layer of cerebellum (Chittajallu et al., 1999). Moreover, Contractor et al. (2001) and Schmitz et al. (2001a) have demonstrated an important role for kainate receptors at mossy
fiber-CA3 synapses in frequency-dependant synaptic facilitation. Bortolotto et al. (1999) also showed that kainate receptors are required for long-term potentiation between mossy fibers and CA3 pyramidal cells. It seems clear that kainate receptors play a role in excitatory transmission, especially in area CA3 of the hippocampus; this may (partly) explain the sensitivity of the hippocampal formation to kainic acid injection. Kainic acid produces excitotoxicity by stimulating AMPA and kainate receptors, which induces a chain reaction with depolarisation of certain neurons and an increase in excitatory amino acid release, leading to a large calcium influx in cells, in turn activating a Ca\(^{2+}\)-dependent enzyme promoting a cascade reaction, which produces cell death and apoptosis (Sperk, 1994), very similar to that of ischemia (figure 6). This excitotoxic response should propagate to CA1 pyramidal cells, taking inputs from area CA3 and/or direct action to kainate receptors of area CA1 cells from kainic acid.

The mechanism of action of these two models of neurodegeneration appears very similar, although they don’t have the same initiating event. This is confirmed by the fact that cerebral ischemia is the most common cause of acquired epilepsy, accounting for 40% of these cases (Hauser et al., 1991). Indeed, similar to ischemia, kainic acid seizures induce the formation of superoxide (Liang et al., 2000) and the production of prostaglandins and thromboxane (which act as indices of inflammation) (Baran et al., 1987a). It also seems clear that these methods induce modifications of gene expression in the brain; Hashimoto et al. (1998) showed that both BDNF mRNA and cyclooxygenase-2 mRNA (in the hippocampus, dentate gyrus, piriform cortex, neocortex and amygdala) and early gene expression change after systemic administration of kainic acid. Comelli et al. (1993) have also observed gene modulation after photochemical stroke, including expression of
BDNF mRNA (in the cortex and the hippocampus) and the expression of immediate early inducible genes (c-fos, c-jun, jun B and zif 268).
**Figure 6:** This diagram shows the different mechanisms putatively involved in ischemia, leading to brain insult.
Figure 7: Kainic acid structure, is a glutamate agonist to the AMPA and kainate receptors

6. Cyclooxygenases

Arachidonic acid, the most abundant precursor of prostaglandins, is cleaved from cell membrane phospholipids by the action of phospholipase A₂ (PLA₂) and phospholipase C (PLC), which are activated by a variety of inter- and intra-cellular mediators (Axelrod et al., 1988). Arachidonic acid is in turn transformed into prostaglandin G₂ (PGG₂) and prostaglandin H₂ (PGH₂) by the action of an enzyme, cyclooxygenase (COX) (figure 8). Prostaglandins have diverse biological functions including the maintenance of vascular and kidney homeostasis, relaxation and contraction of smooth muscle, regulation of gastrointestinal secretion, motility and induction of sleep, pain and inflammation (Cryer and Dubois, 1998).
There are 2 (and possibly 3) cyclooxygenase isoforms, COX-1 and COX-2 (and COX-3); they are membrane-associated enzymes (on the endoplasmic reticulum and the nuclear envelope) with a 70 kDA molecular weight and 60% amino acid sequence identity; they
have a difference of less than 10% of amino acids within the arachidonic acid active sites (Hawkey, 1999). However, COX-1 inhibition is instantaneous and competitively reversible, whereas COX-2 inhibition is time-dependent and essentially irreversible (Hawkey, 1999).

COX-1 is present and is constitutively expressed in most cells and tissues, producing prostaglandins, which under normal conditions regulate essential physiological functions. COX-2 is also expressed constitutively but only in a few tissues (Yamagata et al., 1993; O’Neill et al., 1993) and is barely detectable under normal conditions. However, in response to several pro-inflammatory stimuli such as mitogens, cytokines (Tumor Necrosis Factor-α and Interleukin-1-β; Hansen et al., 1999), growth factors and glutamate (figure 8), the level of COX-2 may be selectively increased over 20 fold (O’Neill et al., 1993) and this may induce a 10 to 80 fold increase in prostaglandin production. Increases in COX-2 can be prevented by glucocorticoids but the inhibitory mechanisms underlying this action of glucocorticoids are unclear as yet (Inoue et al., 1999). Therefore, the anti-inflammatory role of the glucocorticoids may be due to their action in COX-2 expression. There have been recent suggestions of a COX-3 (recently described; Chandrasekharan et al., 2002), which may also be involved in inflammatory conditions (Willoughby et al., 2000).

Prostaglandins are also found after stimulation of the rodent brain by kainic acid (Kim et al., 2001), endotoxin (lipopolysaccharide; Araki et al., 2001), or after ischemia (Nogawa et al., 1997), all of which result in the production mainly of PGD₂, PGE₂ and PGF₂α. Seregi et al. (1987) showed that both neurons and non-neuronal cells such as astroglia can synthesize prostaglandins. There appears to be a basal level of prostaglandins
produced by COX. However, COX-2 seems to be constitutively expressed almost exclusively in neurons, with a level much higher than that of peripheral tissues; it is especially present in the hippocampal formation, amygdala and the neocortex (Kaufmann et al., 1997; Yamagata et al., 1993). Basal COX-2 mRNA expression seems to be maintained by normal synaptic activity, because it is reduced by MK-801 (an NMDA receptor antagonist). Enhancement of synaptic activity, which occurs during high-frequency electrical stimulation of hippocampal afferents, produces enhanced COX-2 mRNA expression (Yamagata et al., 1993). Moreover, Kaufmann et al. (1996) showed that COX-2 protein is found in the proximal dendrites, distal dendrites and dendritic spines and that neurons immunostained for COX-2 are also immunostained for glutamate but not for GABA, which suggests an important role for prostaglandins in the synaptic activation, and perhaps synaptic plasticity, of excitatory neurons (Kaufmann et al., 1997). Furthermore COX-2 seems to belong to the general family of immediate-early genes and can directly modify cellular function (Kaufmann et al., 1996).

A role for COX in memory consolidation has been also shown by several experiments. Holscher (1995a) concluded that inhibitors of cyclooxygenases produce amnesia in a passive-avoidance task in the chick, while Teather et al. (2002) showed that rats who received COX-2 specific inhibitors or a non-selective COX inhibitor were impaired compared to normal animals in the hidden platform watermaze task, whereas rats who received a COX-1 specific inhibitor did not show an impairment in retention.

Although COX-2 is expressed constitutively in some excitatory neurons, it is also markedly induced in central nervous system neurons by excitatory stimuli such as ischemia (Nogawa et al., 1997) or seizures (e.g. kainic acid injection produces an
increase of COX-2 in the brain; Yamagata et al., 1993; Hashimoto et al., 1998; Kim et al.,
2001). Manev et al. (2000) have shown that cyclooxygenase and lipoxygenase mRNA
increase after stimulation of glutamate receptors by kainic acid, whereas COX-2 inhibitor
NS-398 inhibited this effect in the rat hippocampus.

Cyclooxygenase may have actions at several levels in stroke. Prostanoids could play a
role in the disruption of the blood-brain barrier after ischemia; indeed the possible
mechanisms underlying vasogenic oedema have been linked to prostaglandins
(Yamamoto et al., 1996), while COX generates superoxide, which produces many
cellular effects (see section 5: ischemia and kainic acid models in the rat).

Many compounds have been identified in the Alzheimer’s disease brain, which are
known to promote and sustain neuroinflammation such as beta-amyloid protein, c-
reactive protein, the cytokines interleukin-1, interleukin-6 and tumor necrosis factor-
alpha as well as COX-1 and COX-2 (McGeer and McGeer, 2001). The neocortex,
hippocampus and entorhinal cortex are the most affected areas, which exhibit neuronal
loss as well as the characteristic senile plaques.

In the MPTP-mouse model of Parkinson’s disease, Teismann and Ferger (2001) showed
that the inhibition of COX-1 and COX-2 by salicylate or COX-2 by the specific COX-2
inhibitor, meloxicam, provided neuroprotection against MPTP-toxicity in the striatum
and substantia nigra.

It seems clear that inflammation is implicated in acute and chronic neuronal degenerative
diseases in the central nervous system. Nonsteroidal anti-inflammatory drugs (NSAIDs)
such as aspirin or ibuprofen are among the most widely used therapeutics, primarily for
the treatment of pain and inflammation, and are also used as anti-pyretic and analgesics
in humans and animals. In 1971, Vane elucidated the molecular mechanism of NSAIDs by showing that aspirin blocks the biosynthesis of prostaglandins, which play a crucial role in inflammation. The inhibition occurs by blocking the site of cyclooxygenases, preventing the access of arachidonic acid to the enzymatic site. In the 1990’s it was discovered that COX exists in two isoforms, COX-1 and COX-2 (Kujubu et al., 1991; Xie et al., 1991). Until this time, inflammation was treated with NSAIDs, which induced unwanted gastrointestinal and renal effects. With the discovery of COX-2, and its involvement in the inflammatory process, new drugs have been developed specifically to inhibit COX-2 activity. COX-2 is even known to be induced in the kidney in response to sodium depletion or hyperfiltration states; in postsynaptic excitatory neurons in the brain after electroconvulsive stimulation; in the ovary and uterus during ovulation and implantation; in intestinal epithelium after bacterial infection, as well as in colon adenoma and carcinoma cells. The development of a selective COX-2 inhibitor (table 1) could be important for the therapeutic treatment of inflammatory disease and therefore for neurodegenerative disorders. The important action of such drugs is to block the overproduction of COX-2; however, it is also essential to preserve a baseline level of PGE₂ which is involved in synaptic activity.
Table 1: Summary of drug selectivity (Svensson and Yaksh, 2002)

<table>
<thead>
<tr>
<th>COX-1=COX-2</th>
<th>COX-1&gt;COX-2</th>
<th>COX-2&gt;COX-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketorolac</td>
<td>SC58125</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>SC58560</td>
<td>SC384</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td></td>
<td>SC385</td>
</tr>
<tr>
<td>Zomepirac</td>
<td>Celecoxib</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Rofecoxib</td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td></td>
<td>NS398</td>
</tr>
</tbody>
</table>

7. Neurotrophins

Growth factors are substances that stimulate cells to divide or increase in size. Trophic factors are those substances which effect cell differentiation, cell survival, phenotypic expression and plasticity, as well as cell hypertrophy (e.g. neurite extension); neurotrophic factors are a subset of growth factors acting on neural tissues (Fallon and Loughlin, 1993). The discovery of nerve growth factor (NGF) is based on an incidental observation by Bruecker (1948), who noticed that mouse tumor tissue implanted in chick embryos strongly stimulated innervations of the tumor by host sensory neurons. In 1953, Levi-Montalcini and Hamburger showed that soluble factors were responsible for the stimulation of neuronal growth, and Cohen et al. (1954) characterized the protein from
snake venom, which they named nerve growth factor (NGF). It was shown that NGF affects the development of central nervous system neurons (Schwab et al., 1979) and prevents degenerative changes of forebrain cholinergic cells after experimental injury (Hefti et al., 1984). Subsequently, other neurotrophic factors have been isolated, such as brain-derived neurotrophic factor (BDNF; Barde et al., 1982), ciliary neurotrophic factor (CNTF: Barbin et al., 1984), neurotrophin-3 (NT-3; Ernfors et al. 1990b; Hohn et al., 1990) and neurotrophin-4 and 5 (NT-4, NT-5; Hallbook et al., 1991; Berkemeier et al., 1991). The similarity between the structures of NGF, BDNF, NT-3, NT-4 and NT-5 gave birth to the term “neurotrophin family” (Hohn et al., 1990); the “family members” share the core structure, but their exposed regions are different, giving the receptor-binding specificity (Ibanez, 1995). All the members of the neurotrophin family are small, homodimeric polypeptides of 120 amino acids (Ibanez, 1995) and all are involved in the differentiation, survival and regeneration of neurons (Thoenen et al, 1991).

Neurotrophins interact with two classes of receptor: one of them, a protein tyrosine kinase-type receptor, which is a member of the Trk family; the second is a small receptor, which has been characterized as p75^{NGFR} (75 kDa molecular weight) but serves as a receptor for all the neurotrophins. The TrkA receptor binds NGF; the TrkB receptor is specifically activated by BDNF/NT-4/5 and by a high concentration of NT-3 (figure 9); the latter also activates the TrkC receptor most effectively (Meakin et al., 1992).
The neurotrophins are all expressed in neurons of the central nervous system, with the highest mRNA levels in the hippocampus (figure 10). NT-3 mRNA is predominantly found in neurons in areas CA1 and CA2 of the hippocampus, whereas BDNF mRNA is more widely expressed in neurons of fields CA2, CA3, the hilar region of the dentate gyrus and in the cortex (Ernfors et al., 1990b). NGF is produced by hippocampal and cortical neurons (Dreyfus et al., 1989).

Neurotrophins are important regulators of the survival, development and differentiation of multiple neuronal populations (Barde, 1989). During brain development, the neurotrophin family displays distinct stage-specific and tissue specific patterns of expression. The highest level of NT-3 mRNA is found shortly after birth, whereas maximal expression of BDNF and NGF mRNA is observed a few weeks post-natally.
(Ernfors et al., 1990a). Experiments in which endogenous NT-3 is depleted in mutant mice (Farinas et al., 1996) or chick embryos treated with functional blocking antibodies (Gaese et al., 1994) suggest that NT-3 plays an essential role in the differentiation and survival of neuronal precursors, in both the central and the peripheral nervous system. In gene therapy, Haase et al. (1997) showed that adenovirus-mediated gene transfer of NT-3 can produce substantial therapeutic effects in mouse mutant pmn (progressive motor neuronopathy), which is a model of motor neuron disease (such as amyotrophic lateral sclerosis). Tucker et al. (2001) also showed the importance of NGF and BDNF during motor and sensory axon development. The lack of neurotrophins in the peripheral nervous system causes severe deficits during embryonic development. It has been shown by Silos-Santiago et al. (1997) that TrkB(-/-); TrkC(-/-) double-mutant mice were born at the expected frequency, indicating that TrkB and TrkC signalling are not required for embryonic survival. However, these double-mutant mice had a significantly shorter lifespan and displayed more severe sensory defects than their single-mutant TrkB (-/-) and TrkC (-/-) littermates. Analysis of the central nervous system in TrkB (-/-); TrkC (-/-) mutant mice revealed a well-formed hippocampus, cortex and thalamus, though there were dramatic sensory deficits. Subsequently, Martinez et al. (1998) showed that in TrkB (-/-); TrkC (-/-) mutant mice, the main afferents to the hippocampus, the commissural and entorhinal axons, grow into the target hippocampal region with the same timing and pattern of termination as wild-type animals. They also showed that the lack of endogenous TrkB and TrkC receptors alters synaptogenesis in the CNS by reducing the density of synaptic contacts. These observations suggest that in the CNS, neurotrophins are more involved during postnatal development, and are implicated in other aspects of
neuronal function rather than survival. Indeed, neurotrophins have been involved in physiological events such as synaptic efficacy (Thoenen, 1995; Canossa et al., 1997) and in long-term potentiation (Korte et al., 1995). The neurotrophin seems to play a part in the protection of the brain after injury, in both the peripheral and the central nervous system (Haase et al., 1997; Han and Holtzman, 2000).
Figure 10: Coronal section of hippocampus of the right side of the brain, showing the distribution of NGF, BDNF and NT-3. The top panel indicates regions of the hippocampus: DG the dentate gyrus with its molecular (M), granule cell (G) and hilar (H) layers. Ammon’s horn with subfields CA1, CA2 and CA3, (P) pyramidal layer.
Neurotrophins are involved in synaptic plasticity; BDNF has been particularly implicated in long-term potentiation (LTP). Indeed, Korte et al. (1995) showed the influence of the BDNF gene on LTP, using mice with a BDNF gene deletion. They investigated if BDNF-mutant mice could produce LTP in area CA3. LTP was induced 87% of the time in wild-type (+/+) mice, 27% of the time in heterozygous BDNF-mutant mice (+/-) and not at all in homozygous BDNF-mutant (-/-). They concluded that more than 50% of the endogenous or wild type BDNF level is required for LTP. This suggests that an inability to generate BDNF is what causes the failure to induce LTP, rather than developmental or neuronal abnormalities. LTP, evoked by high-frequency afferent stimulation, is typically divided into an early, labile phase dependant on covalent modifications of existing proteins and a late, stable phase requiring synthesis of new mRNA and protein (Bliss and Collingridge, 1993). Korte et al. (1998), using slices from transgenic mice which lack BDNF and slices treated by antibodies against TrkB to block TrkB function, demonstrated that BDNF is required for the late-phase of LTP (e.g. >2 hours). BDNF also blocks the induction of long-term depression in the visual cortex (Kinoshita et al., 1999). The role of BDNF in memory was confirmed by Linnarson et al. (1997) in a watermaze task with mice carrying a deletion in one copy of BDNF gene. Young adult BDNF mutant mice were significantly impaired compared with wild type mice, and in situ hybridization and RNase protection analysis indicated a decrease of the BDNF mRNA expression in the mutant mice. Ma et al. (1998) further reinforced the importance of BDNF in memory consolidation. Their results showed that hippocampal BDNF mRNA levels were significantly increased during the early phase of the memory consolidation process of inhibitory avoidance learning in rats. However, BDNF mRNA
antisense injection in the dentate gyrus impaired retention performance and reduced the amplitude and slope of LTP, demonstrating that BDNF gene expression in the hippocampus is a necessary component for memory processing.

Another important role for BDNF (and NGF) is to protect the neurons of the brain against injuries such as stroke, trauma or seizures. The hippocampus is known to increase its BDNF mRNA expression after kainic acid administration (Lauterbom et al., 1996), brain trauma (Hicks et al., 1997) or ischemia (Lindvall et al., 1992; Miyake et al., 2002). Lauterbom et al. (1996) showed that BDNF mRNA is increased in hippocampal granule and pyramidal cells (especially in CA3) and NGF mRNA in granule cells, by an electrical stimulation, in vivo and by kainic acid, in vitro. In contrast brain-derived neurotrophic factor mRNA is reduced in the substantia nigra pars compacta (Aliaga et al., 1999).

Lauterbom's data also suggest that BDNF is induced as an immediate-early gene (IEG), because its mRNA content is not blocked by protein synthesis inhibition. In fact, only one part of the cellular mechanism to induce the BDNF mRNA can be regulated as immediate-early gene (probably to protect the neurons). Indeed, the rat BDNF gene includes four short 5’ exons (exons I-IV) that are associated with a separate promoter and one 3’exon (exon V) that encodes the BDNF protein (Nakayama et al., 1994). The four different promoters give rise to four predominant transcripts, each containing one of the 5’ exons + exon V. A study by Bishop et al. (1994) has shown that transcripts containing these exons are differentially expressed across brain regions under normal circumstances. Since only the exons III and IV could be regulated as IEGs, Lauterbom's hypothesis would be that these exons are induced with a lower activation threshold, and that there is an increase of exon III-containing mRNA across several models of central nervous
system injury (e.g. seizure and ischemia). Quickly released BDNF probably mediates (at least partly) the synthesis of BDNF mRNA and activates the TrkB receptors, which activates an enhancement of BDNF mRNA via an increase in intracellular calcium (Saarelainen et al., 2001). The up-regulation of BDNF and its receptor TrkB (Hicks et al., 1998) after a brain injury has been suggested to represent an effort by the CNS to protect itself from the effects of the damage. However, there is a discrepancy between the effects of brain injury when exogenous BDNF is used. In experimental models of cerebral ischemia, BDNF has been shown to decrease infarct size when infused either into the ventricles beginning one day prior to ischemia (Schabitz et al., 1997) or directly into the brain immediately after the insult (Yamashita et al., 1997). In contrast Blaha et al. (2000) found that BDNF infused for 2 weeks in the hippocampus or the cortex after traumatic brain injury (TBI, the lateral fluid-percussion brain injury model) had no significant effects on histology (BDNF was ineffective at preventing neuronal loss) nor on cognitive functions (watermaze task). Furthermore, the administration of exogenous BDNF does not affect severity of seizure, but exacerbates the injury caused by kainic acid, specifically to CA3 pyramidal neurons (Rudge et al., 1998). Indeed, Binder et al. (2001) suggest that BDNF can contribute to epileptogenesis.
8. Neurogenesis

All mammals have replicating cells in many organs; blood, skin and gut stem cells have been shown to exist throughout life, contributing to cell replacement. In the CNS also, microglia, astrocytes and oligodendrocytes can divide to respond to an injury. Previously, only neurons were considered to be refractory to replication (Gage, 2002). It is clear now that, contrary to early dogma, the adult nervous system can generate new neurons. It has been shown that in two areas of the brain, the dentate gyrus of the hippocampal formation (Gage et al., 1995; Kempermann et al., 1997; Gould et al., 1999a) and the subventricular zone and its projection through the rostral migratory stream to the olfactory bulb (figure 11; Lois and Alvarez-Buylla, 1994; Doetsch and Alvarez-Buylla, 1996), can generate new neurons.

Hippocampal neurogenesis has been observed in adult animals from birds (Barnea and Notterbohm, 1994) to humans (Eriksson et al., 1998), rodents (Kempermann et al., 1998) and monkeys (Kornack and Rakic, 1999). For the moment, we know that new neurons are only added to the granule cell layer of the dentate gyrus of adult rodents. Neurogenesis was examined by incorporation of bromodeoxyuridine (BrdU) in the DNA of proliferating cells and subsequent immunohistochemical detection of BrdU (Kempermann et al., 1998). Van Praag et al. (2002) confirmed by another technique (they used a retroviral vector expressing green fluorescent protein, which only labels dividing cells) that new neurons are generated in the dentate gyrus and probably arise from progenitors in the subgranular zone. They showed also that newly generated neurons are functionally similar to mature dentate granule cells.
It is unclear why the adult hippocampal formation generates neurons throughout life. One answer could be that new neurons participate in some way in learning and memory. Indeed, Gould et al. (1999a) indicated that after learning a task such as the watermaze task, there is an enhancement of the number of new neurons of the granule cell layer in the hippocampal formation. Kempermann et al. (1997) found that the upregulating effects of enriched environments on adult mice and hippocampal neurogenesis was paralleled by an improvement on tasks that were hippocampal-dependent. This was confirmed by Nilsson et al. (1999) in the rat, and while it seems difficult to establish a definitive coupling between an increased number of neurons and enhanced spatial learning (they used the watermaze task), it is probable that this increase, possibly in combination with augmented numbers of synapses (such as an increase in dendritic spine density in the CA1 area of the hippocampus; Moser et al., 1994) or other factors (e.g. neurotrophins), results in enhanced performance in a behavioural memory test. Animals housed in enriched conditions have significantly higher levels of nerve growth factor in the hippocampus, visual and enthorinal cortices compared with animals housed in isolated conditions (Pham et al., 1999a).

Another possible role for neurogenesis may be in the promotion of functional recovery after hippocampal injury. Indeed, after transient global ischemia in gerbils, it has been shown, using immunohistochemistry to detect the incorporation of BrdU into newly synthesized DNA, that there was neurogenesis in the dentate gyrus, even though ischemia caused a loss of area CA1 neurons, but both may be linked in some way (Liu et al., 1998). Seizures also increase dentate granule cell neurogenesis; these newly born cells contribute to the hippocampal network’s reorganization in the pilocarpine model of
temporal lobe epilepsy, though this reorganization seems to be aberrant (Parent et al., 1997).

