INVESTIGATION OF SECONDARY CEREBRAL DAMAGE IN EPILEPSY

Rebecca Shook Ning Liu, Bsc MBBS MRCP

THE DEPARTMENT OF CLINICAL AND EXPERIMENTAL EPILEPSY

INSTITUTE OF NEUROLOGY, UNIVERSITY COLLEGE LONDON

THESIS SUBMITTED TO THE UNIVERSITY OF LONDON FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

2004
ABSTRACT

Background
This thesis addresses the relationship between epileptic seizures and progressive cerebral damage. It is the first longitudinal community-based quantitative magnetic resonance imaging (MRI) study to investigate the effect of seizures on the hippocampus, cerebellum and neocortex. Two key issues are addressed: whether epileptic seizures result in cerebral damage that can be detected with quantitative MRI, and identification of possible clinical risk factors. The work aims to enhance our understanding of the pathogenesis of cerebral damage in epilepsy and thus assist in the evaluation of neuroprotective strategies.

Methods
One hundred and twenty-two patients with chronic active epilepsy, 68 patients with newly diagnosed seizures and 90 control subjects underwent two MRI brain scans 3.5 years apart. Automated and manual measurement techniques were used to identify global and regional changes in brain volume and alterations in hippocampal T2 relaxometry. Image subtraction of registered scans was used to identify diffuse and focal neocortical atrophy.

Results
Baseline hippocampal, neocortical and cerebellar volumes were significantly reduced in the chronic epilepsy group compared with the newly diagnosed and control group. These differences could be attributed to a history of antecedent neurological insults. Rates of atrophy were similar amongst the three subject groups and primarily determined by age. A history of a prior neurological insult was associated with an increased rate of cerebral and cerebellar atrophy. Individuals with chronic epilepsy were at a significantly greater risk of developing focal and generalized neocortical volume loss. Risk factors included increased age and multiple antiepileptic drug exposure.

Conclusion
Regional and global cerebral atrophy is not an inevitable consequence of seizures. Progressive hippocampal, cerebral and cerebellar atrophy in epilepsy is primarily the result of an initial neurological insult and age. However, subtle neocortical atrophy is common in epilepsy and may occur secondary to an underlying epileptogenic process, multiple antiepileptic drug exposure and individual genetic susceptibility.
OVERVIEW OF THESIS

One of the most debated questions in epilepsy research is whether recurrent seizures lead to progressive cerebral damage. Although experimental studies provide evidence of seizure-induced hippocampal neuronal damage using models of status and perforant pathway stimulation, the applicability of these findings to other regions of the brain, and to humans is unclear. Imaging studies have been largely retrospective and cross-sectional in design, capable only of drawing inferences about intra-individual change from inter-individual data. Such limitations have frequently resulted in contradictory findings and cannot distinguish between cause and effect. Questions regarding the evolution and risk factors for cerebral damage in epilepsy can only be addressed using longitudinal studies. A number of longitudinal MRI studies have been performed, although focussed primarily on individual case reports or relatively small uncontrolled series of patients lacking formal blinding procedures.

In this thesis, a novel quantitative approach to the detection of serial hippocampal, cerebellar and neocortical atrophy is presented. This fully blinded approach incorporating image registration, side-by-side display, and difference image analysis is applied to a community-based study of 90 control subjects, 68 patients with newly diagnosed seizures and 122 patients with chronic active epilepsy. Volumetric changes over 3.5 years are correlated with clinical data and potential risk factors for cerebral damage were addressed. Additional voxel-based analyses identify subtle neocortical changes that are subsequently quantified using a regional brain atlas.

The contents of the thesis have been subdivided into the following sections:

Chapter I presents the relevant background literature starting with classification systems, the aetiologies of epilepsy and basic principles that underlie MR acquisition and MR imaging. The literature on the various approaches to region- and voxel-based analyses is reviewed, together with significant findings from experimental, neuropsychological and imaging studies investigating the effect of seizures on neuronal damage, cognitive dysfunction and volume loss respectively. The section concludes with a review of proposed neuroprotective strategies that may limit the extent of cerebral damage.

Chapter II describes the common methodology used for this work including study design, subject recruitment and acquisition of clinical and imaging data that is applicable to Chapters V to VIII.
Chapter III presents the analytical tools used for regional and voxel-based quantitative assessments that were used to detect focal and global structural changes over the study period.

Chapter IV validates the approach to serial MRI volumetric measurements described in Chapter III. In an initial blinded pilot study of control subjects intermixed with patients with focal epilepsy, the reproducibility of this approach is determined, providing confirmation of its ability to detect subtle volumetric change and its applicability to our study population.

Chapter V studies the volumetric and morphological changes in the control group, providing insight into the rates of brain atrophy seen particularly in younger and middle-aged individuals over 3.5 years. The importance of controlling for such age effects in longitudinal studies of disease states is emphasised.

Chapter VI compares volumetric change in patients with newly diagnosed seizures with changes observed in the control group. The work investigates whether patients are at increased risk of structural damage to the hippocampus, cerebellum and neocortex in the first few years following a diagnosis of seizures.

Chapter VII incorporates region-based data from Chapters V and VI and compares cross-sectional and longitudinal volumetric changes in control subjects, patients with newly diagnosed epilepsy and patients with chronic epilepsy. The results of group and individual analyses are presented, together with a discussion on the role of initial precipitating injuries, epilepsy syndrome and seizures.

Chapter VIII complements the findings in Chapter VII by presenting the results of a voxel-based analysis of progressive neocortical damage in epilepsy. Focal and subtle neocortical changes not detectable using region-based methods are identified and patterns of neocortical damage in epilepsy characterised. Clinical correlates of neocortical damage are proposed.

Chapter IX summarises the intentions and main findings of this thesis, together with the neurobiological implications of the findings, study limitations and scope for future work.
CHAPTER I BACKGROUND

1.1 EPILEPSY.................................................................22
  1.1.1 Seizure classification..................................................22
  1.1.2 Syndrome classification...........................................25
    1.1.2.1 International classification of the Epilepsies........25
    1.1.2.2 International classification of the Epilepsies and
             Epileptic Syndromes..................................................25
    1.1.2.2.1 Criticisms of the ICEES.................................28
  1.1.3 Range of aetiologies of epilepsy...............................31

1.2 STRUCTURAL MRI AND VISUAL ASSESSMENT..............38
  1.2.1 Role of MRI in identifying the aetiology of epilepsy......38
  1.2.2 The impact of improved MRI sensitivity.....................40
    1.2.2.1 Focal epilepsy..................................................40
    1.2.2.2 Generalized epilepsy..........................................43
  1.2.3 MRI negative patients.............................................43
    1.2.3.1 Focal epilepsy..................................................43
    1.2.3.2 Generalized epilepsy..........................................44
  1.2.4 Dual pathology....................................................44

1.3 PRINCIPLES OF MRI.................................................46
  1.3.1 Physics of nuclear magnetic resonance......................46
  1.3.2 T1, T2 relaxation and image contrast..........................47
  1.3.3 MR echoes and contrast modification..........................48
  1.3.4 Image acquisition and reconstruction.......................51
1.3.5 Standard MR sequences used in clinical epilepsy ........................................ 51

1.4 QUANTITATIVE MR IMAGING ....................................................................... 52
  1.4.1 Hippocampal T2 mapping ........................................................................ 52
  1.4.2 Hippocampal volumetry ......................................................................... 55
  1.4.3 Cerebellar volumetry ............................................................................. 61

1.5 NEOCORTICAL IMAGE PROCESSING ............................................................. 62
  1.5.1 Volumes of grey matter, white matter and image post-processing ......... 62
  1.5.2 Image registration ................................................................................ 65
  1.5.3 Brain segmentation .............................................................................. 68
  1.5.4 Subtraction of serial images and difference images ................................ 72
    1.5.4.1 Generation of difference images ...................................................... 72
    1.5.4.2 Visual interpretation ........................................................................ 72
    1.5.4.3 Quantitative analysis ....................................................................... 73
  1.5.5 Voxel based morphometry .................................................................... 74

1.6 SECONDARY CEREBRAL DAMAGE IN EPILEPSY ........................................ 76
  1.6.1 Hippocampal damage following status epilepticus ......................... 76
    1.6.1.1 Experimental and pathological models ............................................ 76
      1.6.1.1.1 Animal studies ...................................................................... 76
      1.6.1.1.2 Human studies ...................................................................... 79
    1.6.1.2 Neuropsychological studies ............................................................ 80
      1.6.1.2.1 Animal studies ...................................................................... 80
      1.6.1.2.2 Human studies .................................................................... 80
    1.6.1.3 Imaging studies ............................................................................... 81
  1.6.2 Hippocampal damage following recurrent epileptic seizures .......... 82
    1.6.2.1 Experimental and pathological models ............................................ 82
      1.6.2.1.1 Animal studies ...................................................................... 82
      1.6.2.1.2 Human studies ...................................................................... 83
    1.6.2.2 Neuropsychological studies ............................................................ 85
      1.6.2.2.1 Animal studies ...................................................................... 85
      1.6.2.2.2 Human studies .................................................................... 86
    1.6.2.3 Imaging studies ............................................................................... 87
      1.6.2.3.1 Cross-sectional studies .......................................................... 87
      1.6.2.3.2 Longitudinal studies .............................................................. 89
  1.6.3 Cerebellar damage in epilepsy ............................................................... 92
    1.6.3.1 Experimental and pathological models ............................................ 92
    1.6.3.2 Neuropsychological studies ............................................................ 94
    1.6.3.3 Imaging studies ............................................................................... 95

1.6.4 Neocortical damage in epilepsy .............................................................. 95
  1.6.4.1 Experimental and pathological models ............................................ 95
  1.6.4.2 Imaging studies ............................................................................... 96
6.4.3 Group analysis of quantitative changes ...................................................... 144
6.4.4 Individual patient analyses of MRI changes ............................................... 144

6.5 DISCUSSION ............................................................................................................... 147
6.5.1 Individuals with significant volume changes .............................................. 147
6.5.2 Group findings .............................................................................................. 148
6.5.3 Incidence of hippocampal sclerosis in newly diagnosed epilepsy ............. 148
6.5.4 Prognostic outcome ....................................................................................... 148

6.6 CONCLUSION ....................................................................................................... 149

CHAPTER VII CEREBRAL DAMAGE IN EPILEPSY: A POPULATION BASED QUANTITATIVE MRI STUDY
7.1 OBJECTIVE ................................................................................................................ 150
7.2 INTRODUCTION ........................................................................................................ 150
7.3 METHODS ............................................................................................................... 151
7.3.1 Study population ............................................................................................ 151
7.3.2 Data acquisition and image processing ....................................................... 151
7.3.3 Statistical analysis .......................................................................................... 151
7.3.3.1 Group analysis ........................................................................................ 152
7.3.3.2 Individual analyses .................................................................................. 152

7.4 RESULTS ...................................................................................................................... 153
7.4.1 Demographic data .......................................................................................... 153
7.4.2 Cross-sectional baseline findings .................................................................. 156
7.4.3 Longitudinal findings .................................................................................... 156
7.4.3.1 Group analysis ........................................................................................ 156
7.4.3.1.1 Hippocampal volume ....................................................................... 156
7.4.3.1.2 HCT2 relaxometry ............................................................................ 158
7.4.3.1.3 Total cerebellar volume ................................................................... 159
7.4.3.1.4 Total brain volume .......................................................................... 159
7.4.3.1.5 Grey matter volume ........................................................................ 160
7.4.3.1.6 White matter volume ....................................................................... 160
7.4.3.2 Quantitative changes in individual subject ............................................ 163

7.5 DISCUSSION ................................................................................................................ 165
7.5.1 Methodological issues and limitations of the study ................................... 165
7.5.2 Cerebral damage in epilepsy ....................................................................... 166
7.5.3 Initial precipitating insults ............................................................................ 167
7.5.4 Seizures ........................................................................................................... 169
7.5.5 Gender ............................................................................................................ 170
7.5.6 Exposure to antiepileptic drugs .................................................................... 171

7.6 CONCLUSION ....................................................................................................... 171
TABLES

Table 1  International Classification of seizures ................................................................. 24
Table 2  The 1989 International Classification of the Epilepsies and Epileptic syndromes (ICEES) .................................................................................. 27
Table 3  The major aetiologies associated with epilepsy ..................................................... 31
Table 4  Principal pulse sequences used for structural imaging ......................................... 49
Table 5  MRI case reports on seizure-related HV changes .................................................. 90
Table 6  Longitudinal MRI studies on seizure-related HV changes ................................... 91
Table 7  Causes of attrition ............................................................................................... 106
Table 8  Group demographics ......................................................................................... 129
Table 9  Correlations between age and ICV-corrected MRI volumes ................................ 130
Table 10 Normative ranges for quantitative brain measures ............................................ 134
Table 11 Structural MRI findings of newly diagnosed patients ....................................... 143
Table 12 Quantitative MRI measures of newly diagnosed patients with significant volume changes ........................................................................................................ 146
Table 13 Demographic and clinical characteristics of study patients ................................ 154
Table 14 Comparison of epilepsy syndromes ................................................................... 155
Table 15 Quantitative data on all subject groups ............................................................. 157
Table 16 Significant quantitative changes in patients with chronic epilepsy .................... 164
Table 17 Patterns of neocortical atrophy according to epilepsy syndrome ....................... 178
Table 18 Neocortical changes in control subjects ............................................................ 180
Table 19 Neocortical changes in newly diagnosed patients ............................................. 181
Table 20 Neocortical changes in patients with chronic epilepsy ....................................... 183
Table 21 Clinical characteristics and patterns of neocortical change .............................. 188
**FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Epilepsy classification based on epidemiology and prognosis</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Schematic representation of a basic pulse sequence</td>
<td>50</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Structure of the hippocampus</td>
<td>59</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Graphical display of cross-sectional hippocampal volume</td>
<td>60</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Anatomical subregions and excitatory pathways of the normal human hippocampus</td>
<td>79</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Image processing steps in hippocampal and cerebellar volumetry</td>
<td>108</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Display of matched scans during hippocampal volumetry</td>
<td>110</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Display of matched scans during cerebellar volumetry</td>
<td>112</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Structured Noise Map</td>
<td>114</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Formation of the genuine change map</td>
<td>115</td>
</tr>
<tr>
<td>Figure 11</td>
<td>Quantification of focal neocortical atrophy</td>
<td>118</td>
</tr>
<tr>
<td>Figure 12</td>
<td>Individual changes in baseline and repeat MRI variables</td>
<td>132</td>
</tr>
<tr>
<td>Figure 13</td>
<td>Distribution of age-related changes according to age epoch</td>
<td>135</td>
</tr>
<tr>
<td>Figure 14</td>
<td>Longitudinal changes in MRI parameters</td>
<td>161</td>
</tr>
<tr>
<td>Figure 15</td>
<td>Initial precipitating injury and longitudinal volume changes</td>
<td>162</td>
</tr>
<tr>
<td>Figure 16</td>
<td>Representative patterns of neocortical change</td>
<td>174</td>
</tr>
<tr>
<td>Figure 17</td>
<td>Patterns of signal change in control subjects</td>
<td>175</td>
</tr>
<tr>
<td>Figure 18</td>
<td>Patterns of signal change in newly diagnosed patients</td>
<td>176</td>
</tr>
<tr>
<td>Figure 19</td>
<td>Patterns of signal change in patients with chronic epilepsy</td>
<td>177</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

AED       Antiepileptic drug
AM-HF     Amygdala–hippocampal formation
APOE      Apolipoprotein E
AVM       Arteriovenous malformation
BZR       Benzodiazepine receptors
CA        Cornu ammonis
CBV       Cerebellar volume
CH        Combined hippocampi
CNS       Central nervous system
CR        Coefficient of repeatability
C-S       Cross-sectional
CSE       Conventional spin echo
CSF       Cerebrospinal fluid
CT        Computerised tomography
CV        Coefficient of variation
DNET      Dysembryoplastic neuroepithelial tumour
EEG       Electroencephalography
FCD       Focal cortical dysplasia
FLAIR     Fluid attenuated inversion recovery
FLE       Frontal lobe epilepsy
FPI       Fluid percussion injury
GABA      \(\gamma\)-aminobutyric acid
GCM       Genuine change map
GMV       Grey matter volume
GTCS      Generalized tonic-clonic seizure
HC        Hippocampus
HCT2      Hippocampal T2
HS        Hippocampal sclerosis
HV        Hippocampal volume
ICEES     International Classification of Epilepsy and Epileptic syndromes
ICES      International Classification of Epileptic Seizures
ICV Intracranial volume
IGE Idiopathic generalized epilepsy
ILAE International League Against Epilepsy
IPI Initial precipitating injury
IR Inversion recovery
ISI Inter-scan interval
JME Juvenile myoclonic epilepsy
MCD Malformations of cortical development
MDM Magnetic dipole moment
MRI Magnetic resonance imaging
NMDA N-methyl-D-aspartate
NMV Net magnetization vector
NSE Neuron-specific enolase
NUC Non-uniformity correction
PET Positron emission tomography
RF Radiofrequency
ROI Region of interest
SDI Structured difference image
SE Status epilepticus
SNM Structured noise map
SNR Signal to noise ratio
SOM-ir Somatostatin immunoreactive
SPGR Spoiled gradient echo
SPM Statistical parametric mapping
SPS Simple partial seizures
TBI Traumatic brain injury
TBV Total brain volume
TCI Transitory cognitive impairment
TE Echo time
TLE Temporal lobe epilepsy
TR Repetition time
VBM Voxel based morphometry
WMV White matter volume
PERSONAL CONTRIBUTION

The creative ideas and driving force behind the work presented in this thesis were derived from a core group of researchers, comprising Professor J Duncan, myself, professors L Sander and S Shorvon; and Drs L Lemieux, S Sisodiya and G Bell. Although the work reflects the contributions and coordinated efforts of the entire team, I have outlined my individual contribution below.

1. Data acquisition

I was responsible for organising the follow-up scans and obtaining clinical data on all 280 study subjects previously identified in Phase I of the Wellcome Study. As part of an initiative to optimise follow-up rates and ensure accuracy of clinical data, I visited all 21 general practices on a six-monthly basis reviewing patient notes and computer records. I created a relational database in which all demographic, clinical and volumetric findings were stored. To ensure the integrity of the data entered, I conducted an audit on 10% of all data fields. By offering a weekly fast-track first seizure clinic and the provision of regular updates in the form of annual newsletters, close contact with general practitioners and specialist nurses was maintained, contributing to high levels of cooperation.

2. Data analysis

All region-based analyses including baseline and repeat manual hippocampal and semiautomated cerebellar volume measurements in both the pilot and main study were performed by myself. I contributed to the development of the side-by-side serial volumetric approach adopted in the study; was responsible for retrieving and archiving scan data; ran all automated programmes including image registration, brain segmentation, generation of difference image, normalisaton and application of the regional brain atlas; and visually interpreted the filtered structural difference images with Professor Duncan.

3. Statistical analysis and data interpretation

I performed all statistical analyses with the assistance of Dr Bell, and contributed to a number of refinements including the improved fit of the regression line in cross-sectional volume changes with age using quadratic function, the advanced application of statistical programs e.g. repeated measures of ANOVA in interpretation of serial
data, and identification of the role of initial precipitating injuries on initial brain volume loss and subsequent atrophy. All initial results and interpretations were presented by myself and developed following internal discussions at regular supervision meetings. I was responsible for all graphical presentations of the data and final interpretation of the results and their biological implications.
ORIGINAL ARTICLES


**ABSTRACTS**


**AWARDS**


*Young Investigator Award* – “Cerebral damage in epilepsy: findings of a longitudinal community-based quantitative MRI study”, American Epilepsy Society, Seattle 2002.
ACKNOWLEDGMENTS

I am grateful to everyone at the Department for Clinical and Experimental Epilepsy for making my period of research such an invaluable and enjoyable experience. I am particularly indebted to the following individuals for their contributions.

Professor John Duncan, for overall supervision of this work. His encouragement and humour renewed me with enthusiasm when I needed it most and his clarity of vision was a source of inspiration.

Dr Louis Lemieux, my co-supervisor, who was responsible for devising the methodological techniques that made this work possible. I am especially grateful to him for his patience, technical guidance and friendship.

Dr Gail Bell, who introduced me gently and expertly to the world of SPSS and was instrumental in helping me overcome my aversion to medical statistics.

Dr Sanjay Sisodiya and Professor Ley Sander for their insightful contributions and conviction in the study.

Philippa Bartlett, Elaine Williams, Jane Burdett and Penny Hitchins for performing all the MRI scans and forsaking valuable Saturday mornings with their families for extra scanning sessions. Particular thanks go to Philippa for tirelessly performing the hippocampal T2 measurements.

Drs John Stevens and Brian Kendall, for their expert radiological interpretations, Joan Blissett for her administrative assistance, and Dr Sam Free for her expert tuition on hippocampal volumetry.

The general practitioners and practice managers for granting me permission to include their patients, the Office for National Statistics for help with tracing individuals and all the patients and control subjects who gave up their time to participate in the study.
Dr Alex Everitt, for recruiting the subjects and providing me with such excellent baseline data.

My colleagues at the Chalfont Centre, in particular, Fergus Rugg-Gunn, Tejal Mitchell, Sofia Eriksson, Rob Simister and Afraim Haddadi, for their support, friendship and thought-provoking discussions.

Final thanks go to my family for their continued support; and to my husband, Alan, for his love, encouragement, and help with the more complicated aspects of information technology.

This work was made possible by a grant from the Wellcome Trust. Support by the National Society for Epilepsy and the UCLH Trust Neuroepidemiology Unit are also gratefully acknowledged.
CHAPTER I BACKGROUND

1.1 EPILEPSY

1.1.1 Seizure classification

The role of the International Classification of Epileptic Seizures (ICES) and the International Classification of Epilepsy and Epileptic Syndromes (ICEES) are two-fold. Firstly, they optimize communication within the clinical and research setting by achieving a common understanding of the terminology used in identifying a heterogeneous spectrum of seizure disorders (So, 1995). Secondly, classification of the seizure type and epilepsy syndrome carries implications for its diagnosis, prognosis and subsequent treatment (Stromme et al., 2002). Both classification systems are based on a dichotomous division between focal and generalized epilepsy disorders and rely on clinical and electrophysiological source data.

The revised ICES (Commission on Classification and Terminology of the International League Against Epilepsy, 1981) was introduced by the International League Against Epilepsy (ILAE) in 1981 (Table 1), and based purely on ictal phenomenology and associated electroencephalography (EEG) features rather than anatomical localization and pathophysiological mechanisms. Such a classification presented the symptoms and signs in epileptology, but provided little information on the underlying aetiology or prognosis (Sander, 1993). The classification employs a double dichotomy, dividing seizures initially into generalized and partial seizures, and partial seizures subsequently into "complex" and "simple", depending on whether consciousness is lost or retained. A seizure is classified as partial when there is EEG or clinical evidence of a focal onset, whilst generalized seizures are those in which the first clinical change indicates involvement of both hemispheres and the EEG shows immediate synchronous spike-wave discharges.

The ICES has been criticized for its unrealistic divisions, its reliance on subsidiary investigations e.g. the EEG and its lack of important localizing material (Benbadis and Luders, 1995). Luders (Luders et al., 1993) argued that although the ICES has been universally accepted by epileptologists for its simplicity, pharmacological implications, and relationship to quality of life; the lack of localizing information has limited its use in the evaluation of pre-surgical patients. The emphasis placed on the division of partial
seizures into simple and complex, fails to emphasise seizure symptomatology that could provide useful lateralizing or localizing information, and ignores information on the origin and spread of the seizure.

Addressing these shortcomings, Luders (Luders et al., 1999) proposed a semiological epileptic seizure classification, modified from traditional seizure classification systems, which dispensed with the double dichotomy and focussed exclusively on ictal semiology. Ancillary investigations e.g. EEG, structural imaging and functional neuroimaging were analyzed separately and then integrated to define the epileptic syndrome. Seizures were classified as: auras, which were ictal manifestations with sensory, psycho-sensory, and experiential symptoms; autonomic seizures in which the main ictal manifestations were objectively documented autonomic alterations; "dialeptic" seizures characterized by an alteration of consciousness independent of ictal EEG manifestation; motor seizures characterized mainly by motor symptoms and subclassified as simple or complex; and special seizures characterized by "negative" features e.g. atonic, astatic, hypomotor, akinetic, and aphasis seizures.

Working groups appointed by the ILAE are in the process of revising the ICES. In addition, the terms "simple" and "complex" are likely to be abandoned, and replaced with neocortical or limbic partial seizures. More recently, the Task Force proposed that all available clinical and laboratory information should be used when making a definitive diagnosis of the seizure type, which would have specific aetiologic, therapeutic, and prognostic implications, and could either supplement syndromic diagnoses or stand alone when syndromic diagnoses could not be made (Engel, 2001).
### Table 1  International Classification of seizures (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). Modified version.

<table>
<thead>
<tr>
<th>Partial seizures (beginning locally)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple partial seizures (consciousness not impaired)</td>
<td></td>
</tr>
<tr>
<td>• With motor symptoms</td>
<td></td>
</tr>
<tr>
<td>• With somatosensory or special sensory symptoms</td>
<td></td>
</tr>
<tr>
<td>• With autonomic symptoms</td>
<td></td>
</tr>
<tr>
<td>• With psychic symptoms</td>
<td></td>
</tr>
<tr>
<td>Complex (with impairment of consciousness)</td>
<td></td>
</tr>
<tr>
<td>• Beginning as simple partial seizure (progressing to complex seizure)</td>
<td></td>
</tr>
<tr>
<td>• Impairment of consciousness at onset</td>
<td></td>
</tr>
<tr>
<td>a) Impairment of consciousness only</td>
<td></td>
</tr>
<tr>
<td>b) With automatism</td>
<td></td>
</tr>
<tr>
<td>• Partial seizures evolving to secondarily generalised seizures (tonic-clonic, tonic or clonic)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Generalised seizures</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence seizures</td>
<td></td>
</tr>
<tr>
<td>• Typical</td>
<td></td>
</tr>
<tr>
<td>• Atypical</td>
<td></td>
</tr>
<tr>
<td>Myoclonic seizures</td>
<td></td>
</tr>
<tr>
<td>Clonic seizures</td>
<td></td>
</tr>
<tr>
<td>Tonic seizures</td>
<td></td>
</tr>
<tr>
<td>Tonic-clonic seizures</td>
<td></td>
</tr>
<tr>
<td>Atonic seizures</td>
<td></td>
</tr>
</tbody>
</table>

**Unclassified seizures**

Inadequate or incomplete data
Studies assessing the relative frequency of seizure types have largely focussed on series of patients with severe epilepsy with a bias towards those with partial epilepsy. The paucity of clinical information and lack of EEG correlates in cases of mild epilepsy, often make it difficult to determine whether the seizure is of primary generalized or partial origin. With these restrictions in mind, most series have suggested that approximately half of epilepsies may be generalized, whilst the other half are partial, most commonly of temporal lobe origin.

1.1.2 Syndrome classification

Initial epilepsy classification systems made no distinction between syndromes and seizures, employing terminology such as grand mal, petit mal and psychomotor epilepsy. These terms were non-discriminatory and made no distinction between patients with generalized and partial epilepsy. The representatives of the International Commission for Classification of the Epilepsies have since proposed three classification systems.

1.1.2.1 International classification of the Epilepsies (Merlis, 1970)

The epilepsies were divided into three major divisions: generalized, partial and unclassifiable. Generalized epilepsies were subsequently subdivided into primary, secondary and undetermined. All partial epilepsies were presumed to be symptomatic, arising from a known or suspected insult. Criticisms of this classification system centred around the terminology, with confusion arising over similar terms such as secondary generalized (meaning secondary to a known illness) and secondarily generalized seizures (implying generalized seizure from a focal onset).

1.1.2.2 International Classification of the Epilepsies and Epileptic Syndromes (ICEES) (1985)

The ICEES aimed to supplement the ICES since the limitation of the latter was that it was confined to the description of individual seizure types, whereas the terminology used in daily communication consists of descriptions of syndromes.

The fundamental dichotomy of focal and generalized epilepsies was retained, although two new categories: the epilepsies and syndromes undetermined whether focal or generalized, and the special syndromes, were included in this classification. The first
acknowledged the often-blurred division between generalized and focal epilepsies, particularly in the case of nocturnal seizures. The special syndromes include febrile convulsions, acute symptomatic (provoked), isolated unprovoked seizures and isolated status epilepticus. The generalized and focal epilepsies were then subdivided into those with a known aetiology (symptomatic or secondary), and idiopathic (primary).

*International Classification of the Epilepsies and Epileptic Syndromes (1989)*

The latest revision of the ICEES (Table 2) differed from its predecessor by the introduction of the term, cryptogenic epilepsies to apply to cases where a symptomatic cause was presumed but supportive evidence was lacking. Idiopathic cases referred only to epilepsies with either a suspected genetic background or those demonstrating characteristic clinical and EEG features. Descriptions of various types of symptomatic partial epilepsies, including the two types of Kojewnikow’s syndrome: Rasmussen’s encephalitis, and epilepsia partialis continua were also included.