Figure 11: The Subventricular Zone-Olfactory Bulb (SVZ-OB) system. Schematic sagittal view of adult rodent brain with the OB to the left and the cerebellum (CB) to the right. The SVZ is along the lateral ventricle (LV). New neurons are constantly produced throughout the SVZ. The young neurons become aligned into long chains that form a complex network of interconnected paths throughout the SVZ. Many of these chains in the anterior SVZ connect with the rostral migratory stream (RMS), with leads young neurons into the core of olfactory bulb. NC, neocortex; cc, corpus callosum (Alvarez-Buylla and Garcia-Verdugo, 2002).

9. Enriched environment and exercise

A stimulating environment has been shown to induce structural changes in the hippocampus (Kempermann et al., 1997) or the subventricular zone (Rochefort et al., 2002). We define the enriched environment as a milieu where the animals will be
stimulated by social interaction (more animals per cage), by exploratory behaviour with objects (toys and tunnels), and physical activity in a running wheel (see figure 12). This type of experience increases neurogenesis in the dentate gyrus of the hippocampus (Kempermann et al., 1997; 1998), whereas enriched odour exposure increased newborn neurons in the adult olfactory bulb and improved odour memory without augmenting the numbers of granule cells in the hippocampus and or enhancing spatial memory in the watermaze task (Rochefort et al., 2002). Van Praag et al. (1999b) separated the components of the enriched environment and demonstrated that voluntary exercise is sufficient for increased neurogenesis in the adult mouse dentate gyrus; furthermore (Van Praag et al., 1999a) running enhances watermaze performance and LTP in medial perforant path to dentate gyrus synapses (but not in the CA1 Schaffer collateral pathway). There are numerous effects on the CNS when animals are exposed to an enriched environment or a running wheel; indeed, high-density oligonucleotide microarrays used to analyse gene expression in the brain indicated that the expression of a large number of genes changes in response to an enriched environment (Rampon et al., 2000). A similar finding is apparent when rats were exposed to voluntary exercise in a running wheel (Tong et al., 2001); gene expression in the hippocampus is either changed, increased or induced.
In particular, Tong et al. (2001, figure 13) found that exercise increases the mRNA levels of BDNF, which is important for neuronal survival and plasticity; levels of NGF are also enhanced. There was an increase of genes such as the Vesl/homer family, which induces proteins playing important roles in structural and functional synaptic changes (Kato et al., 1998). Cox-2 is also substantially elevated, showing the importance of this enzyme in synaptic plasticity (see section 6: cyclooxygenases).

All of these changes in the hippocampus of animals exposed to enriched environments or exercise could explain why these animals perform better in learning tasks than animals in standard housing conditions (Kempermann et al., 1998; Nilsson et al., 1999; Van Praag et al., 1999a). These stimulating conditions can even enhance the recovery of the animal.
after brain damage. Indeed, rats subjected to ischemia and placed in an enriched environment, showed more rapid acquisition of a watermaze task and habituated more quickly to a novel open field than ischemic animals residing in standard cages (Puurunen et al., 1997). It seems probable that enriched environments or exercise causes increased levels of BDNF and other growth factors, stimulating neurogenesis and increasing neuronal resistance to brain insult (Johansson, 2000; Cotman and Berchtold, 2002).

![Figure 13: Effects of exercise on gene transcription. The majority of genes induced by exercise are associated with plasticity and synaptic structure. Reprinted from Cotman and Berchtold, 2002; for details, see Tong et al., 2001.](image-url)
10. The Morris watermaze

The open field watermaze is an apparatus in which rodents are trained to escape from water by swimming to a hidden platform (figure 14) whose location can be identified using a learned sequence of movements, which brings it to the platform (praxis strategy); it can approach the platform using proximal cues (taxis strategy); or it can navigate to the platform using distal extra-maze cues (a spatial strategy; D'Hooge and De Deyn, 2001). However, extra-maze cues seem essential for learning the location of the platform. If curtains are drawn around the watermaze, the animal cannot find the platform after several trials of learning but if curtains are only partially drawn around the watermaze the animals will be able to learn the location of the platform (Steward & Morris, 1993). Normal animals learn where the platform is located very rapidly so that testing can be carried out over short periods of time. Rats are placed in the watermaze facing the side walls and the trial is started as soon as the rat is released. If the rat does not find the platform within a criterion period (say 60 seconds), the animal is guided to the platform by the researcher; the rat will then remain on the platform for a short period of time (say 20 seconds). There are many variations on the basic task, which in this laboratory (Commins et al., 1999; Gooney et al., 2002; Shaw et al., 2001) typically involves a 5 day acquisition phase where the rat learns the location of the hidden platform, followed by a 3 day rest period where the rat remains in the housing unit and then a 3 day retention phase where long-term memory of the platform’s location is assessed. On the final day of the retention phase a probe test is conducted, this is where the platform is removed from the watermaze and the rat is allowed to swim freely for 60 seconds. The probe test allows us to assess if the rat has learned the location of the platform, as indicated by the amount of
time the rat spends in the quadrant where the platform was. There is no universally accepted standard and the task described above is only one example of the many variations on the basic task (Steward & Morris, 1993). Several commercial manufacturers offer complete systems with video equipment and associated computer software. We use the user-friendly EthoVision system by Noldus Information Technology (Wageningen, The Netherlands), which is a perfect example of a modern, sophisticated video tracking system. Data obtained with this system can be further analysed off-line according to the needs of the experimenter.

![Diagram illustrating the arrangement of equipment for automatic tracking of rodents in the watermaze.](image)

**Figure 14:** Diagram illustrating the arrangement of equipment for automatic tracking of rodents in the watermaze.
Most studies have so far examined the role of a few well-defined brain regions in the Morris watermaze learning task. Specific deficits were found in animals with damage to hippocampus (Morris et al., 1982), striatum (Whishaw et al., 1987), basal forebrain (Waite et al., 1994), cerebellum (Lalonde, 1994) and several cortical areas (Commins et al., 1999; Roof et al., 1993; Liu and Bilkey, 1998). It has been well established that the integrity of the hippocampal formation is essential for spatial learning, so the watermaze seems to be an instrument with particular sensitivity to effects of the hippocampal lesions, especially in rats (Morris et al., 1982).

11. Olfactory memory task

In humans, it is widely accepted that hippocampal structures play a critical and selective role in declarative (episodic/explicit) memory. O’Keefe and Nadel’s (1978) “cognitive map” theory predicts that lesions of the hippocampus will selectively disrupt spatial tasks but not non-spatial tasks in non-human subjects. Numerous experiments have been done using animals with hippocampal damage, showing that they are impaired in spatial tasks after hippocampal damage (see section 5: ischemia and kainic acid models). However, there are few reports of non spatial deficits after hippocampal damage, or damage to areas such as the fornix, entorhinal cortex or even a combined hippocampus and subiculum lesion (Eichenbaum et al., 1989; Otto et al., 1991; Bunsey and Eichenbaum, 1995). Burton et al. (2000) did not find deficits in a novel non-spatial task: the social transmission of food preference (Bunsey and Eichenbaum, 1995), where a demonstrator
rat eats a scented food. Subsequently, an observer rat interacts with the demonstrator during which it acquires the association of food odour and the odourous constituents of the demonstrator’s breath. Finally, when given a choice between that scented food and another, the observer will prefer to eat the demonstrator’s food. In another odour task, Li et al. (1999) also did not find an impairment after either unilateral or bilateral hippocampal lesions.

There is, therefore, a discrepancy as to whether the role of rodent hippocampus in memory is limited to the spatial domain. It seems that a purely hippocampal lesion (including Ammon’s horn and dentate gyrus) alone does not impair the animal’s performance in the odour tasks, and that it is necessary to cause more extensive damage (e.g. hippocampus + subiculum) to induce a detectable deficit (Alvarez et al., 2001). This suggestion is moderated by developmental constraints: Hu et al. (1997a) found that young rats (postnatal day 16) learned an odour discrimination task more slowly than older animals (30 and 60-day-olds). Indeed, neurogenesis of granule cells in the dentate gyrus occurs after 15 days post-natally and its interconnections with systems, such as commissural, perforant path and mossy fibers, is fully expressed after about 25 days post-natally. The hippocampus may therefore, not be completely functional until this date, which may explain the functional impairment found in 16-day-old pups.

So, if we admit that the hippocampal formation plays a role in non-spatial memory, then olfactory learning in rodents seems be a good method to evaluate it. Indeed, the olfactory system has projections to the prefrontal cortex, entorhinal cortex and hippocampus, and these connections support the acquisition of simple and higher-order instrumental tasks, as well as a robust memory for odours (figure 15; Slonick, 2001).
Figure 15: Schematic diagram of projections from the main olfactory bulb to the olfactory cortex and projections from this cortex to the thalamic mediodorsal (MD) and submedius (SM) nuclei and to the limbic system (hippocampus, HC). The connections of the accessory olfactory bulb are not shown here. The diagram summarizes results of axonal transport and electrophysiological studies of the forebrain olfactory system.
12. **Object exploration task**

The object exploration task, originally described by Poucet et al. (1989), was proposed as a powerful paradigm for the analysis of the spatial knowledge acquired by animals. During exploration in an open field, where there is an arrangement of 4 different objects, the animals encode both the nature of objects and their position in space (figure 16). The encoding of the object positions is indicated by the renewal of exploration after the objects have been moved. The normal animals such as rodents react to the new environment, increasing their exploration. This reaction suggests that the animals have compared the current spatial arrangement with their memories of the past environment. The specificity of this spatial reaction is shown by the decrease of exploration, i.e. habituation, when the animals are placed again in the same environment. However, if the object positions are changed, the animals will then re-explore the environment, indicating that a change has been noticed. These different reactions suggest that the animal is able to build up a mental representation of the environment that O'Keefe and Nadel (1978) called a "cognitive map". Therefore, object exploration, measuring a spatial component of memory, is another method to evaluate hippocampal damage. It simply uses a spontaneous animal behaviour without induced stress (as probably occurs in the watermaze task) and without any food deprivation (as in the radial arm maze).
Trial 0

Trials 1, 2 and 3

Trials 4 and 5

Figure 16: Schematic representation of series of six consecutive exploratory trials in the open field arena.

Trial 0, rats were exposed to empty novel open field. Trial 1, four objects were introduced. Trial 2 and 3 were a replication of trial 1. In trial 4, two of the four objects were displaced to new positions.


13. Objectives of this thesis

The objectives of this thesis are to understand some of the neuropathological and functional (i.e. learning- and memory-related) changes, which occur after a brain injury, and to investigate some of the mechanisms which may ameliorate these processes. As a first step, using the model of transient global cerebral ischemia, which causes specific damage to CA1 pyramidal cells of the hippocampus, we will investigate the consequences of such damage for learning and memory in the rat. Spatial (watermaze, object exploration) and non-spatial (odour discrimination) tasks will be used to evaluate the effects of focal hippocampal damage on learning and memory. We investigate if a stimulating or enriched environment protects or compensates for the cell loss and if it improves learning after focal damage; an enriched environment is known to induce neuroplasticity (including increases in hippocampal thickness and dendritic arborisation; Kempermann et al., 1997). In parallel, we have measured endogenous BDNF to see if this stimulation regulates the level of BDNF in the hippocampus and to investigate a correlation between BDNF levels and any subsequent recovery.

As epilepsy is a common consequence of transient global ischemia, and many of the molecular and cellular mechanisms underlying transient global ischemia are also believed to underlie those also engaged during epilepsy, we have sought to examine if this is indeed the case. As a second step, we have investigated the role of excitotoxicity induced by overstimulation of glutamate receptors using kainic acid, which is an important tool for studying functions related to excitatory amino acid transmission and for producing cellular death. Kainic acid induces more damage than does ischemia and, indeed, there is a greater loss of pyramidal cells in both CA1 and CA3 areas of the hippocampus,
probably because there is a great expression of kainate receptors in area CA3, which is directly connected to area CA1. It appears clear, however, that ischemia and kainic acid injection share many of the same pathways for inducing neurodegeneration.

In order to protect against these neuropathological and functional consequences, we have isolated a major component of the enriched environment, running (sustained physical activity) and we have examined its impact on BDNF levels. We have also investigated if running reduces cell loss in the hippocampus, as well as the possibility that it might ameliorate the performance of rats after kainic acid injection. Therefore, we hypothesise that exercise alone could protect against cell loss and improve memory (or at least reduce mnemonic deficits) in animals with hippocampal damage. We expect that BDNF levels will increase in this case and that learning will be improved.

We have also isolated one major component of brain injury – neuroinflammation - which involves the formation of arachidonic acid from cell membrane phospholipids, followed by its transformation in prostanglandin, catalysed by the cycloooxygenase-2 enzyme. We hypothesise that by blocking this enzyme after kainic acid injection, that there would be less inflammation and therefore a reduction in cell loss, an enhancement of cognitive function (alternatively, a reduction in deficit) or both. We examined BDNF levels, expecting that there would be a correlation between its level and subsequent recovery. Furthermore, we sought to examine if there is a cumulative improvement of memory and learning after a combination of running and cyclooxygenase 2 inhibitor injection.
Chapter II: Enriched-environment housing enhances performance on odour discrimination, object exploration and spatial learning tasks independently of transient global ischemia.
3.4 Measurement of BDNF  80

3.5 Histology  81

4. Discussion  86
1. Introduction

Ischemic stroke in the brain is a major cause of memory, emotional and other disturbances in humans for which, at present, there is no comprehensive pharmacotherapy (Kidwell et al., 2001). Interruption of cerebral blood flow can produce damage in many brain regions, especially in the hippocampal formation, causing loss of hilar cells in the dentate gyrus (DG) and of hippocampal area CA1 pyramidal cells (Hu et al., 1997b). The excitotoxic hypothesis of ischemic neuronal death (Smith et al., 1997) suggests there are neurotoxic effects of sustained increases in the extracellular concentration of glutamate, which via over-stimulation of N-Methyl-D-Aspartate (NMDA) receptors, causes neuronal damage via neuronal overactivity. Neuronal death occurs progressively over a few days after the ischemic insult (Kondo et al., 1998; Nakayama et al., 1998) and thus, there is a window within which it may be possible to block or slow down the progressive loss or destruction of neuronal tissue.

A substantial literature suggests that prolonged behavioural stimulation can induce hippocampal neurogenesis in rodents, monkeys and humans (Eriksson et al., 1998; Gould et al., 1998, 1999b; Kempermann et al., 1997). Neurogenesis is stimulated by learning (Gould et al., 1999a), physical activity (Kempermann et al., 1997) and enriched environments (Johansson and Ohlsson, 1996) in rodents; there is an increase in new cells in DG and an increase in the density of dendritic spines in area CA1 (Moser et al., 1994). An enriched environment also causes a significant change in the expression of genes (Rampon et al., 2000). Several reports (Mori et al., 1998; Olsen et al., 1994; Sinden et al., 1997) have demonstrated that transient cerebral ischemia causes maturational cell death.
and delayed neuronal death in animals. Long-term potentiation (LTP, Bliss and Lomo, 1973), a popular biological model of the processes that may underlie the consolidation of memory, is inhibited in Schaffer collateral CA1 synapses and in the perforant path-dentate gyrus synapses, 4 days after ischemia (Mori et al., 1998). 4 VO causes cognitive/behavioural impairments: Nelson et al. (1997b) found that rats subjected to 4 VO were not impaired in learning the watermaze 4 weeks after ischemia, but showed significant deficits when tested 43 weeks later. Mori et al. (2001) found that short-term spatial memory in the Y-maze was impaired by ischemic insult.

We evaluated the sequelae of 4 VO ischemia under different housing conditions, using spatial and non-spatial tasks: the watermaze (Morris et al., 1982), object recognition (Galani et al., 1998) and odour discrimination tasks (Roullet et al., 1997). These tasks (more or less) involve the hippocampal formation, which is believed to play a crucial role in spatial learning (O'Keefe and Nadel, 1978) and olfactory memory (Dudchenko et al., 2000; Eichenbaum et al., 1989). We also investigate the question of whether ischemic damage to the hippocampal area CA1 sub-field interacts with housing in an enriched environment to ameliorate the effect of ischemic damage to area CA1. Finally, we investigate the consequences of 4 VO and/or environmental enrichment on BDNF levels in the hippocampus.
2. Materials and Methods

Experiments were conducted in accordance with European Community directive, 86/609/EC, and the Cruelty to Animals Act, 1876 and followed local and international guidelines of good practice.

2.1. Animals

Male Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland) (mean weight of 250-300 g at the start of the experiment) were used. The rats were randomly assigned to two experimental groups after their arrival from the BioResources Unit. The groups were kept under either enriched (see Housing Conditions) or standard conditions. After surgery, ischemic and sham rats were housed in the same cage.

2.2. Housing conditions

24 animals (by 3) were placed in standard cages measuring 44 x 28 x 18 cm, while 28 other animals were placed, in groups of 4, in special cages measuring 55 x 37 x 29 cm. These cages were equipped with running wheels, tunnels and toys, which were changed every week (Kempermann et al., 1997, 1998). Food and water were provided ad lib.
The animals were housed under enriched or standard conditions, 6 weeks before and 4 weeks after the surgery, in a temperature-controlled (20°C), laminar airflow cupboard and maintained on a 12-hr light-dark cycle (lights on at 7 am). All testing was carried out during the light phase.

23. Ischemia surgery

The rats were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg, Sagatal, Rhone-Merieux, Essex, England). Transient global ischemia was induced by the 4-vessel occlusion method of Pulsinelli and Brierly (1979), in which the vertebral arteries were electrocoagulated through the *Alar Foramenae* on the first cervical vertebrae and silastic ties were inserted around the carotids and brought to the surface. After 24 hr, the ties were tightened and clamped for 15 min; the animals were not anaesthetised during the latter procedure. The ties were then removed and the wound was sealed. Head and body temperature were maintained at 37°C using a lamp and the rats were housed in a recovery room at 24°C during 48 hr and received wet mash. On the third day, they were housed again under normal conditions. Sham-operated animals were subject to an identical procedure, except that the ties were not tightened.
2.4. Behavioural testing

2.4.1 Odour discrimination task

Rats were required to learn one odour of three presented, which uniquely identified the presence of food reward, in one corner of a large square wooden box. 2 weeks after surgery, rats were food deprived (11g/day/rat). The following day (Day 1) all rats from the same cage were placed individually in a wooden box (60 x 55 x 55 cm) painted matt black and habituated to the food reward (Cocopops, Kellogg’s, Ireland) for 20 min. On day 2, rats were placed individually into the same box for a second habituation session at the cued food reward and cued environment habituation. On day 3, rats were placed in the wooden box with three sponges placed in three corners. Each sponge had a hole in the centre (for food) and was impregnated with a different odour: Vanilla, Coffee, or Almond (see Figure 1 insert). Each rat was assigned the same reinforced odour. Training consisted of five trials, with a maximum of 5 min duration and 5 min of intertrial interval (ITI). For each, the sponge location was randomly chosen. We recorded the time spent by the rat finding the goal (i.e. sponge with reward).

2.4.2 Watermaze

The watermaze was a black circular pool filled water at room temperature (diameter = 200 cm, height = 35 cm), placed in a room with many extra-maze cues. 4 weeks after the surgery, rats were trained to find the hidden platform (diameter = 9.5 cm, height = 29 cm)
submerged 2 cm below the water surface. The maze was divided in 4 quadrants, North Vest (NW), South West (SW), North East (NE) and South East (SE). The platform was centred in the NW quadrant and the start position was always south. At the start of all trials, the rat was placed in the water facing the maze wall and swam until it found the platform where it waited for 15 sec before removal. The rat was guided to the platform after 60 sec. The velocity, escape latency, total distance swam and the percentage of time spent inside a corridor (called Wishaw’s error) of specified width from starting position to platform (Steward and Morris, 1993) were recorded by an image analysing system (IthoVision, Noldus Information Technology, Wageningen, Netherlands).

Rats were given 5 trials/day for 5 days with a 10 sec ITI.

2.1.3 Object exploration task

3 weeks after the surgery, food was provided ad lib again. Habituation and reactions to spatial changes were evaluated by the exploration of objects placed in an open field, which consisted of a black, circular fibreglass arena (diameter = 200 cm, height = 35 cm). The floor was covered with sawdust and the local environment was homogeneous. The experimenter stood in a fixed position at the side of the arena and recorded the time spent in contact with each object, during each session. The aim of this experiment was to assess the sensitivity of animals to an environment change.

Initially, each rat was allowed to explore the empty arena for a period of 6 min. During the ITI, the rat was confined in its cage outside the experimental room. After this phase, 4
objects were positioned in the centre of the arena and a white and black striped pattern (60 x 30 cm) was attached to the side-wall. The objects were heavy enough not to be displaced by the rats and consisted of a filled plastic jerry-can, a full pitcher, polystyrene box and cement cylinder. The rat was given 5 trials of 6 min duration with 3 min of ITI. For trials 1-3, the objects remained in the same place (Figure 4a); In trial 4 the cement cylinder was substituted for the pitcher, which itself was moved to a new location (moved objects) at the periphery of the open field (Figure 4a), trial 5 was the same as trial 4. In order to mask possible odour trails, the sawdust was raked during the ITI: the non-displaced objects were also manipulated as were the displaced objects. Object exploration was evaluated by the time spent in contact with the objects (i.e., when the rat’s snout touched an object).

2.3 BDNF Elisa procedure

All rats were killed on the last day of the training, their brains removed and tissue taken from the dentate gyrus, hippocampus, and entorhinal cortex. To prepare the samples, dentate gyri were homogenised in ice-cold Krebs 25 times. Samples were centrifuged and supernatant retained. Protein was assessed and samples were diluted to give equal protein concentrations and stored at -80°C. Ninety-six well plates were coated with 100 µl anti-BNF monoclonal antibody diluted (1:1000) in 0.025 M carbonate-bicarbonate buffer. Plates were covered, incubated overnight at 4°C and plates were subjected to interceding washes to remove excess antibody. Plates were blocked for non-specific binding for 1
bur at room temperature and washed (composition of wash buffer in mM: Tris-HCl, 20; NaCl, 150 containing 0.05% Tween (v/v); pH 7.6). Samples of dentate gyrus (50 μl), supernatant (50 μl) or BDNF standards (50 μl; ranging from 0.0078 to 1 ng/ml) were added to the wells, which were covered, incubated for two hours at room temperature with shaking and incubation overnight at 4°C and washed. Aliquots (100 μl) of anti-human BDNF pAb (diluted 1:500) were added to the wells, plates were incubated for 2h at 37°C and washed. Aliquots (100 μl) of anti-IgY HRP (1:2000 dilution) were added to wells and incubated for 1h at 37°C. During this incubation, the enzyme substrate was prepared. Plates were washed and 100 μl of this substrate was added to the wells, incubated for approximately 15 minutes until a blue colour formed in the wells. The reaction was stopped by the addition of 100 μl of 1 M phosphoric acid to the wells. Plates were read a 450 nm in a 96-well plate reader and BDNF concentrations were estimated for the standard curve and expressed as pg/mg protein. (BDNF Emax ™ ImmunoAssay System obtained from Promega UK LTD).

2.1 Quantitation of protein using the Bradford assay

The protein concentration of tissue samples was calculated according to the method of Bradford (1976). The standard curve was run using concentrations ranging from 2 μg/ml to 100 μg/ml prepared in a final volume of 160 μl, from a stock solution of 200 μg/ml bovine serum albumin (BSA). Tissue samples (5 μl) were diluted 1:40 in distilled water (15 μl) and Bio-Rad dye reagent (40 μl), which was added to all samples including the standards. Absorbance was read in a 96-well plate reader at a wavelength of 630 nm,
following gentle agitation. The concentration of protein in samples was calculated with reference to the standard curve plotted from the absorbance of the BSA standards. Protein concentrations were expressed as mg protein/ml tissue homogenate.

27 Histology

Histological evaluation of the brain lesions was carried out 1 day after the last watermaze trial. Rats were killed by decapitation and their brains promptly removed and frozen in chilled isopentane (-30°C). Coronal cryostat sections 10 μm thick were cut and stained with methylene blue 1%. Under light microscopy, sections were examined at different levels, -3.6 mm (level A) and -5.8 mm (level B) posterior to bregma (after the method Cilboume and Corbett, 1995, modified for this experiment). The quantification of ischemic brain damage was done by counting cells in CA1, CA2 and CA3 areas of the left hemisphere, in order to determine the grade of pyramidal cell loss. Counting was performed using x400 magnification with a counting grid measuring 12.5 x 12.5 mm superimposed over the different levels, and the assessment was performed in a blind manner.
2.8 Statistics

The watermaze data were analysed using a 2-way ANOVA with group and day as factors. In cases where variances were unequal, groups were compared using the Kruskal-Wallis one-way ANOVA by ranks, followed by individual comparisons between groups using the Mann-Whitney U test. A p value of 0.05 was considered as the threshold for a significant difference. The object exploration, odour discrimination and the histological damage data were analysed with one or two-way ANOVAs as appropriate; a value of p<0.05 was considered to be significant. Post-hoc comparisons were made using Tukey's method. All data are presented as mean (± sem) %, time or cells.
Normal env. (3 rats/cage)  
Ischemic rats  
Sham rats  
2 weeks post-ischemia:  
odour maze  
4 weeks post-ischemia:  
watermaze

6 weeks

Enriched env.* (4 rats/big cage)  
Ischemic rats  
Sham rats  
3 weeks post-ischemia:  
object exploration task  
Histology and Measurement of BDNF

* Enriched Env : toys changed each week + running wheel
3. Results

31. Odour discrimination task

We compared the average time to find the reward for every group. Figure 1 shows the groups in the standard conditions took more time than the enriched environment groups to find the food reward. There was a significant difference between groups (F(3,261) = 9.513, p<0.001). Post-hoc Tukey tests demonstrated that standard condition groups (mean ± sem: ischemic, 202±18 sec; sham, 185±14 sec) performed worse than the enriched condition groups (ischemic, 113±15 sec; sham, 113±13 sec) (p<0.01).
Odor discrimination task

Figure 1: Odour discrimination task, time to find the reward for every group. The enriched condition groups performed better than the standard condition groups across all comparisons. Post-hoc tests Tukey, * = p<0.05, ** = p<0.01. Insert: odour discrimination task diagram

3.2 Watermaze task analysis

3.2.1 Velocity analysis

There was no difference between the four groups in mean swim speed per day, and therefore no group was likely to have suffered a motor impairment (data not shown).
2.2 Escape latency and total distance swam

There were no escape latency or total distance swam differences between the 4 groups in the watermaze (figure 2a and 2b).

**Figure 2a**: Watermaze escape latency. All groups learned the hidden platform position after 5 days of training.
3.2.3 Direct swim analysis

A direct swim path was defined as a distance swam from the start position to hidden platform inside a corridor 30 cm wide (Figure 3 insert). Rats tend to swim in this corridor more when they have learned the platform position. We recorded the percentage of time spent in this corridor (Wishaw’s error). The two-way ANOVA indicated an overall significant difference across both day ($F(4,1256) = 23.460, p<0.001$) and condition ($F(3, 1256) = 13.178, p<0.001$), but there was no significant interaction between day and
A one-way ANOVA indicated that the standard environment sham (F(4,385) = 8.886, p<0.001), standard environment ischemic (F(4,203) = 5.532, p<0.001) groups and enriched environment sham (F(4,368) = 4.281, p<0.01) and enriched environment ischemic (F(4,316) = 10.876, p<0.001) groups improved over the 5 days of testing. Post-hoc tests indicated that all groups increased the % of time in the corridor significantly between day 1 and day 5 (p<0.001). Similarly, a Kruskal-Wallis one-way ANOVA showed that there were significant differences in % of time spent in the corridor between the groups, on day 1 ($\chi^2 = 18.74$, df = 3, p<0.001) and on day 5 ($\chi^2 = 8.551$, df = 3, p<0.05). On day 1, Mann-Whitney tests indicated the enriched condition sham (39±3) spent more time in the corridor than the enriched condition ischemic (27±2) (p<0.01) and the standard condition sham group (p<0.05) and also that the standard condition sham (31±2) group performed better than the standard condition ischemic (24±2) (p<0.05) group. On day 5, Mann-Whitney tests showed that the standard condition ischemic group (40±3) performed worse than enriched condition sham (54±3) (p<0.01) and standard condition sham (52±3) (p<0.05) groups and that the enriched condition ischemic group (48±3) used the direct route more to the platform than the standard condition ischemic group (40±4) (p<0.05).
Figure 3: Watermaze direct swim path analysis. Percentage of time spent in the corridor to platform for each group. All groups learned the platform position after the 5 days of training. However on day 5, the standard condition ischemic group spent less time in the corridor than the enriched condition, standard condition sham groups and enriched condition ischemic group (Mann-Whitney U test, \(*= p<0.05\)). Insert: diagram shows the corridor between the starting position and the platform.

3.3 Object exploration task

Figure 4 (lower panel) illustrates the mean time of contacts made with non-moved objects. A two-way ANOVA indicated an overall significant difference across only trial (F(4, 245) = 19.951, p<0.001), but there was no significant difference across group (F(3,245) = 2.165, p = 0.093 and no significant interaction between trial and group (F(12,
245) = 0.835, p = 0.614). A one-way ANOVA indicated that there was a significant difference over days for standard environment sham (F(4,79) = 4.411, p<0.01), enriched environment sham (F(4,74) = 5.194, p<0.01) and enriched environment ischemic groups (F(4,64) = 13.192, p<0.001) but not for standard environment ischemic. Post-hoc Tukey tests showed that 3 groups (standard environment sham, enriched environment and enriched environment ischemic groups) decreased exploration over 3 trials, indicating habituation (p<0.01). Post-hoc Tukey tests showed that there was no significant difference between day 3 (habitation) and day 4 (reaction to spatial changes) for all groups.

Figure 4 (upper panel) shows also the mean time of contacts made with moved objects. A two-way ANOVA indicated an overall significant difference across both trial (F(4, 245) = 13.342, p<0.001) and group (F(3, 245) = 9.127, p<0.001), but there was no significant interaction between trial and group (F(12, 245) = 1.448, p= 0.145). A one-way ANOVA indicated that there were significant differences over days for standard environment sham (F(4,79) = 5.287, p<0.01), enriched environment sham (F(4,74) = 5.049, p<0.01) and enriched environment ischemic groups (F(4,64) = 6.074, p<0.001) but not for the standard environment ischemic group. Post-hoc tests showed that 3 groups (standard environment sham, enriched environment sham and enriched environment ischemic groups) decreased in exploration over the 3 trials indicating habituation (p<0.05). Post-hoc Tukey tests showed a significant difference between day 3 (habitation) and day 4 (reaction to spatial changes) (p<0.05) only for sham groups.
Similarly, significant differences in exploration time were found between the enriched condition sham group and the standard condition sham group ($p<0.01$) and between the enriched condition ischemic group and standard condition sham ($p<0.001$) and standard condition ischemic groups ($p<0.05$). On day 1, a one-way ANOVA ($F(3,49) = 6.369$, $p<0.01$) showed a significant differences between the enriched condition ischemic group ($67\pm9$ sec) and the enriched condition sham ($39\pm6$ sec) ($p<0.05$), standard condition sham ($27\pm5$ sec) ($p<0.05$) and standard condition ischemic groups ($31\pm9$ sec) ($p<0.05$).

Figure 4 showed that only the sham groups were sensitive to the object displacement. A one-way ANOVA indicated a significant difference between trials for the enriched condition sham group ($F(4,70) = 5.049$, $p<0.01$) and the enriched condition sham group ($F(4,75) = 5.287$, $p<0.01$). Post-hoc tests revealed an increase in exploration after object displacement, i.e. between trial 3 (enriched, $19\pm5$ sec; standard, $8\pm3$ sec) and trial 4 (enriched, $48\pm7$ sec; standard, $28\pm5$ sec) ($p<0.01$).

There was a significant difference for moved object exploration times between groups (figure 5) ($F(3,261) = 7.425$, $p<0.001$). Post-hoc Tukey tests showed both enriched condition groups (sham, $30\pm3$ sec; ischemic, $35\pm4$ sec) explored significantly more than the standard condition ± sham group ($17\pm2$ sec) (sham versus sham $p<0.05$ and ischemic versus sham $p<0.001$). Post-hoc Tukey tests also showed that the enriched condition ischemic group ($35\pm4$ sec) explored significantly more than the ischemic group in the standard condition ($22\pm3$ sec) ($p<0.05$).
Moved objects

non-moved objects

**Figure 4:** Here, we show the mean time of contact with the objects in the arena for each trial and for each group. Post-hoc tests (Tukey), (*=p<0.05, **=p<0.01) showed that enriched condition sham and standard condition sham groups showed a significant difference between trial 3 and trial 4 in the exploration of displaced objects, whereas both ischemic groups were not sensitive to environmental changes.
Object exploration task: the mean contact time with moved objects

Figure 5: Object Exploration task: the mean time of contact with specific objects in the open field for each group, from first to fifth trials. Post-hoc tests (Tukey), (* = p<0.05, ** = p<0.001) show that the enriched condition groups explored more than standard condition groups.

3.4 Measurement of BDNF

Figure 6 shows the quantity of BDNF in the dentate gyrus at the end of the experiment, 10 weeks after the beginning of the task. A Kruskal-Wallis one-way ANOVA showed significant differences between groups ($\chi^2 = 10.498$, df = 3, p<0.05). Subsequent Mann-Whitney tests indicated that the standard condition sham group had significantly less BDNF than both ischemic groups (enriched and standard environment) and the enriched sham condition.
Figure 6: Measurement of BDNF in the dentate gyrus of the right hippocampus. There is no difference between both ischemic groups (enriched and standard environment) and the enriched condition sham. These groups had significantly greater levels of BDNF compared to the standard condition sham group (Mann-Whitney U test, * = p<0.05, ** = p<0.01).

3.5 Histology

Figure 7 shows the difference between ischemic and sham brains of rats housed under enriched conditions (there was no difference between enriched and standard conditions). We compared the average number of cells in CA1, CA2 and CA3 areas at 2 levels. There was a significant difference between groups in CA1 area at level A (F(3,42) = 61.421, p<0.001) and level B (F(3,41) = 10.372, p<0.001) (Figure 8). At level A, post-hoc tests
demonstrated that both ischemic groups (enriched group, 54±12 cells; standard group, 5±18 cells) had significant cell loss compared to sham groups (enriched group, 295±26 cils; standard group, 359±20 cells). Thus, in the enriched environment, there was 82% cell loss after ischemia, and in the standard condition, there was 84% cell loss. At level B, post-hoc tests demonstrated also that both ischemic groups (enriched group, 342±57 cils; standard group, 429±43 cells) had significant cell loss compared to sham groups (enriched group, 594±48 cells; standard group, 675±40 cells). Thus, in the enriched environment, there was 42% cell loss after ischemia and in the standard condition, there was 36% cell loss.

Figure 9 shows that ischemia induces cell loss, and indeed there is a significant difference between grouped ischemic rats (1381±84 cells) and grouped sham animals (1959±62 cells) (t-test, t = -5.636, df = 44, p<0.001). Thus, the effect of ischemia is to reduce the numbers of cells that can be counted at each of the compared levels.
Figure 7: Photomicrographs of the left hippocampus coronal sections at –3.6 mm (level A) and –5.8 mm (level B) posterior to bregma (magnification x80). There were no differences between the rat brains housed under enriched or standard conditions. We show here only sections from the ischemic (A, C, E) and sham (B, D, F) animals under enriched conditions. At rostral levels (photomicrograph A) ischemic rats showed a substantial decrease in the number of viable pyramidal cell bodies compared to the more caudal levels (photomicrographs C and E) where there was less cell loss.
Figure 8: Densities of the cell bodies in CA1, CA2 and CA3 sub fields of left hippocampus in different groups of rats; level A, is −3.6 mm and level B, is −5.8 mm posterior to bregma. Post-hoc tests Tukey, ** = p<0.01, *** = p<0.001. There is significant decrease in the number of viable cells only in the CA1 sub field. Numbers of animals were: n=13 enriched condition ischemic (Enr Cond Isch) group, n=15 enriched condition sham (Enr Cond Sham) group, n=9 standard condition ischemic (Std Cond Isch) group and n=16 standard condition sham (Std Cond Sham) group.
Figure 9: Sum of cells from both levels (A and B) in CA1, CA2 and CA3 sub fields of left hippocampus in all ischemic and all sham groups of rats. t-test indicates a significant difference between the grouped animals ($p<0.001$).
We investigated the effects of 4VO global transient ischemia on the performance of rats raised under standard housing or enriched environment conditions on object, odour and spatial learning tasks. We wished to determine if enriched environment housing was neuroprotective (i.e., that it would reduce injury and tissue loss), particularly in hippocampal area CA1, and if it would lead to a diminution of any consequent behavioural deficits. Contrary to expectation, we found that the enriched environment improved performance in the non-spatial odour discrimination and the object exploration tasks, independent of ischemia, and that it enhanced the watermaze performance of the enriched environment ischemic rats compared to ischemic rats housed in standard conditions.

We investigated the effects of environmental enrichment on the object recognition task, because exploration has been implicated this task and exploration plays a crucial role in spatial learning (O'Keefe and Nadel, 1978): exploration is the first step for encoding information about an environment and decreases over the time as the environment becomes familiar (Gemmell and O'Mara, 1999; Poucet, 1993). Rats decreased their exploratory activity over repeated exposures to a constant environment; when there was a rearrangement of objects, normal rats explored the environment once again. Rats with hippocampal lesions did not explore environments after object changes (Galani et al., 1998). We found no difference between ischemic and sham groups within any condition; thus, there was no hyperactivity, which can be a consequence of CA1 hippocampal
lesions as described in other reports (e.g. Galani et al., 1998). However, both ischemic and sham enriched environment rats explored more compared to those housed in the standard (non-enriched) environment.

Our results in the moved object exploration task also show that hippocampal-lesioned rats in standard and enriched conditions did not investigate an environmental change, but sham rats did increase their exploration after an object position change. We confirmed previous results (Galani et al., 1998) showing that rats with hippocampal lesions are impaired in exploratory activity. We found that destruction of CA1 alone induces a deficit in this task, as did Galani et al. (1998) with larger hippocampal lesions. Thus, the integrity of the "cognitive map" represented in the hippocampus would be appear to compromised by CA1 damage.

Interestingly, we found that enriched environment, ischemic and sham rats made more object contacts than standard condition rats (ischemic and sham). Thus, the enriched environment did not ameliorate the deficit induced by lesions of CA1, but it increased the mean time contact with specific objects for both ischemic and sham rats. Neurogenesis in dentate gyrus induced by the enriched conditions (Gould et al., 1999b; Kempermann et al. 1997) might compensate for cell loss in CA1, and this possibility should be examined in future experiments. However, we can not rule out the possibility that the animals in enriched housing were used to exploring a new environment, simply, because they were more stimulated in their own cage where every week, the environment was changed by the addition of new objects.
The olfactory system, involved in odour discrimination memory, has projections to the prefrontal cortex, entorhinal cortex and hippocampal formation. The hippocampal formation is weakly involved in olfactory memory (Dudchenko et al., 2000; Otto et al., 1991). We examined if the rat could match a specific odour to a specific reward, as the hippocampus appears to be more involved in this association learning than in olfactory memory per se. Although the enriched condition groups were faster than the standard condition groups at finding the reward, there was no overall difference between ischemic and sham groups in this task. Therefore, area CA1 is either not involved in this type of olfactory discrimination learning or the task was not sensitive enough to detect the effects of injury. However, the better performance of enriched condition groups compared to the standard condition groups at finding the reward, could be explained by neurogenesis in the olfactory bulb, as Rochefort et al. (2002) have shown after enriched odour exposure.

There were no escape latency or total distance swam differences between the four groups in the watermaze. There was, however, a significant difference between the standard condition ischemic group and both sham groups (figure 3) in the Wishaw’s error analysis. Direct swim path analysis is a more sensitive measure of heading or bearing than is velocity, and it shows that all groups used a progressively more direct route between first and fifth day. There was an overall significant difference between the standard condition ischemic group and both sham groups (figure 5); enriched conditions may have improved
the recovery of ischemic rats, but as we did not find any difference between the groups in either escape latency or overall distance swam, it appears that this task is relatively insensitive to area CA1 damage only.

Nilsson et al. (1999) and Puurunen et al. (1997) found that enriched condition groups performed better than standard condition groups in the watermaze, and that ischemic rats had longer escape latencies than sham rats. The discrepancy between these results could be due to the fact that Puurunen et al. (1997) worked with isolated rats, whereas our rats were triple-housed. Social isolation produces stress, which increases levels of corticosterone, itself a possible cause of neuronal damage in the hippocampal formation (Nitta et al., 1999); corticosterone may enhance the injury induced by 4VO, thus producing an escape latency difference between groups. Finally, the different kinds of experimental procedures used to induce ischemia and the tasks used to assess ischemia may explain the considerable discrepancies among different laboratories regarding the effects of ischemic CA1 lesions on watermaze learning: Nelson et al. (1997a) found that ischemic rats performed more poorly than sham-operated animals in the watermaze, although they suggest that the deficit, in terms of latency or heading angle, was difficult to assess (Nelson et al., 1997b). Kiyota et al. (1991) found no impairment in the watermaze (as did Auer et al., 1989) but did find that a significant impairment emerged on other tasks (passive avoidance and 8-arm radial maze). Olsen et al. (1994) and Nunn et al. (1994) found no correlation between CA1 cell loss in rats and behavioural performance in spatial learning tasks.
Eichenbaum et al. (1989) and Otto et al. (1991) have used complex odour tasks (e.g. successive cue, go-no-go tasks with sequenced positive and negative odours, with up to 10 different odours to learn), which may be more sensitive than our task; these tasks may also involve the hippocampus more, so hippocampal lesions may be easier to detect.

In our task, as Nilsson et al. (1999) had shown in the watermaze task, the rats in the enriched conditions (ischemic and sham groups) performed better than the rats in the standard conditions (ischemic and sham groups). Indeed, they spent less time searching for the goal and associated odour and reward than animals in normal housing. A stimulating environment can induce neurogenesis in adulthood (Gould et al., 1999b; Kempermann et al., 1998), particularly in the DG. Our task suggests that there are benefits of environmental enrichment on memory and suggest that the brains of enriched-ischemic rats were able to “compensate” for a lesion of area CA1. However, we could also explain our results with the hypothesis that area CA1 is not involved, or is just weakly involved, in the hippocampal-dependant memory.

Enriched environments modify gene expression in the brain (Rampon et al., 2000) and stimulate the production of multiple neurotrophic factors (Pham et al., 1999b) including BDNF. We found that BDNF levels were increased in enriched condition housed animals compared to those housed in standard conditions. As there is expression of many genes in response to enrichment (Puurunen et al., 2001), which increases the density of dendritic spines (Moser et al., 1994) in area CA1 and induces neurogenesis in DG (Eriksson et al.,
1998; Gould et al., 1999b), so that even if many cells are lost in area CA1, the plasticity of the remaining pyramidal cells and neurogenesis in DG may compensate for ischemic damage. BDNF might play a central role in this improvement. Furthermore, differences in the extent to which differing brain systems benefit from enrichment may account for the pattern of behavioural dissociations, which we observed. Thus, enriched environments enhance recovery after ischemia or brain injuries, and with appropriate drug therapy (e.g. glutamate antagonists or COX2 inhibitors) recovery might be further enhanced.
Chapter III: Running and celecoxib can protect against spatial memory deficits following kainic acid administration.
3.2 Watermaze task analysis

3.2.1 Velocity analysis

3.2.2 KA dose assessment-impairment of learning

3.2.3 Visible platform

3.2.4 Enriched environment

3.2.5 Kainic acid + ibuprofen

3.2.6 Running before KA injection

3.2.7 Kainic acid + celecoxib 6 mg/kg

3.2.8 Running + KA + celecoxib 6 mg/kg

3.2.9 Running + KA + celecoxib 40 mg/kg

3.3 Object exploration task

3.3.1 Running + KA and celecoxib + KA

3.3.2 Running + KA + celecoxib 6 mg/kg

3.3.3 Running + KA + celecoxib 40 mg/kg

3.4 Spontaneous activity in the open field

3.5 Measurement of BDNF

3.5.1 6 hours after injury and after running for 5 nights

3.5.2 Running + kainic acid group

3.5.3 KA + celecoxib 6 mg/kg and Run+ KA + celecoxib groups

3.5.4 Celecoxib 40 mg/kg groups

3.6 Histology
4. Discussion

157
**1. Introduction**

Cerebral ischemia (stroke) is one of the most common causes of neurological and neurodegenerative disorders (Kidwell et al., 2001). The central role of glutamate in neurodegeneration is suggested by the significant increases in the extracellular concentration of glutamate following ischemia (Benveniste et al., 1984), trauma or epilepsy. Glutamate may induce neurodegeneration by the sustained over-activation of calcium-permeable NDMA receptors and by activating phospholipase-coupled metabotropic receptors. Kainic acid (KA), a glutamic acid analogue which acts as an agonist for AMPA and kainate receptors (Bleakman and Lodge, 1998), has been widely used in rodents as a model for epilepsy and neuronal cell death. The excitotoxic hypothesis of ischemic neuronal death (Smith et al., 1997) suggests there are neurotoxic effects of sustained increases in the extracellular concentration of glutamate, which *via* overstimulation of glutamate receptors, causes neuronal damage *via* over activity. The hippocampus is particularly sensitive to systemic KA injection (Sperk, 1994), perhaps because of the high density of glutamatergic receptors present in this structure. High-affinity glutamate receptors are highly localized in the stratum lucidum of area CA3, the termination zone for mossy fibers (Schmitz et al, 2001b), which explains the vulnerability of pyramidal neurons of the CA3 region in the hippocampus to KA application; area CA1 neurons and dentate gyrus granule cells are also vulnerable (Sperk et al., 1998). However, after systemic injection of KA, only about 2-4% of the treatment dose is found in the
hippocampal formation, suggesting that the damage in the brain is not only due to a direct interaction of KA and its glutamate receptors (Ben-Ari et al., 1985; Sperk, 1994). Ben-Ari et al. (1985) found that systemic injection of KA also produces ischemia/trauma, because KA-induced activity leads to subsequent oedema through the release of water and metabolites; this induces compression of blood vessels against the skull, resulting in the disturbance of blood flow. Lassmann et al. (1984) showed that brain oedema plays an important role in the pathogenesis of KA; mannitol (an anti-oedema agent) reduces the neuronal damage induced by KA (Lassmann et al., 1984). More recent experiments with KA (Chen et al., 1995; Sanz et al., 1997; Hu et al., 1998) indicate that immediate early gene products (c-Fos, zif-268, c-Jun, cyclooxygenase-2) and heat shock proteins, are induced and may contribute to cell death, with both apoptotic and necrotic features, in different brain regions (hippocampus, thalamus, piriform cortex and amygdala). Therefore, the KA model appears to be a good model to study neuronal injury, especially damage to the hippocampus and resulting cognitive/mnemonic deficits.