*A proposed diagnostic scheme for people with epileptic seizures and epilepsy (Engel, 2001)*

The task force has recently proposed that the terms partial and localization-related be replaced with “focal”, and that cryptogenic be replaced by “probable symptomatic epilepsy syndrome” to refer to syndromes that are believed to be symptomatic, but no aetiology has been identified.
### Table 2. The 1989 International Classification of the Epilepsies and Epileptic syndromes (ICEES)

#### 1. Localization-related epilepsies and syndromes

1.1 Idiopathic (with age-related onset)
   - Benign childhood epilepsy with centro-temporal spike
   - Childhood epilepsy with occipital paroxysms
   - Primary reading epilepsy

1.2 Symptomatic
   - Chronic progressive epilepsia partialis continua of childhood (Kojewnikow’s syndrome)
   - Syndromes characterized by seizures with specific modes of precipitation.
   - Temporal, frontal, parietal and occipital lobe epilepsies

1.3 Cryptogenic
   - Temporal, frontal, parietal and occipital lobe epilepsies

#### 2. Generalized epilepsies and syndromes

2.1 Idiopathic (with age-related onset)
   - Benign neonatal familial convulsions
   - Benign neonatal convulsions
   - Benign myoclonic epilepsy in infancy
   - Childhood absence epilepsy (pyknolepsy) / Juvenile absence epilepsy
   - Juvenile myoclonic epilepsy
   - Epilepsy with GTCS seizures on awakening
   - Other generalized idiopathic epilepsies not defined above

2.2 Cryptogenic or symptomatic
   - West syndrome (infantile spasms, Blitz-Nick-Salaam Krampfe)
   - Lennox-Gastaut syndrome
   - Epilepsy with myoclonic-astatic seizures
   - Epilepsy with myoclonic absences

2.3 Symptomatic
   2.3.1 Non-specific aetiology
      - Early myoclonic encephalopathy
      - Early infantile epileptic encephalopathy with suppression burst
      - Other symptomatic generalized epilepsies not defined above

   2.3.2 Epilepsies due to specific neurological diseases

#### 3. Epilepsies and syndromes undetermined whether focal or generalized

3.1 With both generalized and focal seizures
   - Neonatal seizures
   - Severe myoclonic epilepsy in infancy
   - Epilepsy with continuous spike-waves during slow wave sleep
   - Acquired epileptic aphasia (Landau-Kleffner-syndrome)
   - Other undetermined epilepsies not defined above

3.2 Without unequivocal generalized or focal features. All cases with generalized tonic-clonic seizures in which clinical and EEG findings do not permit classification as clearly generalized or localization related eg. nocturnal seizures where the patient recalls no aura, and additional studies eg. EEG are not revealing.
4. Special syndromes

4.1 Situation-related seizures
- Febrile convulsions
- Isolated seizures or isolated status epilepticus
- Seizures occurring only when there is an acute metabolic or toxic event due to factors such as alcohol, drugs, eclampsia, nonketotic hyperglycaemia.

1.1.2.2.1 Criticisms of the ICEES
The syndromic classification groups together similar symptoms and signs, providing little information on aetiology or prognosis. Syndromes with a range of diverse aetiologies and clinical course are linked on the basis of the likely site of seizure onset. Although identification of the epilepsy syndrome may have therapeutic and prognostic implications, the range of associated aetiologies results in prognostic heterogeneity (Everitt and Sander, 1999). The ICEES was largely developed for use in tertiary referral epilepsy centres where patients typically have intractable epilepsy with well-defined epileptogenic foci. Such patients are not representative of the majority of people with epilepsy, and consequently the ICEES has been seldom used in any large population study. The lack of access to formative investigations in epidemiological studies may also result in an excess of cryptogenic and unclassifiable epilepsies. In the UK National General Practice Study of Epilepsy (Manford et al., 1992), two-thirds of the newly diagnosed patients fell into the non-specific categories of cryptogenic localization-related, undetermined and isolated unprovoked seizures. The lack of an applicable and functional classification system for population-based studies has affected the validity of comparing the relative frequencies of epilepsy syndromes in different field studies. In response to this problem, the ILAE has published a set of guidelines (Figure 1) for use in epidemiological studies which is more simplistic and utilitarian in its approach and places greater emphasis on clinical risk factors and prognosis (International League Against Epilepsy, 1993).

Further revisions of the classifications will be inevitable as more information is obtained about epileptic disorders from advances in neuroimaging, electrophysiology and molecular genetics. Although the introduction of new MRI techniques e.g. diffusion-weighted, spectroscopic and functional imaging are likely to increase the yield
of structural lesions in MRI in epilepsy, the identified lesion may not represent the site of epileptogenesis. The most recent ICEES makes no reference to the role of neuroimaging in epilepsy syndromic classification, and it remains unclear as to whether EEG or neuroimaging should take precedence in cases where the findings from these investigations are discordant.

A neuroimaging centred approach would currently be impractical in population studies where access to high resolution MR imaging is frequently limited. Neurogenetic studies have described new inherited epilepsy syndromes (Berkovic et al., 1996). However, the impact of such advances on epilepsy classification is limited by their frequently polygenic inheritance and often poor relationship between genetic disturbances and phenotypic expression.

Ultimately, an ideal classification system should be applicable for use in both field studies and in tertiary referral centres. It should also be adaptable enough to incorporate new imaging and neurogenetic developments which have expanded our understanding of the pathogenesis of epilepsy (Everitt and Sander, 1999), and be applicable for specific applications such as pre-surgical evaluation and clinical pharmacology trials.
Figure 1  Simplified diagrammatic representation of the epilepsy classification proposed by the ILAE Commision on Epidemiology and Prognosis [(International League Against Epilepsy, 1993)]
1.1.3 Range of aetiologies of epilepsy
The aetiologies underlying partial epilepsy are diverse and vary according to age and geographical distribution. Endemic infections such as cysticercosis are the commonest identified cause of epilepsy in parts of Latin America, but virtually unknown in Europe (Sotelo et al., 1985). Virtually any condition that causes a disruption to cortical matter has the potential to bring about seizures. The aetiology of epilepsy may be multifactorial, with those patients with an inherited predisposition more prone to the development of acquired conditions (Nashef, 1996). The major aetiologies associated with epilepsy are shown in Table 3.

Table 3 The major aetiologies associated with epilepsy

| Genetic and congenital abnormalities |
| Anoxic and perinatal injury          |
| Trauma                              |
| Cerebral infection                  |
| Cerebrovascular disease             |
| Cerebral tumour                     |
| Cerebral degenerative disorders     |
| Cerebral inflammatory and immunological disorders |
| Toxins and drug withdrawal          |
| Metabolic disorders                 |

By providing a sensitive, non-invasive method of detecting subtle abnormalities such as hippocampal sclerosis (HS) and subtle cortical dysgenesis, high resolution MR imaging has increased the number of patients in whom a positive aetiological diagnosis is possible. In a hospital based MRI study of patients with chronic localization-related epilepsy, aetiologically relevant lesions were identified in 70% of scans (Li et al., 1995). The most common MRI findings were hippocampal asymmetry (32%), cortical
dysgenesis (12%), tumour (12%) and vascular malformations (8%). Hospital based series are, however, subject to selection bias, since the majority of such studies are performed in tertiary referral units with a tendency towards patients with refractory partial epilepsy. In a recent study of patients with focal epilepsy seen in specialist epilepsy and general neurology clinics, computerised tomography (CT) or MRI abnormalities were seen in 54%, which increased to 72% when only patients with focal epilepsy scanned with high resolution MRI were considered (Wieshmann et al., 2003).

The aetiology of epilepsy in population-based studies is mostly unclear, with most epidemiology studies identifying a putative cause in only a quarter to a third of cases. Study design limitations include retrospective case ascertainment, and variable access to formative investigations e.g. MRI scanning. Even in the study from Copparo (Granieri et al., 1983) which has one of the highest percentages of cases with known aetiology, 61% of cases remained cryptogenic. By adopting a prospective study design, and incorporating electro-clinical and high resolution neuroimaging data, Everitt et al., ascertained the likely aetiology in approximately 60% of patients with chronic epilepsy and 75% in patients with newly diagnosed seizures (Everitt et al., 1998). Hippocampal sclerosis, malformations of cortical development and foreign tissue lesions were less frequently observed in the community than in hospital series, although this may be attributed to the lack of selection bias, and the inclusion of both focal and generalized epilepsies.

In the United Kingdom National General Practitioners' study of epilepsy (Sander et al., 1990), the commonest putative aetiology was cerebrovascular disease at 16%. This figure rose to 49% in those aged over 60 years. Other commonly reported causes of partial epilepsy in population-based studies are head trauma, brain tumour, early CNS insults, infectious disease, congenital and genetic abnormalities (Sander and Shorvon, 1996), (Everitt et al., 1998).

Patients with primary generalized epilepsy have traditionally been regarded as having structurally normal brains. However, histopathological post-mortem studies and modern imaging techniques have shown subtle abnormalities that may be of aetiological relevance. These findings will be discussed in more detail in section 1.2.3.2. The more common aetiologies underlying epilepsy are discussed:
Hippocampal sclerosis

Hippocampal sclerosis, refers to a characteristic pattern of neuronal loss, characterized by severe pyramidal neuron loss and gliosis throughout the hippocampus proper, especially in Sommer's sector (cornu ammonis (CA1) and prosubiculum), CA3 and the end folium (including the CA4 pyramids and hilar neurones). It is the commonest MRI lesion observed in patients with chronic focal epilepsy in both hospital based and population-based series accounting for approximately one third of all patients (Li et al., 1995), (Everitt et al., 1998). Surgical specimens from temporal lobectomy series for intractable temporal lobe seizures have found HS in 50 – 75% of operated cases (Babb, 1987), (Bruton, 1988).

CNS Infections

Survivors of a central nervous system (CNS) infection reportedly have a three-fold increased risk for epilepsy (Annegers et al., 1988). The estimated risk of patients developing late epilepsy following viral encephalitis with and without acute symptomatic seizures is 25% and 10% respectively. The risk of developing late epilepsy following bacterial meningitis is approximately 3%. There is no excess risk of epilepsy associated with viral meningitis. A bacterial cerebral abscess may be followed by epilepsy in up to three-quarters of survivors, especially when the infection involves the frontal or temporal lobes (Legg et al., 1973). All ages may develop seizures secondary to infections, ranging from congenital toxoplasmosis in the newborn to Creutzfeldt-Jakob disease in the young and elderly. Common causes in developing countries, accounting for a large proportion of the epileptic patient population are parasitic disorders e.g. cysticercosis, malaria, toxocariasis and tuberculomas (Vasconcelas and Lombardo, 1980).

Cerebrovascular disease

Stroke is the most common risk factor for epilepsy in the elderly (Everitt et al., 1997), (Sander et al., 1990). The reported frequency of seizures after ischaemic stroke varies from 1.8% to 43% depending on seizure type (early- or late-onset), duration of follow-up and study design. The incidence of early seizures in hospital and population-based series ranged from 1 to 6% of ischaemic strokes, and were largely determined by stroke severity, cortical involvement and cortical location (So et al., 1996). More than half of patients developing early seizures following a stroke subsequently develop chronic...
epilepsy. Risk is not elevated with brainstem infarcts and is minimally increased with lacunar infarcts. The likelihood of developing epilepsy is higher following intracerebral haemorrhages than following occlusive insults. Approximately a quarter of survivors of subarachnoid haemorrhage due to ruptured cerebral aneurysm subsequently develop epilepsy (Olafsson et al., 2000). Both acute symptomatic seizures and persistent neurological impairment are associated with an increased risk of epilepsy. Ischaemic lesions may range from subtle cortical destruction to large areas of cystic encephalomalacia from large vessel occlusion. Perinatal anoxic-ischaemic lesions or encephalomalacia may manifest in later life as epilepsy (Fenichel, 1983).

**Brain trauma**

Head injuries are responsible for a significant number of focal epilepsies (Li et al., 1995). The age-specific incidence of head injury with brain involvement has a trimodal appearance, with peaks in early childhood, teenage and young adulthood, and in the elderly population. The chief risk factors for late post-traumatic epilepsy include the presence of intracranial haemorrhage, dural laceration, cortical damage and early post-traumatic seizures, which may be a marker of severity of injury (Jennett, 1995). In the absence of a post-traumatic amnesia of 30 minutes or greater, the risk of developing post-traumatic epilepsy is considered low (Annegers et al., 1980). Late or remote symptomatic seizures follow a latent period ranging from weeks to months, and sometimes years. The risk of late post-traumatic seizures in patients six months after traumatic brain injury was increased two-fold in patients with the Apolipoprotein E ε4 allele (Diaz-Arrastia et al., 2003). The risk of epilepsy following neurosurgery is less than that seen with penetrating head injuries, and depends on the nature and location of surgery, the underlying condition, and the presence or absence of focal brain damage.

**Alcohol**

Reasons for the three-fold increased risk for epilepsy in chronic alcohol abuse are likely to be multifactorial (Little and Gayle, 1980). Seizures may occur in the context of alcohol withdrawal, increased risk of head trauma from falls and road traffic accidents, increased incidence of cerebral infarcts, sleep deprivation, and metabolic effects e.g. hypoglycaemia, acid-base and electrolyte imbalance, state of hydration, and vitamin deficiencies. In incidence studies or series of newly diagnosed cases of epilepsy, chronic alcohol abuse is considered a factor in 10-25%.
Tumours
Seizures are the initial presenting symptom in up to 50% patients with brain tumours (Rasmussen, 1975), and account for 3-8% of epilepsy presenting to tertiary referral units (Li et al., 1995), (Semah et al., 1998). The peak incidence is in middle age, and tumours involving the fronto-parietal or temporal lobes are most likely to cause seizures. Below the age of 20, tumours are more likely to be infratentorial and less epileptogenic. Tumour histology and rate of growth also influence the likelihood of seizures, with oligodendrogliomas, low-grade gliomas, astrocytomas and meningiomas being the most epileptogenic. In contrast, low seizure rates are associated with primary CNS lymphomas and cerebral metastases which tend to be located away from the surface of the cortex at the grey matter / white matter junction. Disinhibitory changes, characterized by loss of function of γ-aminobutyric acid (GABA) receptors, have been demonstrated in neurones in cortex proximal to gliomas (Williamson, 1994).

Cortical dysplasia and migration disorders
With the advent of MRI, malformations of cortical development (MCD) are being increasingly recognized as an important cause of epilepsy (Li et al., 1995). There remains little information about the frequency of such anomalies in the population without either epilepsy or mental retardation. Seizures may be the only overt clinical sign in subtle cases e.g. subependymal heterotopias, whilst more severe MCD e.g. pachygyria, lissencephaly, schizencephaly, and corpus callosal agenesis may also be associated with learning or physical disabilities. Dysembryoplastic neuroepithelial tumours (DNET) exhibit very indolent behaviour but show a very strong association with epilepsy (Daumas-Duport et al., 1988). They typically present with partial seizures starting before the age of 20 years, and are characterized by their cortical location, multinodular architecture composed of astrocytomas, oligodendrogliomas or both cell types and foci of dysplastic cortical disorganization.

Vascular malformations
Seizures are the presenting symptoms in 19% of arteriovenous malformations (Abad et al., 1983). Cavernous angiomas are multiple in about 30% of cases, and the risk of epilepsy is high. Mechanisms of epileptogenesis may include (occult) haemorrhage, steal phenomena (Costantino and Vinters, 1986), or compression of surrounding brain.
Familial and Metabolic Disorders
Neurofibromatosis can be associated with neoplastic lesions and meningocortical hamartomas (angiomatosis) that may be epileptogenic. Mitochondrial encephalomyopathies and leukodystrophies are rare causes of epilepsy.

Chronic Encephalitis/ inflammatory
Rasmussen's encephalitis is a rare cause of epilepsy, usually presenting with epilepsia partialis continua, and associated with progressive hemiparesis and intractable seizures. The syndrome may have an autoimmune basis since antibodies to the glutamate receptor 3 subunit have been identified in the CSF and serum of some patients (McNamara et al., 1999). The role of various viruses e.g. cytomegalovirus and Epstein-Barr virus have been proposed as causal factors (Power et al., 1990).

Genetic causes
Genetic factors may contribute to aetiology in up to 40% of patients (Gardiner, 1999), although genetic or chromosomal syndromes account for only 2-3% of all cases of epilepsy (Nance et al., 1997). Mendelian epilepsies can be divided into symptomatic and idiopathic epilepsies. A diverse group of genes have been identified in symptomatic epilepsies, such as the progressive myoclonic epilepsies. These include the autosomal recessive conditions, Unverricht-Lundborg disease (EPM1) and Lafora body disease (EPM2); the autosomal dominant condition with variable penetrance, dentato-rubro-pallido-luysian atrophy (DRPLA); and the mitochondrial inherited myoclonic epilepsy with ragged red fibres (MERFF). Progress has also been made with the identification of genes in neuronal migration disorders e.g. subcortical band heterotopia (DCX), periventricular nodular heterotopia (FLNI), and isolated lissencephaly (LIS1).

Epilepsy forms part of the phenotype of over 200 single gene disorders, the commonest of which is tuberous sclerosis, an autosomal dominant inherited condition, which is the most frequent pathology underlying the Lennox Gastaut syndrome. Established loci are regions 9q 34 and 16p 13.3. Other Mendelian diseases featuring seizures include neurocutaneous diseases e.g. neurofibromatosis (NF1), xeroderma pigmentosa, neuroichthyosis, von Hippel-Lindau disease, incontinentia pigmentosum, and hereditary haemorrhagic telangiectasia; aminoacidopathies e.g. phenylketonuria and homocystinuria; glycogen storage diseases e.g. glucose-6-PD deficiency;
Chapter I

sphingolipidoses e.g. Gaucher's disease; and miscellaneous conditions e.g. Wilson's disease, acute intermittent porphyria, and the fragile X syndrome (FRAX). Various chromosomal disorders including trisomy 13, 18, 21, and Klinefelter syndrome are also associated with an increased risk (>20%) of seizures.

Idiopathic epilepsies, which account for 40% of all epilepsies (Berkovic et al., 1998) have an almost exclusively genetic aetiology and no clinically identifiable aetiologic factor. The common syndromes e.g. juvenile myoclonic epilepsy, childhood absence epilepsy, and generalized tonic clonic seizures (GTCS) on awakening, display a 'complex' non-Mendelian pattern of inheritance (Gardiner, 1999). A few idiopathic epilepsies are inherited in a Mendelian fashion e.g. benign familial neonatal convulsions (EBN1, EBN2), autosomal dominant nocturnal frontal lobe epilepsy (FLE) (ADNFLE), and generalised epilepsy with febrile seizures plus (GEFS+). There is emerging evidence to suggest that epilepsy susceptibility genes code for voltage-gated and ligand-gated ion channels (see below).

Cryptogenic

Despite optimal MR imaging, an identifiable abnormality cannot be found in 20-30% of patients with refractory focal epilepsy (Duncan, 1997), (Li et al., 1995). Structural correlates of surgically resected epileptogenic areas which have appeared normal on MRI have included mild subpial or white matter gliosis, focal cortical dysplasia, clusters of neuronal aggregates in white matter, impaired cortical lamination and subcortical laminar heterotopia (Theodore et al., 1990), (Palmini et al., 1991a). It has therefore been suggested that occult structural changes e.g. mild gliosis, subtle malformations of cortical development, and microdysgenesis may be a cause of epilepsy in some patients with refractory epilepsy.

Receptor abnormalities have also been proposed as a possible structural / functional basis for cryptogenic epilepsy. \(^{11}\)C Flumazenil-Positron emission tomography (PET) studies have shown regional cortical abnormalities of central benzodiazepine receptors (BZR) in 72% of 'MRI-negative' patients with focal epilepsy (Richardson et al., 1998). Autoradiographic and histopathological studies of surgically removed sclerotic hippocampi have shown reduced central BZR densities that correlated with the degree of neuron loss (Burdette et al., 1995). However, a quantitative in vivo binding study
suggested that there was additional central BZR loss that could not be attributed solely to the loss of hippocampal neurones (Hand et al., 1997). Serial absences have been associated with acute reductions (15-41%) in $^{11}$C diprenorphine binding to association areas of neocortex implying that dynamic changes in ligand-receptor interaction may play a pathophysiological role in the generation of absence seizures (Bartenstein et al., 1993).

Alternative hypotheses propose that certain epilepsies may be channelopathies. Both voltage- and ligand-gated ion channels have been implicated in the genesis of rare dominantly inherited epilepsies (Kullmann, 2002). Mutations in the CHRNA4 or CHRNβ subunits of the neuronal nicotinic acetylcholine receptor lead to familial nocturnal FLE, while defects in the voltage-gated potassium channels KCNQ2 and KCNQ3 have been found to cause benign familial neonatal convulsions. The voltage-gated sodium channel subunits SCN1B, SCN1A and SCN2A as well as the GABRG2 subunit of the GABA$_A$ receptor are involved in generalized epilepsy with febrile seizures plus (Steinlein, 2002). Autosomal dominant juvenile myoclonic epilepsy (JME) has been shown to be a channelopathy associated with a GABA$_A$ receptor, α1 subunit mutation. Ion channel genes are considered strong candidates for susceptibility factors that may underlie the inheritance of common sporadic primary generalized epilepsy.

Two new non-channel related genetic syndromes have recently been uncovered: Leucine-rich, glioma inactivated 1 gene (LGIl), which is associated with an idiopathic form of temporal lobe epilepsy (TLE); and Aristaless related homeobox gene (ARX), associated with severe seizures, intellectual disability and malformations of cortical development (Kalachikov et al., 2002), (Stromme et al., 2002).

1.2 STRUCTURAL MRI AND VISUAL ASSESSMENT
1.2.1 Role of MRI in identifying the aetiology of epilepsy
Since 1984, MRI has largely superseded the use of X-ray computed tomography (CT) scanning in the study of epilepsy and offered new insights into its aetiology (McLachlan et al., 1985). The ability of MRI to provide highly detailed multiplanar anatomical data
without bone artefact or the use of ionizing radiation, has made MR imaging the structural neuroimaging procedure of choice (Kuzniecky et al., 1993).

The higher yield of MRI imaging compared with CT scans may be attributed in part to its better grey-white matter differentiation, better detection of focal brain oedema, and its ability to confer higher contrast between normal and abnormal tissue. Neuroimaging in epilepsy permits the identification of the pathological substrate underlying the epileptic focus, assistance with the formulation of syndromic and aetiological diagnoses, and the selection and presurgical evaluation of patients for epilepsy surgery. Although CT scanning demonstrates high sensitivity for high grade supratentorial neoplasms (Baker et al., 1980), chronic epilepsy is more usually associated with small or low-grade neoplasms in the temporal lobe which are not shown well by Xray CT. Many of the potentially curative lesions e.g. hippocampal sclerosis, indolent gliomas, cavernomas and DNETs, may be located in the temporal lobe and are poorly visualized on CT scanning due to bony artefacts from the middle cranial fossa. Only 60% of MR imaging detected DNETs, 40% of cortical dysplasia and 30% of subependymal heterotopias can be detected on CT (Wieshmann et al., 1996). Situations when a CT scan may be preferable to MRI are: when the patient is acutely unwell, if MRI is contraindicated due to e.g. a pacemaker or cochlear implant, when investigating the possibility of acute intracranial haematomas and skull fractures, and when demonstrating cortical calcification, particularly in patients with congenital or acquired infections. With the improved detection of lesions conferred by structural MRI, the proportion of patients with cryptogenic epilepsy in prevalence studies is diminishing.

Late onset epilepsy is generally associated with a higher yield of structural causes, since MRI scans are superior to CT scans in demonstrating the most common aetiologies seen in this age group i.e. cerebrovascular disease, brain tumours and neurodegenerative disease (Bradley et al., 1984). In a prospective investigation of 32 patients with late onset epilepsy and normal CT scans, MRI revealed irrelevant ischaemic lesions in eight, and the cause of seizures in four patients i.e. left frontal glioma, left hippocampal sclerosis, metastases and temporal lobe haemorrhagic infarct (Kilpatrick et al., 1991). Common findings on the MRI scans of older patients are asymptomatic high-intensity white matter lesions often assumed to be ischaemic. The difficulty lies in determining whether such lesions are incidental findings related to asymptomatic age-related
vascular changes, or whether they represent the epileptogenic focus. An MRI detected lesion may thus only assist in localizing the site of seizure onset, when correlated with ictal semiology and electrophysiological data.

1.2.2 The impact of improved MRI sensitivity

1.2.2.1 Focal epilepsy

High detection rates of structural lesions have been reported in focal epilepsy, in particular TLE. In one series with MRI findings including quantitative assessment of patients with chronic focal epilepsy, a structural lesion was detected in over 90% of patients with temporal lobe symptomatology (Cook et al., 1992). The range of pathologies found in extra-temporal focal epilepsy is more extensive, and the detection of subtle cortical lesions in the frontal lobes more difficult than in the temporal lobes due to volume averaging and high complexity of cortical gyrations (Kuzniecky et al., 1993). Structural lesions detected on high-resolution MRI give an indication of seizure focus, and provide prognostic information since outcome from anterior temporal lobectomy is improved in the presence of identifiable pathology (Jack et al., 1992), (Duncan and Sagar, 1987). The improved detection of hippocampal sclerosis, cortical malformations, vascular malformations, and foreign tissue lesions by MRI are discussed below.

Hippocampal sclerosis

One of the areas in which MRI has had its greatest impact is in the detection of HS. Hippocampal sclerosis is the most common pathological condition underlying intractable partial epilepsy and is amenable to surgical intervention. Seizure outcome following anterior temporal lobe resection is good, with 65% to 80% of patients being rendered seizure-free (Babb, 1987), (Bruton, 1988).

The improved detection of HS can be attributed to: increased knowledge about the anatomy of the hippocampus; optimal imaging planes; and advances in MRI hardware, acquisition sequences and post-processing techniques (Duvernoy, 1998b), (Berkovic et al., 1991), (Press et al., 1989). The sensitivity and specificity for the visual diagnosis of HS ranges from 80 to 93% and 86 to 93% respectively (Jackson et al., 1990), (Jackson et al., 1993a). MRI features of HS include hippocampal atrophy on coronal T1-weighted images, increased signal intensity within the hippocampus on T2-weighted
spin echo images (Jackson et al., 1990), decreased T1-weighted signal intensity and disrupted internal hippocampal architecture (Jackson et al., 1993a). A high correlation is found between MR features / quantitation of HS and pathological findings of neuronal loss and gliosis (Kuzniecky et al., 1987), (Van Paesschen et al., 1997a).

**Malformations of cortical development**

MRI has allowed the recognition of subtle forms of dysgenesis previously only detectable on histological examination. In one study, CT was positive for focal developmental pathology in about 63% of those patients with abnormal MRI (Palmini et al., 1991b). MCDs are being increasingly recognized as causes of both epilepsy and neuro-developmental deficits (Duncan, 1997). Due to the convoluted shape of the cerebral cortex and its variability, it is often difficult to appreciate cortical dysplasia on planar images. Volumetric acquisitions using thin slices permit reformatting in oblique planes, and three-dimensional surface rendering of the cortical surface may enhance the visibility of abnormalities such as, focal areas of polymicrogyria (Sisodiya et al., 1995a).

Common features of MCDs are an alteration in the number, size, and thickness (usually increase) of cortical gyri and the presence of abnormal neuron tissue in heterotopic locations. Occasionally, signal changes are present in the surrounding white matter. Common pathologies visible on MRI include hamartomas, focal cortical dysplasia (FCD), schizencephaly, agyria, diffuse and focal macrogyria, minimal gyral abnormalities, subependymal grey matter heterotopia, focal pachygyria and polymicrogyria, with reported incidences ranging between 6.7% of patients with established epilepsy and 13.7% patients with associated mental retardation (Brodtkorb et al., 1992). The identification of MCDs raise prognostic and therapeutic issues as some forms, such as focal cortical dysplasias and hemimegalencephaly may respond to surgical treatment (Duncan, 1997). Despite significant advances in spatial resolution, subtle forms of cortical dysplasia identified on autopsy may pass undetected using conventional MRI. Novel research techniques aim to identify such occult dysplastic areas.
Chapter I

Dysembryoplastic neuroepithelial tumours

DNETs are common benign developmental tumours, found in both temporal and extra-temporal sites and only recently recognized as a cause of chronic focal epilepsy (Cook et al., 1995). Whether DNETs be regarded as forms of cortical dysplasia or neoplastic lesions, remains a contentious issue. Differentiation from low-grade gliomas or hamartomas relies on the detection of surrounding dysplastic cortex and the presence of bizarre giant neurons within the lesion. Although some are heavily calcified and therefore detected by CT, their frequently small size means that detection is improved by MRI acquisition sequences with thin partitions. The presence of cystic components and blurring of adjacent grey-white matter boundary in temporal lobe lesions are better evaluated on MRI.

Foreign tissue lesions

MRI is more sensitive than CT in the detection of foreign tissue lesions causing focal epilepsy. CT identified lesions are typically either calcified or greater than 2.2cm in diameter (Jackson et al., 1990). MRI detects foreign-tissue lesions with 100% sensitivity, but may not be able to differentiate between tumours of different histologic type e.g. oligodendroglialomas from low-grade astrocytomas. Gangliogliomas and gangliocytomas are rare benign neuronal tumours, with similar MRI appearances to DNETs. Gangliogliomas are important to diagnose, as surgical outcome is often favourable (Duncan, 1997).

Vascular abnormalities

Vascular malformations are well demonstrated by MRI and allow differentiation between an arteriovenous malformation (AVM) and a cavernous angioma. Flow signal, observed in AVMs is often absent in cavernous angiomas. Cavernous angiomas are common vascular causes of focal epilepsy with distinctive MRI appearances, although easily missed on CT. Characteristically, T2-weighted images show a reticulated core of mixed signal intensity with a low signal halo extending into surrounding tissue, attributed to haemosiderin deposition (Duncan, 1997). Surgical removal can be successful with a 40% chance of subsequent seizure remission.
1.2.2.2 *Generalized epilepsy*

A precondition for the diagnosis of primarily generalized epilepsy is the absence of neuroradiological signs (ILAE, 1985). Although visual inspection of routine high resolution MRI is normal in patients with idiopathic generalized epilepsy (IGE), quantitative post-processing techniques (Woermann et al., 1998b), (Woermann et al., 1999) and brain atlas programs (Savic *et al*., 1998) have revealed widespread cerebral structural changes not evident on conventional imaging. These techniques are discussed in section 1.5.1.