Cyclooxygenase-2 (COX-2) activity is an important mediator of inflammation including neuroinflammation (Feng et al., 1993), but it is not known whether COX-2 activity is pathogenic or indeed neuroprotective in the brain. We investigated the effects of COX-2 inhibition after KA-induced damage, using the specific COX-2 inhibitor, celecoxib (Penning et al., 1997) and the non-specific COX inhibitor, ibuprofen (Crofford et al., 2000). In parallel, we investigated whether the effect of housing rats in a complex environment, or of having rats engage in (prolonged) physical activity (i.e. running in a
running wheel), ameliorates the cognitive/mnemonic deficits that result from KA injection. A substantial literature suggests that prolonged behavioural stimulation can induce neurogenesis in the hippocampus of rodents (Kempermann et al., 1997). Neurogenesis is stimulated by learning (Gould et al., 1999a), physical activity (Kempermann et al., 1997) and enriched environments (Johansson and Ohlsson, 1996) in rodents; there is an increase in new cells in DG and an increase in the density of dendritic spines in area CA1 (Moser et al., 1994). Stummer et al. (1994, 1995) have suggested that running in a running wheel is neuroprotective, perhaps because the gene-expression profile changes in the hippocampus after exercise, especially the mRNA levels of brain-derived neurotrophic factor (BDNF), which are substantially increased (Tong et al., 2001); we have measured BDNF levels in the hippocampus for each treatment group. Finally, we have systematically investigated the effects of each of these manipulations on a variety of behavioural tasks (more or less) sensitive to hippocampal damage.
2. Materials and Methods

Experiments were conducted in accordance with European Community directive, 86/609/EC, and the Cruelty to Animals Act, 1876 and followed local and international guidelines of good practice.

2.1 Animals

Male Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland) (mean weight 250-300 g at the start of the experiment) were used. The rats were randomly assigned to different experimental groups after their arrival from the BioResources Unit. The animals were housed under standard conditions, in a temperature-controlled (20°C), laminar airflow cupboard and maintained on a 12-hr light-dark cycle (lights on at 7 am). All testing was carried out during the light phase. Food and water were provided ad lib.

2.2 Housing conditions

Animals (by 3) were placed in standard cages measuring 44 x 28 x 18 cm, while other animals were placed, in groups of 4, in special cages measuring 55 x 37 x 29 cm. These cages were equipped with running wheels, tunnels and toys, which were changed every week (Kempermann et al., 1997, 1998). Food and water were provided ad lib.
A subgroup of animals were also housed under enriched or standard conditions for 6 weeks prior to the kainic acid injection, in a temperature-controlled (20°C), laminar airflow cupboard and maintained on a 12-hr light-dark cycle (lights on at 7 am). All testing was carried out during the light phase.

2. Kainic acid injection

Rats were injected either with 12 mg/kg (or 6 mg/kg) KA intraperitoneal (i.p.) (Sigma-Allrich, Dublin 24, Ireland) dissolved in saline or simply with saline (control). KA administration reliably induced an acute behavioural syndrome, which included "wet dog shakes" and seizures from mild forehead nodding to severe limbic convulsions with rearing and foam at the mouth. In all experiments, rats were tested 3 days after KA injection. We first assessed possible visual and/or motor impairments of the animals after KA injection, using the visible platform version of the watermaze task; this is a non-hipocampal task, which depends on an intact caudate/putamen (Packard and McGaugh, 1992)
2.4 Voluntary physical activity protocol

Rats were assigned randomly to 4 groups: running + KA, running + saline, non-running + KA and non-running + saline. The exercise regimen in the present study consisted of 5 days training in a running wheel (radius: 15 cm) overnight, for 10 hours. In total, rats voluntarily ran between 150 and 2300 meters in 5 days. The non-running groups rats were placed in a non-moving running wheel for similar periods of time, in order to control for the effect of exposure to the apparatus. Food and water were provided *ad lib.* KA or saline were injected after the running wheel exercise or exposure and rats were trained in the watermaze after 3 days.

2.5 COX inhibitors

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) capable of inhibiting the cyclooxygenase activity of COX-1 (the constitutive Cox isoform) and of the inducible isoform COX-2 (Crofford et al., 2000), which is involved in inflammation (Kaufmann et al., 1997) and synaptic plasticity and learning (O’Mara et al., 2001b). Rats were injected with KA or saline and 2 hours later with ibuprofen i.p. (30 mg/kg, Sigma-Aldrich, Dublin 24, Ireland) or saline (ibuprofen vehicle). We injected rats only once (acute treatment) or daily for 5 days (chronic treatment).
We used the same protocol with celecoxib (6 or 40 mg/kg i.p., Celebrex, SEARLE division of Monsanto Ireland Ltd) or its vehicle (10% DMSO). Celecoxib is one of the few specific COX-2 inhibitors (see table 1, section 6, chapter I).

2.6 Voluntary physical activity protocol and COX inhibitor

Rats were assigned to 2 groups: running + KA + celecoxib and running + vehicle KA + celecoxib. The exercise regimen in the present study consisted of 5 days training in the running wheel (radius: 15 cm) overnight, for 10 hours. In total, rats voluntarily ran between 150 and 2300 meters in 5 days. Rats were injected with KA or saline and 2 hours later with a single ibuprofen injection i.p. (30 mg/kg, Sigma-Aldrich, Dublin 24, Ireland). Rats were trained in the watermaze, after 3 days. Food and water were provided ad lib.
### Experimental Schedule

**KA 12mg/kg i.p**
- or vehicle (saline)

+ 2 hours

**Day 1**
- Water Maze 1 to 5 days
- Object exploration

**Day 2**
- Water Maze 9 to 11 d

**Day 3**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 4**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 5**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 6**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 7**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 8**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 12**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 13**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 14**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

**Day 16**
- Water Maze 1 to 5 days
- Water Maze 9 to 11 d

---

**Ibuprofen 30 mg/kg p.o**
- or vehicle (NaCl)

- **Group 1**: KA + ibuprofen Once
- **Group 2**: KA + vehicle ibuprofen
- **Group 3**: KA + ibuprofen Chronic
- **Group 4**: vehicle KA (Control)

**Histology and Measurement of BDNF**
2.7 Behavioural testing

2.7.1 Odour discrimination task

Rats were required to learn one odour of three presented, which uniquely identified the presence of food reward, in one corner of a large square wooden box.

Procedure: 2 weeks after injection, rats were food deprived (11 g/day/rat). The following day (Day 1) all rats from the same cage were placed individually in a wooden box (60 x 55 x 55 cm) painted matt black and habituated to the food reward (Cocopops, Kellogg's, Ireland) for 20 min. On day 2, rats were placed individually into the same box for a second habituation session with the food reward and environment. On day 3 (first training day) rats were placed in the wooden box with three sponges placed in three corners. Each sponge had a hole in the centre (for food) and was impregnated with a different odour: Vanilla, Coffee, or Almond (see Figure 3 insert). Each rat was assigned the same reinforced odour. Training consisted of five trials, with a maximum of five min duration and 5 min of intertrial interval (ITI). For each, the sponge location was randomly chosen. We recorded the time spent by the rat finding the goal (i.e. sponge with reward).

2.7.2 Watermaze

The watermaze was a black circular pool filled with water at room temperature (diameter = 200 cm, height = 35 cm), placed in a room with many extra-maze cues. Three days
After the KA injection, rats were trained to find a platform (diameter = 9.5 cm, height = 2.5 cm) hidden 2 cm below the water surface. The maze was divided in 4 quadrants, North West (NW), South West (SW), North East (NE) and South East (SE). The platform was centred in the NW quadrant and the start position was always south. At the start of all trials, the rat was placed in the water facing the maze wall and swam until it found the platform where it waited for 15 seconds (sec) before removal. The rat was guided to the platform after 60 sec, if it failed to find the hidden platform. Swim path were recorded by an image analysis system (EthoVision, Noldus, Wageningen, Netherlands). Rats were given 5 trials/day for 8 days with a 10 sec ITI: 5 days learning (acquisition phase) with 3 days retention testing after a 3 day-rest period.

2.7.3 Object exploration task

2 weeks after the injection, food was provided ad lib again. Habituation and reactions to spatial changes were evaluated by the exploration of objects placed in an open field, which consisted of a black, circular fibre glass arena (diameter = 200 cm, height = 35 cm). The floor was covered with sawdust and the environment was homogeneous. The experimenter stood in a fixed position at the side of the arena and recorded the number of nose contacts each rat made with the individual objects. The aim of this experiment is to test the sensitivity of animals to environmental change.
Initially, each rat was allowed to explore the empty arena for a period of 6 min. During the ITI, the rat was confined in its cage outside the experimental room. After this phase, 4 objects were positioned in the centre of the arena and a white and black striped pattern (40 x 30 cm) was attached to the side-wall. The objects were heavy enough not to be displaced by the rats and consisted of a filled plastic jerry-can, a full pitcher, polystyrene box and cement cylinder. The rat was given 5 trials of 6 min duration with a 3 min ITI. For trials 1-3, the objects remained in the same place (Figure 4a). In trial 4 the cement cylinder was substituted for the pitcher, which itself was moved to a new location (moved objects) at the periphery of the open field (Figure 4a); trial 5 was the same as trial 4. In order to mask possible odour trials, the sawdust was raked during the ITI: the non-displaced objects were also manipulated as were the displaced objects. Object exploration was evaluated by the time spent in contact with the objects (i.e., when the rat’s snout touched an object).

2.7.4 Open field testing

Rats were tested in a single 3 minute period during the light phase. The open field was a black circular open field (diameter = 200 cm, height = 35 cm). The animals were placed in the centre of the open field and the distance travelled was automatically recorded by an image analysis system (EthoVision, Noldus, Wageningen, Netherlands).
All rats were killed on the last day of the training, their brains removed and tissue taken from the dentate gyrus, hippocampus, and entorhinal cortex. To prepare the samples, dentate gyri were homogenised in ice-cold Krebs 25 times. Samples were centrifuged and supernatant retained. Protein was assessed and samples were diluted to give equal protein concentrations and stored at -80°C. Ninety-six well plates were coated with 100 μl anti-BDNF monoclonal antibody diluted (1:1000) in 0.025 M carbonate-bicarbonate buffer. Plates were covered, incubated overnight at 4°C and plates were subjected to interceding washes to remove excess antibody. Plates were blocked for non-specific binding for 1 hour at room temperature and washed (composition of wash buffer in mM: Tris-HCl, 20; NaCl, 150 containing 0.05% Tween (v/v); pH 7.6). Samples of dentate gyrus (50 μl), supernatant (50 μl) or BDNF standards (50 μl; ranging from 0.0078 to 1 ng/ml) were added to the wells, which were covered, incubated for two hours at room temperature with shaking and incubation overnight at 4°C and washed. Aliquots (100 μl) of anti-human BDNF pAb (diluted 1:500) were added to the wells, plates were incubated for 2h at 37°C and washed. Aliquots (100 μl) of anti-IgY HRP (1:2000 dilution) were added to wells and incubated for 1h at 37°C. During this incubation, the enzyme substrate was prepared. Plates were washed and 100 μl of this substrate was added to the wells, incubated for approximately 15 minutes until a blue colour formed in the wells. The reaction was stopped by the addition of 100 μl of 1 M phosphoric acid to the wells. Plates were read a 450 nm in a 96-well plate reader and BDNF concentrations were estimated.
for the standard curve and expressed as pg/mg protein. (BDNF Emax™ ImmunoAssay System obtained from Promega UK LTD).

2.9 Quantitation of protein using the Bradford assay

The protein concentration of tissue samples was calculated according to the method of Bradford (1976). The standard curve was run using concentrations ranging from 2 μg/ml to 100 μg/ml prepared in a final volume of 160 μl, from a stock solution of 200 μg/ml bovine serum albumin (BSA). Tissue samples (5 μl) were diluted 1:40 in distilled water (155 μl) and Bio-Rad dye reagent (40 μl), which was added to all samples including the standards. Absorbance was read in a 96-well plate reader at a wavelength of 630 nm, following gentle agitation. The concentration of protein in samples was calculated with reference to the standard curve plotted from the absorbance of the BSA standards. Protein concentrations were expressed as mg protein/ml tissue homogenate.
2.10 Histology

Histological evaluation of the brain lesions was carried out 1 day after the last watermaze trial. Rats were killed by decapitation and their brains promptly removed and frozen in chilled isopentane (-30°C). Coronal cryostat sections 10 μm thick were cut and stained with methylene blue 1%. Under light microscopy, sections were examined at -3.6 mm and -5.8 mm posterior to bregma (after the method of Colbourne and Corbett, 1995, modified for this experiment). The quantification of ischemic brain damage was done by counting cells in CA1, CA2 and CA3 areas of the left hemisphere, in order to determine the grade of pyramidal cell loss. Counting was performed using x400 magnification with a counting grid measuring 12.5 x 12.5 mm superimposed over the different levels, and the assessment was performed in a blind manner.

2.11 Statistics

The watermaze and object exploration data were analysed using a 2-way ANOVA with group and day as factors. Post-hoc comparisons were made using Tukey’s method. In cases where tests of homogeneity of variances indicated a significant difference between groups of p<0.05, groups were compared using the Kruskal-Wallis one-way ANOVA by ranks, followed by individual comparisons between groups using the Mann-Whitney U test. A p value of 0.05 was considered as the threshold for a significant difference. The odour discrimination and the histological damage data were analysed with one or two-
way ANOVA as appropriate; a value of $p<0.05$ was considered to be significant. Post-hoc comparisons were made using Tukey's method. All data are presented as mean (±sem) distance, time or cells.
3. Results

3. Odour discrimination task

The two-way ANOVA indicated an overall significant difference across trial (F(4,57) = 9.62, p<0.001) and group (F(1,57) = 16.095, p<0.001), but there was no interaction between trial and group. The one way ANOVA indicated that control (n=6, F(4,26) = 5.94, p<0.01) and KA (n=8, F(4,39) = 5.241, p<0.01) groups found the reward more quickly over trials. Within-day comparisons showed there were no differences between control and KA groups, except on day 2, where a t-test showed that KA group was faster than the control group (t = 4.492, df = 12, p<0.01; figure 1).
Figure 1: Odour discrimination task: there were no differences between control and kainic acid (12 mg/kg) group except on trial 2. The KA group found the reward as fast as the control group over trials.

3. Watermaze task analysis

3.1 Velocity analysis

There was no difference between the four groups in mean swim speed per day, and therefore no group was likely to have suffered a motor impairment.
Figure 2: There were no differences between the kainic acid (12 mg/kg, n=6) and control (n=5) groups, in swim velocity, in the normal version of the watermaze.

3.2.2 KA dose assessment – impairment of learning in the watermaze

Figure 3 demonstrates that escape latencies and distances swam decreased during the training period for all groups. However, the kainic acid 12 mg/kg group (n=6) effectively did not learn the task compared to both controls (n=5) and the kainic acid 6 mg/kg (n=5) group, both of whom performed faster than the kainic acid 12 mg/kg group. A two-way ANOVA indicated an overall significant difference across day (time: $F(4,385) = 38.376$, $p<0.001$; distance: $F(4,365) = 16.606$, $p<0.001$), group (time: $F(2,385) = 139.445$, $p<0.001$; distance: $F(2,365) = 83.619$, $p<0.001$) and a significant interaction between day
The Kruskal-Wallis one-way ANOVA showed significant differences between days 1 to 5 for all groups, control (day 1: 50±3 sec and day 5: 15±3 sec; time: $\chi^2 = 35.406, df = 4, p<0.001$; distance: $\chi^2 = 21.94, df = 4, p<0.001$); KA 6 mg/kg (day 1: 51±4 sec and day 5: 10±1 sec; time: $\chi^2 = 51.873, df = 4, p<0.001$; distance: $\chi^2 = 36.9, df = 4, p<0.001$) and KA 12 m/kg (day 1: 59±1 sec and day 5: 49±3 sec; time: $\chi^2 = 15.204, df = 4, p<0.01$; distance: $\chi^2 = 10.448, df = 4, p<0.05$). A Mann-Whitney test for escape latency and distance swam showed that there were significant differences between day 1 and days 2, 3, 4, 5 ($p<0.05$, KA 6 mg/kg group), between day 1 and days 3, 4, 5 ($p<0.05$, control group) and between day 1 and day 5 ($p<0.05$, for KA 12 mg/kg group). There were significant differences for time between day 2 and days 4, 5 ($p<0.05$, for control and KA 6 mg/kg), for distance between day 2 and days 3, 4, 5 ($p<0.01$, control group), between day 2 and day 5 ($p<0.05$, KA 6 and 12 mg/kg groups). There were also significant differences between days 3 and 5 ($p<0.05$, for time and distance, control and KA 6 mg/kg). The Kruskal-Wallis one-way ANOVA, showed also significant differences between groups on day 1 (time: $\chi^2 = 10.48, df = 2, p<0.01$; distance: NS); day 2 (time: $\chi^2 = 26.468, df = 2, p<0.001$; distance: $\chi^2 = 18.848, df = 2, p<0.001$); day 3 (time: $\chi^2 = 42.72, df = 2, p<0.001$; distance: $\chi^2 = 31.506, df = 2, p<0.001$); on day 4 (time: $\chi^2 = 26.153, df = 2, p<0.001$; distance: $\chi^2 = 29.948, df = 2, p<0.001$); on day 5 (time: $\chi^2 = 36.619, df = 2, p<0.001$; distance: $\chi^2 = 40.994, df = 2, p<0.001$). Finally, Mann-Whitney tests showed that the kaiic acid 12 mg/kg group was slower and swam more than control and KA 6 mg/kg (p<.01), each day except day 1.
Figure 3: There were no differences between both kainic acid (KA) 6 mg/kg (n=5) and control (n=5) groups: they found the hidden platform faster time than the kainic acid (KA) 12 mg/kg (n=6) group, who took more than 50 seconds to find the hidden platform on the final day of training. There were no differences between control and KA 6 mg/kg in distance swam, but significant differences between both groups and the KA 12 mg/kg.
3.2.3 Visible platform

Figure 4: We recorded escape latency and distance swam (n=5) for both control and kainic acid injected (n=5) groups. The two-way ANOVA indicated an overall significant difference across day (time: F(6,336) = 47.07, p<0.001; distance: F(6,336) = 21.726, p<0.001), group (time: F(1,336) = 95.774, p<0.001; distance: F(1,336) = 63.068, p<0.001) and a significant interaction between day and group (time: F(6,336) = 3.323, p<0.01; distance: F(6,336) = 1.4, p<0.01). The Kruskal-Wallis one-way ANOVA showed significant differences between day 1 to day 7 for both groups, control (day 1: 33±3 sec and day 7: 5±1 sec; time: χ² = 123.826, df = 6 , p<0.001; distance: χ² = 103.734, df = 6 , p<0.001), KA (day 1: 55±5 sec and day 7: 10±3 sec; time: χ² = 77.998, df = 6 , p<0.001; distance: χ² = 43.48, df = 6 , p<0.001). Mann-Whitney tests indicated that the time and distance swam were improved significantly between day 1 and days 2, 3, 4, 5, 6, 7 (time and distance swam for control and kainic acid groups: p<0.001), between day 2 and days 4, 5, 6, 7 (time and distance swam for control and kainic acid groups: p<0.05), between day 3 and days 4, 5, 6, 7 (time and distance swam for control and kainic acid groups: p<0.05, except between day 3 and day 4 distance for kainic acid group), between day 4 and days 5, 6, 7 (time and distance swam for control and only time for kainic acid groups: p<0.05, except between day 4 and day 5) and there were no significant difference between day 5 and days 6, 7 and between day 6 and day 7 for any group.

The Kruskal-Wallis one-way ANOVA, following by Mann-Whitney test showed also significant differences in escape latency and distance swam between control and kainic acid groups (p<0.01), for each day (day 1, time: χ² = 15.730, df = 1, p<0.001; distance: χ² = 7.752, df = 1 , p<0.05; day 2, time: χ² = 17.642, df = 1 , p<0.001; distance: χ² = 13.662,
dl = 1, p < 0.001; day 3, time: $\chi^2 = 28.008$, df = 1, p < 0.001; distance: $\chi^2 = 16.646$, df = 1, p < 0.001; day 4, time: $\chi^2 = 26.803$, df = 1, p < 0.001; distance: $\chi^2 = 23.816$, df = 1, p < 0.001; day 5, time: $\chi^2 = 27.798$, df = 1, p < 0.001; distance: $\chi^2 = 19.144$, df = 1, p < 0.001; day 6, time: $\chi^2 = 30.141$, df = 1, p < 0.001; distance: $\chi^2 = 19.485$, df = 1, p < 0.001; day 7, time: $\chi^2 = 30.141$, df = 1, p < 0.001; distance: $\chi^2 = 19.485$, df = 1, p < 0.001).
Figure 4: Both groups found the visible platform more quickly over days, although there is significant difference between both groups. The difference between groups may arise because the KA-treated group (12 mg/kg) tend to be hyperactive relative to the untreated controls.
Sustained exposure to an enriched environment had no neuroprotective effect on rats injected with kainic acid. A two-way ANOVA indicated an overall significant difference across day ($F(7,1084) = 27.568$, $p<0.001$), group ($F(3,1084) = 70.175$, $p<0.001$) and a significant interaction between day and group ($F(21,1084) = 2.940$, $p<0.01$). A subsequent one-way ANOVA indicated that the control standard environment ($n=8$, $F(7,319) = 12.733$, $p<0.001$), KA standard environment ($n=9$, $F(7,334) = 3.402$, $p<0.01$) and control enriched environment group ($n=5$, $F(7,195) = 25.234$, $p<0.001$) groups improved over days of testing, but the KA enriched environment group did not. The one-way ANOVA also showed, on days 1 and 2 that there was no difference between groups, whereas there were significant differences from day 3 to day 11 (day 3: $F(3,144) = 5.859$, $p<0.01$; day 4: $F(3,144) = 9.490$, $p<0.001$; day 5: $F(3,144) = 13.688$, $p<0.001$; day 9: $F(3,139) = 16.545$, $p<0.001$; day 10: $F(3,124) = 27.151$, $p<0.001$; day 11: $F(3,124) = 13.458$, $p<0.001$). Post-hoc Tukey tests indicated that the control groups were faster than all KA groups in finding the platform on all days. There was no difference between KA in the enriched and standard environment groups in distance swam, nor in Wishaw’s error analysis (data not shown). Note, reacquisition in the enriched environment sham group was swifter than standard environment control group. Indeed, 3 days after the break, the animals in the enriched housing continued to improve their performance between day 9 and day 5, while rats in the standard housing had the same performance on days 5 and 9 (figure 5).
Figure 5: Animals housed in an enriched environment did not show improved performance compared to animals housed in a standard environment after KA treatment at 12 mg/kg.

3.2.5 Kainic acid + ibuprofen

A two-way ANOVA indicated an overall significant difference across day (F(7,797) = 8.434, p<0.001), group (F(3,797) = 60.847, p<0.001) and a significant interaction between day and group (F(21,797) = 1.901, p<0.01). A one-way ANOVA showed that all groups, control (n=5, F(7, 201) = 11.657, p<0.001), KA (n=6, F(7,287) = 2.617, p<0.05) improved over days of testing, except the KA + ibuprofen chronic injection (5 days) group (n = 4) and KA + ibuprofen once (n = 3). There was over 50% death in the ibuprofen groups and, moreover, the remaining animals were very sick (rejected food,
were immobile). Within-day comparisons, on day 1, showed there was no difference between groups, but from day 2 to day 11 there were significant differences (day 2: \(F(3,102) = 5.134, p<0.01\); day 3: \(F(3,102) = 4.056, p<0.01\); day 4: \(F(3,102) = 4.83, p<0.01\); day 5: \(F(3,102) = 12.995, p<0.001\); day 9: \(F(3,102) = 11.518, p<0.001\); day 10: \(F(3,102) = 21.089, p<0.001\); day 11: \(F(3,102) = 14.954, p<0.001\)) Post-hoc Tukey tests showed that the control group found the platform faster than the KA groups (\(p<0.05\)). There was no difference between KA + ibuprofen and KA groups in distance swam (data not shown) (figure 6).

**Kainic acid + ibuprofen: escape latency**

![Graph showing escape latency](image)

**Figure 6:** The KA + ibuprofen groups did not show improved performance compared to the KA (kainic acid, 12 mg/kg) group. The KA + ibuprofen had a mortality rate over 50%.
3.1.6 Running before KA injection

Figure 7b demonstrates that escape latencies and distance swam decreased during the training period for all groups (figure 7a shows swim paths taken from the final day of training for each groups): A two-way ANOVA indicated an overall significant difference across day (time: $F(7,958) = 25.262$, $p<0.001$; distance: $F(7,904) = 12.708$, $p<0.001$), group (time: $F(3,958) = 111.843$, $p<0.001$; distance: $F(3,904) = 77.892$, $p<0.001$) but there was no significant interaction between day and group. A Kruskal-Wallis one-ANOVA, showed significant differences between day 1 to day 11 for all groups, control (day 1: $46 \pm 4$ sec and day 11: $14 \pm 2$ sec; time: $\chi^2 = 46.719$, df = 7 , $p<0.001$; distance: $\chi^2 = 37.710$, df = 7 , $p<0.001$), KA (day 1: $60 \pm 0.3$ sec and day 11: $46 \pm 4$ sec; time: $\chi^2 = 20.888$, df = 7 , $p<0.001$; distance: $\chi^2 = 16.441$, df = 7 , $p<0.05$); Run + KA (day 1: $52 \pm 2$ sec and day 11: $36 \pm 4$ sec; time: $\chi^2 = 27.597$, df = 7 , $p<0.001$; distance: $\chi^2 = 16.724$, df = 7 , $p<0.05$); Run + vehicle KA (day 1: $47 \pm 4$ sec and day 11: $18 \pm 2$ sec; time: $\chi^2 = 50.589$, df = 7 , $p<0.001$; distance: $\chi^2 = 43.528$, df = 7 , $p<0.001$). Subsequent Mann-Whitney tests indicated that all groups improved over days, so there were significant differences between day 1 and days 2, 3, 4, 5, 9, 10, 11 (time and distance swam for control (n=5), control-run (n=8), run + KA (n=8) and kainic acid (n=6) groups: $p<0.05$), between day 2 and days 4, 5, 9, 10, 11 (time for control, run-control and run + KA groups: $p<0.05$), between day 2 and days 4, 5, 9, 10, 11 (distance swam for all groups: $p<0.05$).

A Kruskal-Wallis one-way ANOVA also showed significant differences between groups, for each day (on day 1, time: $\chi^2 = 10.424$, df = 3 , $p<0.05$; distance: $\chi^2 = 9.981$, df = 3 , $p<0.05$; on day 2, time: $\chi^2 = 19.563$, df = 3 , $p<0.001$; distance: $\chi^2 = 14.318$, df = 3 ,
p<0.01; on day 3, time: \( \chi^2 = 26.074, \text{df} = 3, p<0.001 \); distance: \( \chi^2 = 28.503, \text{df} = 3, p<0.001 \); on day 4, time: \( \chi^2 = 43.839, \text{df} = 3, p<0.001 \); distance: \( \chi^2 = 39.175, \text{df} = 3, p<0.001 \); on day 5, time: \( \chi^2 = 38.625, \text{df} = 3, p<0.001 \); distance: \( \chi^2 = 32.567, \text{df} = 3, p<0.001 \); on day 9, time: \( \chi^2 = 38.625, \text{df} = 3, p<0.001 \); distance: \( \chi^2 = 40.7, \text{df} = 3, p<0.001 \); on day 10, time: \( \chi^2 = 22.528, \text{df} = 3, p<0.001 \); distance: \( \chi^2 = 38.6, \text{df} = 3, p<0.001 \); on day 11, time: \( \chi^2 = 39.790, \text{df} = 3, p<0.001 \); distance: \( \chi^2 = 38.027, \text{df} = 3, p<0.001 \). Post hoc Mann-Whitney tests showed that KA group was slower and swam less than control and control-run groups (p<0.01) on each day. On days 2, 3, 4, 5, 9, 11, run + KA group was faster than KA group (p<0.05) and on days 3, 4, 5, 9, 10, 11 the run + KA group was slower than run-control and control groups (p<0.05). The distance swam by run + KA group was significantly longer than control and run-control groups on days 3, 4, 5, 9, 10, 11 (p<0.05) whereas the run + KA group swam shorter than the KA group only on days 10 and 11 (p<0.05).

![Control rat](image1.png)  ![KA-treated rat](image2.png)  ![Run + KA-treated rat](image3.png)

**Figure 7a:** Examples of swim paths taken from the final day of training (day 11) in the watermaze task, for each group.
**Figure 7b**: The prior exercise and subsequent kainic acid injection group performed significantly better than kainic acid only group.
3.2.7 Kainic acid + celecoxib 6 mg/kg

Figure 8 shows the escape latencies and distances swam (figure 8c shows examples of swim paths for main groups) for the KA + celecoxib 6 mg/kg (5 days, n=11), KA + celecoxib 6 mg/kg once (n=8), KA + vehicle celecoxib (n=5) and control (n=13). A two-way ANOVA indicated an overall significant difference across day (time: $F(7,1418) = 18.05$, $p<0.001$; distance: $F(7,1336) = 10.131$, $p<0.001$), group (time: $F(3,1418) = 87.474$, $p<0.001$; distance: $F(3,1336) = 52.662$, $p<0.001$) and a significant interaction between day and group (time: $F(21,1418) = 2.832$, $p<0.001$; distance: $F(21,1418) = 2.383$, $p<0.01$). The control (day 1: 49±3 sec and day 11: 12±1 sec; time: $F(7,519) = 30.605$, $p<0.001$; distance: $F(7,502) = 16.354$, $p<0.001$), KA + celecoxib (5 days) (day 1: 51±2 sec and day 11: 38±3 sec; time: $F(7,409) = 3.934$, $p<0.001$; distance: $F(7,375) = 6.623$, $p<0.001$) and KA + celecoxib once (day 1: 52±2 sec and day 11: 36±4 sec; time: $F(7,319) = 3.988$, $p<0.001$; distance: $F(7,303) = 2.579$, $p<0.05$) groups improved over days of testing but not the KA group (day 1: 55±3 sec and day 11: 50±4 sec; time: $F(7,199) = 0.752$, $p = 0.628$; distance: $F(7,184) = 0.945$, $p = 0.473$). Within-day ANOVA comparisons, on days 1 and 2, revealed any significant difference between all groups (time and distance), on day 3, 4, 5, 9, 10, 11 (day 3: $F(3,178) = 9.812$, $p<0.001$; day 4: $F(3,179) = 7.4$, $p<0.001$; day 5: $F(3,178) = 11.043$, $p<0.01$; day 9: $F(3, 178) = 6.238$, $p<0.01$; day 10: $F(3,179) = 14.712$, $p<0.001$; day 11: $F(3,178) = 21.192$, $p<0.001$): the control group swam less than KA and KA + celecoxib-once groups ($p<0.05$, except on day 4 for KA + celecoxib once) and swam less only on days 3, 4, 10, 11 compared to KA + celecoxib 5 days ($p<0.05$). The KA group swam longer than KA + celecoxib once and KA + celecoxib 5 days ($p<0.01$) on days 10 and 11.
The control group also found the platform faster than all other groups from day 3 to day 11 (day 3: F(3, 178) = 15.977, p<0.001; day 4: F(3,179) = 8.31, p<0.001; day 5: F(3,178) = 13.973, p<0.001; day 9: F(3, 178) = 18.771, p<0.001; day 10: F(3,179) = 17.053, p<0.001; day 11: F(3,178) = 21.192, p<0.001), (post-hoc: p<0.05). The KA group was slower than the KA + celecoxib groups on days 3, 4, 10, 11 (p<0.05, celecoxib 5 days) and on days 10, 11 (p<0.05, celecoxib once).

**Figure 8a:** The kainic acid (KA) + celecoxib groups found significantly faster the platform than kainic acid group, but slower than control group.
Figure 8b: The KA (kainic acid) + celecoxib groups swam significantly shorter distances than KA only group, on days 10 and 11.

Figure 8c: Examples of swim paths taken from the final day of training (day 11) in the watermaze task, for each group (KA dose was 12 mg/kg).
3.2.8 Running + KA + celecoxib 6 mg/kg

Figure 9 shows the escape latencies and distances swam for Run+ KA+ celecoxib 6 mg/kg, 5 days, (n=7), Run + KA + celecoxib 6 mg/kg once (n=6), KA + vehicle celecoxib (n=11) and Run + vehicle KA + celecoxib (n=10, control). A two-way ANOVA indicated an overall significant difference across day (time: F(7,1313) = 23.383, p<0.001; distance: F(7,1264) = 7.045, p<0.001), group (time: F(3,1313) = 318.569, p<0.001; distance: F(3,1264) = 199.889, p<0.001) and a significant interaction between day and group (time: F(21,1313) = 4.221, p<0.001; distance: F(21,1264) = 3.234, p<0.01). A one-way ANOVA indicated that no KA groups showed a reduction in distance swam to find the platform over days, whereas the control group (F(7,399) = 29.026, p<0.01) improved over days of testing. Otherwise, except for day 1, the control group swam less than all other KA groups (from day 2 to day 11: day 2: F(3,163) = 10.288, p<0.01; day 3: F(3,163) = 28.173, p<0.001; day 4: F(3,163) = 36.92, p<0.001; day 5: F(3,163) = 25.605, p<0.001; day 9: F(3, 163) = 45.226, p<0.001; day 10: F(3,163) = 37.708, p<0.001; day 11: F(3,163) = 58.040, p<0.001). Moreover, the run + KA+ celecoxib once group swam more than the KA group on day 4, 5, 9, 11 (p<0.05).

All groups improved over day the time spent to find the platform (Run+ KA+ celecoxib 5 days: F(7,179) = 4.565, p<0.001; Run+ KA+ celecoxib once: F(7,239) = 2.541, p<0.05; KA+ vehicle celecoxib: F(7,424) = 4.937, p<0.001 and Run+ vehicle KA+ celecoxib: F(7,399) = 31.242, p<0.001. The control group (day 2: F(3,169) = 24.335, p<0.001; day 3: F(3,169) = 58.9, p<0.001; day 4: F(3,169) = 54.936, p<0.001; day 5: F(3,169) = 47.319, p<0.001; day 9: F(3, 169) = 46.827, p<0.001; day 10: F(3,164) = 42.990,
p<0.001; day 11: F(3,159) = 47.311, p<0.001) also found the hidden platform faster than all other groups from day 2 to day 11 (post-hoc, P<0.05).
**escape latency**

- RUN+KA +Celecoxib 5days (n=7)
- RUN+ Veh KA + Celecoxib (n=10)
- KA (n=11)
- RUN+KA +Celecoxib Once (n=6)

**distance swam**

- RUN+KA +Celecoxib 5days (n=7)
- RUN+ Veh KA + Celecoxib (n=10)
- KA (n=11)
- RUN+KA +Celecoxib Once (n=6)

**Figure 9:** There were no differences in escape latency and in distance swam between the KA (12 mg/kg) group and the Run + KA (12 mg/kg) + celecoxib (6 mg/kg) groups.

131
3.2.9 Running + KA + celecoxib 40 mg/kg

Figure 10 shows the escape latencies and distances swum for Run+ KA+ celecoxib 40 mg/kg 5 days (n=5), Run+ KA+ celecoxib 40 mg/kg once (n=4), KA + vehicle celecoxib (n=5), celecoxib 40 mg/kg (n=6) and Run+ vehicle KA+ celecoxib (n=7, control). A two-way ANOVA indicated an overall significant difference across day (time: F(7,1005) = 30.952, p<0.001; distance: F(7,1005) = 17.477, p<0.001), group (time: F(4,1005) = 117.538, p<0.001; distance: F(4,1005) = 92.730, p<0.001) and a significant interaction between day and group (time: F(28,1005) = 1.580, p<0.001; distance: NS). The one-way ANOVA indicated that KA and Run+ KA+ celecoxib 40 mg/kg 5 days groups did not reduce their swim distance to find the platform across days, the control (F(7,279) = 20.328, p<0.001), celecoxib 40 mg/kg (F(7,239) = 16.963, p<0.001) and Run+ KA+ celecoxib 40 mg/kg once (F(7,124) = 3.806, p<0.01) group improved over day of testing. Otherwise, except on day 1, there were significant differences between groups (day 2: F(4,129) = 7.25, p<0.001; day 3: F(4,129) = 7.934, p<0.001; day 4: F(4,129) = 15.37, p<0.001; day 5: F(4,129) = 19.887, p<0.001; day 9: F(4,129) = 16.381, p<0.001; day 10: F(4,129) = 22.627, p<0.001; day 11: F(4,129) = 17.132, p<0.001). The control and celecoxib 40 mg/kg groups swam less than KA, Run+ KA+ celecoxib once and Run+ KA+ celecoxib 5 days groups from day 2 to day 11 (p<0.05). However, post-hoc tests indicated that there was no significant difference on days 2, 3, 9, 11 between control, celecoxib 40 mg/kg and Run+ KA+ celecoxib once groups, while on days 3, 5, the Run+ KA+ celecoxib once group swam significantly less than the KA group (p<0.05).

All groups improved over days the time spent finding the hidden platform (Run+ KA+ celecoxib 5 days: F(7,199) = 2.129, p<0.05; Run+ KA+ celecoxib once: F(7,124) =
3.622, p<0.01; KA+ vehicle celecoxib: F(7,199) = 3.094, p<0.01, celecoxib: F(7,239) = 21.432, p<0.001 and control: F(7,279) = 23.33, p<0.001. There were significant differences between groups over days (day 1: F(3,169) = 7.304, p<0.001; day 2: F(3,169) = 8.054, p<0.001; day 3: F(3,169) = 8.084, p<0.001; day 4: F(3,169) = 17.531, p<0.001; day 5: F(3,169) = 23.038, p<0.001; day 9: F(3, 169) = 19.863, p<0.001; day 10: F(3,164) = 31.125, p<0.001; day 11: F(3,159) = 16.118, p<0.001). Post-hoc tests showed that the control and celecoxib groups also found the platform faster than all other groups from day 1 to day 11 (p<0.05), except on day 1, when the control group was faster than only the KA group (p<0.05) and on day 3, there was no difference between control, celecoxib and Run + KA + celecoxib 5 days. On day 5, the Run + KA + celecoxib once was faster than the KA group (p<0.01).
There were no differences between the KA (12 mg/kg) group and Run+KA (12 mg/kg) + celecoxib (40 mg/kg) and also no differences between control and the celecoxib 40 mg/kg group in either escape latency or distance swam.
3. Object exploration task

3.1 Run + KA and celecoxib + KA

Figure 11 illustrates the mean time of contacts made with moved objects or all objects. A two-way ANOVA indicated an overall significant difference across trial (moved objects: $F(4, 74) = 7.43$, $p<0.001$, non moved objects: $F(4, 74) = 4.364$, $p<0.003$) but there was no significant difference across groups or significant interaction between trial and group. A Kriskal-Wallis one-way ANOVA showed that there were significant differences between trials for the control (moved objects: $\chi^2 = 16.33$, df = 4, $p = 0.003$), Run+ KA (moved objects: $\chi^2 = 14.498$, df = 4, $p = 0.006$) and KA+ celecoxib (once and 5 days) (moved objects: $\chi^2 = 17.684$, df = 4, $p = 0.001$) groups but there was no significant difference between trials for all non moved object groups. Post hoc Mann-Whitney tests showed that the 3 groups decreased exploration over the 3 trials, indicating habituation ($p<0.05$); there was also a significant difference between controls, Run + KA and celecoxib groups, between trial 3 (habituation) and trial 4 (reaction to spatial changes) ($p<0.05$).
**moved objects**

- Open squares: control
- Pink squares: run + KA 12 mg/kg
- Black triangles: COX-2 + KA 12 mg/kg
- Pink triangles: KA 12 mg/kg

**non moved objects**

- Open squares: control
- Pink squares: run + KA 12 mg/kg
- Black triangles: COX-2 + KA 12 mg/kg
- Pink triangles: KA 12 mg/kg

**Figure 11:** All groups except the KA group decreased the exploration over trials; all groups showed a significant difference between trial 3 (habituation) and 4 (spatial change). Mann-Whitney test, *=p<0.05, **=p<0.01.
3.3.2 Running + KA + celecoxib 6 mg/kg

Figure 12 illustrates the mean time of contacts made with (specific) moved objects or non moved objects. A two-way ANOVA indicated an overall significant difference across trial (moved objects: $F(4, 110) = 6.485, p<0.001$, non moved objects: $F(4, 110) = 7.538$, $p<0.001$) but there were no significant differences across group or interaction between trial and group. A Kruskal-Wallis one-way ANOVA showed that there was a significant difference between trials for the control (non moved objects: NS; moved objects: $\chi^2 = 11.328, df = 4, p = 0.023$), Run+ KA + celecoxib 5 days (non moved and moved objects: NS) and Run + KA + celecoxib once (non moved and moved objects: NS) groups. Post hoc Mann-Whitney tests showed that the 3 groups decreased exploration over 3 trials indicating habituation ($p<0.05$). Post-hoc tests showed a significant difference between trial 3 (habituation) and trial 4 (reaction to spatial changes) only for the control group ($p<0.05$).
Moved objects

Non moved objects

Figure 12: Control and Run + KA + celecoxib (Cxb) groups decreased exploration over trials, but only the control group demonstrated a significant difference between trial 3 (habituation) and 4 (spatial change). Mann-Whitney test, * = p < 0.05.
Running + KA + celecoxib 40 mg/kg

Figure 13: A two-way ANOVA indicated an overall significant difference across trial (moved objects: $F(4, 100) = 4.044, p<0.01$, non moved objects: $F(4, 100) = 6.400, p<0.001$) and across group (moved objects $F(4, 100) = 2.497, p<0.05$, non moved objects: $F(4, 100) = 3.172, p<0.05$) but there was no significant interaction between trial and group. A Kruskal-Wallis one-way ANOVA showed that there were significant differences between trials for the control (non moved objects: $\chi^2 = 12.513, df = 4, p = 0.001$; moved objects: $\chi^2 = 11.328, df = 4, p = 0.023$); Run+ KA + celecoxib 5 days (non moved objects: NS; objects moved: $\chi^2 = 12.151, df = 4, p = 0.016$) and celecoxib 40 mg/kg (non moved objects: $\chi^2 = 13.473, df = 4, p = 0.009$; objects moved: $\chi^2 = 7.972, df = 4, p = 0.033$) groups. Post hoc Mann-Whitney tests showed that the 3 groups decreased exploration over the 3 trials indicating habituation ($p<0.05$). Post-hoc tests showed a significant difference between trial 3 (habituation) and trial 4 (reaction to spatial changes) only for control and the celecoxib 40 mg/kg group ($p<0.05$).
Moved objects

- control
- celecoxib (Cxb) 40mg
- run+KA+Cbx 40 mg ONCE
- run+KA+Cbx 40 mg 5 days
- KA 12 mg/kg

Non moved objects

Figure 15: The control and celecoxib 40 mg/kg groups demonstrated a significant difference between trial 3 (habituation) and 4 (spatial change). Mann-Whitney test, *=p<0.05.
3.4 Spontaneous activity in the open field

Figure 14 shows the spontaneous activity of rats in the open field: a one-way ANOVA indicated that there were significant differences between the groups ($F(6,41) = 8.301, p<0.001$). Post-hoc Tukey tests indicate that KA and KA + Run groups covered significantly great distances than controls, celecoxib + KA, celecoxib 40 mg and Run + KA + celecoxib 40 mg groups ($P<0.05$), whereas the celecoxib group walked less than Run + KA + celecoxib group ($P<0.05$).

![Graph showing distance travelled in 3 min in the open field arena.](image)

Figure 14: Distance travelled in 3 min in the open field arena. Post-hoc tests Tukey, $*=p<0.05$, $**=p<0.01$, $***=p<0.001$. Cxb: celecoxib. Unless otherwise indicated, the celecoxib dose was 6 mg/kg.
3.5 Measurement of BDNF

3.5.1 BDNF 6 hours after injuries and after running for 5 nights

Figure 15 shows the quantity of BDNF (picogram/mg protein) in dentate gyrus of the hippocampus for each group, 6 hours after kainic acid injection or ischemia or 5 days training in running wheel overnight for rats. A Kruskal-Wallis one-way ANOVA showed that there were a significant differences between the groups ($\chi^2 = 12.643$, df = 3, $p = 0.005$). Post hoc Mann-Whitney tests showed that the kainic acid, ischemic and running groups had increased levels of BDNF compared to the control group ($p<0.05$).

Figure 15: The graph shows the quantity of BDNF in the dentate gyrus of the right hippocampus of the rat 6 hours after KA injection or 5 days after running. Mann-Whitney U test, * = $p<0.05$. 
3.5.2 Running + kainic acid group

Figure 16 shows the quantity of BDNF in the dentate gyrus of the right hippocampus of the rat after 5 days training in the running wheel overnight, watermaze task and object exploration task. A Kruskal-Wallis one-way ANOVA showed that there was a significant difference between the groups ($\chi^2 = 8.206, df = 3, p = 0.042$). Post hoc Mann-Whitney tests showed that kainic acid + running group had 4 times more BDNF than the control and 5 times more than the run groups ($p<0.05$); moreover, the KA (kainic acid) group had more BDNF than the run group ($p<0.05$).

![Graph showing BDNF levels in different groups](image)

**Figure 16**: The kainic acid + run and KA groups had more BDNF in the dentate gyrus in the right hippocampus than run and control (animals in the running wheel without running) groups at the end of the experiment (4 weeks after KA or saline injection). Mann-Whitney U test, * = $p<0.05$. 

143
3.3 Kainic acid + celecoxib 6 mg/kg and run + KA+ celecoxib groups

Figure 17 shows the quantity of BDNF in the dentate gyrus of the right hippocampus of the rat after 5 days training in running wheel overnight + kainic acid and celecoxib 6mg/kg, and the watermaze and object exploration tasks. The Kruskal-Wallis one-way ANOVA indicated that there were significant differences between the groups ($\chi^2 = 29197$, df = 5, $p<0.001$). Post hoc Mann-Whitney tests showed that the kainic acid group (KA+ vehicle celecoxib) had more BDNF than the control, KA (kainic acid)+ celecoxib (once and 5 days), control, Run+ KA+ celecoxib and Run+ vehicle KA+ celecoxib groups ($p<0.05$). Moreover KA+ celecoxib (once and 5 days) and Run + KA+ celecoxib groups had also more quantity of BDNF bigger than control and Run+ vehicle KA+ celecoxib groups group ($p<0.05$). There was no difference between the 3 celecoxib + KA groups.
Figure 17: The graph shows the BDNF in the dentate gyrus in the right hippocampus of rats at the end of the experiment (4 weeks after KA, celecoxib or saline injection). Mann-Whitney U test, *= p<0.05, **=p<0.01 (celecoxib dose was 6 mg/kg).

3.5.4 Celecoxib 40 mg/kg groups

Figure 18 shows the quantity of BDNF in the dentate gyrus of the right hippocampus of the rat after 5 days training in the running wheel overnight + kainic acid and celecoxib 40 mg/kg, and the watermaze and object exploration tasks. A Kruskal-Wallis one-way ANOVA indicated that there were significant differences between groups ($\chi^2 = 9.975$, df = 3, $p = 0.019$). Post hoc Mann-Whitney tests showed that Run + KA + celecoxib (once
and 5 days) groups and celecoxib (Cxb) 40 mg/kg group had more BDNF than the control (p<0.05), 2 weeks after the first injection of celecoxib at 40 mg/kg.