1.2.3 *MRI negative patients*

1.2.3.1 *Focal epilepsy*

Despite higher spatial resolution in data acquisition and multiplanar reformatting of volumetrically acquired data, up to 25% of patients with chronic focal epilepsy remain cryptogenic with no demonstrable lesion on conventional two-dimensional images (Li *et al*., 1995). Approximately 20% TLE patients have been found to have normal MRI scans despite the use of quantitative hippocampal and amgdala T2 measurements (Van Paesschen *et al*., 1996). This is consistent with the finding that a definite structural abnormality is not detected in 20% of histological specimens taken from patients undergoing surgery for intractable TLE (Corsellis, 1970).

Further processing of volumetric scan data may extract additional data and increase the yield of structural lesions. Abnormal gyral patterns e.g. subtle focal cortical dysplasia (FCD), may be better displayed using curvilinear reformatting (Bastos *et al*., 1999) and texture analysis (Bernasconi *et al*., 2001), and are discussed in section 1.5.1.

In one series, MRI and PET studies revealed MRI abnormalities in only 46% of cases of FLE (Swartz *et al*., 1989). Despite volumetric analysis and improved reformatting, approximately 50% of cases of FLE remained MRI negative (Laskowitz *et al*., 1995). Post-processing methods including 3D-reconstructive techniques have improved the detection of gyral abnormalities and are discussed in section 1.5.1 (Sisodiya *et al*., 1995a).

Diffusion-weighted MR, in particularly, increased diffusivity has been shown to be a sensitive index in identifying abnormalities in patients with epilepsy that are not evident...
using conventional MRI. Voxel-by-voxel analysis of anisotropy and diffusivity revealed abnormalities of diffusion in regions concordant with EEG localization in 23% patients with normal conventional MRI (Rugg-Gunn et al., 2001). Abnormalities of diffusion were thought to represent occult dysgenesis, acquired damage or atrophy, or gliosis secondary to repeated seizures. Other novel MRI contrasts such as magnetization transfer ratio imaging (Rugg-Gunn et al., 2003), double inversion recovery and fast fluid attenuated inversion recovery T2 mapping are also showing potential in identifying brain abnormalities missed on conventional MRI.

The improved detection of occult structural lesions provided by these novel techniques play an important role in increasing the number of patients with focal epilepsy that are amenable to surgery. Formal pathological correlation for these techniques is in progress.

1.2.3.2 Generalized epilepsy

Although visual inspection of high resolution MRI in generalized epilepsy is normal, quantitative MRI using semi-automated segmentation has identified widespread cerebral structural changes in patients with various IGE syndromes (Woermann et al., 1998b). This finding is concordant with pathological studies showing that cortical and subcortical ectopic neurones and microdysgenesis may be seen in autopsies of cases with IGE (Meencke and Janz, 1984). The structural changes may reflect functional abnormalities demonstrated by PET studies, such as increased volume of distribution of 11C flumazenil binding that may reflect increased number of neurone cell bodies, and an increase of cortical and thalamic blood flow during absence seizures (Koepp et al., 1997b), (Savic et al., 1994). In addition, a computerized anatomic brain atlas has demonstrated morphological abnormalities in patients with primary GTCS where conventional structural neuroimaging has been normal (Savic et al., 1998).

1.2.4 Dual pathology

Dual pathology refers to the presence of more than one pathology and is important to diagnose since its presence may dictate treatment options and influence surgical outcome. Dual pathology is detected in about 5-30% of patients with refractory focal epilepsy assessed for surgical evaluation using high resolution MRI (Cascino et al., 1993), (Cendes et al., 1995), (Li et al., 1997). Extrahippocampal lesions frequently found in association with HS are developmental abnormalities such as cortical
dysgenesis (CD), tumours, contusions / infarcts, vascular malformations and gliotic lesions acquired in early childhood. In a study by Raymond et al. (Raymond et al., 1994), the most common dysgenetic lesion found in association with HS was subependymal heterotopia. In contrast, cortical dysplasia amongst normal subjects is rare. In two separate histological series (Meencke and Veith, 1992), (Raymond et al., 1994), marked migrational abnormalities were observed in only 1-2% of normals.

Dual pathology is found in 87% of patients demonstrating evidence of unilateral temporal lobe developmental malformations. In these patients the amygdala formation is commonly involved and hippocampal (HC) atrophy is often bilateral (57% compared with 18% of patients with pure HS without extrahippocampal pathology) (Ho et al., 1998). The detection of unilateral and bilateral amygdala and hippocampal formation (AM-HF) damage by quantitative MRI is important as it is associated with a significantly higher risk of seizure recurrence if only the developmental lesion alone is resected (Kuzniecky et al., 1999).

Only 13% of patients with dual pathology give a history of childhood febrile convulsions compared with 36% of patients with isolated HS (Raymond et al., 1994). Several studies have reported that both the extrahippocampal lesion and the atrophic lesion are likely to be involved in seizure generation and consequently removal of both are necessary to achieve seizure freedom (Li et al., 1997), (Cascino et al., 1993). However, this is only feasible if the pathologies lie in close proximity. In one series, 73% patients undergoing removal of both the lesion and the atrophic hippocampus became seizure free compared with 20% of those who had resection of the atrophic hippocampus alone and only 12.5% of those who had a lesionectomy (Li et al., 1999).

The presence of HS in dual pathology exerts a negative effect on the surgical outcome independent of the severity of the hippocampal atrophy (Li et al., 1997). Therefore, careful quantitative evaluation with volumetric measurement of mesial temporal structures should be performed in all patients with developmental lesions referred for surgery (Raymond et al., 1994), (Cendes et al., 1995).

Three mechanisms have been proposed to explain the dual occurrence of HS and CD: (1) the presence of CD predisposes to prolonged febrile convulsions in childhood,
leading to HS; (2) repeated seizures from CD results in secondary damage to the hippocampus; and (3) a common pathogenesis during gestational development of the brain is responsible for both lesions. Pertaining to the kindling theory, extrahippocampal lesions vary in their ability to create dual pathology. Gliomas are less commonly found in association with HS compared with vascular malformations or hamartomas, suggesting differences in the threshold of hippocampal susceptibility to seizure-induced damage (Mathern et al., 1995a). Although Ho et al. (Ho et al., 1998) found no correlation between amygdala–hippocampal (AM-HF) formation volume and duration of seizure history or age at seizure onset, hippocampal specimens showed sclerotic changes classical of mesial temporal sclerosis. This observation would suggest that the abnormal HF and AM are likely to be atrophic rather than developmentally small. The presence of bilateral AM-HF abnormalities is not discordant with seizure-related changes, since mesial temporal structures receive anterior commissural fibres from the contralateral temporal neocortex. The projections between the mesial temporal structures and ipsilateral temporal neocortex could explain the unilateral AM-HF damage (Amaral and Price, 1984).

1.3 PRINCIPLES OF MRI
1.3.1 Physics of nuclear magnetic resonance
The principles of MRI rely on nuclei with spin or angular momentum, that is nuclei capable of aligning their axis of rotation to an external magnetic field. If the nucleus has a net charge, it also has a magnetic dipole moment (MDM). The strength of the magnetic moment varies according to each nucleus and determines the sensitivity to magnetic resonance. In clinical MRI imaging, the strongest signal is obtained if the hydrogen nucleus is chosen, due to its solitary proton which gives it a relatively large magnetic moment, and its abundance in the human body. When a subject is placed in a static external magnetic field (B₀), typically 0.5 to 4 Tesla, the magnetic moments of the hydrogen nuclei align either parallel or anti-parallel to B₀, constituting the net magnetization vector (NMV). B₀ exerts a secondary spin (precession) on the natural spin of each hydrogen nucleus where the precessional frequency (Larmor frequency) is proportional to B₀.
Larmor frequency \((\omega_0) = B_0 \gamma\)

where \(\omega_0\) = the precession frequency of the magnetic dipole of a spinning nucleus in a magnetic field in revolutions per second, \(B_0\) = the strength of the magnetic field in Tesla, and \(\gamma\) = the gyro-magnetic ratio for the nucleus in question and expressed in MHz / T.

For resonance of hydrogen to occur, a radio frequency (RF) pulse of energy at exactly the Larmor frequency of the hydrogen NMV must be applied. In a 1.5T MRI scanner, hydrogen nuclei MDMs are precessing at 63.76MHz. The RF pulse is an electromagnetic wave produced from the brief application of an alternating electric current through a transmitter coil, which is applied perpendicular to the \(B_0\). Resonance causes the NMV to move into a transverse plane that is in phase. The degree to which the NMV is tipped (flip angle) is directly proportional to the amplitude and duration of the RF pulse. Following the application of the RF pulse, the NMV precesses in the transverse plane and hence generates the MR signal.

1.3.2 T1, T2 relaxation and image contrast

T1 and T2 are time constants that refer to the reinstatement of thermal equilibrium of the NMV following a RF pulse. The reduction in magnitude of transverse magnetisation results in a fall in the magnitude of the voltage induced in the receiver coil, the free induction decay signal. T1 recovery (spin lattice relaxation) refers to the recovery of longitudinal magnetisation and T2 decay (spin spin relaxation) describes the decay of transverse magnetisation. The T1 relaxation time is the time it takes 63% of the longitudinal magnetisation to recover, and the T2 relaxation is the time taken for transverse magnetisation to decay to about 37% of their maximum value.

MRI has excellent soft tissue discrimination. Tissues with a large transverse component produce areas of high signal, whereas a small transverse component of magnetisation produces areas of low signal. The Larmor frequency of hydrogen in water is significantly higher than hydrogen in fat, thereby accounting for the extremes of contrast in MRI. The free water molecules tumble rapidly and relax ineffectively. The magnetic moments take longer to regain their longitudinal magnetisation and have a
long T1. In contrast, the slow molecular tumbling in fat allows for a relatively rapid recovery process, a faster recovery of longitudinal magnetisation and a shorter T1 time.

Proton or spin density contrast, the basic MRI contrast, is determined by the result of the relative number of protons per unit volume. Brain tissue has a high proton density, whereas cortical bone has a low proton density. An image acquisition with a long repetition time (TR) and a short echo time (TE,) would result in an image that was essentially neither T1 nor T2 weighted, hence the only factor left to influence image contrast would be the number of hydrogen nuclei per mm$^3$.

1.3.3 MR echoes and contrast modification

A spin echo pulse sequence uses a 90° excitation pulse followed by one or more 180° rephrasing pulses to generate one or more spin echoes. The 180° pulse eliminates external magnetic field inhomogeneity and allows only the tissue characteristics to prevail as the main dephasing cause. Multiple echo pulse sequences are produced when a spin-echo pulse cycle contains more than one signal measurement. Spin echo pulse sequences are used to generate T1, T2 or proton density weighting. T1 and T2 weighted images are images in which the intensity contrast between two tissues is due mainly to the T1 and T2 relaxation properties of the tissues respectively (Figure 2). T1 and T2 weighted images are generated by the manipulation of TR (time interval between two successive pulse cycles) and TE (time interval from a pulse to the measurement of the MR signal). T1 weighting requires a short TE to eliminate the effect of T2 and a short TR in order not to eliminate the effect of T1, whereas T2 weighting requires a long TR to eliminate the effect of T1 and a long TE not to eliminate the effect of T2.

Proton density and T2 weighting is acquired if two RF rephasing pulses are applied generating two spin echoes. The first echo has a short TE and a long TR (proton density), and the second has a long TE and a long TR to achieve T2 weighting.
A gradient echo pulse sequence uses a variable RF excitation pulse that is able to flip the NMV through any angle. On withdrawal of the RF pulse, the magnetic moments are rephased by a gradient, inducing a maximum signal in the receiver coil called a gradient echo. With low flip angles, full recovery of the longitudinal magnetisation occurs sooner allowing for a shorter TR. Compared with spin echo pulse sequences, gradient echo pulse sequences have the advantage of shorter scan times, but are more susceptible to magnetic field inhomogeneities. These inhomogeneities may be the result of either intrinsic defects in the magnet itself or of susceptibility-induced field distortions produced by tissue or haemoglobin. A range of pulse sequences is shown in Table 4.

Table 4 Principal pulse sequences used for structural imaging

<table>
<thead>
<tr>
<th>Spin echo pulse sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional spin echo</strong></td>
</tr>
<tr>
<td><strong>Fast spin echo</strong></td>
</tr>
</tbody>
</table>

**Inversion recovery**
- STIR (short T1 inversion recovery)
- FLAIR (fluid attenuated inversion recovery)

**Gradient echo pulse sequences**
- Conventional gradient echo

**Ultra-fast sequences**
Figure 2  Schematic representation of a basic pulse sequence.

**Repetition time (TR)** is the interval between two successive pulse cycles in milliseconds (ms). The TR determines the amount of T1 relaxation.

**Echo Time (TE)** is the time taken from the application of the RF pulse to the measurement of the MR signal in ms. The TE determines the degree of transverse magnetisation allowed to occur before the signal is read. The TE determines the amount of T2 relaxation.
1.3.4 Image acquisition and reconstruction
The application of different gradients (slice selecting, frequency encoding and phase encoding) results in a mixture of signals with different frequencies and phases according to their specific location. The subsequent generation of an image from these signals involves three conceptual stages. The first stage is called digitization, in which the scanner stores signal amplitude measurements as a matrix of data known as the “k-space”. This matrix has no correspondence to spatial coordinates, but provides information on the amplitude of the total signal at each instant of time. The Fourier transform is the mathematical formula that allows the translation of this description into a set of amplitudes at specific frequencies and phases. These phase-frequency matrices are then transformed into images expressed in Cartesian format, with X and Y referring to the linear dimensions of the image slice inplane and Z referring to the slice depth. The position and linear dimensions of each voxel are known, and each voxel is assigned a 16-bit grey-scale level. This grey-scale value represents the MRI signal intensity of the tissue at that spatial location.

1.3.5 Standard MR sequences used in clinical epilepsy
An optimal MRI scanning protocol should comprise T1 and T2-weighted sequences in at least two orthogonal planes using minimum slice thickness. Commonly, a T1-weighted image in the sagittal plane is acquired at the level of the interhemispheric fissure that allows optimal visualization of anatomy and pathology at a 90° perspective to the other pulses and acts as a localizer for subsequent pulse sequences. Most institutions advocate that T1-weighted volume acquisitions with a slice thickness equal to or less than 1.5mm should be included in any epilepsy protocol. Volume sequences provide whole brain coverage in a short period of time, and allow 3-D reconstruction and reformatting in any orientation. Such acquisitions are of value in the detection of pathology either by visual detection or by post-processing methods, and are a regular feature in most presurgical MRI protocols. Typically, the sequence is acquired in an oblique coronal orientation, orthogonal to the long axis of the hippocampi. In our institution, we use a three-dimensional gradient recalled echo sequence, IRP-SPGR (GE) with strong T1-weighting rather than a spin echo sequence as it provides the best
grey-white matter differentiation and takes about 8 minutes to acquire. This pulse sequence allows a highly detailed view of the mesial temporal lobes and frontal lobes.

The third sequence is a double spin echo sequence comprising an oblique coronal spin-echo sequence with proton density and T2-weighted acquisitions. Although fast spin-echo sequences improve image quality and reduce image time, heavily T2-weighted spin echo acquisitions are required for the quantitation of regional T2 values. This sequence is optimal in detecting foreign-tissue lesion and in identifying increased T2 signal in the hippocampal formation.

Other pulse sequences e.g. the fluid inversion recovery sequence in the coronal plane are possible which provide excellent anatomical definition of the mesial temporal structures and increase the conspicuity of subtle brain changes by suppressing cerebrospinal fluid (CSF) signal (Wieshmann et al., 1998).

1.4 QUANTITATIVE MR IMAGING

1.4.1 Hippocampal T2 mapping

The visual assessment of T2-weighted signal abnormalities in TLE patients with HS is subjective, with a reported prevalence of visible T2 signal abnormalities ranging from 8% to 70% (Jackson et al., 1993b). The quantitative measurement of hippocampal T2 (HCT2) relaxation time provides an objective measure of the severity of hippocampal signal abnormalities and improves the detection of subtle and bilateral hippocampal disease. It has been postulated that elevations in HCT2 have a different neuropathological basis from reductions in hippocampal volume (HV), such that these two methods of quantitation provide complementary information in longitudinal studies and the presurgical evaluation of TLE (Van Paesschen et al., 1997c).

Until recently, HCT2 was measured on one slice using a 16-echo sequence, or on a few thick slices with interslice gaps (Van Paesschen et al., 1997a), (Jackson et al., 1993b), (Van Paesschen et al., 1995). Although these pulse sequences generated precise HCT2 relaxometry data, the use of only a few thick coronal slices with inter-slice gaps meant that this method was unable to detect localised change.
More recently, interleaved multi-slice, standard dual echo sequences covering the whole brain have been used (Duncan et al., 1996). Pixel by pixel T2 maps are calculated from the images, using the following expression:

\[
T2 = \frac{(TE2-TE1)}{\ln(S1/S2)}
\]

in which S1 and S2 are the signal intensities in the early and late echo images, and TE1 and TE2 are the respective echo times.

T2 relaxation time is calculated for each pixel, and an image constructed in which pixel intensity corresponds to the calculated T2 relaxation time. Conventional spin echo (CSE) data has been shown to be more reliable than fast spin echo data in the discrimination of normal from abnormal hippocampi (Duncan et al., 1996). The reliability of HCT2 measures on repeated acquisitions was superior for the CSE sequence with echo time 30,120 than for the 30, 80 sequence. Complete coverage of the hippocampus in contiguous 5mm thick sections compensates for misalignment of the patient in the scanner, whilst quantification of HCT2 on contiguous slices permits the assessment of the severity and topographical extent of hippocampal sclerosis (Woermann et al., 1998a).

HCT2 relaxation times are measured by placement of the largest possible elliptical region of interest within the hippocampus, avoiding boundaries between hippocampus and CSF e.g. hippocampal or uncal sulcus, where partial volume effects might occur (Jackson et al., 1993b). The application of a fast fluid attenuated inversion recovery (FLAIR) dual-echo technique to suppress CSF has been used to distinguish between partial volume effects from CSF, and genuine parenchymal changes in the hippocampus (Woermann et al., 2001).

Whereas a pathologic diagnosis of hippocampal sclerosis is based on greater than 50% neuronal loss in Sommer's sector (Bronen et al., 1991), a significantly elevated HCT2 signal may represent clinically significant but less severe hippocampal damage (Dam, 1979). HCT2 values in HS correlate inversely with MRI-based HCV (Van Paesschen et al., 1995). Good interobserver reliability has been achieved in the quantification of T2 relaxation times in normal and abnormal hippocampi (Jackson et al., 1993c). The range of HCT2 in normal controls is narrow suggesting that HCT2 measurements may be
useful absolute measures, capable of detecting bilateral hippocampal pathology (Jackson et al., 1993b).

A high correlation is observed between HCT2 and the ratio of glial to neuronal density, particularly in the CA1 and CA4 subregions of the hippocampus (Van Paesschen et al., 1997c). A recent paper (Briellmann et al., 2002b) incorporating absolute cell counts suggested that an increased HCT2 signal reflected the severity of dentate gliosis, with many astrocytes showing GFAP-positive properties indicative of recently occurring or ongoing abnormal processes. Increased free water associated with the relative expansion of extracellular space following the loss of neurons, may also contribute to the raised HCT2.

A significantly elevated HCT2 correlates strongly with the presence of HS on pathologic or MRI evidence, although quantitative neuropathological investigations have shown that proven HS may be associated with a normal HCT2. HCT2 and HV ratios are often normal in patients with histologically proven end folium sclerosis, a variant of HS characterized by neuron loss and gliosis confined to the end folium (Van Paesschen et al., 1995).

Patients with slightly increased HCT2 relaxation times do not usually have visually detectable abnormal T2-weighted signal intensity and are often found contralateral to a clearly sclerotic hippocampus. Minor degrees of T2 abnormality may represent less severe degrees of gliosis (Margerison and Corsellis, 1966) or mild oedema secondary to seizures (Takamatsu et al., 1991). Experimental studies have used T2 relaxometry to demonstrate the development of intramyelinic oedema and astrocystosis in rats treated with vigabatrin (Jackson et al., 1994c) although this finding has not been replicated in humans (Jackson et al., 1994a).

The most common predictor for the development of HCT2 signal abnormality is a history of prolonged early childhood convulsions. Grünewald et al. showed that HCT2 remained stable over 10 months in patients with chronic epilepsy and was not affected by recent seizure activity (Grünewald et al., 1994). The same study failed to show any correlation with seizure frequency or duration of epilepsy, suggesting that hippocampal sclerosis was established before adulthood and was a static phenomenon. Furthermore,
patients with IGE had no evidence of HCT2 signal change despite recurrent GTCS. Other studies have produced conflicting results, demonstrating a significant correlation between ipsilateral and contralateral HCT2 values, and seizure frequency (Namer et al., 1998). More recently, T2 relaxometry has been applied to the identification of hippocampal pathology and the lateralization of epileptic foci in patients with intractable TLE, who have normal conventional MRI and hippocampal volumetry (Bemasconi et al., 2000). Studies on limited numbers of patients have suggested that HS without hippocampal atrophy may still be associated with a good surgical outcome (Jackson et al., 1994b).

1.4.2 Hippocampal volumetry
The MRI correlate of the cell loss seen in pathological specimens of mesial temporal sclerosis is atrophy. The use of hippocampal volumetry to improve the detection of hippocampal atrophy was first introduced in the late 1980’s (Press et al., 1989), (Jack et al., 1990a). Studies show that hippocampal volume measurements are significantly correlated with neuronal densities in the dentate gyrus and all hippocampal subfields with the exception of CA-2 (Lencz et al., 1992), (Bronen et al., 1991). Other studies have shown that hippocampal volume loss correlates well with clinical / EEG seizure lateralization (Bronen et al., 1991), (Cascino et al., 1991) and neuropsychological abnormalities (Lencz et al., 1992).

It has been reported that visual interpretation of hippocampal abnormalities using optimal MR sequences can reveal the presence of mesial temporal sclerosis with around 90% accuracy (Jackson et al., 1993a). However, others (Reutens et al., 1996) have observed a threshold for reliable visual detection of asymmetry only in those with hippocampal volume ratios below 0.7. MRI-based volumetry allows the quantification of the magnitude of hippocampal atrophy in MTS (Cook et al., 1992), (Cendes et al., 1993a). The use of corrected absolute hippocampal volumes and their relationship to the normal control group permits the detection of bilateral hippocampal atrophy (Watson et al., 1997).

Hippocampal volumetry plays an important role in the preoperative evaluation of patients with TLE since pre-operative hippocampal formation volume measurements correlate well with the severity of HS on pathological examination and outcome after
temporal lobectomy (Jack et al., 1992), (Watson et al., 1997). When unilateral hippocampal atrophy can be identified on MR images for patients with intractable TLE, approximately 70% will be rendered seizure free following surgical removal of the affected hippocampus, and 80-90% will experience a significant improvement (Engel, 1996). It has also been suggested that the extent of hippocampal atrophy may be useful as a guide to the extent of hippocampal resection (Jackson et al., 1990). Unilateral hippocampal atrophy concordant with localization of the seizure focus obtained by clinical, scalp EEG, and neuropsychological means reduces the need for invasive EEG, and its associated risks (Spencer, 1987). A significant relationship between hippocampal volume and decline in postoperative decline in neuropsychological function has also been demonstrated (Trenerry et al., 1993a).

However, the greatest utility of formal volumetry probably lies in clinical research, particularly in evaluating the relationship between hippocampal volumes and biological variables.

The range of "normal" hippocampal volumes in young healthy individuals described in the literature is broad. This variability has been attributed to inter-institutional differences in MR acquisition technique, hippocampal boundary criteria, subjective in-plane boundary tracing and software employed for counting pixels in a defined region of interest (Jack et al., 1995). Hippocampal volume increases linearly with total intracranial volume (ICV) in normal young adults. Therefore, when comparing across groups, the absolute hippocampal volume should be corrected for ICV (Free et al., 1995). This correction process reduces the variance in absolute hippocampal volumes and enables identification of unsuspected bilateral HV loss. The ICV can be obtained using automatic segmentation (Lemieux, 1999), or by the manual delineation of the inner surface of the intracranial cavity in every tenth coronal slice (Free et al., 1995). The HV can then be adjusted via the covariance method using a linear regression equation. Although ICV correction can be used cross-sectionally to reduce inter-individual variation, it can also be applied longitudinally, to adjust for subtle image distortions e.g. voxel size variations due to drifts in imager calibration that may affect the measurement of change in individuals. Correction of HV for total brain volume results in a less consistent reduction in variance compared with correction using ICV.
Chapter I

(Free et al., 1995), but allows an assessment of hippocampal atrophy independent of the reduction in whole brain volume.

Historically, optimal MRI pulse sequences for hippocampal volume measurements have improved along with technical advances. Since 1990, researchers have used 3D volumetric gradient-echo sequences that can be customized to permit a direct oblique coronal acquisition producing sharper hippocampal boundaries (Jack, 1994). Spatial resolution is improved with contiguous fine slices, since thick slices or large interslice gaps may reduce the accuracy of cross-sectional areas, and encounter problems with head tilt. It has been recommended that the minimum sampling interval should be no greater than 3mm in hippocampal volumetry studies (Cook et al., 1992). Further precision of hippocampal anatomy may be obtained by including an inversion recovery pulse prior to the gradient echo acquisition by increasing T1 contrast.

Typically, hippocampal surface area is measured in sequential contiguous slices, using a manual contouring function because of the complexity of the structures involved. The oblique coronal section in which the fornical crura are seen in full profile lifting away from the hippocampal tail, marks the posterior boundary of the hippocampus. The hippocampus proper (Ammon’s horn), uncus, alveus, intralimbic gyrus and fimbria, are included in the measurements (Figure 3). Fornix is excluded where it can be identified as a distinct structure posteriorly. Laterally, the hippocampal boundary is determined by CSF in the temporal horn, superiorly by the fornices and the CSF in the choroidal fissure, medially by CSF in the uncal and ambient cistern and inferiorly by the white matter of the parahippocampal gyrus (Jack, 1994). The uncal recess of the temporal horn and the alveus provide superior landmarks for the head of the hippocampus. Although disarticulation of the anterior hippocampus from the amygdala is prone to measurement error, it is important to delineate the head of the hippocampus accurately, as the pathology frequently lies exclusively in this region.

The hippocampal area is obtained by pixel counting, and an estimate of the hippocampal volume obtained from the product of slice interval and the sum of all hippocampal cross-sectional areas (Cavalieri’s principle). According to stereological criteria, a narrow slice thickness is essential for supplying an unbiased and efficient estimation of brain compartment volume (McNulty et al., 2000). The cross-sectional
surface area at successive slices can be plotted against slice number, allowing a graphical display of cross-sectional volume (Figure 4). This permits the morphological analysis of the hippocampus throughout its length, revealing regional atrophy, by slice by slice comparison with that of the contralateral hippocampus (Cook et al., 1992).
Figure 3  **Structure of the hippocampus.** Structures 1-8 were included in volumetric measurements of the hippocampus. Modified from Duvernoy HM. The human hippocampus. Berlin Heidelberg: Springer, 2nd edition, 1998 (Duvernoy, 1998a).

1. cornu Ammonis  
2. gyrus dentatus  
3. hippocampal sulcus (vestigial part)  
4. fimbria  
5. prosubiculum  
6. subiculum proper  
7. presubiculum  
8. parasubiculum  
9. entorhinal area  
10. parahippocampal gyrus  
11. collateral sulcus  
12. collateral eminence  
13. temporal horn of the lateral ventricle  
14. tail of the caudate nucleus  
15. stria terminalis  
16. choroid fissure and choroid plexuses  
17. lateral geniculate body  
18. wing of ambient cistern  
19. ambient cistern  
20. mesencephalon  
21. pons  
22. tentorium cerebelli
Figure 4  Graphical display of cross-sectional hippocampal volume. The cross-sectional area of the hippocampus in each slice (y-axis) is plotted against the slice number (x-axis), shown posterior to anterior. Graph shows focal anterior right hippocampal volume loss. Reproduced from Cook MJ (Cook, 1994)

Other studies have used non-biased methods, such as point counting, in which a grid is randomly thrown onto the hippocampus, and the number of points falling within a defined boundary calculated (Sheline et al., 1999), (Webb et al., 1999).

Automation of this process is difficult due to the small volume of the structure and the lack of grey scale differences between the hippocampus and adjacent tissue along its inferomedial border. A number of groups have attempted automatic segmentation of the hippocampus. Webb et al. (Webb et al., 1999) describe a method to enable the automatic evaluation of hippocampal atrophy through an intensity-based image registration technique. The presence of an anomaly in the manually segmented mask region caused by the encroachment of CSF or white matter arising from morphological changes in the hippocampus, (i.e. atrophy), formed the basis for the automatic determination of hippocampal atrophy. Shortcomings of this technique relate to problems with misregistration in patients with global brain distortion, and an over-reliance on the presence of intensity anomalies in the mask region.

Other groups have applied high-dimensional brain mapping (HDBM) to the study of hippocampal volume and shape (Csernansky et al., 1998). Electronic atlases of the
hippocampus are used as a deformable template. General pattern matching computes shape transformation as the atlas is iteratively adjusted to assume the shape of the target hippocampus. The advantage of this method over manual contouring is that hippocampal shape eigenvectors yielded by HDBM allow the identification of subtle neuroanatomic deformity as well as change in volume. However, one of the limitations of this method is the potential bias introduced by different template atlases (Haller et al., 1997). Researchers investigating Alzheimer’s disease have used fluid registration to detect hippocampal change. However, the hippocampus is manually delineated prior to propagation between images, and analysis remains qualitative (Crum et al., 2001).

1.4.3 Cerebellar volumetry

Due to the difficulty of defining criteria for cerebellar atrophy, a visual diagnosis is often subjective and takes into account indirect measures such as enlargement of cerebellar sulci and size of ventricles. These visual cues are dependent on normal variation and age-related changes, leading to an inaccurate estimate of the true prevalence of cerebellar atrophy. Cerebellar volumetry overcomes these problems, permitting the accurate detection and quantification of cerebellar atrophy and its relation to clinical variables.

Although cerebellar atrophy is a frequent finding in patients with chronic epilepsy (Dam, 1987), (Botez et al., 1988), the factors responsible for its pathogenesis are not clear. Proposed mechanisms are discussed in section 1.6.3.1. Volumetric findings have been corroborated by autopsy findings of purkinje cell loss in the cerebellum of patients with severe epilepsy (Dam, 1979), (Salcman et al., 1978).

Earlier studies used planimetric techniques, focused on a single midsagittal slice of the vermis area using manual tracing or automated algorithms (Raz et al., 1992). However, such measurements have been criticised for their misrepresentation of a three-dimensional structure and susceptibility to head positioning and partial volume effects. Three-dimensional evaluation is superior but time-consuming as the structure has to be segmented on many sections. Current practice is to perform cerebellar volumetry on T1-weighted volumetric series acquired contiguously in the coronal plane (Sandok et al., 2000), whilst measurements of the cerebellar vermis are performed on
sagittal acquisitions (Raz et al., 1998). Contrast-defined boundaries are automatically segmented using a seed and region-growing algorithm according to a determined pixel intensity threshold (Lemieux et al., 2000), (Luft et al., 1999), (Hagemann et al., 2002). Anatomical boundaries are defined in the methodology section. Cerebellar volumes are subsequently calculated using the Cavalieri principle (Mayhew and Olsen, 1991).

Normalization of cerebellar volume for total brain volume allows the detection of cerebellar atrophy independent of generalized brain volume reduction. Studies using normalized cerebellar volumes suggest that up to one third of patients with apparent cerebellar atrophy have a proportionate degree of generalized cerebral atrophy (Sandok et al., 2000).

1.5 NEOCORTICAL IMAGE PROCESSING

1.5.1 Volumes of grey matter, white matter and image post-processing

Developments in data processing after acquisition of volumetric scan data have increased the yield of useful information. Using quantitative analysis of the regional distribution of grey and subcortical white matter volumes, Sisodiya et al., demonstrated occult structural cerebral changes beyond the margins of the visualized lesion in patients with cortical dysgenesis (Sisodiya et al., 1995b) and HS (Sisodiya et al., 1997b), which could at least partially explain why a significant proportion of patients with histologically confirmed dysgenesis, and approximately 30% of patients with refractory partial epilepsy due to HS, do not become seizure free following lesion resection. Neuropsychological correlates of grey and white matter regional abnormalities in patients with histologically proven HS have revealed preoperative global memory deficits, which may explain the association between preoperative cognitive dysfunction and poor surgical outcome in some TLE patients with unilateral HS (Baxendale et al., 1999).

To analyze the regional distribution of volume, volumetric datasets were semi-automatically segmented into cortical grey matter and subcortical matter (white matter and basal nuclei excluding the caudate). Hemispheric grey matter and subcortical matter volumes of interest were subsequently divided into “blocks” spanning one-tenth of the entire coronal extent of each hemisphere. Individual blocks were normalized for brain
volume by dividing each measure by the total segmented volume, allowing comparison of volume distribution measurements between subjects. Ratios of ipsilateral cortical to subcortical matter in volumes of interest and ratios of ipsilateral to contralateral volumes of homologous volumes of interest were calculated, with each brain yielding 80 volumetric variables. The presence of two or more abnormal values out of the 80 variables in a subject classified that brain as structurally abnormal on the basis of distribution of volume proportion. The definition of normality was an empirical one based on the observation that none of the controls studied had more than one abnormal value (Sisodiya et al., 1995b). Using this method, abnormal cortical structure was shown to extend beyond the lesion in 24 of 29 patients with cortical dysgenesis visible on 2D and 3D MRI. The authors hypothesized that underlying structural changes detected in MRI quantification were due to changes in neuronal connectivity and subtle structural abnormalities (Sisodiya et al., 1995b). The demonstration of widespread structural abnormalities in epileptic patients with HS (Raymond et al., 1994) and hypothalamic hamartomata, (Sisodiya et al., 1997a) beyond the apparent lesional and resection margin in those with persistent seizures postoperatively, suggest that these structural changes may partake in generating seizures after, or before surgery.

Similar "block" methodology was used to demonstrate subtle widespread cerebral structural changes in patients with IGE. A marked difference in mean normalized total cortical and subcortical matter was observed between normal subjects and IGE patients, particularly juvenile absence epilepsy patients. A third of IGE patients showed volume of interest based abnormalities of cerebral structure. Based on this observation, it was proposed that MRI quantification of grey and white matter volumes could contribute to the identification of distinct phenotypes of patients with IGE and thus aid genetic analysis (Woermann et al., 1998b). Grey and white matter segmentation may be operator dependent (Sisodiya et al., 1997a) or fully automated (Mitchell et al., 2001), (Lemieux et al., 1999) and is discussed in section 1.5.3.

Three-dimensional reconstructions from conventional 2D slices, where grey matter reconstructions represent the cortical surface, allow the in vivo visualisation of surface gyral patterns (Sisodiya et al., 1995a). 3-D reconstructions have been applied to neurosurgical planning (Jack et al., 1990b), and to the more precise localisation and delineation of known lesions (Damasio and Frank, 1992). By comparing the gyral
anatomy of patients with control subjects, abnormal gyral patterns were observed in 47% of 30 patients with extra-temporal lobe epilepsy with previously normal conventional MRI scans (Sisodiya et al., 1996). However, comparisons were subjective, and required an extensive knowledge of gyral anatomy and its normal variability. The identification of structural abnormalities might not only influence the type of surgical intervention, including the placement of depth electrodes, but also prevent inappropriate surgical intervention. Despite reconstruction, lesions located in the occipitotemporal and medial aspects of the brain remain poorly visualised.

Attempts to overcome artifactual cortical thickening caused by volume averaging when using thick slices, and obliquity of the slice in relation to the inward folding of gyri, have been made using curvilinear reformatting (Bastos et al., 1999). In this method, thin serial curved slices were obtained along the hemispheric convexities, producing an approximately perpendicular orientation of the slice in relation to the inward folding gyri. Anatomical display of the gyral structures particularly of the hemispheric convexities, and asymmetric sampling of grey-white matter was therefore improved, allowing the detection of small cortical lesions e.g. FCDs. Subtle alterations in the gyral pattern are thought to relate to underlying cytoarchitectonic changes (Barth, 1987).

Another post-processing technique incorporated voxel-based methods including first-order texture analysis and morphological processing modelled on known MRI features of FCD to improve the detection of subtle cortical malformations (Bernasconi et al., 2001). Ratio maps based on grey matter thickness, blurring of the grey matter (GM)-white matter (WM) junction, and the hyperintense signal of the lesion, were used to increase the sensitivity of lesion detection by 37.5% over conventional MRI analysis.

Three-dimensional fractal analysis has studied the alteration of gyral patterns rather than the volume distribution, by quantifying the complexity of the underlying subcortical matter (Free et al., 1996). The fractal description of a structure depends upon the variation in the metrics of the structure with respect to the size of the measuring elements that derive the metrics. For example, a reduction and increase in fractal dimension may be observed with pachgyria and polymicrogyria respectively.
Further postprocessing of the data available in volumetric MRI scans can therefore be of value in cases of refractory chronic focal epilepsy where two-dimensional MRI has been non-contributory.

1.5.2 Image registration

Applications of image registration in medicine include the fusion of subject data taken from different modalities, the alignment of serial scans to compensate for non biological changes between scans, and the alignment of images from multiple subjects (Pelizzari et al., 1989), (Woods et al., 1993). Precise anatomical alignment allows separate sets of images to be constituted into a single unified spatiotemporal dataset in which individual voxel intensity values can be compared in space and time. Since patients often move during examinations, and cannot be repositioned perfectly on subsequent visits, registration is an essential prerequisite for detecting subtle change. Image registration allows the meaningful subtraction of serial data e.g. in digital subtraction angiography and the precise and consistent transposition of regions of interest from one scan to another (Lemieux et al., 1998).

It is frequently assumed that anatomical and pathological structures of interest do not distort or deform, thus most medical image registration algorithms usually register images related by a rigid body transformation i.e. six degrees of freedom: three translations and three rotations. The rigid body assumption can however, be violated by scanner-induced geometrical distortions that differ between scans. The brain is reasonably non-deformable, provided the skull remains intact and there is no substantial change in pathology. Some registration algorithms increase the number of degrees of freedom by allowing for anisotropic scaling (giving nine degrees of freedom) and skew (giving 12 degrees of freedom). The latter, referred to as an affine transformation, has limited application, as tissues usually deform in complicated ways, and do not only stretch or shear. However, affine transformations can be used to overcome scanner introduced errors e.g. to correct inaccurate voxel dimensions due to miscalibrated scanner gradients (Freeborough et al., 1996).

The main requirements of a registration software are: two or more images, a measure of similarity which may be voxel or feature-based, an optimisation algorithm and a reslicing method e.g. linear or sinc interpolation. Early attempts at rigid body...
registration, including patient fixation devices, such as head holder devices, or geometric features e.g. homologous landmark and surface matching, resulted in positional matching to within 1 to 2 voxels (Jiang et al., 1992), (Hill et al., 1991). Consequently any change in size or shape smaller than this magnitude could not be accounted for. Rigid body registration algorithms that relied on feature matching were prone to error for convoluted surfaces.

More accurately matched brain images are now possible using voxel similarity measures which are capable of aligning images with subvoxel precision. The registration software program, MRreg is able to coregister to an accuracy of less than 0.06mm in each linear dimension using cross correlation as a measure of similarity (Lemieux et al., 1998), (Lemieux and Barker, 1998). One of the simplest voxel similarity measures is the sum of squared intensity differences between images, SSD or $\chi^2$, which is minimized during registration.

$$\chi^2 = \frac{\sum \text{voxels} \ (I_B - I_A)^2}{N \text{ voxels}}$$

where $I_A$ and $I_B$ are the intensities of corresponding pixels in the images A and B that are to be positionally matched and $N$ voxels is the number of voxels used in the calculation.

The SSD measure is widely used for serial MR registration (Hajnal et al., 1995b), and is also used in the registration algorithm within Friston’s statistical parametric mapping software (Ashburner and Friston, 1999). The registration algorithm iteratively reduces the value of $\chi^2$ by rigid body translation and rotation of the follow-up image dataset. The SSD measure is very sensitive to a small number of voxels with large intensity differences between images e.g. post contrast injection. Other similarity measures include the standard deviation of the ratio of voxel intensities (Woods et al., 1992). Image registration for brain applications is best with initial segmentation prior to registration. This ensures that deformable extraneous regions, such as the mandible, cranium and earlobes, which might undergo alterations in position relative to the brain, are precluded from affecting the similarity measure.
All these approaches require an optimisation scheme, in which an initial estimate of the transformation is gradually refined by trial and error. Once the parameters required to optimally position the follow-up image have been determined, the follow-up image is reformatted (respliced) to match the baseline set using image interpolation. The most widely used image interpolation function is probably trilinear interpolation, in which a voxel value in the transformed coordinates is estimated by taking a weighted average of the nearest eight neighbours in the original dataset (Hill et al., 2001). Sinc interpolation is also commonly used, and allows image shifts without distorting voxel signal intensity values but is slower than linear interpolation (Hajnal et al., 1995a).

Non-rigid or non-affine registration algorithms normally either include an initial rigid body or affine transformation, or are run after an affine algorithm has provided a starting estimate. Non-rigid registration allows the alignment of images of organs that deform, the alignment of images from different subjects, and can be used to address the correction of scanner-induced geometric distortions. Approaches include the use of pseudo-physical models in which the deformation of one image with respect to another is modelled as elastic deformation or fluid flow (based on the physical model of a compressible viscous fluid) (Freeborough and Fox, 1998), (Crum et al., 2001). The transformation determined can be regarded as a deformation field that records the displacement vector at each voxel in one image needed to align it with the corresponding location in the second image. A recent study that applied a nonlinear warping algorithm to serial MRI scans in Alzheimer’s disease demonstrated a scan-rescan hippocampal volumetric consistency of ~2%, which was superior to human serial manual segmentation (Crum et al., 2001). The study used manual segmentation of the baseline scan to drive an automated segmentation of the same structure on the repeat scan by fluid registration. However, fluid registration may perform suboptimally if scan quality is impaired by motion artifact or if there are significant topological differences between registered scans e.g. the appearance or resolution of lesions.

Coregistration is particularly useful in serial scanning when applied to repeated acquisitions of MR data from the same individual under similar conditions. Applications include comparisons of pre- and post- contrast enhancement images (Hajnal et al., 1995a), clinical follow-up and serial volumetric studies (Wieszmann et al., 1997), (Liu et al., 2000). Image registration has been used in Alzheimer’s disease as
Chapter I

a diagnostic tool and surrogate marker of progression (Fox et al., 1996a), (Fox et al., 1999a). Small volume and signal changes in low-grade brain lesions are of considerable value in monitoring early response to therapy and have allowed the early detection of tumour progression or recurrence. Progressive changes in multiple sclerosis have been derived directly from registered scan pairs using the brain boundary shift integral (BBSI) which represents the total volume traversed by brain boundaries in going from the first scan to the second scan (Fox et al., 2000b). Using the BBSI, the rate of cerebral atrophy in multiple sclerosis patients was over twice that of controls (0.8% compared with 0.3%) after one year (Fox et al., 2000b).

The assessment of signal intensity change within a structure, after contrast enhancement has also been improved by coregistration, since the act of injecting a contrast agent may alter patient position in the scanner. Registration is therefore useful in assessing change in signal intensity when enhancement is subtle or at boundary regions, and in increasing the sensitivity of contrast-enhanced MRI. Registration is also required in studies of brain activation, where images are acquired serially in control and activated states by the use of subtraction and / or statistical analyses (Bydder, 1995).

Intersubject normalization describes the practice of registering images from different individuals into a standard space, in order to study variability between subjects, or to compare normal subjects with volunteers. Commonly images are aligned to the Talairach stereotactic space (Talairach and Tournoux, 1988), using an affine transformation (Collins et al., 1994), although more degrees of freedom can be used (Ashburner and Friston, 1999). Many studies using PET have employed averaging across subjects and so have required inter-subject normalization (Friston et al., 1991).

Applications of cross-modality registration include the overlay of MR and CT images for surgical planning, and the matching of MR and PET images to identify the anatomical correlate associated with functional changes (Woods et al., 1993).

1.5.3 Brain segmentation

Segmentation of MR data is the process by which natural elements e.g. tissues and organs are defined and extracted. Segmentation of the brain from MRI scans has widespread application in neuroimaging. These include its use as a preliminary step in
image registration (Hajnal et al., 1995a), visualisation and quantification of the shape of the cortex (Maudgil et al., 1998), and the analysis of the spatial distribution of grey and white matter (Woermann et al., 1998b). Segmentation may be used to extract whole brain or to segment individual tissues, allowing quantitative analysis. Segmentation of GM, WM and CSF can be used to refine the quantitative analysis of magnetic resonance spectroscopy and positron emission tomography by correcting for partial volume effects due to the mixture of tissues in each voxel (Labbe et al., 2002).

A range of methods has been published on whole-brain segmentation from volume MRI data. These range from observer-dependent methods of manually tracing the region of interest (ROI) on contiguous brain slices, to semi-automated region-growing methods and fully automated techniques that require no user interaction. One method consists of a morphological algorithm that relies on automatic edge detection followed by morphological operations (Sandor and Leahy, 1997). As with many approaches to segmentation, a human observer is required to select a seed point inside the brain by visual inspection and the technique is therefore not totally automatic.

It is possible to isolate brain tissue by imaging tissue types directly, according to the selective suppression of signal from specific tissues (Bedell and Narayana, 1998). However this technique involves long acquisition times and the data obtained from multiple acquisitions may require image registration prior to segmentation (Lemieux et al., 1999).

The first step in many segmentation algorithms is the generation of a mask image outlining the contours of the brain, which is usually obtained from a proton density weighted image. The interface between GM and WM regions is complex, therefore a substantial number of voxels sample both GM and WM, creating a partial volume effect. Partial volume effects blur the distinction between closely adjacent surfaces in deep sulci, leading to a segmentation error. Attempts at extracting morphometric information e.g. cortical thickness may therefore be inaccurate, depending on how partial volume effects are modelled.

Manual tracing of brain boundaries on contiguous brain slices, and semiautomatic techniques using region-growing (Robb and Hanson, 1991), are time and labour
intensive, and prone to errors caused by operator subjectivity. It is therefore preferable to perform segmentation using automated techniques, although the accuracy of segmentation is dependent on the model used to define voxels of homogeneous signal and the quality of the data provided.

Numerous computational techniques for brain extraction from 3D MR images have been described. An efficient intensity non-uniformity correction is a useful pre-processing stage for effective automatic thresholding and segmentation in 3D, although this can be incorporated into the segmentation.

The most popular methods of brain segmentation have entailed the use of multiple, coregistered pulse sequences to increase the dimensionality of the discrimination problem, allowing multispectral statistical techniques to improve the reliability of segmentation (Bedell and Narayana, 1996). Held et al. (Held et al., 1997) demonstrated a method to segment GM, WM and CSF based on Markov Random fields (MRFs) in multi-echo and proton density-weighted volume images. Segmentation using the MRF’s takes into account information from neighbouring voxels, for example by increasing the probability of a particular voxel being assigned GM if all its neighbours are GM. However, high resolution multispectral scans are not acquired routinely as part of a clinical protocol.

A number of techniques are based on the principle of histogram analysis, which attributes intensities to tissue classes, based on the assumption that the histogram is composed of a GM distribution and a WM distribution. Voxels of the intensity of GM are then segmented as GM and the same applied to voxels of WM intensity. The advantages of histogram-based methods are their ease of implementation. Common methods to separate the two distributions are the “variance optimization method” which finds the best separation threshold in terms of variances between the two distributions (Otsu, 1979), and the “nadir algorithm” which takes the position of the local minimum between the two distributions (Kennedy et al., 1989). The method of Otsu has been applied to the automatic segmentation of the brain and CSF in T1-weighted volume scans (Lemieux, 2001). This approach is particularly reliant on non-uniformity correction.
One limitation of many of the above approaches is that they classify each pixel as belonging to one tissue category, and fail to reflect the partial volume nature of the underlying data (Grabowski et al., 2000). This is particularly problematic at ventricular surfaces where voxels containing WM and CSF may be erroneously assigned as GM. The subcortical nuclei are also less well segmented as they often have an intensity closer to WM than GM due to the numerous myelinated axons contained within the nuclei. Volumetric measurements using a continuous classification of tissue composition, based on tissue mixing segmentations are likely to be more sensitive and robust. Techniques incorporating partial volume effects are usually based on a model of intensity probability distribution. In the segmentation program, Exbrain (Lemieux, 2001), brain and CSF are initially segmented using a combination of intensity and histogram analysis, and morphological operations. The intensity probability distribution for the combined brain and CSF masks is subsequently modelled as a penta-Gaussian distribution, which includes two partial volume classes. Local rather than global histograms can be applied to provide a more accurate estimation of GM in subcortical structures e.g. the thalamus and globus pallidus.

A further method is that available as part of SPM 99 package (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, UK). Prior probability images derived from the MR images of 152 subjects segmented into GM, WM and CSF are used to provide a priori knowledge of the probability of each voxel belonging to a particular tissue class. This combines an intensity-based and neuroanatomical model for tissue classification that assumes that all voxels in the image have been drawn from a known number of distinct tissue classes. The distribution of voxel intensities within each tissue class is normally distributed.

Other techniques are based on multiple-surface deformation algorithms. MacDonald et al. proposed a deformable surface technique called anatomic segmentation using proximities (MacDonald et al., 2000b). Topology constraints ensure that the cortical mantle is properly identified in areas susceptible to partial volume errors, resulting in more accurate sulcal penetration and a measure of GM thickness over the whole cortical mantle. Most of the deformation models have been based upon the active contour method of Kass et al. (Kass et al., 1988), commonly referred to as “Snakes”. In this
model, shape constraints are imposed to reduce sensitivity to noise, and to impose an expected class of shapes on data.

1.5.4 Subtraction of serial images and difference images

1.5.4.1 Generation of difference image

Difference images are produced by the digital voxel-by-voxel subtraction of pairs of registered image datasets and play an important role in the detection of small changes in the brain in serial imaging studies (Hajnal et al., 1995a), (Lemieux et al., 1998). However, careful coregistration is required to avoid misregistration artefacts that are often greater than the changes being sought (Hajnal et al., 1995a). With precise image registration, it is possible to produce a difference image with virtually complete cancellation of signals from unchanged structures. Difference image analysis can be applied to the study of the brain in a range of physiologic and clinical situations.

Automated image subtraction allows all areas of the brain to be examined simultaneously and objectively (Fox et al., 1996a). Signal intensity on difference images may be due to an intrinsic signal change, a change in signal intensity of a tissue or fluid (e.g. following contrast enhancement), change in site, shape, or size of a tissue or fluid, or a combination of these (Hajnal et al., 1995a). Changes in the difference images may be either positive or negative signals, and areas of atrophy may be quantified to give regional or global measures of atrophy (Fox et al., 1996a). The detection of signal intensity changes is usually easier to interpret on difference images than on source images because signals from unchanged tissues and fluids are reduced to a common background level. This can be particularly useful at boundary regions where there are variable partial volume effects.

1.5.4.2 Visual interpretation

With T1-weighted images, a steep signal intensity gradient is found at brain interfaces i.e. between brain (either grey or white matter) and CSF. Therefore, a shift in brain boundary to encroach on an area previously occupied by CSF produces an increase in signal intensity on the subtraction image i.e. a white line. Conversely, a shift away from a boundary with brain matter being replaced by CSF generates a dark line or negative signal. The magnitude of the change in signal intensity on the difference image is
determined by the size and direction of the signal intensity gradient as well as the size and direction of the shift (Hajnal et al., 1995a).

Physiological studies investigating diurnal changes and different phases in the menstrual cycle have revealed minimal changes confined to the straight sinus, and subtle ventricular changes respectively (Hajnal et al., 1995a). Difference image analysis has also been applied to the study of normal growth and development in children, however, global changes may be minimised by the registration process.

In T1-weighted gradient echo images, differences in matched images can be generated by head movement during scanning, and pulsation and susceptibility artefacts causing random signal variations. Head movement is usually recognized by a biphasic pattern of signal change and their predominant location in the boundary region.

1.5.4.3 Quantitative analysis

Using spatial normalization (Friston et al., 1995a), it is possible to construct structured noise maps (SNM) or anatomical distribution maps of artefact occurrence from a database of images from different controls. This can then be used to filter data from individual subjects and statistical tests of significance performed on a voxel-by-voxel basis (Lemieux et al., 1998). The statistical significance of the changes in a normalized structured difference image in a patient is assessed according to the probability of a given voxel being classified as changed in the SNM (see section 3.5.3). The product of this classification process is the genuine change map (GCM), which can be quantified by determining the total number of structured difference voxels, the total number of genuine change voxels, and the total number of normal structured voxels.

Quantification of brain atrophy by calculating the integral of the change, including loss and gain, in normalised signal intensity in each voxel, over the whole brain has been applied to the study of Alzheimer's disease (Fox et al., 1996b), where greater rates of atrophy are observed in individuals with Alzheimer's disease and those at risk of familial Alzheimer's disease compared with controls. Image subtraction has revealed subtle atrophy in patients with Alzheimer's disease over intervals as short as three months (Freeborough et al., 1996).
Chapter I

1.5.5 Voxel based morphometry

Voxel based morphometry (VBM) allows the comparison of image datasets at a voxel level. Problems inherent in ROI methods e.g. mixed tissue sampling, partial volume effects, and the subjective placement of ROIs are avoided. VBM is therefore not biased to a particular structure and allows the comprehensive assessment of anatomical differences throughout the entire brain (Ashburner and Friston, 2000). There are two principle approaches to characterising differences in the shape and configuration of different brains.

The first approach includes deformation-based morphometry (DBM) and tensor-based morphometry (TBM), and deals with the study of brain shapes by examining the deformation fields required to map individual brains onto a standard reference using non-linear registration. DBM is used to formulate group comparisons by identifying differences in the relative positions of brain structures, whereas TBM identifies local differences in brain structure (Ashburner et al., 1998), (Ashburner and Friston, 2000).

The second approach allows the identification of residual anatomic differences by comparing spatially normalized images on a voxel basis after deformation fields have been applied. The most widely used technique based on this approach is Statistical Parametric Mapping (SPM) (Friston et al., 1995b), a fully automated and objective three step technique that studies the whole brain. SPM has been used to demonstrate sex-related differences in cerebral structure (Good et al., 2001a), normal ageing, (Good et al., 2001b) and differences in brain structure among a range of patient populations (Woermann et al., 1999), (May et al., 1999), (Shah et al., 1998), (Woermann et al., 2000).

All images are initially transformed into a common stereotactic space. The use of nonlinear normalization to a common 3D space minimises the global variability between brains, and increases sensitivity to regional differences by comparing data from homologous regions. The first step of the spatial normalization process involves estimating the optimum affine transformation to match each image to the template by minimizing the residual sum of squared differences between the image and the template (Ashburner and Friston, 1997). The second step accounts for global nonlinear shape differences, modelled by a slice-by-slice transformation employing predetermined
cosine basis functions (Ashburner and Friston, 1999). The template usually chosen is the ICBM 152 template (Montreal Neurological Institute), derived from 152 normal subjects which conforms to the Talairach space (Evans et al., 1993). However, to minimize bias, a customized template can be created which is appropriate to the population being studied. This should consist of the average of a large number of MR images registered to within the accuracy of the spatial normalization technique (Ashburner and Friston, 2000), (Good et al., 2001a).

The image noise is then reduced by filtering or smoothing the images using a simple Gaussian filter (Friston et al., 1995b). Convolving the data with a smoothing kernel renders the data more normally distributed, increasing the validity of parametric statistical tests. It also helps to compensate for the inexact nature of the spatial normalization (Ashburner and Friston, 2000), although may reduce the spatial resolution of the image data.

The normalised MRI can then be segmented using a clustering algorithm identifying voxel intensities of particular tissue types, combined with a priori knowledge about the spatial distribution of these clusters in normal subjects (Ashburner and Friston, 1997).

Independent voxels from groups of images can then be statistically analysed using the General Linear Model which performs the appropriate univariate test at each and every voxel. Based on a null hypothesis of no difference between the two groups of images and via a “Gaussianisation” of the t-distribution to a Z-distribution, a p-value is calculated for each voxel. A voxel-by-voxel map of p-values is constructed, constituting the SPM. By thresholding at a suitable p-value, a map of anatomical areas which differ between the groups of images at that statistical threshold is produced (Friston et al., 1995b). Caution is required when interpreting SPM data as the output is highly dependent on the threshold used.

Voxels from the same image are not entirely independent of one another due to partial volume effects, smoothing and physiological connections between brain regions. Two approaches are often used to overcome errors arising from multiple dependent comparisons. Firstly, specific regions can be examined based on a priori hypotheses, and extraneous regions ignored regardless of their corresponding p-values.
Alternatively, p-values can be corrected using the theory of Gaussian random fields (Friston et al., 1996), allowing an examination of the entire brain. The spatial extent (κ) and peak height (μ) of foci of supra-threshold voxels may be characterised (Friston et al., 1994). This correction describes the probability that a region of the observed size could have occurred by chance over the entire volume analysed (Ashburner and Friston, 2000). However, since the non-stationary smoothness of T1 volumetric data leads to inexact p-values, the use of peak height rather than the voxel-based extent statistic should be used in VBM.

SPM has become one of the standard tools in functional MRI studies and has been used extensively in the analysis of data from PET, diffusion and functional MRI activation studies.

1.6 SECONDARY CEREBRAL DAMAGE IN EPILEPSY

1.6.1 Hippocampal damage following status epilepticus

1.6.1.1 Experimental and pathological models

Animal studies

A number of animal models have shown similar patterns of neuronal damage to humans, with selective neuronal death from excessive neuronal activation in the absence of systemic complications (Meldrum et al., 1973). One advantage of these experimental paradigms is that they can control for confounding variables e.g. aetiology, age at seizure onset, seizure type and duration and antiepileptic drug (AED) use and allow seizures to be accurately counted and assessed.