Figure 18: The diagram shows the BDNF in the dentate gyrus in the right hippocampus of rats at the end of the experiment (4 weeks after KA, celecoxib or saline injection). Mann-Whitney U test, *= p<0.05.
3. Histology

Figure 19 shows the average number of cells in CA1, CA2 and CA3 areas of the hippocampus at 2 levels (-3.6 mm and -5.8 mm posterior to bregma) in the left hemisphere. We included all animals of different experiments, except those that died before the end of our experiments. A one-way ANOVA indicated that there was a significant difference between groups in area CA1 (F(7,76) = 5.127, p<0.001), CA2 (F(7,76) = 3.98, p<0.01) and CA3 (F(7,76) = 3.023, p<0.01) areas at level -3.6 mm and in CA1 (F(7,71) = 7.294, p<0.001), CA2 (F(7,71) = 4.211, p<0.01) and CA3 (F(7,71) = 5.413, p<0.001) areas at level -5.8 mm. At level -3.6 mm (figure 19a), post-hoc Tukey tests demonstrated that KA group (CA1: 145±18 cells, CA2: 50±5 cells, CA3: 169±21 cells) had significant cell loss compared to the control group (CA1: 270±17 cells, CA2: 80±3 cells, CA3: 257±18 cells). The Run+ KA (only CA1: 152±38 cells), KA+ celecoxib 5 days (CA1 only: 208±17 cells) and Run+ KA+ celecoxib once (CA3 only: 151±19 cells) groups also had significant cell loss compared to the control group. Moreover, the KA group had significant cell loss compared to the Run+ vehicle KA + celecoxib group (CA1: 286±30 cells, CA2: 90±8 cells).

At level -5.8 mm (figure 19b), post-hoc tests demonstrated also that KA group (CA1: 291±36 cells, CA2: 75±12 cells, CA3: 336±29 cells) had significant cell loss compared to the control group (CA1: 608±30 cells, CA2: 140±8 cells, CA3: 482±26 cells). The KA+ celecoxib 5 days (CA1 only: 402±34 cells), Run+ KA+ celecoxib once (CA2 only: 73±21 cells, CA3: 220±38 cells) and Run + KA+ celecoxib 5 days (CA3 only: 331±59 cells) groups also had significant cell loss compared to the control group. The Run + Vehicle KA- celecoxib (CA1 only: 727±48 cells) group had more cells than KA (CA1 only: 727±48 cells).
291±36 cells), Run+ KA (CA1 only: 440±34 cells), KA+ celecoxib 5 days (CA1 only: 402±34 cells), KA+ celecoxib once (CA1 only: 447±85 cells) and Run+ KA+ celecoxib once (CA1 only: 383±100 cells).

Figure 20: A one-way ANOVA indicated that there was a significant difference between groups in areas CA1 (F(4,21) = 14.693, p<0.001), CA2 (F(4,21) = 5.121, p<0.01) and CA3 (F(4,21) = 2.739, p<0.05) areas at level −3.6 mm and in CA1 (F(4,21) = 8.834, p<0.001), CA2 (F(4,21) = 1.753, NS) and CA3 (F(4,21) = 4.029, p<0.05) areas at level −5.8 mm. At level −3.6 mm, post-hoc tests demonstrated that KA group (CA1: 145±18 cells, CA3: 169±21 cells) had significant cell loss compared to control group (CA1: 378±15 cells, CA3: 228±9 cells). The Run + KA+ celecoxib 40 mg/kg 5 days (CA1 only: 219±19 cells) and Run + KA+ celecoxib once (CA1 only: 259±28 cells) groups also had significant cell loss compared to control group. Moreover, the KA group had significant cell loss compared to the celecoxib 40 mg/kg group (CA1: 289±19 cells, CA2: 78±3 cells, CA3: 169±21 cells).

At level −5.8 mm, post-hoc tests demonstrated also that KA group (CA1: 291±36 cells) had significant cell loss compared to control (CA1: 611±48 cells) and celecoxib 40 mg/kg (CA1: 481±42 cells) groups; the Run + KA+ celecoxib once (CA1 only: 341±21 cells) and Run + KA+ celecoxib 5 days (CA1 only: 331±25 cells) groups also had significant cell loss compared to the control group. The celecoxib 40 mg/kg (CA3 only: 413±56 cells) group had more cells compared Run+ KA+ celecoxib 40 mg/kg once (CA1 only: 225±16 cells) group.
We grouped all animals to measure the correlation between number of cells counted at the 2 levels and the time finding the platform in the watermaze task (escape latency) for each animal. Figure 21 shows that the correlation coefficient is significant beyond the 0.001 percent level (Pearson correlation: $r = -0.681$, $n = 87$, $p<0.001$).

Figures 22, 23 and 24 show photomicrographs of the left hippocampus (coronal sections) at $-3.6$ mm and $-5.8$ mm posterior to bregma. There are several photomicrographs for the kainic acid group, which show the different degree of cell loss in the same group. There were no overall differences in histology between all kainic acid groups, so a sample of control and kainic acid groups are shown.
Figure 19a: Numbers of cell bodies in CA1, CA2 and CA3 areas of left hippocampus at -3.6 mm level posterior to the bregma. Post-hoc tests Tukey, \(*=p<0.05, **=p<0.01, ***=p<0.001\). There are significant decreases in the number of viable cells after KA (kainic acid) injection in CA1, CA2 and CA3 sub-fields compared to control group and in CA1, CA2 compared to Run+ vehicle KA+Cxb (celecoxib) group. The Run + KA (only CA1), KA+ celecoxib 5 days (only CA1) and Run+ KA+ celecoxib once (only CA3) groups also had significant cell loss compared to control group. There is no difference between all KA groups in the 3 areas. KA dose was 12 mg/kg and the celecoxib dose was 6 mg/kg.
**Figure 19b:** Numbers of cell bodies in CA1, CA2 and CA3 areas of left hippocampus at the -5.8 mm level posterior to the bregma. Post-hoc tests Tukey, * = p<0.05, ** = p<0.01, *** = p<0.001. There were significant decreases in the number of viable cells after KA (kainic acid) injection in CA1, CA2 and CA3 sub fields compared to control group and in CA1 compared to the Run+ vehicle KA+Cxb (celecoxib) group. The KA + celecoxib 5 days (only CA1), Run+ KA+ celecoxib once (only CA2, CA3) and Run+ KA+ celecoxib 5 days (only CA3) groups also had significant cell loss compared to controls. There were no differences between all KA groups in the 3 areas. KA dose was 12 mg/kg and the celecoxib dose was 6 mg/kg.
Figure 20: Numbers of cell bodies in CA1, CA2 and CA3 areas of left hippocampus at -3.6 mm and -5.8 mm levels posterior to the bregma. Post-hoc tests Tukey, * = p<0.05, ** = p<0.01, *** = p<0.001, (Cxb: celecoxib). KA dose was 12 mg/kg.
Figure 21: correlation between number of cells counted in 2 levels (in CA1, CA2 and CA3 areas of left hippocampus at -3.6 mm and -5.8 mm levels posterior to the bregma) and the time spent to find the platform in watermaze task (escape latency) for each animal. (Pearson correlation: r = -0.681, n = 87, p<0.001).
Figure 22: Photomicrographs of the left hippocampus coronal sections at -3.6 mm posterior to bregma (magnification x200). There were no differences between the rat brains of the different groups injected with kainic acid (KA). We showed here only sections from the kainic acid and sham animals. In CA1, CA2 and CA3, KA rats (B, C, E, F, H, I: example different degrees of cell loss with kainic acid (i) and (ii)) showed a substantial decrease in the number of viable pyramidal cell bodies compares to the control group.
Figure 23: Photomicrographs of the left hippocampus coronal sections at -5.8 mm posterior to bregma (magnification x200). There were no differences between the rat brains of the different groups injected with kainic acid (KA). In CA1, KA rats (B, C, E, F: different degrees of cell loss with kainic acid (i) and (ii)) showed a substantial decrease in the number of viable pyramidal cell bodies compares to the control group.
Figure 24: Photomicrographs of the left hippocampus coronal sections at -5.8 mm posterior to bregma (magnification x200). We show here only sections from the kainic acid and sham animals. KA rats (H, I, J, L, M, N: different degrees of cell loss with kainic acid (i), (ii) and (iii), in the same group) showed fewer viable pyramidal cell bodies than the control group.
4. Discussion

The apparent obligatory role of the hippocampus in spatial orientation and learning has been the subject of intensive experimental investigation and theoretical speculation for many years (e.g. O'Keefe and Nadel, 1978; Kessels et al., 2001; Warburton et al., 2001; Burgess et al., 2001; Gaffan et al., 2000; O'Mara, 1995; Rolls and O'Mara, 1993). Here, we have shown that KA-induced neuronal destruction in areas CA1 and CA3 of the hippocampus (Baran et al., 1994) leading to a major deficit in spatial learning. KA induces injury in the brain by over-stimulating glutamate receptors, leading to the chronic depolarisation of cells, thereby provoking a cascade of reactions resulting in apoptosis and necrosis, an inflammatory response, and ultimately leading to the production of prostaglandins and also to oedema, probably induced by excessive activation of arachidonic acid (AA) (Rao et al., 1999; Winkler et al., 2000). Indeed, AA induces oedema probably via free radical production (Ohnishi, 1992), which induce a chain reaction causing disruption of membrane integrity (via a dislocation of Na⁺/K⁺-ATPases) and leading to cell glial swelling (Winkler et al., 2000). Thus, mannitol reduces KA-induced irreversible brain damage and inhibits propagation of epileptic seizure (Baran et al., 1987b). Superoxide dismutase also reduces AA-induced cell swelling, demonstrating the involvement of superoxide anions in this process; by contrast, BW 755C, a dual inhibitor of cyclo- and lipoxygenases does not reduce the cell swelling (Winkler et al., 2000).

We tested different doses of KA (6 and 12 mg/kg), in the spatial version of the watermaze task and we found that only the 12 mg/kg group was impaired in finding the hidden platform; they swam longer than controls and the 6 mg/kg group and did not
meaningfully learn the task. We also investigated if the KA-treated animals had motor problems, using the non-spatial and non-hippocampal visible platform version of the watermaze task; the KA group learned the location of the visible platform over days, confirming that this group did not have any visual or motor impairment; furthermore, the analysis of the swim velocity (in the hippocampal version of the watermaze) between control and KA groups did not show any difference. However it is clear that the KA group performed worse than the control group (figure 4) indicating some impairments, but these do not appear to be sensory or motor in nature. Thus, it seems clear that the deficiency of the KA group in the spatial task was due to the lesions of the hippocampus, mainly in the CA1 and CA3 regions and not to visual or motor deficits. It is less clear if these animals have an odour discrimination deficit because, perhaps due to hyperactivity, the KA group found the food as quickly as the control group, and the task may not have been sufficiently sensitive to see if the animals were able to associate odour and reward. By contrast, when we evaluated our animals in the watermaze and object exploration tasks, we found that in both tasks the KA group was impaired, showing the important role of hippocampus in spatial learning and exploration.

To investigate how the brain might recover after injury and how to protect it after injury we used several different pharmacological and physical activity-based protocols. Indeed, we showed in the preceding chapter that an enriched environment increases BDNF levels in the dentate gyrus; environmental enrichment also induces neurogenesis (Eriksson et al., 1998; Kempermann et al., 1998; Kornack and Rakic, 1999). However, we found that an enriched environment was without any effect on the KA group, irrespective of the measures used: escape latency, distance swam or direct path swam. The damage induced
by KA in the CA fields was probably too great for it to be compensated by any positive action of the enriched environment. Furthermore, the histological analyses did not indicate any difference between the KA-enriched and standard condition groups (data not shown). Rutten et al. (2002) found similar results to ours regarding histological evaluation, but also found an enriched environment also improved performance in the watermaze when young rats (20 days postnatal) were raised in environmentally enriched conditions. It remains possible that if our animals had been raised in enriched conditions for a much greater period of time then there might have been an ameliorative or neuroprotective effect of such enrichment.

Inflammation is one of the components leading to neuronal death after KA injection. COX-2 is implicated in this mechanism, as it catalyses the first reaction leading from arachidonic acid to prostaglandins. We investigated the effect of the broad spectrum (COX-1 and COX-2 inhibitor) COX inhibitor, ibuprofen on KA-induced damage. Contrary to what we had expected, no difference was found in any of the behavioural tasks or in cell viability or BDNF measurement (data not shown), but the KA + ibuprofen group animals were sicker and their death rate was increased relative to the other groups. Baik et al. (1999) reported that COX inhibitors, especially COX-2 inhibitors, aggravated KA-induced seizures and mortality in mice; endogenous prostaglandins appear to possess some anticonvulsant properties and inhibiting these may explain the aggravation of KA-induced seizures. However, Baran et al. (1994) found the opposite effect for COX-2 inhibitors: they protected against the KA-seizure and its consequent neurotoxic effects. Rapoport and Bosetti (2002) found that lithium and anticonvulsant drugs might target the arachidonic acid cascade by reducing arachidonate turnover. Sodium salicylate (aspirin
mtabolite) has a similar effect to ibuprofen when given simultaneously with KA (Najbauer et al., 2000). Najbauer et al. suggest that KA activates KA-type glutamate receptors, leading to depolarisation and further release of glutamate, which activates NMDA receptors, therefore causing massive intracellular influx of Ca^{2+}. High levels of intracellular Ca^{2+} in turn trigger calcium uptake by mitochondria. During this uptake of calcium by mitochondria, mitochondrial membrane potential is attenuated because the permeability transition pore (PTP) megachannels are opened leading to a drop in ATP synthesis. Under lesser stimulation, the cells may recover their homeostasis. However, because salycilate inhibits the opening of the PTP channel, it leads to an increase in the excitotoxicity seizure activity. It may be that ibuprofen (a broad spectrum COX inhibitor) has an action similar to salicylate. Our results agree with those of Baran et al. (1994), i.e. that the COX-2 selective inhibitor has a neuroprotective effect. Indeed, for KA-treated rats, celecoxib, a specific COX-2 inhibitor, improved spatial learning in the watermaze task, in both escape latency and distance swam, and especially in the consolidation of memory (i.e. after 3 days break) as well as in object exploration. This confirmed our expectations: by blocking inflammatory processes with a COX-2 inhibitor, we stopped one of mechanisms leading to injury after KA injection. There was an improvement in spatial learning and the animals were also sensitive to environment change (object exploration), although we did not find a difference in histology, as there was no significant difference between the KA and KA + celecoxib groups. After 12 mg/kg KA injection, there were 25% more cells in the celecoxib + KA than KA groups; it may be that there was a better recovery of the viable cells under these conditions, than a particular protection of neurons against cell death. The quantity of BDNF found in the
KA+ celecoxib group was still increased, 2 weeks after KA injection, compared to the control group. We did not expect to find this result: Chen et al. (2002), however, found that in normal conditions that COX-2 inhibition reduces membrane excitability because the production of prostaglandin E₂ (PGE₂) was decreased. Thus, PGE₂ seems involved not only in inflammation but also in synaptic signalling. Extrapolating this finding to our experiments could explain why firstly, our animals injected with celecoxib were less excitable than those injected only with KA (as shown by the open field task) and secondly that the level of BDNF was less increased in the KA + celecoxib group, compared to the KA group, because if PGE₂ signalling modulates postsynaptic membrane excitability (Chen et al., 2002), then decreasing the production of PGE₂ could prevent the propagation of depolarisation, hence attenuating the excitotoxicity. Therefore celecoxib might act via blocking inflammation and membrane excitability.

Sustained physical activity such as running modifies the expression of numerous genes. The majority of genes induced are associated with plasticity and synaptic structure (Tong et al., 2001). We demonstrated here that BDNF protein is augmented after 5 days of sustained exercise in the running wheel; furthermore, we have shown that BDNF has a probable role in neuroprotection, as our results show that after a brain injury, levels of BDNF were augmented (figures 15 and 16). Our results suggest that 5 days running permitted these animals to perform in spatial learning better than KA-induced group, even after KA injection, and that they were also sensitive to environmental change. If after 2 weeks, there was any difference in BDNF levels between the run only and control groups, the augmented levels of BDNF may have come back to normal levels without physical activity. We did not find any differences in cell counts during subsequent...
histological analysis. Thus, it is possible that running did not protect against cell loss directly, but instead protects viable cells, and BDNF increases permit a better connection between these surviving cells, increasing the density of dendritic spines (such as can occur in an enriched environment; Moser et al., 1994). Okazaki et al. (1995) and Kotti et al. (1997) found that there was a reorganization of mossy fibres in the hippocampus innervating novel target neurons, including granule cells after inducing status epilepticus. Scharfman et al. (1999) hypothesized that if BDNF's effects were specific for mossy fibres, then responses of granule cells to their new mossy fibres input would be enhanced by BDNF. We did not find an improvement in histologic evaluation after running compared to controls despite the fact that BDNF blocks caspase-3, an enzyme primarily involved in apoptosis (Han et al., 2000b).

We expected a further improvement in recovery when we added pre-running and celecoxib together after KA injection. However, the combined treatment did not cause an improvement or enhancement in recovery at all compared to either the KA group, the Run + KA + celecoxib (6 mg/kg), either in the spatial watermaze task or in the object exploration task or in subsequent histological evaluation, but BDNF levels were increased compared to the control group. In fact, this “hope of double protection” or “synergistic expectation”, negated the single effects of running and celecoxib together without blocking an increase in BDNF. As stated above, running increases numerous mRNA types, some of which are involved in plasticity, and particularly BDNF mRNA, which is more involved in neurotrophic and neuroprotective action (Cotman and Berchtold, 2002). This may explain the improvement in the spatial task performance after KA injection, which increases both BDNF and COX-2 mRNA (here we have shown there
was BDNF protein increase also). The BDNF increase permits recovery of cells and attenuates the effect of increased COX-2 and of PGE₂, which enhances membrane excitability (Chen et al., 2002). COX-2 inhibitors block the PGE₂ production induced after KA injection, attenuate both inflammation and increased membrane excitability, permitting an improvement also in the watermaze task. We hypothesise that in these two cases, BDNF production plays a role in tissue repair, permitting viable cells to compensate for any tissue loss. Other experiments have shown that BDNF can under some conditions exacerbate the injury caused by KA (Rudge et al., 1998); Lahteinen et al. (2000) showed that transgenic mice overexpressing truncated TrkB (a dominant negative receptor of BDNF) had less severe KA-induced seizures with later onset and lower mortality; and Croll et al. (1999) have found that transgenic mice overexpressing BDNF exhibit an increased severity of KA-induced seizure; BDNF can also induce hyperexcitability in the mossy fibres of an epileptic hippocampus (Scharfman et al., 1999). These data all suggest that under certain conditions BDNF may contribute to epileptogenesis.

Our data tend to confirm these prior results (run + KA + Celecoxib 6 mg/kg) but with an increased dose of celecoxib (40 mg/kg); there was no effect of the run + KA + celecoxib 40 mg/kg: there was no amelioration of the spatial learning deficit or neuronal viability. Injection alone of celecoxib at 40 mg/kg enhanced BDNF protein levels significantly compared to controls while this group learned the task as well as controls in the watermaze task and were also sensitive to environment change in the object exploration task, contrasting with experiments showing that COX-2 inhibitors block LTP (long-term potentiation) (O’Mara et al., 2001b; Chen et al., 2002). This suggests that any
straightforward mapping of electrophysiological experiments concerned with the neuropharmacological analyses of synaptic plasticity and of behavioural manipulations is not straightforward at all (Martin et al., 2000).

Why did combined running and celecoxib together not improve the performance of the animals after KA-induced brain insult? To summarize:

1) After KA injection, there are increases of COX-2 mRNA, BDNF protein, BDNF mRNA and truncated TrkB (especially on astrocytes; Frisen et al., 1992), while full-length TrkB protein is unchanged (Rudge et al., 1998). However, the increase in BDNF is not enough to compensate for the brain insult.

2) When animals are exercised before the KA-induced insult, the expression of BDNF protein, BDNF mRNA and other plasticity genes is enhanced (Cotman and Berchtold, 2002). Thus, levels of full-length TrkB protein, which could be located preferentially at mossy fibre buttons (Scharfman et al., 1999) may be enhanced more quickly. The recovery of animals after KA treatment may be due to increases in both BDNF, its active receptor, TrkB and their involvement in the reorganization of cells innervating novel target neurons (Okazaki et al., 1995)

3) Celecoxib acts by blocking COX-2, which in turn is increased after KA injection; it decreases PGE$_2$ production, preventing brain inflammation, and reducing postsynaptic membrane hyper-excitability. COX-2, in normal conditions, participates in hippocampal synaptic plasticity through regulation of PGE$_2$ (O'Mara et al., 2001b; Chen et al., 2002), but after an injury, PGE$_2$ might also be
excitotoxic. This could explain the positive action of COX-2 inhibitors on brain insult.

4 We hypothesized that the combination of running and celecoxib together after KA-induced seizure did not improve the performance of the animals in different tasks, because celecoxib reduced postsynaptic membrane excitability via PGE$_2$ down-regulation, and also prevented the BDNF-induced formation of new synaptic contacts (sprouting). This increase in BDNF levels (running + KA induced) may therefore be neuropathological, increasing neurotransmitter release and inducing an inconsistent alteration of synaptic strengths (Binder et al., 2001).

In conclusion, we found two ways to ameliorate KA-induced insult, and we therefore have shown that there are several distinct mechanisms involved in this injury. We suggest that there is a relation between COX-2 and BDNF, probably linked to synaptic transmission. Finally, although BDNF is involved in the survival, maintenance and growth of neurons; it may also have a negative role under several conditions following brain insult.
Chapter IV: Evaluation of celecoxib pre-administration in kainic acid-induced seizure
# Contents

1. Introduction .................................................. 168

2. Materials and methods ....................................... 171
   2.1 Animals .................................................. 171
   2.2 Kainic acid and celecoxib injections ................. 171
   2.3 Behavioural testing ...................................... 172
     2.3.1 Watermaze ........................................... 172
     2.3.2 Object exploration task ............................ 172
     2.3.3 Open field testing .................................. 174
   2.4 BDNF Elisa procedure .................................. 174
   2.5 Quantification of protein using the Bradford assay 175
   2.6 Histology ............................................... 176
   2.7 Statistics .............................................. 176

3. Results ..................................................... 178
   3.1 Watermaze task analysis ............................... 178
   3.2 Object exploration task ................................ 180
   3.3 Spontaneous activity in the open field .............. 183
   3.4 Measure of BDNF ....................................... 184
   3.5 Histology .............................................. 185

4. Discussion .................................................. 187
1. Introduction

Following ischemia or seizure, tissue damage continues to occur as a result of inflammation (Perry et al., 1998), excitotoxicity (Lipton et al., 1994) and apoptosis (Simonian et al., 1996). The COX-2 enzyme seems to play an important role in brain injury: after an ischemia-induced or KA-induced seizure, COX-2 mRNA and protein (and also its metabolite, prostaglandin E₂ (PGE₂); Kim et al., 2001; Nogawa et al., 1997; Yanagata et al., 1993) are all increased. KA-treatment increases COX-2 and PGE synthase (the enzyme that catalyses the formation of PGE₂ from PGH₂) levels in the whole brain, and these increases are greater in hippocampus and cortex than the whole brain, as Western blot analyses indicate that COX-2 and PGE synthase bands were more intense for hippocampus than cortex (Ciceri et al., 2002). PGE₂ is involved in inflammatory processes (Cryer and Dubois, 1998); Takadera et al. (2002) showed also that PGE₂ is implicated in apoptosis in cortical cells, acting on one of its 4 receptors (the EP₂ receptor), which, in turn activates caspase-3, itself centrally implicated in apoptosis (Takadera et al., 2002). PGE₂ also participates in the regulation of membrane excitability (Baga et al., 2001) and long-term synaptic plasticity in hippocampal perforant path-denate gyrus synapses (O’Mara et al., 2001b; Chen et al., 2002). After brain insult, among others, there is an increase in both COX-2 and certain neurotrophins, especially BDNF. By these responses, the brain tries to compensate for the damage caused; there is, indeed, synaptic sprouting and new connections formed between neurons (Okazaki et al., 1995). However, animals with KA-induced injury show impairments in spatial learning and in object exploration (see previous chapter); therefore, these neuroprotective
responses do not seem sufficient to ameliorate the injury. We demonstrated in the previous chapter that by blocking COX-2 with an inhibitor after KA induced seizure, the performance of animals improves in both spatial learning and in object exploration. It seems possible, therefore, that excess PGE$_2$ induced by the increase of COX-2, is harmful. However, COX-2 and PGE$_2$ are also involved in membrane excitability and long-term synaptic plasticity in the hippocampus. Moreover, exogenously applied prostaglandins seem to protect neuronal cultures following glutamate or hypoxic injury: Otaki et al. (1994) found that PGE$_1$ protects rat hippocampal cells against hypoxic injury, while Akaike et al. (1994) demonstrated that PGE$_2$ protects cultured cortical neurons against glutamate cytotoxicity. Prostaglandins, however, do not seem to inhibit free radical-related membrane damage (Cazevieille et al., 1994). Kim et al. (2001) found that in cortical neurons, COX-2 inhibitors (NS398 and celecoxib) failed to inhibit PGE$_2$ induced by kainic acid administration, while COX-2 inhibitors block PGE$_2$ production in kainic acid-treated and in basal condition hippocampal neurons. We ask here, what is the effect of completely blocking prostaglandin production in the hippocampus prior to KA-induced seizure? It may be the case, for example, that the transient production of prostaglandins is neuroprotective, whereas the sustained production of prostaglandins is neuropathological. Therefore, one prediction is that preventing the production of prostaglandins prior to the KA-induced insult may result in neuropathological consequences that are equivalent to the sustained overproduction of prostaglandins arising from KA-administration.