Animal models of epileptogenesis include kindling and perforant pathway stimulation. Kindling refers to a progressive increase in electrographic and behavioural seizures evoked by initially subconvulsive, periodic electrical or chemical stimulation of neural pathways that eventually evolve into a permanent epileptic state (Goddard et al., 1969). The progressive development of seizures in kindling, through well-defined stages, enables a detailed assessment of cellular degeneration during epileptogenesis. Experimental models including kainic acid-induced status and fluoroethyl-induced status in well oxygenated rats have demonstrated hippocampal and extrahippocampal damage,
including neuronal loss in the amygdala, entorhinal and perirhinal cortices (Tuunanen et al., 1996), (Du et al., 1998). The pattern of neuronal loss in qualitative histologic studies in animal models are dependent on the convulsive agent used. Fluorothyl-induced SE results in neuronal injury in CA1 and CA4 subregions (Nevander et al., 1985), whilst kainic acid-induced SE causes primarily CA3 necrosis. The latter may be attributed to a site-specific effect, related to the location of kainate receptors in the CA3 region (Collins, 1986), (Ben Ari et al., 1980).

A sustained stimulation of the perforant pathway, a fibre bundle that conveys information from the entorhinal cortex to the dentate gyrus, produces damage to the CA1 and CA3 pyramidal cell layers. This model has the advantage in that any effects can be attributed to seizure activity and not to extrinsic chemoconvulsants. Experimental studies in rats demonstrate signs of ongoing neuronal damage even after the cessation of electrographic and behavioural seizure activity (Tuunanen et al., 1999). A recent study showed that brain aconitase and α-ketoglutarate dehydrogenase activity (enzymes comprising part of the Krebs cycle in the mitochondrial matrix) and glutathione levels were significantly reduced in the first 16-44 hours following status (Cock et al., 2002). The findings suggested that reversible mechanisms i.e. mitochondrial dysfunction and loss of brain glutathione, were involved in the initial mechanisms of excitotoxic cell damage.

Other morphological changes in the hippocampus induced by kindling include reorganization of the axons of the granule cells, the mossy fibres, i.e. synaptic reorganization (Cavazos et al., 1991). Instead of projecting to hippocampal neurons in CA3 and hilus (Figure 5), during kindling, granule cell axons (mossy fibres) sprout into the inner molecular layer of the dentate gyrus where they are believed to establish both excitatory and inhibitory synapses (Cavazos et al., 1991). The significance of these aberrant synapses remains unclear. Similar patterns of mossy fibre synaptic reorganization have been observed in the resected hippocampi of patients with TLE and HS (Babb et al., 1991; Mathern et al., 1995b; Mathern et al., 1996). Animal models of epileptogenesis are associated with loss of specific cell types, gliosis, axonal sprouting, neuronal neogenesis, synaptic reorganisation and alterations in neurotransmitter receptors (Sutula et al., 1988), (Holmes et al., 1998a); however, it is not clear whether
these processes are simply epiphenomena, and whether they are beneficial or detrimental.

The characteristic patterns of seizure-induced damage may be caused by excitotoxic damage, since the presynaptic release of glutamate activates postsynaptic N-methyl-D-aspartate (NMDA) and non-NMDA-receptors. Using intrahippocampal microdialysis, During et al. (During and Spencer, 1993) demonstrated that during and prior to seizures, there was a sustained increase in extracellular glutamate in the epileptogenic hippocampus despite a loss of glutamatergic neurones, reflecting decreased uptake or increased release. Due to their high density of NMDA receptors, hippocampal structures are particularly vulnerable to damage. Excessive activation of NMDA and kainate receptors (heavily distributed in the CA1 region and CA3 region respectively) in hypoxia / ischaemia, hypoglycaemia, and SE, results in excessive calcium influx and accumulation of intracellular water. A high level of intracellular calcium leads to generation of reactive oxygen species via activation of nitric oxide synthase, uncouples oxidative phosphorylation in mitochondria, and activates a range of enzymes e.g. lipases, proteases, endonucleases, and other catabolic enzymes that adversely affect cell function. Calbindin (Sloviter et al., 1991) and chromogranin A (Munoz, 1990) are capable of binding to calcium and may confer protection against detrimental intracellular release. These proteins are distributed predominantly in the CA2 hippocampal subregion and the dentate gyrus.

Patients who have succumbed to domoic acid (a kainate analogue) poisoning typically present with prolonged limbic seizures and acute confusion. Subsequent neuropathologic studies have revealed bilateral neuronal loss in the dentate gyrus and zones CA1 and CA3 of the hippocampus, as well as the amygdala and thalamus. Similar patterns of damage are reproduced by the systemic injection of domoate or kainate in rodents. It has been proposed that the pathology is likely to be a consequence of the seizure activity rather than the direct excitotoxic action of domoate, as the administration of an NMDA receptor antagonist (which abolishes kainate-induced seizure activity) prevents the development of neuronal loss except for CA3 and some damage to the amygdala. It is therefore likely that only the damage to the CA3 neurones is a direct result of the excitotoxic action of the domoate. Following intra-amygdaloid kainic acid injection, the administration of diazepam has produced a similar
neuroprotective effect (Ben Ari et al., 1980). Prior transection of the perforant path ipsilateral to the kainate injection decreased the extent of “remote” pathological brain damage. It was thus concluded that seizure propagation plays a crucial role in the induction of “remote” brain damage after focal intracerebral injections.

Figure 5  Diagram of the normal human hippocampus showing the anatomical subregions and excitatory pathways. Excitatory axons from the entorhinal cortex form the perforant pathway that terminates in the outer dentate molecular layer and excites the dendrites of the dentate granule cells. Granule cells excite hilar neurones via axon collaterals and CA3 pyramidal cells via the mossy fibre pathways. CA3 pyramidal cells then excite CA1 pyramidal cells via the Schaffer collateral pathway. During kindling, aberrant mossy fibre sprouting occurs into the inner molecular layer of the dentate gyrus where they are thought to form excitatory synapses. Modified from Sloviter, RS (Sloviter, 1994).

1.6.1.1.2 Human studies

Status epilepticus (SE) is usually defined as greater than 30 minutes of continuous seizure activity or two or more sequential seizures without full recovery of consciousness between seizures (Working Group on Status Epilepticus, 1993).

Autopsy studies on patients who have died following severe SE have shown a characteristic pattern of neuronal death with significantly reduced neuronal densities in Sommer’s sector (prosubiculum and CA1) and the CA3 subregion of the hippocampus (DeGiorgio et al., 1992). The CA2 region appeared relatively resistant to damage.

Neuron-specific enolase (NSE), a sensitive marker for neuronal injury, can be measured in serum and CSF. Reports of increased NSE levels have been observed in both
generalized convulsive and complex partial status epilepticus, although higher levels have been associated with the former. The rise in NSE has been significantly correlated with outcome and seizure duration (DeGiorgio et al., 1995).

1.6.1.2 Neuropsychological studies

1.6.1.2.1 Animal studies

Several studies have demonstrated long-term deficits in memory, learning, and behaviour in mature rats after kainate and pilocarpine-induced SE (Albala et al., 1984), (Stafstrom et al., 1993). These neuropsychological deficits were often not replicated among immature animals (Stafstrom et al., 1993), (Thurber et al., 1992). Similarly, other experimental models including continuous hippocampal stimulation have produced long-term adverse effects on cognition in pubescent and mature rats but not in prepubescent animals (Thurber et al., 1992). Consistent with pathological reports, it has been inferred that the age at which SE occurs is crucial in determining whether or not long-term impairment in learning and memory will occur. Contradictory inferences were suggested by Lynch et al. (Lynch et al., 2000) who demonstrated that seizures evoked by kainic acid during early postnatal development (postnatal days 1-14), induced a long-term loss of hippocampal plasticity which manifested as impaired spatial learning. Since the dentate gyrus undergoes the majority of its development in the early postnatal period, it has been suggested that seizures during this period might affect the strength of the GABAergic inhibitory circuitry in the dentate gyrus and subsequently influence hippocampal function in adulthood.

1.6.1.2.2 Human studies

Information on neuropsychological function in humans following SE is limited. In a serial MRI study of a patient who developed progressive hippocampal atrophy and chronic epilepsy after SE due to herpes encephalitis, memory deficits were observed two months after the acute episode coinciding with the development of HS. Progressive hippocampal atrophy occurring over the 58-month follow-up period corresponded with continued neuropsychological decline characterised by a decline in Warrington recognition test for words and faces. The possibility that the observed decline in cognitive function might have been a sequelae of the presumed encephalitic illness rather than the direct consequence of the prolonged convulsive seizure must however be considered.
Conversely, a prospective study of nine patients showed that when SE was treated according to a predetermined protocol, no deterioration in clinical neurological status or socio-functional capacity was observed after one year (Salmenpera et al., 2000a). In a retrospective study of non-convulsive SE, Cockerell et al. (Cockerell et al., 1994) showed that despite frequent recurrent episodes of complex partial status secondary to a range of epilepsy aetiologies, none of the 20 patients developed marked evidence of cognitive or neurological decline.

1.6.1.3 Imaging studies

Serial MRI provides a tool for following the temporal progression of cerebral damage following an episode of SE. A number of case reports have demonstrated acute increases of T2 signal intensity after SE and complex febrile convulsions followed by progressive hippocampal atrophy (Nohria et al., 1994), (Tien and Felsberg, 1995), (Van Landingham et al., 1998). The initial transient oedematous signal changes have been attributed to vasogenic oedema (secondary to blood-brain barrier breakdown with abnormal vascular permeability) or cytotoxic oedema (Bouilleret et al., 2000). The report of Wieshmann et al. (Wieshmann et al., 1997) of progressive hippocampal atrophy in an adult for up to 58 months after generalized SE secondary to presumed herpes encephalitis, includes the possibility that the underlying aetiology might have influenced subsequent hippocampal damage. In a patient with extratemporal focal SE, progressive MRI changes were confined to the epileptogenic region of the superior frontal gyrus and pre-frontal gyrus which appeared normal prior to SE (Meierkord et al., 1997). This observation suggests that focal SE not accompanied by systemic changes may also result in neuronal damage.

Status epilepticus does not invariably lead to progressive damage to medial temporal lobe structures. In a quantitative MRI study, volumes of the hippocampus, amygdala, entorhinal and perirhinal cortices were measured three weeks, six months and 12 months after an episode of SE. The study showed that when nine adult SE patients were treated promptly, no significant volume losses were observed after one year (Salmenpera et al., 2000a). Possible explanations for the favourable outcome in this study were: the relatively short duration of SE (mean duration 1 hour, 44 minutes); the fact that chronic rather than acute processes were the underlying cause of SE; and the aggressive and effective management of SE.
1.6.2 Hippocampal damage following recurrent epileptic seizures

1.6.2.1 Experimental and pathological models

1.6.2.1.1 Animal models

Although there is evidence to suggest that SE may result in the death of hippocampal neurons through necrosis and apoptosis, it is unclear if brief repeated seizures, may also lead to neuronal damage.

A number of animal studies have provided evidence that even a few seizures can cause neuronal damage in the hippocampus. Using a rat hippocampal kindling stimulation model and in situ DNA fragmentation analysis, Bengzon et al. (Bengzon et al., 1997) demonstrated apoptotic neuronal death in the granule cell layer and the dentate gyri bilaterally after single and recurrent kindling-evoked seizures. Higher numbers of apoptotic cells were observed within the dentate gyrus after forty seizure events, than with single focal hippocampal seizures, suggesting that degeneration was correlated to the severity and duration of epileptic activity. Immunolabelling for the neurone-specific antigen NeuN and TUNEL histochemistry demonstrated that the majority of apoptotic cells were neurones. The NMDA receptor antagonist MK-801 did not influence the number of labelled nuclei in the dentate gyrus after 40 kindling-induced seizures, suggesting that this model of degeneration may not be triggered by the activation of NMDA receptor-operated channels. Subsequent work, however, has demonstrated that apoptosis occurs primarily to subgranular immature cells in contrast to established neuronal tissue.

Using perforant pathway kindling and a quantitative stereological method, Cavazos et al. (Cavazos et al., 1994) demonstrated selective vulnerability of hippocampal neurones to seizure-induced injury, with neuronal loss in the hilus of the dentate gyrus and CA1 after three GTCS; damage to CA3, entorhinal cortex, and the rostral endopiriform nucleus after 30 seizures; and damage to the granule cell layer and CA2 after 150 seizures. Similarly, Zhang et al. (Zhang et al., 1998) reported a 30% increase in the number of ApopTag-positive cells (a marker of apoptotic cell death) in the hippocampus after one Class V kindled seizure and 82.5% increase after 20 seizures. Conversely, Gorter et al. (Gorter et al., 2003) recently showed that hippocampal cell loss was related to the duration of the initial SE and not the frequency of spontaneously occurring seizures. However, the relevance of seizures induced by direct electrical
stimulation of the perforant pathway to habitual complex partial seizures in human TLE is unclear.

There is increasing evidence to suggest that epileptic brain damage results from a combination of excitotoxic-induced necrosis (During and Spencer, 1993) and apoptosis (Sloviter et al., 1996). It has been proposed that following seizures, the dentate gyrus may become functionally abnormal if apoptotic cells are replaced by the subsequent neurogenesis of immature neurons, which are ectopically located or have formed aberrant connections (Bengzon et al., 1997).

Using an amygdala-kindling model of TLE, Tuunanen et al. (Tuunanen et al., 1997) found a 30-40% loss of somatostatin immunoreactive (SOM-ir) neurones in several amygdaloid nuclei after five generalized seizures. Such decreases in SOM-ir neurones (a subpopulation of GABAergic inhibitory interneurones) may contribute to epileptogenesis by increasing the local excitability of the amygdala.

In his dentate lamellar hypothesis, Sloviter (Sloviter, 1994) proposed that endfolium sclerosis was the initial pathological lesion, caused by an initial precipitating factor e.g. status epilepticus, prolonged febrile seizures, head trauma, encephalitis, or genetic factors. This initial dentate hilar cell loss and dysfunction might deafferent GABA-mediated lateral inhibition in the dentate gyrus, abolishing the functional lamellar organization of hippocampal excitatory pathways, resulting in granule cell hyperexcitability and the generation of hippocampal seizures. He further hypothesized that the duration of the latent period between the initial insult and the onset of spontaneous seizures i.e. the "ripening of the scar" may be proportional to the severity of the initial neuronal loss.

It has therefore been postulated that chronic epilepsy arises from either the loss of inhibitory drive (Sloviter et al., 1996), a sprouting-induced increase in granule cell hyperexcitability (Schmid et al., 1999), or loss of GABA_B-mediated inhibition (Tuunanen et al., 1997).
1.6.2.1.2 Human studies

Early autopsy studies from epilepsy patients showed that severe hippocampal damage in a distribution termed “Ammon’s horn” or “hippocampal” sclerosis was strongly associated with complex partial TLE, while minor hippocampal neuron loss to other regions, such as the end folium were associated with other seizure types (Margerison and Corsellis, 1966). In one series, neuronal loss in CA3, CA1, and the hilus of the dentate gyrus was observed in more than 90% of temporal lobectomy and hippocampectomy specimens from patients with medically intractable TLE, and more extensive involvement of all hippocampal subfields seen in 2.8% (Thom et al., 2002). Resected hippocampi also showed reduced density and branching of dendritic spines, sprouting of mossy fibres in the dentate gyrus, dispersion of granule cells in the dentate gyrus and astrocytosis. With the advent of surgical resections for TLE, pathological studies have compared pre-mortem specimens with pre-operative clinical data. Some studies have suggested that although HS probably starts with an initial precipitating injury (Meyer et al., 1954), (Sloviter, 1994), repeated temporal lobe seizures or generalized seizures may contribute to additional hippocampal neuron loss, since longer seizure durations in TLE were associated with decreased neuronal densities in all hippocampal subfields (Mathern et al., 2002). The additional damage was, however, mild, non-specific, occurred over a long time interval (>30 years), and found in specific subfields, e.g. the endfolium and granule cell layers (Sagar and Oxbury, 1987), (Mathern et al., 2002). In other words, hippocampal neuron loss could be considered both a “cause” and a “consequence” of limbic seizures.

Pathological studies have further provided support in evidence of seizure-induced damage by demonstrating the increased expression of HLA-DR-immunoreactive microglia in CA1 and CA3 subregions of hippocampi removed at temporal lobectomy for intractable seizures. Since microglial activation has been found to be an acute or sub-acute response to injury, the results of this study are more in favour of the concept that HS is a progressive lesion arising from ongoing seizure activity (Beach et al., 1995). Human studies have also demonstrated that a single secondarily generalized hippocampal seizure may be associated with a six-fold increase in intrahippocampal glutamate concentration (During and Spencer, 1993), and an elevation in the level of serum γ-enolase, a marker of neuronal damage (Rabinowicz et al., 1995).
One study found a higher frequency of gestational or birth complications in TLE patients who had suffered prolonged febrile convulsions, suggesting that such perinatal or other preexisting factors may make a child with a genetic predisposition for febrile convulsions more vulnerable to prolonged or complex seizures (Abou-Khalil et al., 1993). The theory that seizures alone, do not lead to HS is supported by the pathologically proven existence of HS in association with alzheimer's dementia, dementia with Lewy bodies, and frontotemporal dementia (Dickson et al., 1994), (Ala et al., 2000), (Jellinger, 2000). Although such patients gave no history of seizures, cardiac disease was a frequent concomitant of HS, suggesting that hypoxic injury may be contributory.

1.6.2.2 Neuropsychological studies

1.6.2.2.1 Animal studies

The theory that seizure-induced cellular alterations in the hippocampus can contribute to memory dysfunction has been supported by animal models showing that memory function is dependent on the integrity of hippocampal pathways. Sutula et al. (Sutula et al., 1995) demonstrated that kindled rats show a long-lasting deficit in radial arm maze performance (a visuo-spatial learning and memory task) compared with controls, and that the severity of the deficit was directly related to the number of kindling evoked seizures. Neuronal loss was detected in the temporal hilus of the dentate gyrus, CA1, and CA3 of the hippocampus after greater than 69 secondary generalized tonic-clonic seizures, and was associated with progressive memory dysfunction (Kotloski et al., 2002). A similar deficit in spatial memory was seen in rats subject to kindled seizures in the early postnatal development (P1-P14) (Lynch et al., 2000). This memory deficit was accompanied by impaired induction of long-term potentiation, which has been implicated as a synaptic mechanism for information storage and memory (Bliss and Collingridge, 1993).

There is increasing evidence that there is an inverse relationship between the degree of CA3 innervation by mossy fibre terminals and learning. Landrot and colleagues (Landrot et al., 2001) demonstrated an inverse relationship between water maze performance and mossy fibre sprouting in the CA3 subregion i.e. the rats with the greatest degree of mossy fibre sprouting had the worse performance in the water maze. Mossy fibres release glutamate, the primary excitatory neurotransmitter of the mossy
fibres (Toth et al., 2000). Glutamate binds to three types of ionotropic receptors: NMDA, AMPA and kainic acid, which play a critical role in the initiation and maintenance of epileptic seizures (Meldrum et al., 1999). In a study of neonatal rats exposed to flurothyl-induced seizures, increased expression of AMPA receptors and NMDA receptors was seen in the CA3 region and dentate gyrus at 20 and 35 days postnatally. Alterations in cognition and seizure susceptibility were paralleled by sprouting of mossy fibres and increased expression of glutamate receptors (Sogawa et al., 2001).

1.6.2.2.2 Human studies

Human studies have produced inconsistent findings concerning cognitive impairment and seizures. Studies are often confounded by the underlying aetiology for the seizures, multiple drug effects and the lack of appropriate age-matched control groups.

A longitudinal prospective study following 35 adults with intractable complex partial seizures over a 10 year period, showed that global measures of intelligence and neuropsychological functions remained reasonably stable (Holmes et al., 1998b). Even patients with frequent secondarily generalized seizures were not associated with a deterioration in global cognitive measures (Holmes et al., 1998b). When intellectual decline has been documented in epilepsy, a history of generalized convulsive SE or histories of >100 GTCS can usually be elicited (Dodrill, 1986). A longitudinal study of children with epilepsy during test / re-test intervals of 3.5 and 11 years demonstrated mean IQ declines or failure to demonstrate age expected IQ gains, suggesting that recurrent seizures were a considerable risk factor for intellectual decline. A recent longitudinal study compared memory and non-memory function in 147 surgically and 102 medically treated patients with TLE, followed-up for a median period of 52 and 49 months respectively (Helmstaedter et al., 2003). Both surgically and medically treated groups were associated with cognitive decline, preferentially affecting memory function. Risk factors were frequency and severity of seizures, left sided temporal lobe surgery, and the extent of the resection. In those who became seizure free, memory stabilized and even reversed, emphasizing the importance of early and complete control of seizures.
Memory complaints are common in patients with epilepsy and there is objective evidence of impairment in many patients (Corcoran and Thompson, 1993), (Thompson, 1991). Patients with seizures that arise from the temporal lobes seem to be at particular risk of episodic memory loss, which may be seen in approximately one-third of such patients. However, it is difficult to differentiate between the effects of seizures and interrelated factors, such as, antiepileptic medication, recurrent head injuries, subclinical discharges, and underlying cerebral pathology.

Amnesia during the seizure is a feature of complex partial and generalized seizures and a variable period of anterograde amnesia follows these seizures. It has been proposed that ictal and interictal epileptic activity may transiently disturb memory encoding and retrieval processes (Zeman et al., 1998). It is known that memory traces of newly learned material are fragile for extended periods during the process of memory consolidation. In a study investigating the effect of seizures on memory for recently learned material, Bergin et al. (Bergin et al., 1995) showed that there was no correlation between memory performance and the timing or frequency of seizures. The authors concluded that isolated seizures do not generally cause patients to forget material they have recently learned. Similarly, a study investigating the effect of temporal lobe seizures on the retention of newly learned information also showed no general effect of seizures, although seizures impaired the 24-hour retention of recently learned information in patients with left-sided TLE (Jokeit et al., 2001). There is evidence to suggest that the mesial temporal lobe structures are critically involved for several hours to weeks in consolidation processes that require new protein synthesis and gene expression (McGaugh, 2000). Antiepileptic drugs may result in impairments of attention and cognitive slowing (Meador, 2002), which may interfere with encoding and retrieval processes.

Patients with refractory TLE frequently complain of accelerated memory loss that is not detected by conventional memory tests with short retention intervals of less than an hour (Blake et al., 2000). The authors thus suggested that retention problems in patients with TLE were probably underestimated by standard assessment techniques.
1.6.2.3 Imaging studies

1.6.2.3.1 Cross-sectional studies

Imaging studies investigating the association between the severity of HS, and seizure frequency and duration have largely focused on cross-sectional (C-S) studies. These studies are usually retrospective in design and frequently produce inconclusive and conflicting results since they infer intraindividual changes from interindividual differences (Kalviainen et al., 1998), (Cendes et al., 1993b), (Cook et al., 1992).

Several C-S studies have failed to find a relationship between MRI hippocampal volume, and the frequency of habitual seizures (Cendes et al., 1993b) or duration of epilepsy (Cendes et al., 1993b), (Trenerry et al., 1993b). Harvey et al. (Fox et al., 2000b) demonstrated HS in 57% of children with new-onset TLE, suggesting that its presence at an early age was evidence against recurrent seizures being the predominant cause of HS.

In contrast, a C-S analysis of a community-based cohort showed that the mean HV, corrected for ICV, in patients with chronic focal epilepsy was 6% less than in those with newly diagnosed partial seizures and 10% less than the control group (Everitt et al., 1998). Another study demonstrated an 18% and 14% reduction of the left and right HV respectively on the side ipsilateral to the seizure focus in patients with chronic drug resistant cryptogenic TLE (Kalviainen et al., 1998). They also demonstrated that seizure frequency was related to the extent of hippocampal volume loss and prolongation of T2 relaxation time. Using linear correlation, they calculated that approximately 6500 seizures would be required to cause a 50% reduction in hippocampal volume. Other volumetric MRI studies have reported a similar relationship between epilepsy duration and ipsilateral hippocampal volume (Spencer et al., 1993), (Theodore et al., 1999). Some studies have found a relation with a prior history of complicated febrile convulsions but not duration of epilepsy (Cendes et al., 1993b), (Kuks et al., 1993).

Possible explanations for the discrepant findings in C-S studies include the inaccuracy of recording seizures retrospectively, and failure to correct for age of onset or the effect of TBV on HV in many of the studies reporting an inverse relationship between hippocampal size and duration of epilepsy (Lawson et al., 2000). Inferences that can be drawn on disease progression from C-S studies are limited since (1) small changes in
anatomical structure over time may be masked by large biological variability across subjects, and (2) cross-sectional studies are unable to give direct information on the causal relationship between seizures and structural brain damage. Since studies of a cross-sectional design study only a single time point in different patients, they do not exclude the possibility that those people with chronic drug-refractory had the most severe hippocampal volume loss prior to the onset of habitual epilepsy. Another possible explanation is that patients with drug-resistant TLE may have some intrinsic progressive hippocampal disease that is responsible for the reduction in hippocampal volume.

1.6.2.3.2 Longitudinal studies

The longitudinal development of hippocampal damage in patients with TLE have been described in several case reports (Table 5). Briellmann and colleagues (Briellmann et al., 2001) described the development of HS over 33 months in a patient following seven secondarily generalised tonic-clonic seizures and frequent simple partial temporal lobe seizures. Although there were no major precipitating events, minor head injuries between the scans and a possible developmental abnormality in the ipsilateral hippocampus may have contributed to the subsequent development of HS. In a separate case study, visual and quantitative progressive hippocampal changes following brief generalized seizures were detected after 4.25 years of habitual seizures (O'Brien et al., 1999). More recently, Worrell and colleagues (Worrell et al., 2002) reported the rapid development of hippocampal formation atrophy over 5.5 months in a patient with newly diagnosed temporal lobe seizures following acute thrombosis of an ipsilateral parietal venous angioma. Since, the patient suffered only a single brief generalized tonic-clonic seizure, the authors concluded that partial seizure activity could cause rapid progressive hippocampal atrophy in some patients.
## Table 5 Summary of MRI case reports on seizure-related hippocampal volume change

<table>
<thead>
<tr>
<th>Longitudinal imaging studies Authors (date of publication)</th>
<th>Details</th>
<th>Findings</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case reports</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worrell GA et al. (Worrell et al., 2002)</td>
<td>43 year old female. New onset of NCSE and SGS following acute thrombosis of an ipsilateral parietal venous angioma detected on MRI 2 days after the event. Subsequently developed TLE with 1-5 CPS/month.</td>
<td>Day 2: No hippocampal atrophy but increased signal Lt HC. Day 143: Left HS (LHV diminished by 26%). RHV unchanged. Slight reduction in LHV between days 2 and 21</td>
<td>Rapid progression of HA can occur in new onset seizures over 5.5 months. HC was not involved in the acute stroke event.</td>
</tr>
<tr>
<td>Wieshmann UC et al. (Wieshmann et al., 1997)</td>
<td>30 year old female with convulsive SE. due to presumed herpes encephalitis.</td>
<td>During status, both mesiotemporal lobes returned a high signal on T2-weighted images. Bilateral HA detected 2 months after SE. Progressive HA occurred over 58 months.</td>
<td>HA can continue after the end of SE, and was first noted only 2 months after the acute event.</td>
</tr>
<tr>
<td>O'Brien TJ et al. (O'Brien et al., 1999)</td>
<td>28 year old man with intractable TLE. Patient had 4-5 seizures per month, with 50% secondarily generalizing. No episodes of SE.</td>
<td>MRI scans 4 years apart showed progressive decrease in ipsilateral HV. Surgical resection confirmed mesial temporal sclerosis.</td>
<td>Poorly controlled TLE seizures may lead to progressive hippocampal atrophy in the absence of SE.</td>
</tr>
<tr>
<td>Briellmann RS et al. (Briellmann et al., 2001)</td>
<td>18 year old. Onset of SPS aged 16.5. First GTCS aged 18 years following minor head injury. 7 GTCS between the first two scans. MRS performed at the 2nd scan.</td>
<td>Initial MRI after 1st GTCS quantitatively normal. 2nd MRI scan performed 2.75 years after 1st scan and after 7 GTCS showed reduced HV and increased HCT2 signal (30% ipsilateral HV loss, 10% contralateral). 3rd MRI 9 months after 2nd MRI, after 3 more GTCS and no further HC changes seen. Low NAA/Cr ratio seen in ipsilateral temporal lobe.</td>
<td>HS may be acquired in adulthood after brief GTCS in absence of major precipitating event over 2.75 years. Possible contributory factors were two minor head injuries and pre-existing relatively small ipsilateral HC.</td>
</tr>
<tr>
<td>Nohria et al. (Nohria et al., 1994)</td>
<td>32 month old child with focal SE. CPS started one month later, and 3 further episodes of SE between 33 and 37 months of age.</td>
<td>MRI performed &lt;24 hours after seizures showed increased T2 signal in ipsilateral HC, but no atrophy. Repeat MRIs at 34 and 45 months showed progressive HA with resolution of T2 signal.</td>
<td>SE is associated with acute increases in HCT2 signal, followed by HS after several months in early childhood SE.</td>
</tr>
<tr>
<td>Group studies</td>
<td>Details</td>
<td>Findings</td>
<td>Conclusion</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Hospital-based studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Paesschen W et al. (Van Paesschen et al., 1998)</td>
<td>36 patients with newly diagnosed partial seizures. Serial MRI scans performed one year apart. HV volumetry and HCT2 measurements performed. 12 control subjects also scanned one-year apart.</td>
<td>11% patients had HS at baseline. No patients developed HS. 3 patients developed significant HC changes over the study period. Differences in HV or HCT2 between controls and patients were not significant.</td>
<td>Subtle HC changes seen in 8% patients, attributed to resolution of oedema / effect of seizures.</td>
</tr>
<tr>
<td>VanLandingham KE et al. (VanLandingham et al., 1998)</td>
<td>MRIs performed after complex FC in 27 infants.</td>
<td>6/15 patients with focal CFCs and 0/12 with generalized CFCs had MRI abnormalities. 2/6 patients with focal CFCs and abnormal MRIs had pre-existing bilateral HA due to perinatal insults. Remaining 4 had increased HCT2 signal intensity and increased HV predominantly on ipsilateral side - Follow-up of 2 of these infants showed development of HA after 8-10 months.</td>
<td>Prolonged and focal CFCs can produce acute hippocampal injury that evolves into hippocampal atrophy.</td>
</tr>
<tr>
<td>Briellmann RS et al. (Briellmann et al., 2002a)</td>
<td>24 patients with newly diagnosed TLE (mean age 30 +/- 14 years). Baseline and repeat MRI scans 3.5 years apart on different scanners. No control group.</td>
<td>Corrected ipsilateral HV decreased by 9% (range -30% to +0.5%, p=0.002). Contralateral HV decreased by 5% (-6% to -17%). Ipsilateral HV loss correlated significantly with number of GTCS between scans. One patient developed HS between the two scans.</td>
<td>Even a few GTCS can have a harmful effect on the hippocampus in some patients with mild TLE.</td>
</tr>
<tr>
<td>Fuerst D et al. (Fuerst et al., 2003)</td>
<td>12 patients with refractory TLE and unilateral HS were scanned 3.4 years apart. No control group.</td>
<td>Significant change in mean ipsilateral HV but not contralateral HV between scans. Ipsilateral HV decline was correlated with the number of partial seizures but not generalized seizures. Ipsilateral HV changes were not seen in patients who became seizure free.</td>
<td>Patients with refractory TLE and unilateral HS may develop progressive hippocampal atrophy that is related to the frequency of partial seizures.</td>
</tr>
<tr>
<td><strong>Community-based studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liu RSN et al. (Liu et al., 2002b),</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter I

Prospective longitudinal MRI studies comparing patients with epilepsy with control subjects will more definitively address whether seizures cause progressive brain atrophy (Table 6). In a hospital-based study, one-year hippocampal changes were explored in 36 adult patients with newly diagnosed partial seizures (Van Paesschen et al., 1998). Group comparisons did not find significant differences between the patient and control group although individual analyses identified three patients with either a significant reduction in hippocampal volume or increase in hippocampal T2 signal. These subtle changes were considered to be due either to the resolution of oedema or to the consequence of frequent seizures. None of the patients developed HS de novo. More recently, Briellmann and colleagues (Briellmann et al., 2002a) investigated the effect of convulsive seizures on the hippocampus by following-up 24 patients with newly diagnosed cryptogenic TLE over a 3.5-year period. A strong correlation was found between the number of convulsive seizures and the extent of volume loss in the hippocampus ipsilateral to the putative epileptic focus. However the results may have been influenced by a number of methodological limitations including the use of different MRI scanners for the baseline and repeat scans, lack of normal controls and the relative lack of blinding. In another longitudinal study of 12 patients with drug refractory TLE and unilateral HS, a 10% ipsilateral HV loss was seen in association with recurrent complex partial seizures (Fuerst et al., 2003).

1.6.3 Cerebellar damage in epilepsy

1.6.3.1 Pathological and experimental studies

Neuropathological and neuroimaging studies have shown that cerebellar atrophy is a common finding in patients with chronic epilepsy, although its pathogenesis remains unclear (Dam, 1970), (Spielmeyer, 1930), (Botez et al., 1988). Potential aetiologial factors include: seizure mediated cellular damage, phenytoin toxicity, seizure-induced anoxic-ischaemic injury, excitotoxic damage via cerebro-cerebellar projections, post traumatic lesions, pre-existing brain injury resulting from the initial epileptogenic insult or a combination of these factors (Ney et al., 1994), (Savic and Thorell, 1996), (Specht et al., 1997), (Crooks et al., 2000).

Histological examinations in epileptic patients with cerebellar atrophy have shown Purkinje cell loss, preservation of basket cells, Bergmann gliosis and focal granule cell
depletion (Crooks et al., 2000). In their quantitative neuropathological study, Crooks et al. (Crooks et al., 2000) did not find a significant difference in mean Purkinje cell linear densities between patients with chronic partial seizures and those with generalized seizures, or between patients with partial epilepsy suffering from partial or secondary generalized seizures. Hypoxic-ischaemic cellular injury alone, appeared unlikely to be solely responsible for the occurrence of cerebellar atrophy, since greater cell loss in the watershed zone and concomitant neuronal changes in other brain structures vulnerable to hypoxia e.g. Ammon’s horn would have been expected (Crooks et al., 2000), (Ghatak et al., 1976). Furthermore, previous studies have shown that cerebellar atrophy can occur in patients with focal seizures in the absence of generalized seizures, and in those on phenytoin for seizure prophylaxis with no history of seizures (Ney et al., 1994), (Savic and Thorell, 1996), (Salcman et al., 1978b), (Masur et al., 1989).

Both acute intoxication and chronic phenytoin usage has been associated with cerebellar atrophy (Botez et al., 1988), (Ney et al., 1994), (Luef et al., 1996). However, it seems unlikely that phenytoin alone could account for the variable patterns of cerebellar atrophy observed. Spielmeyer (Spielmeyer, 1930) and Zimmerman (Zimmerman, 1938) both described cerebellar atrophy in post-mortem studies of patients with frequent convulsive seizures and marked hypoxia, which predated the introduction of phenytoin in 1938. Proposed mechanisms for phenytoin toxicity have included alterations in Purkinje cell axon morphology (Luef et al., 1996) and diffuse Purkinje cell loss (Gessaga and Urich, 1985). Immunofluorescent studies have implicated a specific binding site for phenytoin in the vicinity of Purkinje cells and granule cells (Hammond and Wilder, 1983). The increased firing rates in cerebellar neurones induced by phenytoin (Julien and Halpern, 1972), although potentially protective against cortical seizures via the cerebello-thalamic-cortical circuit, may be harmful to cerebellar neurones (Ney et al., 1994).

Cerebrocerebellar diachisis has also been implicated in the development of cerebellar atrophy in focal epilepsy (Tien and Ashdown, 1992). Diaschisis is defined as a disturbance in a brain region secondary to a focal disturbance in another remote but anatomically connected region. It has been proposed that cerebellar atrophy may result from excessive excitatory discharges propagated via glutamatergic cortico-pontine-cerebellar pathways (Stubgen, 1995). Baudrimont et al. (Baudrimont et al., 1983)
reported two autopsy cases with supratentorial lesions and contralateral cerebellar atrophy. Animal experiments involving penicillin-induced cortical focal discharges have demonstrated firing in cerebellar Purkinje cells and dentate nucleus neurons (Julien and Laxer, 1974).

Some studies have postulated that the cerebellum may exert an inhibitory effect on seizures. Specht et al. (Spect et al., 1997) demonstrated that cerebellar atrophy was associated with a poorer seizure outcome following temporal lobectomy. This finding is consistent with reports from animal studies of bidirectional cerebro-cerebellar connections, with the anterior lobe of the cerebellum exerting an inhibitory effect on cortical activity (Middleton and Strick, 1997).

Ultimately, the evidence suggests that cerebellar atrophy in patients with epilepsy is unlikely to result from a single aetiological agent but is likely to be the culmination of genetic susceptibility and multiple factors acting synergistically.

1.6.3.2 Neuropsychological studies

Anatomical, physiological and functional neuroimaging studies have suggested that as well as its recognized role in motor coordination (Holmes 1939), the cerebellum may also be involved in the control of cognitive and behavioural function. A complex of symptoms has been described in patients with acquired cerebellar lesions involving executive, spatial, linguistic and affective functions, known as the “cerebellar cognitive affective syndrome” (Schmahmann and Sherman, 1997). This is characterized by disturbances of executive function including deficient planning, abstract reasoning, working memory and decreased verbal fluency; impaired spatial cognition including visuospatial organization; personality change characterized by flattening of affect and disinhibited behaviour; and linguistic difficulties including nominal aphasia. Lesions of the vermis were in particular associated with dysregulation of affect. These cognitive deficits would be consistent with disruption to the feedback limb of the cerebrocerebellar anatomical circuitry i.e. the cerebellothalamic and thalalmocortical systems (Middleton and Strick, 1994).
Compatible with these findings is the observation of cerebellar activation during functional neuroimaging tests of language function and cognitive planning (Petersen et al., 1989).

1.6.3.3 Imaging studies

MRI studies have shown that cerebellar atrophy may be found in approximately 16 to 47% of patients with chronic focal epilepsy, depending upon patient selection and the criteria with which cerebellar atrophy was diagnosed (Ney et al., 1994), (Specht et al., 1997). Studies have proposed that age at scan, a history of GTCS, intellectual impairment, and duration of epilepsy may be significantly correlated with its presence (Specht et al., 1997), (Sandok et al., 2000).

Imaging studies have been mostly C-S in design, and results have been inconclusive. Whilst Bohnen (Bohnen et al., 1998) found a positive correlation between seizure frequency and cerebellar volume (corrected for total brain volume), other investigators (Ney et al., 1994), (Bekkelund et al., 1996) failed to find a correlation between cerebellar atrophy and, duration of epilepsy, duration of phenytoin therapy or maximum dose of phenytoin. However, in these latter studies, cerebellar volumes were not assessed quantitatively. The contribution of phenytoin to the pathogenesis of cerebellar atrophy is difficult to resolve in retrospective studies since high dose phenytoin treatment and severe seizures often coexist in the same patient. In his MRI study, Ney (Ney et al., 1994) demonstrated cerebellar atrophy in 44% of patients exposed to chronic phenytoin, who had experienced only 0-5 lifetime convulsions.

Contralateral cerebellar diaschisis and atrophy have been demonstrated in patients with partial epilepsy using PET and MRI studies respectively (Tien and Ashdown, 1992). Several studies using periictal single photon emission computed tomography have demonstrated that partial seizures may be associated with transient contralateral cerebellar hyperperfusion which may contribute to neuronal cell injury and death (Duncan et al., 1987). Reversed cerebellar diaschisis with hypoperfused regions in cerebral association areas has also been observed in the PET/SPECT studies of cerebellar patients (Botez et al., 1991).
1.6.4 Neocortical damage in epilepsy

1.6.4.1 Experimental and pathological models

Although the hippocampus is recognized as an essential structure in initiating and maintaining epileptogenesis, less is known about neocortical structures in TLE. Experimental studies (Meldrum and Brierley, 1973), (Margerison and Corsellis, 1966) have demonstrated that prolonged convulsions can produce pathological changes in the neurones of the neocortex as well as the hippocampus and cerebellum.

A number of mechanisms have been proposed regarding the pathogenesis of neocortical abnormalities in TLE. One proposition is that neocortical developmental abnormalities may predispose a patient to febrile convulsions and the subsequent development of HS (Hardiman et al., 1988) or arise in response to the same initial insult that produced the HS (Lawson et al., 2000). This notion has been supported by a study that demonstrated that the degree of extrahippocampal atrophy was correlated with the severity of hippocampal atrophy but not with the occurrence of generalized seizures or duration of epilepsy (Hardiman et al., 1988). Another possibility is that both HS and neocortical abnormalities are the result of prolonged childhood febrile convulsions or acquired lesions arising from recurrent seizures or medication.

1.6.4.2 Imaging studies

Quantitative MRI studies of patients with intractable mesial TLE have demonstrated widespread extrahippocampal temporal lobe volume losses, ranging from 8.3% in the parahippocampal gyrus and medial occipitotemporal gyrus, to 18.4% in the temporal pole and a 13% mean loss in temporal lobe volume (Moran et al., 1999). Similarly, Lee (Lee et al., 1998) demonstrated reduced grey matter volume in both temporal lobes of patients with TLE. The volume loss correlated with epilepsy duration, and was most marked on the side ipsilateral to the seizure focus.

Volume losses have been reported in the entorhinal cortex, contralateral temporal lobe, extratemporal cortical region, and cerebral hemisphere (Bernasconi et al., 1999), (Bernasconi et al., 2003), (Salmenpera et al., 2000a), (DeCarli et al., 1998), (Marsh et al., 1997). Subtle neocortical abnormalities have been identified in patients with a variety of epilepsy syndromes using voxel-based analyses of automatically segmented grey matter distribution (Woermann et al., 1999).
The implication of these neocortical deficits in TLE is unclear. Sisodiya and colleagues (Sisodiya et al., 1997b) showed that the presence of occult extrahippocampal cerebral structural defects was significantly correlated with poorer surgical outcomes in patients with HS and cerebral dysgenesis. They went on to postulate that these widespread cerebral structural abnormalities might possess epileptogenic properties that became apparent after surgery.

Extrahippocampal temporal lobe abnormalities in patients with TLE have also been demonstrated in PET studies (Henry et al., 1993) which have reported interictal hypometabolism in the ipsilateral lateral temporal lobe, whilst MR spectroscopy findings have demonstrated changes in N-acetyl aspartate, creatine/phosphocreatine, and choline-containing compounds in the contralateral temporal lobe (Connelly et al., 1994).

1.7 CAUSES OF SECONDARY CEREBRAL DAMAGE / DYSFUNCTION AND THEIR PREVENTION

1.7.1 Seizures

Epileptic brain damage is thought to be caused by excitotoxic effects produced by glutamate or aspartate-activating NMDA and other receptors with contributions by increased free-radical production and activation of apoptotic mechanisms (Meldrum, 1986) (see sections 1.6.1 and 1.6.2).

The identification of agents that attenuate injury after prolonged seizures may be of value in the management of refractory SE. The efficacy of vigabatrin monotherapy, carbamazepine monotherapy and the combination of both for the prevention of neuronal loss has been addressed in a perforant pathway model of SE in rats (Pitkanen, 1996). Although either independently and a combination of the two drugs were equally effective in preventing the severity and reducing the duration of seizures, neuroprotection (evaluated on a functional level by counting the number of SOM-ir neurones in the hilus), was greatest in the vigabatrin-treated group (92% of neurones remaining), and least in the carbamazepine group (47% of SOM-ir neurones left). Combination therapy resulted in intermediate neuronal protection (79% SOM-ir neurones left). Pyramidal cell damage in the CA1 and CA3 regions, assessed using silver-stained
sections, showed that damage was significantly more severe in the carbamazepine group than in the vigabatrin group. Furthermore, the neuronal preservation observed with vigabatrin was associated with preservation of function in rats assessed using the Morris water-maze test (Ylinen et al., 1991). Vigabatrin was not shown to confer protection against damage to the lateral and basal nuclei in the amygdala (Pitkanen et al., 1996). A recent study based on the lithium-pilocarpine model of SE in rats provided confirmatory evidence of the neuroprotective effects of vigabatrin with almost complete protection in CA3, efficient protection in CA1 and partial protection of the hilus of the dentate gyrus (Andre et al., 2001). However, neuroprotection of the Ammon’s horn of the hippocampus was not sufficient to prevent epileptogenesis, suggesting that the hilus and extra-hippocampal structures that were not protected in this study might also contribute to epileptogenesis.

The intraperitoneal injection of topiramate has also been associated with a significant reduction in silver staining density bilaterally in the CA1 area and the dentate hilus (Niebauer and Gruenthal, 1999). This may be related to the ability of topiramate to suppress voltage-sensitive sodium channels and non-NMDA receptors, and its ability to enhance GABA-mediated inhibition (Yang et al., 1998).

Oestrogen (beta-estradiol benzoate, EB) has also been shown to protect against kainic acid-induced damage to the CA3 hippocampal subfield and dentate gyrus following an episode of SE (Veliskova et al., 2000). Pre-treatment with tamoxifen abolished the neuroprotective effect of oestrogen suggesting that protection was mediated through intracellular EB receptors; and synthesis of 17 beta-oestradiol by hippocampal cells is completely inhibited in vitro by letrozol, an aromatase inhibitor (Prange-Kiel et al., 2003).

Animal models have shown that felbamate (Shuaib et al., 1996) and lamotrigine (Crumrine et al., 1997) may confer cerebral structural protection following experimentally–induced brain ischaemia. However, the significance of these findings is difficult to extrapolate to cerebral ischaemia and epilepsy in humans.

It therefore appears that certain agents can prevent structural damage and memory impairment in animal models. However, further studies are needed to determine the
time window for successful implementation of neuroprotective strategies, to identify those at greatest risk of seizure-induced damage, and to compare the relative neuroprotective properties of the different antiepileptic drugs.

1.7.2 Interictal epileptiform activity

Many patients with chronic epilepsy display cognitive deficits and consequent psychosocial dysfunction. A number of investigators (Binnie et al., 1987), (Aarts et al., 1984) have described a phenomenon, transitory cognitive impairment (TCI), in which subclinical / interictal generalized and focal EEG discharges result in momentary disruption of cognitive function. TCI has been detected in approximately 50% (Aarts et al., 1984) of patients exhibiting subclinical epileptiform EEG discharges during appropriate psychological testing, and may be found during brief focal discharges including single spikes. With focal discharges, the effects are material specific: verbal memory deficits associated with a dominant hemisphere focus (Hermann et al., 1987), while visuospatial deficits are associated with non-dominant hemisphere focus (Binnie et al., 1987). This would suggest that TCI is not necessarily a consequence of a general impairment of attention but reflects disruption of specific psychological functions located in the region where the epileptiform discharges arise (Aarts et al., 1984). Discharges during stimulus presentation were most disruptive of performance whereas there was no demonstrable effect when patients were responding (Aarts et al., 1984). In addition to causing TCI, interictal discharges occurring after the learning experience can disrupt consolidation processes (Binnie et al., 1990). Currently, the impact of such TCI on psychosocial function in daily life is uncertain, but it is probable that TCI contributes to some of the cognitive problems associated with chronic epilepsy.

It has been shown in selected case histories that anti-epileptic drugs can improve cognition and suppress EEG discharges which may be beneficial, provided the drugs themselves do not carry a cognitive penalty (Aarts et al., 1984). However, most anti-epileptic drugs, with the exception of the benzodiazepines (which themselves adversely affect cognition) and lamotrigine, do not suppress interictal discharges (Binnie, 1994). The surgical removal of a discharging focus may lead to an improvement of test performance and may account for the occasional improvement in cognitive function, seen after selective amygdalohippocampectomy (Wieser, 1985). A meta-analysis of 33 studies of verbal and non-verbal memory performance pre- and post-anterior temporal
lobectomy showed a trend for contralateral improvement in non-verbal memory after a left anterior temporal lobectomy but no evidence of verbal memory improvement after resection from the right temporal lobe (Lee et al., 2002).

1.7.3 Antiepileptic drugs
Certain authors have reported loss of cerebellar Purkinje cells with phenytoin therapy (Dam, 1970), although much of the data on this topic remains controversial. Purkinje cell loss is a common finding in patients with epilepsy, regardless of whether or not they are taking AEDs. Permanent dysfunction is only occasionally seen with phenytoin toxicity and therefore may be related to the duration of acute phenytoin toxicity or to pre-existing pathological damage in the cerebellum from seizures (Reynolds, 1975). Children with epilepsy and severe brain damage on multiple drugs are especially susceptible to the neurotoxic effects of phenytoin (Iivanainen and Savolainen, 1983).

The use of AEDs has been implicated in the cognitive deficits commonly observed in patients with chronic epilepsy. In patient studies, the evaluation of results is confused by practice effects and changes in the frequency of seizures and interictal discharges (Binnie, 1994). The effects of carbamazepine and valproate appear modest when dosages are kept within standard therapeutic ranges and polypharmacy is avoided. There are however, rare reports of valproate-induced cognitive deterioration in the absence of hyperammonemia, and visual reports of pseudo-atrophy on MRI which are reversible on discontinuation of valproate (Guerrini et al., 1998), (McLachlan, 1987). Evidence suggests that alterations in arousal, attention, memory, psychomotor functioning and higher cognitive processes are relatively greater for phenytoin, benzodiazepines, phenobarbitone and topiramate (Blennow et al., 1990), (Thompson et al., 2000).

1.7.4 Cerebral trauma
A number of experimental studies have shown that the hippocampus is selectively vulnerable to experimental traumatic brain injury (TBI) (Carbonell and Grady, 1999). Studies incorporating an acceleration model of brain injury have shown that up to 94% of non-human primates demonstrate hippocampal damage after sustaining severe injuries and prolonged posttraumatic coma (Kotapka et al., 1991). These lesions typically involved the CA-1 hippocampal subfield and were bilateral in approximately
half the animals studied. Contrary to conventional mechanistic theories of head injury, hippocampal involvement was not associated with marked elevation of intracranial pressure or depression of cerebral perfusion pressure. Furthermore other brain regions considered selectively vulnerable to hypoxic insults were spared. The authors hypothesized that glutamate-mediated excitotoxicity might have been responsible for the demonstrated lesions.

Subjects with head injuries frequently experience respiratory distress that results in a secondary hypoxic insult. Experimental studies in rats have shown that a secondary hypoxic insult following a parasagittal fluid-percussion injury exacerbates contusion and damage to the CA1 and CA2 subregions of the hippocampus, which emphasizes the importance of controlling for post-traumatic hypoxia (Bramlett et al., 1999). A separate study demonstrated selective neuronal damage in the CA3 region after mild closed head injury combined with hypoxia (Katoh et al., 1997). Quantitative autoradiography techniques showed that this selective hippocampal damage might have been mediated through an increase in NMDA receptor activation and the further release of glutamate; and that treatment with the NMDA antagonist, MK-801 could be beneficial in preventing secondary neuronal damage in hypoxia.

Other authors have disputed claims that glutamate-mediated excitotoxicity might be responsible for post-traumatic hippocampal damage. Carbonell et al. (Carbonell and Grady, 1999) showed that when two different strains of mice shown to be susceptible and resistant to kainic acid-induced excitotoxic hippocampal damage, were subject to a severe parasagittal fluid percussion injury (FPI), dystrophic neurones were evident in the hippocampi of both strains after 10 minutes. Damaged hippocampal neurons were absent at 4 and 7 days, with no significant difference between neuronal survival between the two different strains of mice, suggesting that hippocampal cell death after traumatic brain injury is hyperacute, and that excitotoxicity does not significantly contribute to hippocampal neurone loss after FPI.

Studies of fatal human non-missile injuries have shown a similar distribution of hippocampal lesions to that reported in experimental acceleration head injury. An imaging study comparing patients with TBI with healthy volunteers showed significant
bilateral atrophic changes in the hippocampus and temporal horn enlargement in the former (Bigler et al., 1997).

The cerebellum has also been implicated in TBI, with pathological evidence of microglial activation and purkinje cell loss in the cerebellum secondary to an impact to the forebrain (Crooks et al., 2000).

### 1.7.5 Hypoxia

Experimental models of unilateral common carotid artery ligation followed by systemic hypoxia in 7-day postnatal rats, have demonstrated selective neuronal necrosis or apoptosis commencing after 1 hour of hypoxia-ischaemia, followed by infarction after 90 minutes (Rice et al., 1981). Microdialysis techniques have shown a late rise in CSF / extracellular fluid glutamate corresponding temporally with infarction rather than selective neuronal death (Vannucci et al., 1999). Similar increases in extracellular glutamate concentrations have also been noted in striatum and hippocampus of the same immature rat model after 90 minutes of hypoxia-ischaemia (Gordon et al., 1991).

Both in vitro and in vivo experiments have shown that excitotoxic neuronal death caused by excessive glutamate concentrations, occurs only when cellular energy levels (ATP) are at least partially depleted and not able to facilitate the reuptake of glutamate from the synaptic cleft (Novelli et al., 1988).

A therapeutic approach has been adopted, investigating the action of drugs at various sites e.g. glutamate release, postsynaptic glutamate receptors and the secondary events following receptor activation (Meldrum, 1990). Glutamate receptor antagonists e.g. MK-801 have been shown to decrease infarct size in cerebral cortex, striatum, and hippocampus by up to 75% (Ford et al., 1989), although it is not clear whether glutamate antagonists would prevent selective neuronal death in the absence of infarction.

A novel calcium channel blocker, LY393615, has recently been shown to protect against neuronal damage caused by hypoxia-hypoglycaemia in vitro and both global and focal cerebral ischaemia in vivo (O'Neill et al., 2001). Neuroprotection was achieved when the agent was administered post-occlusion, suggesting that it might be a
useful anti-ischaemic agent. The delayed secondary elevation of ECF glutamate observed six hours after 2 hours of hypoxia-ischaemia in adult rats may be attributed either to progressive neuronal damage during the reperfusion period, or simply reflect the leakage of neurotransmitter from dying cellular elements into the extracellular space.

The neuroprotective properties of diazepam have also been investigated in rats subject to transient global ischaemia (Schwartz et al., 1995). The administration of intraperitoneal diazepam conferred substantial protection of the striatal neurones and pyramidal neurones in the CA1 region of the hippocampus. In addition, diazepam prevented the loss of GABA_A receptors in the striatum and the dendritic fields of the CA1 region. This observation led the authors to propose that the enhancement of GABAergic neurotransmission directly at the site of vulnerability following ischaemia may protect vulnerable neurones from cell death. A dose-dependent neuroprotective effect has also been observed with intraperitoneal topiramate, administered after middle carotid artery embolization in a rat model of ischaemia (Yang et al., 1998).

A variety of other agents including phenytoin (Hayakawa et al., 1994), Zonisamide (Owen et al., 1997), and platelet-activating factor antagonists (Frerichs et al., 1990) have demonstrated neuroprotective properties against hypoxic-ischaemic damage to the hippocampus. The neuroprotective effects of lamotrigine are discussed earlier (see section 1.7.1).

Hypothermia has been shown to have a neuroprotective effect in cerebral ischaemia, with up to 50 to 100% preservation of rodent hippocampi after lowering of the temperature by 2°C in experimental models (Barone et al., 1997). However, evidence of the efficacy and safety of therapeutic hypothermia in humans from clinical trials is needed.
CHAPTER II COMMON METHODOLOGY

This chapter describes the methodological approach that was applied to the study of all control subjects and patients in this study, and is referred to in subsequent chapters. Information is provided on inclusion and exclusion criteria, study design, collection of clinical data, acquisition sequences used for baseline and follow-up scans and the image processing steps involved in acquiring serial quantitative data. Volumetric techniques will be described in Chapter III.

2.1 SUBJECT RECRUITMENT AND STUDY DESIGN
The initial phase of this study, performed between June 1995 and May 1997, identified 153 patients with chronic active epilepsy, 90 patients with newly diagnosed seizures and 90 age-and sex-matched control subjects. Patients were prospectively recruited from a local population of 207,553, and case ascertainment established through the regular active surveillance of 21 local general practices within a 15-mile (24-km) radius of the National Society for Epilepsy (Everitt et al., 1997).

Chronic active epilepsy was defined as a minimum seizure history of four years with at least one seizure in the year prior to study recruitment. A new diagnosis of epilepsy was made in any person over the age of 14 with a new presentation of seizures. Any person suspected of previously undiagnosed epileptic seizures was referred to a fast-track clinic at the National Society for Epilepsy, and fully evaluated with clinical assessment and interictal EEG within two weeks of their clinical presentation. Identification of cases was optimised through an intensive manual and electronic audit of primary care patient records documenting either the new prescription of antiepileptic drugs or epilepsy-related diagnostic codings. For the 10% additional patients identified from the audit, the median latency between clinical presentation and their recruitment was 5 months (range 1-12 months). Any seizure occurring within seven days of a brain insult was defined as a provoked or an acute symptomatic seizure. Patients with acute symptomatic seizures or a history of febrile convulsions were included in the study. Further EEG assessment with sleep or ambulatory EEG study was performed when indicated. Three experienced epileptologists identified patients suffering from definite or probable seizures and classified them, utilising clinical and EEG information, according to epilepsy syndrome
and aetiology (ILAE, 1989). Seizures were classified as generalised in the absence of localising or lateralising electro-clinical information.

Control subjects were healthy adult volunteers free of active neurological or psychiatric disease who were recruited from the same community base. All subjects were contacted by letter and invited back for follow-up MRI brain scans 3.5 years after their initial scan. Control subjects were rescanned over the same period to control for age-related atrophy and temporal fluctuations in scanner performance.

Subjects with significant cerebrovascular disease at baseline (defined as greater than two high signal white matter lesions on either T2-weighted or FLAIR images), were excluded from the second phase of the study since cerebrovascular disease was considered an independent risk factor for cerebral atrophy.

The study was approved by the Joint Research Ethics Committee of the National Hospital of Neurology and Neurosurgery and the Institute of Neurology, and informed consent was obtained from each subject.

2.2 PATIENT FOLLOW-UP AND RETENTION RATES

Patient recall was optimised through an active monitoring system. Contact details for patients were regularly updated through six monthly visits to all participating general practices. Patients who had changed their family physicians were traced via a central UK health register (the Office for National Statistics) and those who had emigrated were flagged in case they subsequently returned and registered with a new medical practice. This tracing process has been successfully used to optimise follow-up rates in other large community-based longitudinal studies (Sander et al., 1990). The assistance of local general practitioners, practice managers and community epilepsy nurse specialists in tracing individuals was also enlisted.

The second phase of the study was performed between February 1999 and August 2001. One hundred and twenty-two patients with chronic active epilepsy (80% of the original cohort), 68 patients with newly diagnosed seizures (76%) and 90 control subjects were re-scanned after 3.5 years. Causes of attrition are provided in Table 7.
2.3 ACQUISITION OF CLINICAL DATA

At their follow-up assessment, subjects were questioned on head injuries, alcohol consumption, drug consumption, medication (in particular, steroid use), and any significant medical or psychiatric morbidity that might have occurred between the two scans that could affect cerebral structure. Cerebellar function was assessed on the basis of gait and upper limb coordination and dichotomously ranked as either normal or abnormal. Patients were asked to record their seizures prospectively using a seizure diary that provided an accurate estimation of seizure number. They were also specifically questioned on seizure type, seizure clustering, episodes of SE and use of AEDs. Clinical data were supplemented with information obtained from a thorough review of general practice and hospital records and the syndromic classification updated. A subsequent audit of 10% of all demographic and quantitative data fields entered into a relational database showed an error rate of 0.6%.

Table 7 Causes of attrition

<table>
<thead>
<tr>
<th>Causes</th>
<th>Newly diagnosed epilepsy</th>
<th>Chronic active epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>5 (5.6%)</td>
<td>6 (3.9%)</td>
</tr>
<tr>
<td>Infirmity</td>
<td>2 (2.2%)</td>
<td>3 (1.9%)</td>
</tr>
<tr>
<td>Untraceable</td>
<td>2 (2.2%)</td>
<td>5 (3.2%)</td>
</tr>
<tr>
<td>Emigration</td>
<td>2 (2.2%)</td>
<td>2 (1.3%)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>-</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Not tolerate scan</td>
<td>-</td>
<td>2 (1.3%)</td>
</tr>
<tr>
<td>Refusal</td>
<td>11 (12.2%) *</td>
<td>12 (7.8%)</td>
</tr>
</tbody>
</table>

* Ten of the 11 patients had isolated seizures with no further recurrences. Untraceable referred to patients who could not be traced by the Office for National Statistics since they were no longer registered with a general practice.

2.4 IMAGE ACQUISITION

2.4.1 Scan acquisition

All subjects underwent baseline and repeat standard MRI sequences on a 1.5T GE Signa Horizon Echospeed MR scanner (GE Medical Systems, Milwaukee WI, USA).
Identical scan acquisition sequences were used at baseline and follow-up. Scan acquisition time for each subject was 45 minutes. The acquisitions comprised:

- a fast inversion recovery prepared 3D spoiled gradient echo (IRP-SPGR), T1 weighted volume sequence TI/TR/TE, 450/15/4.2 (msec), flip angle 25 degrees; 124 1.5mm-thick coronal slices; matrix, 256 X 192 voxels, 24 X 18cm field of view; scan time, 7 minutes. This covered the whole brain with voxel sizes of 0.9375 X 0.9375 X 1.5mm.

- 5mm thick contiguous oblique coronal proton density and T2 weighted spin echo images orthogonal to the long axis of the hippocampi, TR/TE 2000/30 (proton density), 2000/120 (T2-weighted)

- 5mm thick contiguous oblique coronal FLAIR images, orthogonal to the long axis of the hippocampi, TR/TE/TI 11000/2600/144, 8 echo train length.

Scan pairs were visually compared for qualitative change on the T1, T2 weighted and FLAIR images (Wieshmann et al., 1996) by two consultant neuroradiologists (JS, BK) who were blinded to all clinical information.

### 2.4.2 Image processing

Prior to volumetry, a series of automatic processing steps were performed on the T1-weighted volume datasets (Figure 6). After an initial automatic brain segmentation of the baseline and repeat scan using a 2D version of our segmentation software Exbrain (Lemieux et al., 1999), non-uniformity correction (NUC) was performed, using the automatic method, N3 (Sled et al., 1998), a publicly available software (http://www.bic.mni.mcgill.ca/brainweb/). Automatic brain segmentation of the NUC baseline scan was then performed using the 3D version of Exbrain, resulting in an accurate delineation of the brain (Lemieux et al., 1999) and cerebrospinal fluid (CSF) (Lemieux, 1999). In the segmented scans all voxels outside the brain are set to zero intensity. The repeat scan was then co-registered and intensity matched to the segmented baseline scan using locally developed image analysis software MRreg (Lemieux et al., 1998), (Lemieux and Barker, 1998). In MRreg, a 9-parameter rigid body transformation (three rotation, three translation and three scaling), was used to register images with an accuracy of <0.06mm in each linear dimension and correct for variations in voxel dimensions. The matched repeat scan was then resampled using...
sinc-based interpolation with a kernel radius of 5 voxels. A final automatic segmentation of the brain and CSF in the matched repeat scan was then performed using *Exbrain*.

Figure 6 Flow diagram showing image processing steps involved in serial hippocampal and cerebellar volumetry [(Lemieux et al., 2000)].

NUC refers to non-uniformity correction.
CHAPTER 111 ANALYTICAL TOOLS

This chapter describes the quantitative approach to hippocampal and cerebellar volumetry used to detect subtle volumetric change over 3.5 years. Existing volumetric approaches were modified to incorporate co-registration and refined for the purposes of the study. Details of our approach to hippocampal T2 relaxometry, and the detection and quantification of subtle neocortical change using voxel- and region-based analyses are also provided.

3.1 HIPPOCAMPAL VOLUMETRY

Volume measurements of both baseline and follow-up scans were performed by the same trained observer (RSNL) on a Sun Ultra workstation using the IRP-SPGR sequence. Contiguous slices of the two datasets of each subject were displayed alongside each other. To eliminate bias, patient and control data were intermixed and the operator was blinded to the clinical status of the subject and whether the dataset displayed in the left-hand window was the baseline or the matched repeat scan. All volumetry was performed using the volumetry tool in MRreg (Lemieux et al., 2000). An in-plane magnification of X4 was used and the same standardized intensity window setting applied to both display windows.

Manual delineation of the entire hippocampus was performed using a mouse-driven cursor according to published anatomical guidelines (Watson et al., 1992). Measurements included the hippocampus proper, subiculum, dentate gyrus, fimbria, uncinate gyrus and intralimbic gyrus. The oblique coronal section displaying the full profile of the fornical crura lifting off the tail of the hippocampus marked the posterior boundary. In each case, the right hippocampus in the left window was measured first. The completed traces were displayed in the left window and exhibited for visual reference whilst measurement of the right hippocampus in the right window was performed (Figure 7). The procedure was then repeated for the left hippocampus. MRreg automatically calculated the hippocampal volume by summing the trace area of each slice and multiplying by the slice thickness. After unblinding, the difference in hippocampal volume (d HV) was calculated for each subject.

\[ d \ HV = HV_{repeat} - HV_{baseline} \]
3.2 HIPPOCAMPAL T2 RELAXOMETRY

Hippocampal T2 (HCT2) measurements reflect the glial / neurone ratio in the CA1 subregion (Van Paesschen et al., 1997c) and the glial cell count in the dentate gyrus (Briellmann et al., 2002b). When used in conjunction with hippocampal volume measurements, it acts as a useful marker of the severity of HS (Van Paesschen et al., 1997c).

HCT2 maps were acquired from the conventional spin echo sequence obtained from two interleaved acquisitions covering the entire brain (Duncan et al., 1996). Both baseline and follow-up HCT2 measurements were performed by the same observer (PAB) using DisplImage image analysis software (Plummer, 1992). Measurements were performed on contiguous tilted coronal slices perpendicular to the longitudinal axis of the hippocampi to minimize partial volume effects. The first slice was defined as the one anterior to the slice in which the fornix was seen in its greatest length. On average, the hippocampus was visible on 4-6 slices. The largest possible elliptical region of interest was placed in each hippocampal slice, avoiding partial volume effects from the CSF.
Chapter III

3.3 CEREBELLAR VOLUMETRY

Cerebellar volume measurements were semi-automated and performed on the matched segmented coronal images. The observer was blinded to the chronological order of the scan pair and the subject status as for HV measurements. An in-plane magnification of X2 was used and identical intensity pre-sets applied to both display windows. Using the seed and region-growing tool in MRreg, the threshold level was set to unity as all non-brain voxels are set to zero by the segmentation. Single or multiple seeds were deposited in the cerebellum by mouse clicking, and automatic 2D region growing was used to connect the seed point to all neighbouring voxels with an intensity value equal to or above the threshold value. "Spillage" of regions of similar intensity across anatomical boundaries e.g. between the cerebellum and cerebrum, and cerebellum and brainstem were manually edited. The seeds were automatically carried to the next slice. Measurements were performed on alternate slices (Lemieux et al., 2000).

The rostral border of the cerebellum was defined as the first section in which cerebellar grey matter was visible and distinguishable from the cerebellar peduncles. Only grey matter was included, with exclusion of the fourth ventricle, venous sinuses and cerebellar peduncles in slices rostral to an arbitrary point that was defined as the most rostral section in which both superior and inferior vermis were visible. Thus, hemispheric GM, WM and part of the cerebellar peduncles caudal to this arbitrary point were included. Measurements were performed on alternate slices, progressing in a rostro-caudal fashion with sagittal reconstructions displayed simultaneously to clarify anatomical orientation. The completed traces of the cerebellum were displayed on the left window whilst measurement of the matched segmented scan were performed in the active trace in the right window (Figure 8). To account for slice skipping, the cerebellar volume was calculated by doubling the product of the slice area and slice thickness (Hagemann et al., 2002).
3.4 NEOCORTICAL VOLUMETRY

Automatic segmentation of the baseline and matched repeat scan was performed using Exbrain, a fully automated three-dimensional segmentation algorithm (Lemieux, 2001). Separation of the brain from the rest of the head was based on thresholding at an optimal level, followed by erosion and connected component analysis to identify the largest component. Since GM/WM segmentation relies on thresholding at the optimal level as determined using the minimum error segmentation for a bi-modal distribution, parts of the basal ganglia which have a voxel intensity above the GM/WM threshold will be classified as WM. Baseline and repeat measurements were obtained for TBV, ICV, GMV and WMV. The TBV was the sum of GM and WM, whilst the ICV was the cumulative sum of the TBV and CSF. All baseline and repeat volumes were normalized to account for variations in physiognomy, using a linear regression equation that corrected for baseline ICV (Moran et al., 2001). The test-retest reliability of TBV, GMV and WMV derived using Exbrain for coregistered scans is 1%, 3.3% and 2.2% respectively (Lemieux, 2001).
3.5 DIFFERENCE IMAGE ANALYSIS

3.5.1 Generation of the structured difference image

A difference image for each subject was generated by subtraction of the baseline image from the matched repeat image. Pixels that had changed in signal intensity from GM to CSF or WM, or WM to CSF were identified. \textit{MRreg} estimated the noise level across the whole dataset and created a structured difference image (SDI) by identifying deviations with intensity differences at least three times the noise level and cluster thresholds greater than seven voxels (Lemieux \textit{et al.}, 1998). The significance of changed voxels in the SDI was determined by thresholding the SDI against a spatially normalized structured noise map.

3.5.2 Structured Noise Map (SNM)

The SNM is an anatomical map reflecting the artifacts seen in pairs of MRI scans acquired in individual subjects e.g. pulsation artifacts within the temporal lobe and differences in magnetic susceptibility at the base of the temporal and frontal lobes. The SNM for this study was derived from the SDIs of 40 healthy adults (mean age, 37.4 years, range 20-60 years; mean ISI, 7 months) that were spatially normalised to a stereotaxic T1-weighted MRI template (the Montreal Neurological Institute / International Consortium for Brain Mapping 152 standard), using the Statistical Parametric Mapping package (SPM 99, Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, UK). There was no overlap between these 40 subjects and the 90 control subjects in our study.

The initial step in the generation of the SNM was re-orientation of the 40 baseline T1-weighted images along the anterior-posterior commissure line. Each baseline scan was matched to the SPM T1-template and a normalisation matrix determined using the default functions in the SPM99 package (12-parameter affine transformation followed by nonlinear steps utilising basis functions). The normalisation matrix was subsequently applied to the smoothed SDI using nearest neighbour interpolation. The normalised SDIs were subsequently merged to create a SNM (Figure 9).
Figure 9  Structured Noise Map  Coronal and sagittal views of the SNM. The slices have been selected for their concentration of structural differences, which are maximal in the temporal lobes, inferior frontal lobe, occipital regions and around the ventricles. The concentration of structured noise in the temporal lobes reflects susceptibility and pulsation artifact (associated with the middle cerebral artery).

3.5.3 Automatic thresholding
Normalized SDIs (nSDI) were generated for each of the study subjects and filtered against the SNM to remove changes due to physical artifacts. Persistent differences were defined as biological. A threshold of 1:40 was selected i.e. if a voxel in the spatially normalized scans changed intensity in any of the 40 pairs of control subjects used to construct the SNM, changes in that equivalent voxel would be disregarded in the study population. Voxels in the resulting genuine change map (see Figure 10) were displayed as no genuine change (grey), decrease in signal (red), and increase in signal (green).
**Figure 10** Formation of the genuine change map. The upper path shows the sequence of post-processing steps involved in the generation of the difference image whilst the lower path shows the formation of the structured noise map. SNM, structured noise map; SPM, statistical parametric mapping.
Chapter III

Thus a change of GM to CSF, WM to CSF or GM, would appear as red; and GM or CSF to WM as green.

3.5.4 Genuine change map
The resulting maps of changed voxels for all patients and controls were visually assessed by two raters (JSD, RSNL), who were blinded to all clinical information. The raters scrolled through a coronal display for each subject and reached a consensus on the pattern of signal change. The corresponding baseline and matched repeat images were displayed simultaneously to allow cross-referencing of changed voxels back to the source images. Assessments were rated according to the nature and distribution of change. Increases or decreases in signal intensity were rated semiquantitatively as mild, moderate or marked, based on the concentration of changed voxels. “Mild” was used to describe change in individuals in which changed voxels were sparsely distributed and non-contiguous, “moderate” referred to contiguous voxels, and “severe” to clusters of contiguous voxels that were greater than 1 voxel thick.

The pattern of signal change was either focal, generalized, a combination of focal and generalized or artifactual. “Generalized” changes referred to patterns in which changed voxels were symmetrical, multilobar and distributed at ventricular and cortical surfaces. “Focal” changes referred to voxel changes that were localized to discrete lobar regions. Reproducibility confirmed by the blinded re-assessment of 20 datasets was 100%.

3.5.5 Quantification of neocortical change
The extent of focal neocortical changes identified visually was quantified using a probabilistic anatomical template (Hammers et al., 2002a), produced by the spatial transformation of 20 atlases of normal subjects into standard stereotaxic space (MNI/ICBM 152 template in SPM 99). The template comprised 49 manually delineated 3D volumes of interest grouped into eight regions for our purpose (right and left temporal, frontal, occipital and parietal lobes). The template was spatially transformed to fit the native T1-weighted volume MRI space of each individual (Hammers et al., 2002b)(Figure 11).

Quantitative analysis was restricted to areas of signal change highlighted by visual assessment of the maps of changed voxels. Application of the atlas to segmented
baseline and repeat T1-weighted images resulted in baseline and repeat GMV and WMV within defined anatomical regions. The change in lobar GMV and WMV was calculated by subtracting the baseline volume from the repeat volume. Generalized changes in the neocortex detected on visual assessment were compared with changes in TBV, and total GM and WM that were determined using the same segmentation algorithm (Liu et al., 2001). Visually identified cerebellar atrophy was quantified using semiautomated measures of baseline and repeat cerebellar volumes.
Chapter III

Figure 11  Quantification of focal neocortical atrophy. Flow chart showing the image processing steps involved in the normalization of the regional brain atlas to native MRI space. Lobar grey and white matter volumes were obtained by application of the atlas to segmented T1-weighted images.
CHAPTER IV REPRODUCIBILITY OF SERIAL HIPPOCAMPAL AND CEREBELLAR VOLUME MEASUREMENTS

This chapter reports on a pilot study that confirms the validity of our side-by-side approach to serial hippocampal and cerebellar volume measurements described in the previous chapter.

4.1 OBJECTIVE
The aim of this initial study was to establish a methodology for serial hippocampal and cerebellar volumetric measurements that could be applied to our study population. Reproducibility measures were determined through the blinded volumetric assessment of 20 control subjects and five patients with focal epilepsy measured on two separate occasions.

4.2 INTRODUCTION
Longitudinal imaging studies offer a unique opportunity to study the morphological changes associated with epilepsy. Currently, hippocampal volume measurements are largely performed by manual delineation in individual slices from volumetric MRI data since automation of hippocampal volume measurements is difficult (refer to section 1.4.2). Similarly, although a large proportion of the cerebellum’s boundary, i.e. cortex / CSF, is clearly and easily defined, cerebellar volumetry requires the operator to make subjective decisions to define the boundary with the brainstem (Hagemann et al., 2002).

The sensitivity of the manual technique to change is limited by its subjectivity. In serial imaging where the interest lies mainly in the difference between repeated measurements, the variability of repeated measurements may be larger than that of single-scan repeated measurements. Co-registration of the serial scans should lead to an improvement in the variability of repeated measurements by correcting for differences in scan position, orientation and intensity prior to the analysis.

The aim of this study was to determine the reproducibility of hippocampal and cerebellar volume measurements in serial MRI data after co-registration and intensity matching. The reproducibility of hippocampal volume measurements following co-registration has been previously documented (Jack et al., 1998), however certain
methodological details (e.g., whether the operator could visualise the two datasets side-by-side) were not clearly presented. Furthermore, since only controls were used when determining the reproducibility of their technique, observers were not blinded to the nature of the subject.

In this work, the reproducibility of the volumetric measurements in normal subjects was assessed in a group of subjects containing a (blinded) mixture of normal subjects and patients with chronic focal epilepsy. The operator was also blinded to the chronological order of the scans during the measurements.

4.3 METHODS
4.3.1 Subjects
Twenty normal subjects (9 males; mean age at baseline: 31.7 years) were scanned twice with a mean inter-scan interval (ISI) of 9.1 months using a 1.5T GE Signa Horizon Echospeed (GE Medical Systems, Milwaukee WI, USA) MRI scanner. The sequence used was a fast, inversion recovery-prepared, spoiled gradient-recalled (IRP-SPGR) T1-weighted volume sequence (TI/TR/TE): 450/17.4/4.2msec, flip angle: 20°, matrix size: 256×192, 24×18cm FOV, 124, 1.5mm thick coronal slices). Five patients (2 males; mean age at baseline, 29.4 years) with chronic focal epilepsy were scanned twice using the same sequence, at a mean interval of 22.9 months.

The scans were processed as described in section 2.4.2. The identity of the resulting baseline and matched repeat scans was hidden and the images displayed side-by-side to the operator (RSNL) in a blinded fashion. The operator was naïve to whether the images were from a normal subject or patient, or whether the scan in the left-hand window was the baseline or repeat image (section 3.1).

4.3.2 Volume measurements and repeatability measures
Hippocampal and cerebellar volume measurements were performed according to our protocol (see section 3.1, 3.3).

The difference between repeated measurements, \( d \), was defined as \( d = V_{\text{repeat}} - V_{\text{base}} \)
Assuming that the mean of $d$ was not significantly different from zero (i.e. small relative to the standard error), we used the coefficient of repeatability (CR), which was defined as 1.96 times the standard deviation of the differences, $\sigma$ (Bland and Altman, 1986).

Another measure, the coefficient of variation (CV), is sometimes used and is defined as the standard deviation of the repeated measurements (pair-wise) expressed as a percentage of the mean measurement (Jack et al., 1998). However, the CV is most useful when the variability of the measurements is expected to be related to the mean, which is not the case for our measurements (Moran et al., 1999). However, the value of CV has been calculated to allow comparisons with previously published results using that measure.

4.4 RESULTS

Regarding the blinding of the images, the operator noted that in one case it was obvious that the scans were from a patient (the repeat scan showed signs of surgical resection) and therefore this data was removed from the study. In all other cases, the operator was unable to determine the nature of the subject (control or patient) or the chronological order of the scans.

4.4.1 Hippocampal Volumetry

In the patients, the mean hippocampal volumes in the baseline scans were 2.500cm$^3$ and 2.542cm$^3$ for the RH and LH respectively. The mean volume change, $d$, was $-0.080$ cm$^3$ and $-0.116$ cm$^3$ respectively.

The mean hippocampal baseline volumes in the control group were 2.497cm$^3$ and 2.501 cm$^3$ for the RH and LH respectively. The mean value of $d$ was 0.003cm$^3$, -0.006cm$^3$ and -0.003cm$^3$ for the RH, LH and combined hippocampi (CH), respectively. The value of $\sigma$ was 0.032cm$^3$, 0.039cm$^3$ and 0.058cm$^3$ for the RH, LH and CH, respectively, corresponding to CR values of 0.064cm$^3$, 0.078cm$^3$ and 1.16cm$^3$. Regarding the CV, the median value was 0.38%, 0.57% and 0.36% (range: 0.01%-1.30%) for the RH, LH and CH, respectively.
4.4.2 Cerebellar volumetry

For the patients, the mean cerebellar baseline volume was $112.0\,cm^3$ and the mean volume change was $+0.48\,cm^3$. The mean baseline volume in the controls was $127.7\,cm^3$ and the mean value of $d$ was $-1.21\,cm^3$. The value of $\sigma$ was $1.90\,cm^3$ corresponding to a CR value of $3.8\,cm^3$. Regarding the CV, the median value was $0.66\%$ (range: $0.01\%-1.54\%$).

4.5 DISCUSSION

4.5.1 Methodological issues

Our approach to serial measurements is based on the use of co-registration and side-by-side display of the matched images. The display of the boundaries drawn in one of the scans while measuring in the other is designed to help the user in making the subjective drawing decisions more consistently. Whilst this approach could introduce bias by reducing the sensitivity to genuine differences through imitation, we validated our approach by requiring the operator be blinded to the clinical status of the subject and the chronological order of the scans. This was achieved by randomly mixing normal controls and patients of a similar age range and randomly re-ordering the scans chronologically for each subject. In the small group of patients chosen, there was a suspicion that some hippocampal and cerebellar damage may have taken place in the inter-scan interval. The measurements revealed significantly larger differences in the patients than in the controls; in particular one patient had significant volume losses in both hippocampi ($0.277\,cm^3$ in the RH and $0.426\,cm^3$ for the LH). This indicated that the operator was performing the task correctly. We have also found significant hippocampal and cerebellar volume increases in a subject with previous excessive alcohol intake who abstained from alcohol after the baseline scan, using the methodology presented (Liu et al., 2000).

4.5.2 Coefficient of repeatability

The usefulness of CR as a measure of repeatability in our study relies on the assertion that the mean difference in the repeated measures in the control group was small. The choice of an inter-scan interval of 7 months was chosen as a compromise between the need to minimise any biological effect, i.e. due to natural volume loss; and the need to test the ability of the scan matching process to compensate for variability in scanner
performance over a prolonged period. Fluctuations in the performance of the scanner's gradients can lead to variations of the order of 1% in the voxel dimensions (Lemieux and Barker, 1998). Although the linear scaling within registration process should compensate for this, it is likely that the registration error depends on the amount of scaling correction.

Our results indicate that individual hippocampal volume changes greater than $0.078 \text{cm}^3$ can be detected and measured reliably in individual subjects using our method; for CH, the threshold of detectability is $0.116 \text{cm}^3$; these are equivalent to 3.1% and 2.3% of the mean baseline individual and combined volumes, respectively. For the cerebellar volume, changes greater than $3.8 \text{cm}^3$ can be detected reliably, which is equivalent to 3.0% of the mean baseline normal volume.

### 4.5.3 Coefficient of variation

Regarding the CV as a measure of repeatability, we found that our hippocampal results were comparable with those obtained by Jack et al. (median CV for CH was 0.28%, range 0.02 to 0.70% in their study) The slightly higher median CV for CH may be explained by a higher degree of scanner variability (signal to noise, geometric distortion, etc) due to the much longer inter-scan interval in our study and our more stringent approach to blinding. We also note that the CV, as a measure or repeatability, appears superficially to give much more favourable results than the CR.

### 4.6 CONCLUSIONS

The study demonstrates that our approach to serial MRI volumetric measurements is valid and can be applied to the detection of subtle hippocampal and cerebellar volume changes in epilepsy. Based on the results of our control group, we reliably detected volume changes greater than $0.078 \text{cm}^3$ (3.1%) for the hippocampus and $3.8 \text{cm}^3$ (3.0%) for the cerebellum. Our study confirms that vigorous blinding and side-by-side display increases the reproducibility of serial measurements yet maintains sensitivity to change.
CHAPTER V  A LONGITUDINAL STUDY OF NORMAL AGEING

Chapters II to IV focus on the methodological approach used throughout this study. This chapter uses the afore-mentioned approach to study the effect of 3.5 years on our 90 control subjects, and provides an insight into the effects of ageing in our control population.

5.1 OBJECTIVE
To quantify and characterize longitudinal changes in the hippocampus, cerebellum and neocortex in younger and middle-age individuals by studying change in brain morphometries in our control group over 3.5 years. We thus demonstrate the importance of controlling for age effects when studying pathological brain changes over a wide age range.

5.2 INTRODUCTION
Serial MRI of the brain and image registration has provided insight into the evolution of structural abnormalities associated with a range of neurodegenerative conditions (see section 1.5.2). However, the application of serial imaging to the study of pathological change demands a greater understanding of the morphometric changes associated with normal brain development and physiological ageing.

There is considerable literature on brain volume changes associated with age, however the majority of these studies have been cross-sectional. By comparing the brains of individuals at discrete time points, these studies perform a useful function in generating hypotheses about brain structure and age. However, they do not distinguish between secular changes and changes directly attributed to the ageing process. In addition, subtle age-related changes may be obscured by wide inter-subject variability.

Cross-sectional studies fall into two main categories: voxel-based morphometry and region-based methods. The application of VBM to the study of 465 normal individuals, aged between 18 and 79, revealed a global age-related decline in grey matter and regional grey matter volume reduction in the superior parietal gyri, insula, and central
and cingulate sulci (Good et al., 2001b). The study did not find significant age effects in white matter or limbic structures.

Region-based studies have produced similar findings, and describe a curvilinear decline in the relative proportion of cortical grey matter volume after a peak around the age of four (Pfefferbaum et al., 1994). Differential rates of decline in GM are described, with greater losses in the frontal cortex than in the parietal or insular cortices (Jernigan et al., 2001).

Cross-sectional studies on cerebellar volume and ageing have produced conflicting results. Although a recent study reported no change in cerebellar GMV with age (Jernigan et al., 2001), Luft and colleagues (Luft et al., 1999) described an exponential model which predicted that cerebellar volume remained stable until 50 before declining, with the most striking loss being in the vermis. In contrast to the observations of Good and colleagues (Good et al., 2001b), several papers have shown accelerated age-related losses in the hippocampus (Jernigan et al., 2001). In the largest cross-sectional study addressing change in hippocampal volume with age, Mu and colleagues (Mu et al., 1999) found significantly smaller hippocampal and amygdala volumes amongst individuals over the age of 60. A quadratic relationship with age has been described for white matter volumes, with volumes typically peaking at the age of 20 (Pfefferbaum et al., 1994), although there are reports that frontal white matter volume increases until 44 and temporal white matter continues to increase until the age of 47 (Bartzokis et al., 2001). The subsequent decline in WM has been at a reportedly greater rate than the loss in GMV (Guttmann et al., 1998).

Several longitudinal neuroimaging studies have been applied to the study of ageing, although the majority have involved small number of individuals used as age-matched controls in the study of comparative rates of brain atrophy in Alzheimer's disease (Fox et al., 1996a), (Jack et al., 1998), mild cognitive impairment (Jack et al., 2000) and pre-clinical dementia (Fox et al., 1999b). Other longitudinal studies have focussed on older age groups. In a study of 46 healthy subjects over the age of 65, Mueller and colleagues (Mueller et al., 1998) reported no significant difference in the annual rates of change of cortical regions over a 3 to 6 year-interval, suggesting that the rate of volume loss remained static in the elderly if individuals with pre-clinical dementia were excluded. In
this study, volumetric measures were obtained using semi-automated segmentation of multiecho images. A study of 116 controls over the age of 59, using semi-automated brain segmentation, non-linear normalization and statistical analyses on automatically determined Talairach “boxels”, similarly found no significant one-year changes in global and regional brain volumes. Only ventricular volumes increased significantly by an average of 1.53cm$^3$ (Resnick et al., 2000).

To reduce secular influences inherent in cross-sectional studies, we aimed to characterize and quantify the structural cerebral changes associated with normal ageing using a longitudinal MRI study. We included a wide age spectrum, as significant structural changes may occur prior to the more advanced ages previously investigated in longitudinal studies (Mueller et al., 1998). We hypothesized that normal ageing has a differential effect on the rates of regional brain atrophy and that significant loss of brain matter occurs before the age of 60.

5.3 METHODS
5.3.1 Subjects
The study group comprised 90 healthy adult volunteers who were recruited from the community and were participating in our longitudinal study of cerebral damage (see section 2.1). Subjects were excluded if they had any active neurological or psychiatric disorder. Other exclusion criteria included a history of substance dependence, significant CNS impairment, and major head injury (skull fracture or loss of consciousness more than 30 minutes). Exclusion criteria were minimized to represent a continuum of “normal” ageing; consequently subjects with co-morbid illnesses such as well-controlled hypertension and stable treated coronary artery disease were not excluded. Baseline MRI brain scans performed on all subjects between June 1995 and May 1997, were examined by two experienced neuroradiologists, and individuals with significant cerebral structural abnormalities were excluded from the study.

At the three and a half-year follow-up visit, individuals were rescanned and questioned about head injuries, significant medical or psychiatric illnesses, medication, and alcohol and drug intake during the follow-up period.
5.3.2 MRI acquisition and image processing

MRI acquisition and image processing (including hippocampal and cerebellar volumetry) were performed according to our common protocol (refer to sections 2.4, 3.1, 3.3, 3.4). Difference image analysis was performed according to section 3.5.

5.3.3 Statistical methods

Statistical analysis was performed using SPSS for Windows, release 9 (SPSS, Inc., Chicago, IL).

5.3.3.1 Cross-sectional analysis

The associations between age and ICV-corrected baseline MRI volumes were examined using hierarchical polynomial regression analyses. Linear and curvilinear age trends were assessed, and the contribution of higher order age trends evaluated by comparing $R^2$ values with corresponding values derived using linear functions. Non-linear regression plots were used to determine age at peak volumes of WM and GM/WM ratios (Giedd et al., 1999). Significance levels for F statistics were set at $P < 0.05$. Handedness was entered into a stepwise regression model with age to predict its contribution to each MR variable. All handedness contributions to the model were non-significant and therefore excluded from the analysis.

5.3.3.2 Longitudinal analysis

For the group analysis, subjects were subdivided into three epochs according to age at baseline scan: persons under 35, persons between 35 and 54, and persons greater than 54 years of age. A comparison of group demographics was performed using one-way analysis of variance (ANOVA).

The percentage volume change (% dVol) in each MRI variable over three and a half years was calculated for each subject:

$$\% \text{dVol} = \frac{\text{Corrected repeat volume (cm}^3\text{)} - \text{corrected baseline volume (cm}^3\text{)}}{\text{Corrected baseline volume (cm}^3\text{)}} \times 100$$
To assess whether change in volumes over time was significantly different from zero, corrected baseline and repeat volumes in each age band were compared using paired t-tests. All tests were two-tailed, and the $\alpha$ level of significance was 0.05. The Levene test was used to test for homogeneity of variance of the normalized volumes across the three age bands. Gender effects on the volume changes in each age band were explored using independent t-tests. The mean volumetric changes in the three age bands were compared using one-way ANOVA.

To identify individuals with significant volume changes over the 3.5 years, age-band specific normative ranges based on the study group were calculated for each MR variable. Since GMV and WMV were significantly correlated in our control subjects, a Bonferroni correction was used to correct for three independent parameters (hippocampus, cerebellum, and neocortex) where 2.4SD corresponds to an $\alpha$ level of 0.05/3 (Liu et al., 2001).

5.4 RESULTS
5.4.1 Subject characteristics
The demographic characteristics of the 90 subjects in each age band are shown in Table 8. Apart from age, sample characteristics were statistically comparable. Visual comparison of the scans revealed one subject with new small white matter lesions in the left frontal lobe. No other visual changes were reported.

5.4.2 Cross-sectional analysis of age on MRI volumes
The Pearson correlation coefficients for the relationships between age and ICV-corrected baseline MRI volumes are tabulated in Table 9. Only WMV and GMV/WMV ratio showed an improved fit using a quadratic function compared with a linear function. Ages at peak volumes of WM were 38 years for the males and 34 years for the females. Troughs of GMV/WMV ratio were seen at approximately 44 and 39 years, respectively. Increasing age was significantly associated with a reduction in all brain volumes except combined hippocampal volume. There was no significant correlation between cerebellar volume and age in females, and WMV or GMV/WMV ratio and age in males. The strongest association was observed between age and TBV, with age accounting for 41% of the variance in TBV.
Table 8  Group demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>&lt;35 years</th>
<th>35-54 years</th>
<th>&gt;54 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>44</td>
<td>37</td>
<td>9</td>
</tr>
<tr>
<td>Age at baseline scan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (SD), y</td>
<td>24.5 (6.6)</td>
<td>44.5 (5.7)</td>
<td>67.9 (6.4)</td>
</tr>
<tr>
<td>Age range, y</td>
<td>14 - 34</td>
<td>35-53</td>
<td>57 - 77</td>
</tr>
<tr>
<td>Gender ratio (Males:Female)</td>
<td>26:18</td>
<td>17:20</td>
<td>6:3</td>
</tr>
<tr>
<td>Interscan interval</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD), months</td>
<td>42.8 (1.5)</td>
<td>42.3 (1.0)</td>
<td>42.4 (1.1)</td>
</tr>
<tr>
<td>Range, months</td>
<td>40 - 48</td>
<td>41 - 46</td>
<td>41 - 45</td>
</tr>
<tr>
<td>Number of R handers : non R handers</td>
<td>34 : 10</td>
<td>33 : 4</td>
<td>7 : 2</td>
</tr>
<tr>
<td>Units of alcohol consumed per week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>8 (0-50)</td>
<td>8 (0-72)</td>
<td>5 (0-36)</td>
</tr>
<tr>
<td>Medical disorder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>35</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>Depression</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Treated hypertension</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>History of malignancy</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 9  Correlations between age and baseline MRI volumes corrected for ICV

<table>
<thead>
<tr>
<th>ROI</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV</td>
<td>0.02</td>
<td>-0.27</td>
<td>-0.10</td>
</tr>
<tr>
<td>CBV</td>
<td>-0.51**</td>
<td>-0.16</td>
<td>-0.37**</td>
</tr>
<tr>
<td>TBV</td>
<td>-0.68**</td>
<td>-0.62**</td>
<td>-0.64**</td>
</tr>
<tr>
<td>ICV</td>
<td>-0.34*</td>
<td>-0.38*</td>
<td>-0.30**</td>
</tr>
<tr>
<td>GMV</td>
<td>-0.37**</td>
<td>-0.33*</td>
<td>-0.36**</td>
</tr>
<tr>
<td>WMV+</td>
<td>0.24</td>
<td>0.43*</td>
<td>0.32*</td>
</tr>
<tr>
<td>GMV/WMV+</td>
<td>0.22</td>
<td>0.40*</td>
<td>0.28*</td>
</tr>
</tbody>
</table>

HV = combined hippocampal volume; CBV = cerebellar volume; TBV = total brain volume; ICV = intracranial volume; GMV = grey matter volume; WMV = white matter volume.

Figures expressed in Pearson Correlation Coefficients. + Quadratic coefficients were used for WMV and GMV/WMV; all other figures are linear coefficients.

*P < 0.05; **P < 0.01; two tailed.
5.4.3 Longitudinal analysis of age-related volume changes

5.4.3.1 Group analysis

The baseline and follow-up data for the whole cohort are shown in Figure 12. Although males had significantly larger baseline ICVs compared with females (P<0.0001), no gender effects were observed in the mean rates of change in any of the MRI parameters.

5.4.3.1.1 Combined hippocampal volume.

A mean HV change of -0.39% and -2.3% was observed in individuals under 35, and over 55 years respectively, with the former being significantly different from zero at the p<0.05 level. A significant difference in the rate of change of combined HV was observed between the three age bands (p = 0.001).

5.4.3.1.2 Total cerebellar volume.

There were decreases in cerebellar volumes in the two older age groups that reached significance in the 35-54 year age group.

5.4.3.1.3 Neocortical volume.

A significant loss in TBV was observed in the age bands 35-54 and >54 years, with mean percentage brain volume losses of 0.6% and 1.35% respectively. A 1.5% and 2.0% reduction in WMV was observed in the 35-54 years and >54 year groups respectively, being greatest in the oldest groups (p=0.007). Rate of change in GMV and GMV/WMV ratio remained relatively stable with age, with none of the changes being statistically different from zero. The increase in ICV observed in the <35 group was statistically different from zero, but the changes in ICV were not significantly different between between the three groups.

5.4.3.2 Individual analysis

Limits of agreement for quantitative brain measures over 3.5 years in the three age epochs are shown in Table 10. Individual analysis revealed five (5.6%) subjects with volume changes in at least one MR variable that fell outside the normative range. The significant changes comprised: one subject with a significant reduction in HV and TBV, one with an increase in TBV, two with a reduction in WMV (one of whom had a
Figure 12 Individual pairs of baseline and repeat (a) intracranial volume, and volumes corrected for ICV, are displayed for (b) combined hippocampal volume, (c) total cerebellar volume, (d) total brain volume, (e) grey matter volume, and (f) white matter volume. Individual volume changes and regression lines for males are shown in blue and females in red. The reduced corrected baseline TBV and increased rate of volume loss with age is clearly seen in figure (d). Apart from one subject with a significant increase in TBV and another with a significant increase in WMV, all observed volume increases were non-significant and within the coefficient of reliability. Rate measurement errors were similar between different structures.
Chapter V

corresponding reduction in GMV) and one with an increase in WMV. These covered all age bands, and no causal factors could be identified.

Table 10  Normative ranges for quantitative brain measures

<table>
<thead>
<tr>
<th>MRI parameter</th>
<th>Limits of agreement</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Lower range</strong></td>
<td><strong>Upper range</strong></td>
</tr>
<tr>
<td>Hippocampal volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>$\geq -0.085$</td>
<td>$\leq +0.065$</td>
</tr>
<tr>
<td>35-54</td>
<td>$\geq -0.097$</td>
<td>$\leq +0.089$</td>
</tr>
<tr>
<td>&gt;54</td>
<td>$\geq -0.242$</td>
<td>$\leq +0.126$</td>
</tr>
<tr>
<td>Cerebellar volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>$\geq -5.91$</td>
<td>$\leq +6.24$</td>
</tr>
<tr>
<td>35-54</td>
<td>$\geq -7.26$</td>
<td>$\leq +4.87$</td>
</tr>
<tr>
<td>&gt;54</td>
<td>$\geq -8.10$</td>
<td>$\leq +5.93$</td>
</tr>
<tr>
<td>Total brain volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>$\geq -48.70$</td>
<td>$\leq +43.85$</td>
</tr>
<tr>
<td>35-54</td>
<td>$\geq -42.93$</td>
<td>$\leq +27.33$</td>
</tr>
<tr>
<td>&gt;54</td>
<td>$\geq -61.11$</td>
<td>$\leq +29.39$</td>
</tr>
<tr>
<td>Cerebral grey matter volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>$\geq -59.12$</td>
<td>$\leq +53.14$</td>
</tr>
<tr>
<td>35-54</td>
<td>$\geq -54.56$</td>
<td>$\leq +53.68$</td>
</tr>
<tr>
<td>&gt;54</td>
<td>$\geq -57.32$</td>
<td>$\leq +43.62$</td>
</tr>
<tr>
<td>Cerebral white matter volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>$\geq -27.00$</td>
<td>$\leq +29.18$</td>
</tr>
<tr>
<td>35-54</td>
<td>$\geq -43.15$</td>
<td>$\leq +28.35$</td>
</tr>
<tr>
<td>&gt;54</td>
<td>$\geq -30.13$</td>
<td>$\leq +12.09$</td>
</tr>
<tr>
<td>Hippocampal T2 relaxometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>$\geq -2.49$</td>
<td>$\leq +1.76$</td>
</tr>
<tr>
<td>35-54</td>
<td>$\geq -2.15$</td>
<td>$\leq +1.70$</td>
</tr>
<tr>
<td>&gt;54</td>
<td>$\geq -1.69$</td>
<td>$\leq +2.01$</td>
</tr>
</tbody>
</table>

Limits of agreement = ±2.4SD of mean difference of control subjects.
All volumes expressed in cm$^3$, T2 relaxometry expressed in msec.
Epoch sizes: 44 subjects <35 years, 37 subjects between 35 and 54 years, and 9 subjects >54 years.
5.4.4 **Morphological changes**

Figure 13 illustrates the distribution of age-related changes associated with the three age bands. Each image represents the summation of all normalized structured difference images in that age band. Each summed difference image was filtered against the SNM. The intensities have been adjusted for variations in group size and reflect the proportion of individuals in a cohort showing change at a given voxel. Figures (a) and (b) are comparable, and comprise differences corresponding to susceptibility artefact at the base of the temporal lobes, and mild changes around the ventricles and base of the pons. Figure (b) shows increased cortical changes compared to figure (a). In contrast, an increase in ventricular cerebrospinal fluid is observed in figure (c), along with more dramatic changes around the ventricles which have caused an upward shift in the corpus callosum. Diffuse cortical changes are also apparent in figure (c).

![Figure 13](image.jpg)

**Figure 13** Distribution of age-related changes in each age band: (a) individuals under 35 years, (b) individuals between 35 and 54 years, and (c) individuals over 54 years of age. The absence of visible hippocampal changes may reflect structural heterogeneity, as well as limitations in the precision of the normalization process.

5.5 **DISCUSSION**

Our cross-sectional findings support previous findings of age differences in brain structure. However, we have expanded on these findings and elucidated them with the study of longitudinal brain changes.
5.5.1 Cross-sectional findings

In our cross-sectional analysis, we found a significant linear association between advancing age and reduction in ICV, TBV, GMV and CBV. It is implausible that ICV should change significantly over time in adults; therefore our observations of smaller ICV in the older age group are likely to reflect secular changes due to constitutional and environmental trends over the past century (Miller and Corsellis, 1977). The age-related reduction in other brain volumes may reflect the development of atrophy since baseline and follow-up volumes were corrected for ICV and therefore less vulnerable to cohort effects. These findings were concordant with previous cross-sectional studies of linear age-related changes (Raz et al., 1997), (Mueller et al., 1998), (Luft et al., 1999), (Good et al., 2001b). The most substantial decline in GMV has been localized to the prefrontal cortex (Raz et al., 1997). Although our finding of a relatively preserved combined hippocampal volume with age was in agreement with previous studies (Raz et al., 1997), (Good et al., 2001b), other researchers have frequently produced conflicting results (Landfield, 1988), (Schuff et al., 1999), possibly reflecting measurement error and wide variability in hippocampal volume across individuals. Although maximal WM was comparably delayed at 37 years, our observation that the regression line was significantly improved by a quadratic function is consistent with reports of cortical white matter volumes steadily increasing until the age of 20 before reaching a plateau (Pfefferbaum et al., 1994), (Good et al., 2001b). A quadratic regression line was also the optimal fit for GMV/WMV ratio, supporting earlier observations that there is preferential loss of GM between the ages of 20 and 50, with a subsequent predominance of WMV loss (Miller et al., 1980).

5.5.2 Longitudinal findings

Although there were significant age-related associations with baseline ICV, cerebellar (CBV), TBV, GMV and GMV/WMV ratio, there were no significant differences in the rate of change of these variables among the groups. The cross-sectional findings are therefore likely to reflect either secular trends or the end result of uniform rates of atrophy. In contrast to the cross-sectional findings, the rate of change in hippocampal volume was significantly different between the three groups, with the most accelerated loss occurring in the eldest age group. This observation probably relates to the increased sensitivity afforded by the use of longitudinal studies where individuals act as their own
control. The subtle HV changes seen in normal individuals over 3.5 years may be easily obscured by the variance in HV seen across individuals in cross-sectional studies. Our rates of hippocampal volume loss were lower than those obtained from other longitudinal studies, however this is likely to be due to our younger study population (Jack et al., 1998). Pathological studies suggest that ageing is associated with region-specific loss of neurones in the subiculum and hilum of the dentate gyrus, with typical sparing of the CA1 region. This discriminates from the hippocampal changes accompanying Alzheimer’s disease in which the CA1 region is characteristically involved (West, 1993).

The mean rates of brain atrophy were similar to annualized rates of TBV loss in controls using the brain boundary shift integral method (Fox et al., 2000a). Increased rates of WM loss and accelerated rates of brain atrophy were observed in subjects in the 35-54 and >55 groups. We did not adjust for WM signal hyperintensities, as we excluded subjects with significant WM disease. The preferential loss of WM with advancing age in the absence of GM loss, supports earlier imaging and neuropathological reports (Double et al., 1996), (Guttmann et al., 1998) and may be explained by the selective loss of myelin with preservation of axons. The onset of accelerated TBV, WM and HV loss occurred before the age of 59, which may explain why previous longitudinal studies concentrating on the elderly failed to find increased rates of global and regional atrophy (Mueller et al., 1998), (Resnick et al., 2000). In contrast to cross-sectional studies suggesting that men suffer greater age-related atrophy than women (Coffey et al., 1998), (Xu et al., 2000), (Good et al., 2001a), we found no significant gender effects in any of the volume changes.

5.5.3 Methodological issues

Our choice of age epochs was influenced by the observation that pre-analysis error plots of change in volume against age in 10-year age bands, consistently demonstrated step reductions around the age of 55. In addition, imaging and autopsy studies (Torvik et al., 1986), (Luft et al., 1999) showed that age-related degeneration of the cerebellum starts between 50 and 60 years of age. To make the groups more comparable in terms of subject numbers, the under 55’s were divided into those under 35, and those between 35 and 54. Our subjects comprised members of staff, relatives or friends of epilepsy
patients, and volunteers prospectively recruited from the local population. Our criteria enabled us to study individuals that were fairly representative of the general population. We would therefore expect to see greater volume changes than obtained in previous longitudinal studies in which highly selected individuals were used as reference groups for disease states (Fox et al., 1999b).

Previous longitudinal studies of ageing have focussed primarily on individuals beyond the sixth decade (Resnick et al., 2000), (Mueller et al., 1998). The current study allows the study of morphometric changes over a much broader spectrum of ageing and confirms that significant loss of brain matter occurs at an earlier age.

Approximately 5-7% people over the age of 54 are at risk of developing pre-Alzheimer’s or Alzheimer’s disease (McDowell, 2001). Therefore, although we did not make a formal assessment of cognitive function, the likelihood that elderly subjects with pre-Alzheimer’s or mild dementia may erroneously exaggerate the rate of brain / hippocampal atrophy (Fox et al., 1996b), (Jack et al., 1997) is likely to be negligible. None of our subjects reported or showed overt signs of cognitive decline.

In this study, we have maintained longitudinal data integrity by scanning all subjects on the same scanner using identical acquisition sequences at baseline and follow-up. Fluctuations in voxel dimension are corrected by scaling factors in the coregistration program and monitored using phantom-based quality assurance programs. The obscuration of biological atrophy by the scaling process is unlikely since brain atrophy is best modelled as erosion and does not conform to a linear shrinking or stretching pattern. The change in ICV was not used for correction of voxel dimension fluctuations since the error (two standard deviations of the difference) in ICV with our segmentation software is between 0.5 and 1.0% of the baseline value, which is of the same order as the fluctuations.

Analysis of the subtraction images not only allows the detection of changes outside the pre-defined ROIs, but also checks the fidelity of the registration programme. The summation of structured difference images in each age epoch provides an overview of the common patterns of morphometric changes associated with ageing. The thresholding of structured difference images against a structured noise map highlights
biological change due to ageing. However, whilst random signal variation due to physiological and scanner-related artefact are filtered out by the thresholding process, genuine differences may also be minimized.

5.6 CONCLUSION

In conclusion, our findings support the hypothesis that different rates of regional brain atrophy are seen over 3.5 years in younger and middle-aged individuals. Total brain volume, white matter volume and hippocampal volume were particularly vulnerable, with incremental rates of generalized atrophy occurring from the age of 35 years. Increased rates of hippocampal atrophy were seen a little later, in those over the age of 54. Although the annualized rate of volume loss remained low (0.2-0.4% TBV, HV, WMV loss), the finding of significant volume losses with age and the identification of five individuals with changes lying outside the normative range, stress the importance of controlling for age when studying morphological change in neurological disease. Accounting for age effects should therefore be considered in all individuals over the fourth decade. This would be particularly pertinent when monitoring therapeutic responses or when predicting the onset of a neurodegenerative illness on the basis of longitudinal brain changes.
CHAPTER VI THE STRUCTURAL CONSEQUENCES OF NEWLY DIAGNOSED SEIZURES

In the previous chapter, we showed that increasing age was associated with accelerated rates of hippocampal and cerebral atrophy, emphasizing the need for a control population with which to identify pathological change. In this chapter, we compare volumetric change in our newly diagnosed patients with our control group to determine the effect of newly diagnosed seizures on brain volume.

6.1 OBJECTIVE

Intractable epilepsy may be associated with widespread structural cerebral damage, however little is known about when in the natural history of the disease this damage occurs. In this study, we determined whether structural damage occurs to the hippocampus, cerebellum and neocortex in the first few years following a diagnosis of seizures.

6.2 INTRODUCTION

Hippocampal damage, cerebral atrophy and cerebellar atrophy are frequently observed in patients with chronic epilepsy, and less commonly seen in patients with newly diagnosed seizures (Everitt et al., 1998), (Kalviainen et al., 1998). Cross-sectional studies cannot determine whether pre-existing cerebral damage determines the refractoriness of epilepsy or whether neuronal damage occurs progressively through the result of repeated seizures. To address these issues, we performed a longitudinal blinded quantitative MRI study to identify whether structural damage to the hippocampus, cerebellum and neocortex occurs in the first few years following a diagnosis of epilepsy.

A number of case studies have described the rapid development of HS in adults and children following an episode of status epilepticus (Nohria et al., 1994), (Wiesmann et al., 1997), (Perez et al., 2000). These typically describe an episode of SE secondary to an event, such as fulminant encephalitis, which results in a biphasic change in MRI appearance. An acute phase characterised by hippocampal enlargement and increased hippocampal T2 signal (representing the development of edema), is followed by the
gradual development of hippocampal atrophy and HS (Tien and Felsberg, 1995). Single adult case reports have described the development of HS in a patient with TLE (Briellmann et al., 2001), and worsening HS over a four year period in a patient with frequent temporal lobe seizures in the absence of SE or encephalitis (O'Brien et al., 1999).

6.3 METHODS

6.3.1 Subjects
Sixty-eight patients (37 men and 31 women) with a median age of 31 years (range 15-70 years) had a repeat MRI brain scan. Reasons for the observed attrition rate (22 / 90, 24%) are shown in Table 7. Of the five deaths, four were directly related to the aetiology of the seizures (neoplasia, alcohol, multi-system atrophy), with the other resulting from a stroke-related pneumonia. Ten of the 11 patients who refused had not experienced further seizures since their index seizure and declined further participation in the study.

The median interval between baseline and follow-up scan in the patients was 42 months (range 38-54 months). Patients were compared with 90 control subjects, as previously described in section 2.1. The median ISI for the controls was 42 months (range 42-48 months).

6.3.2 Study design and quantitative assessment
See common methodology in Chapters II and III.

6.3.3 Statistics
Independent t-tests were used to (1) determine whether the changes in quantitative measures between patient and control groups were significantly different, and (2) to study the relationship between structural change and seizures by comparing volume and signal change in patients with and without recurrent seizures. Clinical variables distinguishing patients with recurrent seizures from those experiencing isolated seizures were examined using Chi-square and Mann-Whitney U test.

Individual analysis compared the results of a single patient with the limits of agreement defined by the control group. A Bonferroni correction was applied to account for
multiple comparisons (see section 5.3.3.2). Normative ranges were calculated for three age epochs (<35, 35-54, >54 years) to account for the differential effects of atrophy associated with normal ageing (see Table 10). Volume changes exceeding the normative range were considered significant.

6.4 RESULTS

6.4.1 Patient characteristics

Sixty-eight patients with newly diagnosed seizures were included in the analysis. Two patients had a meningioma excised prior to the baseline scan, and four patients had resective surgery in the interval between the two scans. Forty-two (62%) patients had focal epilepsy and 26 (38%) patients had a generalised seizure disorder. Of the patients with focal epilepsy, 18 (43%) had cryptogenic and 24 (57%) had symptomatic epilepsy. Four (15%) of the patients with generalised epilepsy had idiopathic generalised epilepsy, 16 (62%) had symptomatic, and 6 (23%) had cryptogenic generalised epilepsy. The range of structural MRI abnormalities at the baseline assessment is displayed in Table 11. One patient with a learning disability, and a history of premature birth, complicated febrile convulsions and meningitis at 18 months, satisfied our MRI criteria for bilateral HS at baseline.

Thirty-eight patients (56%) with newly diagnosed seizures had continuing seizures. Of these, 26 (68%) had focal epilepsy and 12 (32%) had a generalised seizure disorder. Excluding the 15 patients with provoked seizures, 34 (64%) patients experienced further seizures and 19 (36%) patients remained seizure free. The 34 patients with recurrent unprovoked seizures had a median of 1.5 convulsive seizures (range 0 - 233) and 12 non-convulsive seizures (range 0-1821). Only five patients experienced more than 10 convulsive seizures.

6.4.2 Baseline MRI findings

Baseline volumetric data were missing from one patient with multiple sclerosis who was unable to comply with the full imaging sequence at baseline scan. Three cases (4% of scans) were excluded from the analysis due to sub-optimal image registration and brain segmentation: in one patient, image registration was rendered inaccurate by the excision of an extensive meningioma between the baseline and repeat scan, and the
quality of the two other baseline scans was substantially impaired by movement artefact. Age distribution, mean corrected baseline volumes and baseline HCT2 values were not significantly different between patient and control groups. At baseline, three patients had significant cerebral atrophy, four patients had hippocampal atrophy and

Table 11  Structural MRI findings of newly diagnosed patients on baseline scan

<table>
<thead>
<tr>
<th>Structural MRI findings</th>
<th>Numbers of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>44 (64.7)</td>
</tr>
<tr>
<td>Focal cortical dysplasia</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Hippocampal sclerosis</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Meningioma</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Focal brain damage, infarct</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Postoperative changes from resection of meningioma</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Demyelinating plaques</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Multiple intracerebral haemorrhage, subdural haematoma</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Cavernoma</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Foramen magnum decompression, diffuse cerebellar atrophy</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Cystic lesion</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Dysembryoplastic neuroepithelial tumour</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Neurosarcoïdosis</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Others</td>
<td>6 (8.8)</td>
</tr>
</tbody>
</table>

Others; non-specific focal white matter lesions, mild cerebellar ectopia, tiny infarcts.
five patients had cerebellar atrophy (defined as a baseline ICV-corrected volume less than 2 SD of the mean control volume). Age, gender, history of febrile convulsions, alcohol dependence, epilepsy syndrome, cerebellar atrophy and history of head or birth injury were not significantly associated with seizure recurrence. However, eighteen of the 20 (90%) patients with TLE experienced recurrent seizures compared with 20 out of 48 (42%) patients with extra-temporal lobe epilepsy or generalised epilepsy (difference 48%, 95% CI 29% to 68%; \( \chi^2, p = 0.0003 \)).

6.4.3 Group analysis of quantitative changes
The mean interscan interval was statistically comparable between the two groups. Quantitative changes in HV, HCT2, CBV, GMV, WMV, TBV and ICV were not significantly different between patient and control groups. The Mann-Whitney U test did not show significant differences in HV or HCT2 change between patients with TLE and those with extratemporal lobe epilepsy. There were also no significant differences between those who had a seizure recurrence and those who remained seizure-free in terms of change in any of the MRI parameters over 3.5 years. The numbers of convulsions and non-convulsive partial seizures considered independently were not significantly correlated with any volumetric or signal change. Further analyses of groups of patients with idiopathic generalized epilepsy, focal epilepsy with secondary generalization and focal epilepsy without secondary generalization demonstrated only an increase in the combined HCT2 signal with the estimated number of partial seizures in patients with focal epilepsy without secondary generalization (adjusted \( R^2 = 0.248, p=0.013 \)). No other correlations were observed between numbers of seizures and MRI volumetric or signal change in any of the groups.

6.4.4 Individual patient analyses of MRI changes
Seventeen patients had changes in quantitative values that exceeded the normal range. Ten of these were attributable to confounding factors that could affect brain atrophy or signal change; of these, five patients had structural lesions that were either progressive or had previously received surgical treatment: (1) craniotomy and evacuation of extradural clot prior to baseline scan, (2) active neurosarcoïdosis treated with high dose steroids, (3) excision of left fronto-parietal meningioma prior to baseline scan, (4)
recurrence of frontal lobe meningioma and osteomyelitis of skull flap between the two scans, and (5) a progressive inoperable meningioma in the left medial temporal lobe. The other five patients had a history of alcohol abuse. The remaining seven patients (13.5% of the patient cohort excluding surgical cases and patients with explicable changes) had at least one MRI parameter that changed significantly. A summary of these cases is provided in Table 12.

The patient with pre-existing bilateral HS had frequent secondarily generalised seizures (20-30 annually) despite treatment, but did not suffer progressive volume or signal change. Status epilepticus occurred in one patient during the observation period and it was associated with a significant reduction in HCT2 signal. This patient had a progressive inoperable meningioma in the left temporal lobe and the reduction in signal was thought to have represented haemorrhage within the tumour.

In comparison, the control group had 5 subjects (5.6%) with significant volume or signal change that could not be attributed to obvious causative factors e.g. alcohol, head injury, other medical illness or medication. These changes are described in more detail in section 7.4.3.2. The number of individuals with inexplicable changes was not significantly different between the two groups ($\chi^2$ with Yates correction=1.74, df=1, p=0.19). The magnitude of the changes was comparable in the two groups, and none of the changes was detected on visual assessment.
Table 12  Quantitative MRI measures and clinical features of 7 newly diagnosed patients with significant changes over 3.5 years

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>HV</th>
<th>HCT2</th>
<th>CBV</th>
<th>TBV</th>
<th>GMV</th>
<th>WMV</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>-0.14cm³</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Right TLE treated with CBZ, then VPA. Presented with an episode of NCSE characterised by déjà vu and visual hallucinations a fortnight before the baseline scan. Frequent SPS between scans, but no prolonged seizures.</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>-</td>
<td>+2.5msec</td>
<td>(+2.9%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Right TLE treated with PHT. Clusters of SPS characterised by stereotypical smell and intense visual colours 2-3 times a week.</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>-0.09 cm³</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Juvenile absence epilepsy treated with CBZ, then VPA. Seizure free for 18 months, relapse on withdrawing CBZ. Patient experienced 23 GTCS between baseline and repeat scan.</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-8.4cm³</td>
<td>(-6.5%)</td>
<td>-</td>
<td>-</td>
<td>Left FLE treated with VPA. Cluster of SGS 5 days prior to baseline scan, and subsequently seizure free. Baseline and repeat HCT2 measures within normal limits.</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-34.6cm³</td>
<td>(-5.7%)</td>
<td>Right TLE on VPA. Head injury