Other processes are involved independently of PGE$_2$ production after KA administration, such as the release of the serine protease tissue plasminogen activator (tPA), which as
expressed in both neurons and microglia. Glutamate agonists can induce the release of tPA and it converts plasminogen to plasmin. tPA may play a role in synaptic plasticity and in the growth of new synaptic connections (Huang et al., 1996).

We gave celecoxib (the specific COX-2 inhibitor) at 6 mg/kg to rats, for 5 days prior to induced KA-seizure and we then tested animals in the watermaze task and object exploration task, and then evaluated the histological and biochemical outcomes to test the prediction stated above.
2. Materials and Methods

Experiments were conducted in accordance with European Community directive, 86609/EC, and the Cruelty to Animals Act, 1876 and followed local and international guidelines of good practice.

2.1 Animals

Male Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland) (mean weight 25-300 g at the start of the experiment) were used. The rats were randomly assigned to different experimental groups after their arrival from the BioResources Unit. The animals were housed under standard conditions, in a temperature-controlled (20°C), laminar airflow cupboard and maintained on a 12-hr light-dark cycle (lights on at 7 am). All testing was carried out during the light phase. Food and water were provided ad lib.

2.2 Kainic acid and celecoxib injections

Rats were injected with celecoxib (6 mg/kg i.p., Celebrex, SEARLE division of Monsanto Ireland Ltd) or its vehicle (10% DMSO) once a day/5 days or once 2 hours before KA injection (12 mg/kg intraperitoneally (i.p.), Sigma-Aldrich, Dublin 24, Ireland) dissolved in saline or simply with saline (control). Rats were tested 3 days after KA injection, using the watermaze task, followed by the object exploration task.
2.3 Behavioural testing

2.3.1 Watermaze

The watermaze was a black circular pool filled with water at room temperature (diameter = 200 cm, height = 35 cm), placed in a room with many extra-maze cues. 3 days after the KA injection, rats were trained to find a platform hidden (diameter = 9.5 cm, height = 29 cm) 2 cm below the water surface. The maze was divided in 4 quadrants, North West (NW), South West (SW), North East (NE) and South East (SE). The platform was centred in the NW quadrant and the start position was always south. At the start of all trials, the rat was placed in the water facing the maze wall and swam until it found the platform where it waited for 15 seconds (sec) before removal. The rat was guided to the platform after 60 sec, if it failed to find the hidden platform. Swim path were recorded by an image analysis system (EthoVision, Noldus, Wageningen, Netherlands). Rats were given 5 trials/day for 8 days with a 10 sec ITI: 5 days learning (acquisition phase) with 3 days retention testing after a 3 day-rest period.

2.3.2 Object exploration task

2 weeks after the injection, food was provided ad lib again. Habituation and reactions to spatial changes were evaluated by the exploration of objects placed in an open field, which consisted of a black, circular fibreglass arena (diameter = 200 cm, height = 35 cm). The floor was covered with sawdust and the environment was homogeneous. The
experimenter stood in a fixed position at the side of the arena and recorded the number of nose contacts each rat made with the individual objects. The aim of this experiment is to see the sensitivity of animals after a environment changing.

Initially, each rat was allowed to explore the empty arena for a period of 6 min. During the ITI, the rat was confined in its cage outside the experimental room. After this phase, 4 objects were positioned in the centre of the arena and a white and black striped pattern (40 x 30 cm) was attached to the side-wall. The objects were heavy enough not to be displaced by the rats and consisted of a filled plastic jerry-can, a full pitcher, polystyrene box and cement cylinder. The rat was given 5 trials of 6 min duration with 3 min of ITI. For trials 1-3, the objects remained in the same place (Figure 4a). In trial 4 the cement cylinder was substituted for the pitcher, which itself was moved to a new location (moved objects) at the periphery of the open field (Figure 4a), trial 5 was the same as trial 4. In order to mask possible odour trials, the sawdust was raked during the ITI: the non-displaced objects were also manipulated as were the displaced objects. Object exploration was evaluated by the time spent in contact with the objects (i.e., when the rat’s snout touched an object).
2.3.3 Open field testing

Rats were tested in a single 3 minute trial at the same time during the light phase. The open field was a black circular arena (diameter = 200 cm, height = 35 cm). The animals were placed in the centre of the arena and the distance they travelled was automatically recorded by an image analysis system (EthoVision, Noldus, Wageningen, Netherlands).

2.4 BDNF Elisa procedure

All rats were killed on the last day of the training, their brains removed and tissue taken from the dentate gyrus, hippocampus, and entorhinal cortex. To prepare the samples, dentate gyri were homogenised in ice-cold Krebs 25 times. Samples were centrifuged and supernatant retained. Protein was assessed and samples were diluted to give equal protein concentrations and stored at -80°C. Ninety-six well plates were coated with 100 µl anti-BDNF monoclonal antibody diluted (1:1000) in 0.025 M carbonate-bicarbonate buffer. Plates were covered, incubated overnight at 4°C and plates were subjected to interceding washes to remove excess antibody. Plates were blocked for non-specific binding for 1 hour at room temperature and washed (composition of wash buffer in mM: Tris-HCl, 20; NaCl, 150 containing 0.05% Tween (v/v); pH 7.6). Samples of dentate gyrus (50 µl), supernatant (50 µl) or BDNF standards (50 µl; ranging from 0.0078 to 1 ng/ml) were added to the wells, which were covered, incubated for two hours at room temperature.
with shaking and incubation overnight at 4°C and washed. Aliquots (100 μl) of anti-human BDNF pAb (diluted 1:500) were added to the wells, plates were incubated for 2h at 37°C and washed. Aliquots (100 μl) of anti-IgY HRP (1:2000 dilution) were added to wells and incubated for 1h at 37°C. During this incubation, the enzyme substrate was prepared. Plates were washed and 100 μl of this substrate was added to the wells, incubated for approximately 15 minutes until a blue colour formed in the wells. The reaction was stopped by the addition of 100 μl of 1 M phosphoric acid to the wells. Plates were read a 450 nm in a 96-well plate reader and BDNF concentrations were estimated for the standard curve and expressed as pg/mg protein. (BDNF Emax ™ ImmunoAssay System obtained from Promega UK LTD).

2.5 Quantitation of protein using the Bradford assay

The protein concentration of tissue samples was calculated according to the method of Bradford (1976). The standard curve was run using concentrations ranging from 2 μg/ml to 100 μg/ml prepared in a final volume of 160 μl, from a stock solution of 200 μg/ml bovine serum albumin (BSA). Tissue samples (5 μl) were diluted 1:40 in distilled water (155 μl) and Bio-Rad dye reagent (40 μl), which was added to all samples including the standards. Absorbance was read in a 96-well plate reader at a wavelength of 630 nm, following gentle agitation. The concentration of protein in samples was calculated with
reference to the standard curve plotted from the absorbance of the BSA standards. Protein concentrations were expressed as mg protein/ml tissue homogenate.

2.1 Histology

Histological evaluation of the brain lesions was carried out 1 day after the last watermaze trial. Rats were killed by decapitation and their brains promptly removed and frozen in chilled isopentane (-30°C). Coronal cryostat sections 10 µm thick were cut and stained with methylene blue 1%. Under light microscopy, sections were examined at different levels, -3.6 mm and -5.8 mm posterior to bregma (after the method of Colbourne and Cobett, 1995, modified for this experiment). The quantification of ischemic brain damage was done by counting cells in CA1, CA2 and CA3 areas of the left hemisphere, in order to determine the grade of pyramidal cell loss. Counting was performed using x400 magnification with a counting grid measuring 12.5 x 12.5 mm superimposed over the different levels, and the assessment was performed in a blind manner.

2.7 Statistics

The watermaze and object exploration data were analysed using a 2-way ANOVA with group and day as factors. Post-hoc comparisons were made using Tukey's method. In cases where test of homogeneity of variances was <0.05, groups were compared using the
Kruskal-Wallis one-way ANOVA by ranks, followed by individual comparisons between groups using the Mann-Whitney U test. A p value of 0.05 was considered as the threshold for a significant difference. The odour discrimination and the histological damage data were analysed with one or two-way ANOVAs as appropriate; a value of p<0.05 was considered to be significant. Post-hoc comparisons were made using Tukey’s method. All data are presented as mean (± sem) distance, time or cells.
3. Results

3.1 Watermaze task analysis

Figure 1 shows the escape latencies and distances swum for celecoxib 6 mg/kg (5 days, n=5) + KA, celecoxib 6 mg/kg once (n=5) + KA (all celecoxib groups are grouped because there was no significant difference between them; we point out here that about 50% of animals treated with celecoxib prior to KA-induced seizure were dead after 2-3 days; and thus, PGE₂-inhibition aggravated the consequences of KA-induced seizure), KA + vehicle celecoxib (DMSO) (n=5) and control (n=6). The two-way ANOVA indicated an overall significant difference across day (time: F(7,641) = 16.27, p<0.001; distance: F(7,641) = 6.638, p<0.001), group (time: F(2,641) = 182.741, p<0.001; distance: F(2,641) = 126.92, p<0.001) and a significant interaction between day and group (time: F(14,641) = 3.157, p<0.001; distance: F(14,641) = 1.794, p<0.05). The one-way ANOVA indicates that Control (time: F(7,232) = 23.401, p<0.001; distance: F(7,232) = 17.233, p<0.001), celecoxib + KA (time: F(7,217) = 3.305, p<0.01; distance: NS) and KA (time: F(7,192) = 3.094, p<0.01; distance: NS) groups improved over days. For within-day comparisons, ANOVA showed significant difference between groups (time and distance); on day 1, 2, 3, 4, 5, 9, 10, 11 (day 1: F(2,82) = 5.081, p<0.01; day 2: F(2,82) = 7.988, p<0.01; day 3: F(2,82) = 19.228, p<0.001; day 4: F(2,82) = 24.747, p<0.001; day 5: F(2,82) = 29.267, p<0.001; day 9: F(2,77) = 13.678, p<0.001; day 10: F(2,77) = 25.169, p<0.001; day 11: F(2,77) = 21.418, p<0.001), control group swam less than KA and celecoxib + KA groups (p<0.001), while post-hoc shows any difference between KA and celecoxib + KA groups. The control group also found the platform
faster than all other groups from day 1 to day 11 (day 1: $F(2,82) = 11.249$, $p<0.001$; day 2: $F(2,82) = 4.393$, $p<0.05$; day 3: $F(2,82) = 22.275$, $p<0.001$; day 4: $F(2,82) = 33.971$, $p<0.001$; day 5: $F(2,82) = 49.504$, $p<0.001$; day 9: $F(2,77) = 20.413$, $p<0.001$; day 10: $F(2,77) = 50.737$, $p<0.001$; day 11: $F(2,323) = 26.323$, $p<0.001$), (post-hoc: $p<0.001$).

Thus, the KA group was as slow as the celecoxib + KA groups on each day of training.

Figure 1a: The control group (celecoxib vehicle) found the hidden platform significantly faster than the kainic acid (KA, 12 mg/kg) and the celecoxib + KA groups.
**distance swam**

- Cxb before KA (n=6)
- control (n=6)
- KA (n=5)

![Graph showing distance swam](image)

*Figure 1b:* The control group swam significantly shorter distances between the start point and the hidden platform than the KA (12 mg/kg) and the celecoxib + KA groups.

### 3.2 Object exploration task

*Figure 3:* A two-way ANOVA indicated an overall significant difference across trials (moved objects: $F(4, 65) = 3.348$, $p<0.05$, non moved objects: $F(4, 65) = 3.618$, $p<0.05$) and across groups (moved objects: $F(2, 65) = 7.571$, $p<0.01$, non moved objects: NS) but...
there was no significant interaction between trial and group. A Kruskal-Wallis one-way ANOVA showed that there was significant difference between trials for the control (non moved objects: $\chi^2 = 15.473$, df = 4, p<0.01; moved objects: $\chi^2 = 20.064$, df = 4, p<0.001) and celecoxib before KA (non moved objects: $\chi^2 = 10.559$, df = 4, p<0.05; moved objects: NS). Post-hoc Mann-Whitney tests showed that these groups decreased exploration over the 3 trials indicating habituation (p<0.05) for control and over the 5 trials for celecoxib before KA (p<0.05). Post-hoc tests showed a significant difference between trial 3 (habituation) and trial 4 (reaction to spatial changes) (p<0.05), only for moved object control group.
Figure 2: The control group showed a significant difference in exploration between trial 3 (habituation) and 4 (spatial change). Mann-Whitney test, **=p<0.01.
3.3 Spontaneous activity in the open field

Figure 3 shows the spontaneous activity of rat in the open field; a one-way ANOVA indicates that there was a significant difference between groups (F(2,23) = 9.254, p<0.01). Post-hoc Tukey tests indicate that the KA group covered significantly more distance than the control group in the open field.

![Graph showing distance travelled in 3 min in the open field arena. Post-hoc tests Tukey, **=p<0.01, Cxb: celecoxib. KA dose was 12 mg/kg.](image)

**Figure 3**: Distance travelled in 3 min in the open field arena. Post-hoc tests Tukey, **=p<0.01, Cxb: celecoxib. KA dose was 12 mg/kg.
3.4 Measurement of BDNF

Figure 4 shows the quantity of BDNF in the dentate gyrus of the right hippocampus of the rat after one injection per day/5 days celecoxib injection at 6 mg/kg (or a single injection 2 hours before KA) + kainic acid and the watermaze and object exploration tasks. A Kruskal-Wallis one-way ANOVA indicated that there were significant differences between the groups ($\chi^2 = 12.182$, df = 3, $p = 0.007$). Post-hoc Mann-Whitney tests showed that celecoxib (single and 5 days injections) prior to KA, celecoxib (Cxb) before saline and kainic acid groups had more BDNF than the control group ($p<0.05$).

![Figure 4](image)

**Figure 4**: The graph shows all groups had more BDNF in the dentate gyrus in the right hippocampus than control group at the end of the experiment (4 weeks after KA or saline injection) Mann-Whitney U test, *= p<0.05 (Cxb-celecoxib).
3.5 Histology

Figure 5: A one-way ANOVA indicated that there was a significant difference between groups in areas CA1 (F(3,17) = 20.233, p<0.001) and CA2 (F(3,17) = 4.068, p<0.05) at level -3.6 mm and in areas CA1 (F(3,17) = 7.557, p<0.01), CA2 (F(3,17) = 3.452, p<0.05) and CA3 (F(3,17) = 8.674, p<0.001) at level -5.8 mm. At level -3.6 mm, post-hoc tests demonstrated that KA (CA1: 145±18 cells) and celecoxib before KA (single and 5 days injections were grouped) (CA1: 218±30 cells) groups had significant cell loss compared to control (CA1: 431±23 cells) and celecoxib before saline (CA1: 370±57 cells) groups.

At the -5.8 mm level, post-hoc tests also demonstrated that KA (CA1: 291±36 cells, CA3: 336±29 cells) and celecoxib before KA (CA1: 339±45 cells, CA3: 238±18 cells) groups had significant cell loss compared to control (CA1: 670±115 cells, CA3: 479±22 cells) and celecoxib before saline (CA1: 339±45 cells, CA3: 430±10 cells) groups.
Figure 5: Densities of the cell bodies in CA1, CA2 and CA3 areas of left hippocampus at -3.6 mm and -5.8 mm levels posterior to the bregma. Post-hoc tests (Tukey), * = p<0.05, ** = p<0.01, *** = p<0.001 (Cxb-Celecoxib, KA dose was 12 mg/kg).
4. Discussion

The expression of COX-2 in selected neurons and its upregulation after ischemia and KA-induced seizure, indicate that after these brain insults, COX-2 is one major component of cell death via the production of PGE$_2$ and reactive oxygen species (Kaufmann et al., 1997; Koroshetz and Moskowitz, 1996). Ciceri et al. (2002) showed that (24 hours post KA-treatment) dexamethasone inhibits COX-2 synthesis and PGE$_2$ levels. However, dexamethasone administration 6 hours post-KA injection had little effect on PGE$_2$ levels, possibly because it blocks the formation of the new COX-2 enzyme. Thus, in the normal rat brain, there are constitutive levels of COX-1 and low constitutive levels of COX-2 (Kaufmann et al., 1997), producing a low but constant basal quantity of PGE$_2$, which was not affected by dexamethasone. Basal levels of PGE$_2$ are probably important for neuron membrane excitability and synaptic activity (Yamagata et al., 1993; Chen et al., 2002): we found that, however, 40 mg/kg of celecoxib (a selective COX-2 inhibitor) did not impair learning in the watermaze task, nor did it affect behaviour in the object exploration task, compared to normal controls. Moreover, celecoxib appears to have little effect on COX-2 mRNA levels in the KA-treated rat brain (Ciceri et al., 2002). Most prostaglandins, particularly PGE$_2$, have increased expression post-brain injury, and they seem to also have a neuroprotective role by preventing neuronal apoptosis and by limiting microglial activation (Akaike et al., 1994; Perry et al., 1998; Zhang and Rivest, 2001). Paradoxically, however, Takadera et al. (2002) have shown the participation of PGE$_2$ in apoptosis via a direct activation of caspase-3; Pepicelli et al. (2002), found that infusion of N-Methyl-D-Aspartate (NMDA) in hippocampus increases PGE$_2$ levels and free radicals, suggesting a link between NMDA
receptors and COX-2 activity. Pepicelli et al. (2002) further suggest that free radicals mediate the toxicity associated with COX-2 activity, and suggest that PGE$_2$ may play a neuroprotective role. They measured the level of isoprostane 8-epi-PGF$_{2\alpha}$, which is a specific and reliable marker of in vivo lipid peroxidation and oxidative damage (Greco et al., 2000). It is produced either via the COX-2 pathway or possibly via some non-cyclooxygenase mechanism (Morrow et al., 1990). The existence of these two pathways may explain our results; indeed, celecoxib is given alone at 6 mg/kg (for 5 days) it increased levels of BDNF; if celecoxib inhibits constitutive COX-2 in the hippocampus, then normal arachidonic acid levels should increase, leading to the formation of prostanoids such as PGF, by a mechanism involving free radical-catalysed peroxidation of AA (via the non-cyclooxygenase pathway; see figure 6). The increase of free radicals may induce some level of minor damage and the augmented levels of BDNF consequently should have a neuroprotective role. Our experiments indicate that celecoxib administered for 5 days or single injection 2 hours prior KA-induced seizure, strongly increased BDNF levels in the rat hippocampus and that these animals did not learn the platform position in the spatial version of the watermaze task, and these groups were insensitive to environment change in the object exploration task. There was no difference between the 5 day celecoxib treated or single celecoxib treated groups, probably because the most significant neuropathological events had occurred in the first few hours post KA-treatment. Indeed, COX-2 mRNA levels in the hippocampus are strongly induced within 30 minutes after KA injection, peak within 1-2 hours and remain elevated for as long as 8 hours after KA administration, and return to normal levels within about 24 hours (Yamagata et al., 1993). Celecoxib reduces prostaglandin levels to near baseline
levels 4 hours after kainic acid administration (Ciceri et al., 2002); plasma levels of celecoxib decline by about 90% in 24 hours (Paulson et al., 2000). The pre-injection of the COX-2 inhibitor appears to completely block the production of PGE₂. Thus, the celecoxib treatment prior to KA injection has the opposite effect to celecoxib treatment after kainic acid administration (see previous chapter). It seems clear that the window of opportunity for the treatment is very important and, indeed, that a total inhibition of PGE₂ production is as harmful as a large and sustained increase in PGE₂ levels. Kunz and Oliw (2001) found similar results to ours, using nimesulide (a COX-2 inhibitor); mortality increased when nimesulide was given before rather than after kainic acid administration. We found that the celecoxib prior to KA group had the same level of cell loss as the KA-treated group. Thus, a transient increase in PGE₂ levels may have a neuroprotective role, whereas a prolonged augmentation in PGE₂ levels may aggravate brain injury. In both situations, there are increases in BDNF levels (even without injury, when we used celecoxib alone 40 mg/kg) probably indicating the harmful effect of inhibition of PGE₂ production. It is probable that the inhibition of PGE₂ at the beginning of brain insult generates an overproduction of free radicals via non-cyclooxygenase pathways; the increase of glutamate release induced by injury stimulates an increase in intracellular calcium concentrations via NMDA receptor activation. Therefore, phospholipase A₂ activity is enhanced, producing large quantities of free radicals, while COX-2 is inhibited (figure 6). We can test this hypothesis in further experiments, by giving celecoxib prior to KA administration + a free radical scavenger and subsequently testing their performance in the spatial learning task, or by co-administering an NMDA receptor blocker, such as AP-5 or CPP.
NMDA receptors $(+)$ \( \rightarrow \) [Ca\(^{2+}\)]_i $(+)$ \( \rightarrow \) PLA\(_2\)

Arachidonic acid

COX-1

COX-2

PGG\(_2\)

PGH\(_2\)

PGE\(_2\)

8-epi-PGF\(_{2\alpha}\)

Peroxidation of AA

Peroxidated phospholipids

Phospholipids

O\(_2^·\)

Figure 6: Schematic drawing illustrating how the hypothesised non- and cyclooxygenase pathways lead to the formation of prostanoids. Grey lines indicate the cyclooxygenase pathway, black lines indicate the non-cyclooxygenase pathway and dotted lines indicate activation of phospholipase A\(_2\) (PLA\(_2\)) via Ca\(^{2+}\) intracellular augmentation. Modified from Pepicelli et al. (2002).
Chapter V: General discussion
We investigated the consequences of brain insult after ischemia and kainic acid using different behavioural tasks, because both ischemia or KA model induce a similar neurodegenerative process to that resulting from traumatic brain injury, as well as some aspects of Alzheimer's and Parkinson's diseases. We have investigated some of the mechanisms which might be engaged by these pathologies; we have shown possible mechanisms which are involved in the process of recovery, focusing attention on prostaglandin production involved in the inflammation pathway generated from arachidonic acid via the action of the cyclooxygenases (COX), especially COX-2 (Cryer and Dubois, 1999). We also examined BDNF protein levels (one of most important trophic factors in the hippocampus, and which is augmented during brain injury); we examined if increases in BDNF protein levels could protect against cell death, following brain insult.

1. Summary of our results (table 1)

Chapter II:

- Ischemic rats were not sensitive to an environment change in the object exploration task, after exposure to an enriched or standard environment, compared to sham animals raised in the same conditions. However, the enriched environment rats spent more time in contact with the objects over trials, compared those in the standard environment. Our ischemic animals did not show any evidence of hyperactivity.
In the odour discrimination task, we did not find any overall difference between the groups, but we did find that animals housed in an enriched environment found the reward faster than those housed in a standard environment, independently of the treatment (ischemic or sham lesion groups).

All groups swam the same distance to the hidden platform and with similar escape latencies (so there was no motor impairment in any of the treatment groups), whereas in the direct route analysis (Wishaw's error analysis), the ischemic enriched environment groups and the sham animals performed better than those in normal environment.

Dentate gyrus BDNF protein levels were greater in the sham enriched environment and ischemic rats compared to the sham standard housing group.

The ischemic animals had significantly greater cell loss compared to the sham rats, independent of the housing conditions.

Chapter III and IV:

The results of the odour discrimination task suggested that there was no difference between control and kainic acid-administered groups; probably as a result of
hyperactivity induced by a hippocampal lesion, the KA-treated animals found the reward as quickly as the normal rats. Hyperactivity was found in the open field, where KA-treated rats were significantly more active and traversed a significantly greater distance than did control animals.

- The non-spatial version of the watermaze task (with visible platform) showed that there was no performance difference between the KA-treated and control rats, indicating that KA administration does not induce a visual or motor impairment. A KA dose of 6 mg/kg did not produce impairments in spatial learning either, so there is a clear dose-dependant effect of the KA dose.

- The enriched environment did not diminish the impairments found in the KA-treated rats, a similar result to that of ibuprofen treatment. Ibuprofen actually aggravated the condition of the animal and increased the rate of death in KA-treated animals.

- Extensive exercise prior to KA administration enhanced performance in the spatial version of the watermaze task as well as in the object exploration task, but did not return performance to such levels as that of the normal rats. Celecoxib administration post KA-treatment also improved the performance of the rats, whereas the addition or combination of both running and celecoxib (at 6 mg/kg) did not further enhance the behavioural performance of animals in both tasks; increasing the celecoxib dose to 40 mg/kg did not give any such addition effect
either. At this dose, celecoxib alone had no effect on spatial learning in the watermaze or object exploration tasks.

- The KA-treated animals were hyperactive compared to control animals, as were the KA-treated animals who engaged in prior running, whereas all the celecoxib-treated alone groups had normal levels of activity.

- The histological analysis did not show any reduced cell loss after treatment with extended exercise (running) or celecoxib in KA-administered animals; there was a clear difference between control and KA-treated rats in both CA1 and CA3 sub-regions of the hippocampus.

- The BDNF evaluation indicated that, at 6 hours post-insult (ischemia or KA-administration) and with prior exercise for 5 nights, the dentate gyrus BDNF protein levels were significantly increased compared to control animals. Whereas at the end of each experiment (2 weeks post-KA injection), the running group did not show different levels of BDNF from controls; the running + KA, KA, KA + celecoxib and running + KA + celecoxib (6 and 40 mg/kg) groups had higher BDNF levels than controls, or the celecoxib at 40 mg/kg alone group. When celecoxib was given for 5 days before KA or saline administration, BDNF protein levels were increased in these animals compared to control rats.
• In contrast with the KA+ celecoxib group, the celecoxib at 6 mg/kg prior the KA group did not improve their performance compared to controls, in either the watermaze or object exploration tasks, and had similar numbers of cells present after histological evaluation.
<table>
<thead>
<tr>
<th>Technique</th>
<th>group</th>
<th>watermaze</th>
<th>odour maze</th>
<th>object exploration</th>
<th>measurement BDNF pg/mg protein</th>
<th>Histology (number of cells) level -3.6 mm</th>
<th>Histology (number of cells) Level -5.8 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td>Std cond sham group</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>224</td>
<td>359 87 299</td>
<td>675 130 54</td>
</tr>
<tr>
<td></td>
<td>Std cond isch group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>458</td>
<td>56 64 258</td>
<td>429 122 565</td>
</tr>
<tr>
<td></td>
<td>Enr cond sham group</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>567</td>
<td>295 71 301</td>
<td>594 120 490</td>
</tr>
<tr>
<td></td>
<td>Enr cond isch group</td>
<td>++ direct swim</td>
<td>+</td>
<td>-</td>
<td>470</td>
<td>54 59 248</td>
<td>343 103 484</td>
</tr>
<tr>
<td>KA 6 mg/kg</td>
<td>KA</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td>injection</td>
<td>control</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td></td>
<td>Std cond sham group</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td></td>
<td>Std cond isch group</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td></td>
<td>Enr cond sham group</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td></td>
<td>Enr cond isch group</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td></td>
<td>&quot;+ ibuprofen</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>+</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no no no</td>
<td>no no no</td>
</tr>
<tr>
<td>KA 12 mg/kg</td>
<td>control</td>
<td>+</td>
<td>no</td>
<td>+</td>
<td>134/ 200</td>
<td>270 80 257</td>
<td>8 140 482</td>
</tr>
<tr>
<td>injection</td>
<td>KA</td>
<td>-</td>
<td>no</td>
<td>-</td>
<td>1381</td>
<td>144 51 169</td>
<td>291 75 337</td>
</tr>
<tr>
<td></td>
<td>run+ KA</td>
<td>+</td>
<td>no</td>
<td>+</td>
<td>595</td>
<td>152 67 197</td>
<td>440 110 367</td>
</tr>
<tr>
<td></td>
<td>KA+ celecoxib</td>
<td>+</td>
<td>no</td>
<td>+</td>
<td>535</td>
<td>208 64 191</td>
<td>402 94 347</td>
</tr>
<tr>
<td></td>
<td>run+ KA+ celecoxib</td>
<td>-</td>
<td>no</td>
<td>-</td>
<td>720</td>
<td>200 72 141</td>
<td>383 74 220</td>
</tr>
<tr>
<td></td>
<td>run+ veh KA+ celecoxib</td>
<td>+</td>
<td>no</td>
<td>+</td>
<td>98</td>
<td>287 90 231</td>
<td>727 137 523</td>
</tr>
<tr>
<td></td>
<td>Celecoxib 40 mg</td>
<td>+</td>
<td>no</td>
<td>+</td>
<td>247</td>
<td>289 78 257</td>
<td>481 114 413</td>
</tr>
<tr>
<td></td>
<td>run+ KA+ celecoxib 40 mg</td>
<td>-</td>
<td>no</td>
<td>-</td>
<td>529</td>
<td>259 68 190</td>
<td>341 91 227</td>
</tr>
<tr>
<td></td>
<td>Celecoxib before KA</td>
<td>-</td>
<td>no</td>
<td>-</td>
<td>749</td>
<td>218 56 148</td>
<td>339 92 238</td>
</tr>
</tbody>
</table>

Table 1: Summary of our results, -: impairment, +: improvement, no: no results
2. The hippocampus

The hippocampus is clearly involved in some aspects of memory and learning, and has been particularly implicated in spatial learning in rodents and primates (O'Keefe and Nadel, 1978; Burgess et al., 2001; Maguire et al., 1998; Morris et al., 1982); it also has a major role in object exploration (Poucet et al., 1989; Gemmell and O'Mara, 1999). We have shown here that the CA1 sub-region is involved in spatial learning, but not in non-spatial versions of the same task. There are some discrepancies, as some previous experiments did not find an impairment in spatial learning in ischemic animals (Kiyota et al., 1991; Auer et al., 1989). Our experiment in the spatial version of the watermaze task with ischemic animals indicated that when only area CA1 is damaged, that the behavioural evaluation of the insult was difficult; indeed, the only impairment found was in the Wishaw's error score. Animals seem to be able to compensate for the area CA1 injury, in finding the hidden platform in the watermaze task, by using a different strategic approach to those of normal rats. However, an analysis of the directness of the approach to the hidden platform (Wishaw's error) demonstrates a subtle impairment. This may explain the divergence in findings between laboratories, as indeed it was not easy to find the more subtle effects of the lesion. In contrast, the impairment was clear in object exploration, probably because exploration is the first step in encoding spatial memories and the object exploration task does not require any directed strategy. A lesion induced by kainic acid is not so subtle in its behavioural effects (because there is destruction of both the CA1 and CA3 sub-regions), and deficits are found in both latency and distance swam to reach the goal platform. KA-treated animals were significantly worse than
control animals, and they did not show any recovery as a result of an enriched environment as did the CA1-lesioned rats. This finding does not indicate that the CA3 sub-region of the hippocampus is more important than area CA1, where spatial learning is concerned, but it does indicate that KA-induced lesion is more difficult to recover from than 15 minutes of ischemic-insult, because the extent of the insult is more substantial.

3. Enriched environment and running

It is now clear that both enriched environments and extensive voluntary exercise can stimulate neurogenesis, improve learning and increase resistance to brain insult. Numerous changes derive from the type of stimulation at the level of genes, mRNA and protein expression in brain tissue (Rampon et al., 2000; Kempermann et al., 1998); in our experiments, these modifications produce a rescue of the lesioned regions, depending (more or less) on the lesion size: the enriched environment had a positive effect on our ischemic animals but was without effect on KA-injured rats (who had a greater area of lesion). Running alone, however, significantly ameliorated the performance of our KA-injured rats in both the spatial learning and object exploration tasks. We suggest, therefore, that exercise (running) might be one of the more important components of enriched environments, possibly even more than the exploratory behavioural and social interactions that arise as a consequence of the housing conditions. However, in an enriched environment, the animals exercise less than animals maintained in a running wheel overnight. Voluntary activity in running wheel is concentrated for a short but
intense period, whereas in the enriched environment the exercise is less intense but is for a longer period of time. The fact that the running ameliorated the performance of KA-injured animals in object exploration while the enriched environment had no effect on KA-injured animals, reinforces our idea that exercise is a major (if not the major) component of enriched housing. Tong et al. (2001) showed, using high-density oligonucleotide microarrays, that gene changes occur after exercise in the hippocampus: the most important modifications involve the genes associated with neuronal activity, synaptic structure and neuronal plasticity, particularly the growth factors and neurotrophins such as BDNF.

4. Brain-derived neurotrophic factor (BDNF)

We have shown that BDNF levels are increased after exercise, but our experiments also indicate that BDNF levels were augmented after brain injuries (see chapter II, III, IV). It is probable that the mechanisms involved are different. The administration of pentylenetetrazole (a GABA receptor antagonist) stimulates BDNF production in postsynaptic granule neurons (Katoh-Semba et al., 2001); KA-induced BDNF production has been observed in presynaptic granule neurons via voltage-dependant calcium channels (Katoh-Semba et al., 2001; Perry et al., 1994). In synapses between granule neurons and pyramidal cells, TrkB receptors are less densely distributed on the presynaptic rather than on the postsynaptic granule neurons (Katoh-Semba et al., 2001). Pyramidal neurons in the CA1 sub-region also show an augmented level of BDNF after
kainic acid injection (Katoh-Semba et al., 1999). Therefore, we have observed that after a brain insult, augmentation of BDNF is not sufficient to stop the progression of the injury, which, in turn, induces an impairment in the behavioural tasks tested here. However, running prior to the KA-induced insult improved the performance of the rats in these same tasks. Our hypothesis is that physical activity increases BDNF, which is present at the beginning of injury, and this BDNF can, therefore, play a neuroprotective role. Indeed, after a brain insult, there is a period of latency (a few hours) before new BDNF protein production occurs (Katoh-Semba et al., 2001). Subsequently, numerous harmful processes such as necrosis, apoptosis, excitotoxic cascade, inflammation and oedema could be activated and BDNF may not be able to counteract these. Although BDNF is one of the most important neurotrophins, other neurotrophins such as NGF and NT-3 are also implicated after brain injury and NGF mRNA and protein are increased after brain insult, while NT-3 is unique in its down-regulation following seizure (Jankowsky and Patterson, 2001). It must also be kept in mind that running modifies not only BDNF but also a great number of other factors involved in plasticity such as NGF (which also increases in the hippocampus of rats exposed to environmental enrichment; Pham et al., 1999a). Another example of such factors are the extracellular matrix adhesion molecules, which are also implicated in neuronal growth and synaptic plasticity (see for review Tong et al., 2001).

BDNF is considered to be a vital factor for promoting the survival, maintenance and growth of neurons. However, it seems also to be implicated in epileptogenesis (Binder et al., 2001). Exogenous BDNF infusion post KA-induced seizure can exacerbate hippocampal injury, especially in the CA3 sub-region (Rudge et al., 1998). These results
anc other experiments, which show that exposing hippocampal neurons to BDNF in vitro downregulates the TrkB receptor (the BDNF receptor; Frank et al., 1996) could provide another explanation for the beneficial effects of running after brain insult. The hypothesis here is that running increases levels of BDNF protein, and that this increase induces a downregulation of TrkB receptors, producing a decrease of BDNF responsiveness. Therefore, the post-insult BDNF augmentation should have less impact on the hippocampus, inducing less damage than otherwise might be the case. Moreover, BDNF could be responsible for the sprouting, which happens in mossy fibers after ischemic and KA-induced insult, producing hyperexcitability of mossy fiber-CA3 synapses (Binder et al., 2001). Kotti et al. (1997) found that 2 months after KA administration, there were target cells for aberrant mossy fiber collaterals in the dentate gyrus; such neurogenesis does not have a positive effect on the brain. With both hypotheses, we suggest that BDNF plays an important role in brain injury and that BDNF is probably more subtle in its effects than just being a simple neurotrophic agent (see chapter below).

5. Cyclooxygenases

The 2 isoforms of cyclooxygenase could play a role in inflammation in tissues throughout the body; COX-1 however mediates “housekeeping” functions and the inducible COX-2 enzyme makes the more important contribution to the synthesis of prostaglandins, which mediate inflammation (Cryer and Dubois, 1998). Prostaglandin E2 (PGE2) is downstream of the cascade of COX-2 activity on arachidonic acid. After brain insult, there is an
augmentation of neuronal COX-2. Other prostaglandins are also produced via the non COX-2 pathway, such as PGE_{2a}, which is a reliable marker of oxidative stress (see chapter IV), and also PGL_{2}, 6-keto-PGF_{1a} and thromboxane B_{2} (see figure 5 chapter I). However, COX-2 is also constitutively expressed in neurons where PGE_{2} participates in hippocampal long-term synaptic plasticity (O'Mara et al., 2001b; Shaw et al., 2001; Chen et al., 2002). COX2 inhibition blocks the induction of LTP in the hippocampus, whereas exogenous application of PGE_{2} restores LTP (Chen et al., 2002).

We have shown that KA-treated animals improve their performance in spatial learning and object exploration when we administered celecoxib 2 hours post-KA injection. Celecoxib blocks the pro-inflammatory processes leading to PGE_{2} production (PGE_{2} is also involved in apoptosis; Takadera et al., 2001), and we therefore suggest that stopping PGE_{2} production may enhance recovery after brain injury induced by KA administration. However, when celecoxib was given before KA to rats, it did not enhance the performance of KA-treated animals in behavioural tasks. We conclude that PGE_{2} (induced by COX-2) plays an important role at the beginning of insult, which is at worst benign and at best neuroprotective, confirming previous in vitro experiments, which are suggestive of a protective role of prostaglandins against glutamate toxicity (Akaike et al., 1994; Cazevieille et al., 1994). Thus, we hypothesise that, as with many bioactive molecules, PGE_{2} has dichotomous effects, where its production at the beginning of the insult is beneficial whereas a sustained augmentation in its production has harmful consequences (figure 1). It is more difficult to explain the harmful effect of ibuprofen, when it was administered after KA-treatment (it aggravated the effect of KA administration). It may be that COX-1 activity alone has also some beneficial or
neuroprotective consequences - an idea not testable at present because of the lack of selective COX-1 inhibitors. In spite of its anti-inflammatory activity, ibuprofen could have another action such as that Najbauer et al. (2000) have shown for aspirin, which aggravates neuronal damage in KA-treated rats. Another hypothesis is that ibuprofen completely blocks the production of PGE$_2$ (induced by COX-1 (?) and COX-2) whereas celecoxib blocks only the PGE$_2$ production via COX-2 enzyme. This reinforces the idea that total inhibition of PGE$_2$ production should be harmful after brain insult as would be the massive overproduction of prostaglandins.

![Graph showing PGE$_2$ production and functional recovery](image)

**Figure 1:** Hypothesis of time and insult dependence of PGE$_2$ production in the neurons.
Our results show that celecoxib at 40 mg/kg did not impair performance in spatial learning, whereas ibuprofen-treated rats did not learn the watermaze task as quickly as controls (O’Mara et al, 2001b). These data on normal animals might explain why it is necessary to totally block the production of PGE$_2$ with non-specific COX inhibitors (like ibuprofen or aspirin) to impair learning. There may also be another cyclooxygenase isoform (COX-3?), which might be inhibited by ibuprofen and not by celecoxib. This new and putative cyclooxygenase might play a role in LTP and in learning and memory. Such a novel COX isoform has been suggested by Willoughby et al (2000), and Botting (2000), using acetaminophen (also known as paracetamol) as the pharmacological tool. Definitive evidence for COX-3 has recently been presented by Chandrasekharan et al. (2002), who indicated COX-3 and 2 smaller COX-1-derived proteins (partial COX-1) made from the COX-1 gene but retaining intron 1 in their mRNAs. COX-3 seems most abundant in cerebral cortex and heart (it is important to note that COX-2 is variably expressed after stimulation in hippocampus, which could be the same case for COX-3). Chandrasekharan’s studies show that COX-3 is sensitive to drugs that are analgesic/antipyretic, but which have low anti-inflammatory activity. However, acetaminophen was found to as stimulate the synthesis of prostaglandins in low concentration and inhibiting this production in high concentration (for review: Botting, 2000). It will be important to test acetaminophen (paracetamol) in behavioural tasks, and prior to and after brain insult to better our understanding of COX-3 in the neuroinflammatory process. Moreover, Landolfi et al. (1998) indicated that in an Alzheimer’s in vitro inflammatory model, acetaminophen completely inhibited PGE$_2$ production when cells were stimulated with interleukin-1β (a major pro-inflammatory
cytokine in the brain; Laye et al., 1999). The mechanism of action of acetaminophen in inflammatory processes is unclear at this time, but it could be helpful to understanding the functions of COX-3.

6. BDNF and cyclooxygenases

Our unexpected result shows that the combination of running and celecoxib together did not protect against KA-induced injury; indeed, the animals did not learn the platform position as well as the controls, in watermaze task. This experiment indicated to us the possibility that BDNF could be neurotoxic under some conditions, as has been suggested by some previous experiments (Rudge et al., 1998; Binder et al., 2001). Our hypothesis in chapter IV was based on our results and the literature review, but further investigations will necessary to understand the underlying molecular mechanisms. Nevertheless, this result indicates that BDNF may have dichotomous role in the brain – either neurotoxic or neuroprotective, depending on the state of the tissue. We might also suggest that biology is not like mathematics and that the addition of one positive effect to another one does not equal to the sum of both, but sometime can give rise to a negative outcome (i.e. $1 + 1 = -1$).
7. Histology

None of our interventions (enriched environment, running or COX-2 inhibitor) offered any protection against cell death, even when these interventions resulted in enhanced spatial learning. We suggest that the positive effect of these treatments are more involved in the enhancement of connections between cells, making new synapses, rather than in the diminution of cell loss.

However, it seems us that another technique, more sensitive for the evaluation of neuronal death, should be used, such as TUNEL staining.

8. Concluding remarks

In conclusion, we have shown that BDNF and PGE$_2$ can play dichotomous roles in the brain, that they can have positive or negative effects on spatial learning, object exploration task and other behavioural tasks depending on the prior state of brain tissue. We have demonstrated the importance of the CA1 and CA3 sub-regions of the hippocampus in spatial learning tasks, and have suggested why there are possibly discrepancies between different experiments. We have suggested that there is a vital role for physical activity in an enriched environment, especially in the amelioration of the performance of injured animals. We showed the importance of BDNF in brain insult, especially in the hippocampus, indicating that its augmentation before an injury is able to reduce the harmful effects of the injury on spatial learning and memory. Furthermore, we
hypothesise that under some conditions that BDNF could have a negative effect on the neuronal viability.

However, further investigations must be undertaken, for example to demonstrate the involvement of COX-3 in the neuroinflammation, using acetaminophen in different models of brain insults, such KA-induced seizure or ischemia. Finally, it will be interesting to investigate if BDNF protein levels increase when celecoxib is co-administered with free radical scavengers to normal animals to understand the importance of free radical production in the injured brain.
References


Contractor, A.; Swanson, G., and Heinemann, S. F. Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. Neuron. 2001 Jan; 29(1):209-16.


Galani, R.; Weiss, I.; Cassel, J. C., and Kelche, C. Spatial memory, habituation, and reactions to spatial and nonspatial changes in rats with selective lesions of the hippocampus, the entorhinal cortex or the subiculum. Behav Brain Res. 1998 Nov; 96(1-2):1-12.


Mori, K.; Togashi, H.; Ueno, K. I.; Matsumoto, M., and Yoshioka, M. Aminoguanidine prevented the impairment of learning behavior and hippocampal long-term potentiation following transient cerebral ischemia. Behav Brain Res. 2001 May; 120(2):159-68.


236


Moser, M. B.; Trommald, M., and Andersen, P. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc Natl Acad Sci U S A. 1994 Dec 20; 91(26):12673-5.


Poucet, B. Spatial cognitive maps in animals: new hypotheses on their structure and neural mechanisms. Psychol Rev. 1993 Apr; 100(2):163-82.


Rapoport, S. I. and Bosetti, F. Do lithium and anticonvulsants target the brain arachidonic acid cascade in bipolar disorder? Arch Gen Psychiatry. 2002 Jul; 59(7):592-6.

Rochefort, C.; Gheusi, G.; Vincent, J. D., and Lledo, P. M. Enriched odour exposure increases the number of newborn neurons in the adult olfactory bulb and improves odour memory. J Neurosci. 2002 Apr 1; 22(7):2679-89.


Rudge, J. S.; Mather, P. E.; Pasnikowski, E. M.; Cai, N.; Corcoran, T.; Acheson, A.; Anderson, K.; Lindsay, R. M., and Wiegand, S. J. Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. Exp Neurol. 1998 Feb; 149(2):398-410.


Shaw, K. N.; Commins, S., and O'Mara, S. M. Lipopolysaccharide causes deficits in spatial learning in the watermaze but not in BDNF expression in the rat dentate gyrus. Behav Brain Res. 2001 Sep 28; 124(1):47-54.


Squire, L. R. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev. 1992 Apr; 99(2):195-231.


Publications
Published article:


Articles submitted:

Gobbo O. & O'Mara S. M. Enriched-environment housing enhances performance on odour discrimination, object exploration and spatial learning tasks independently of transient global ischemia.


Gobbo O. & O'Mara S. M. Celecoxib can aggravate or ameliorate kainic acid-induced seizures in the rat, depending on the timepoint of administration.

Reviewed Abstracts:
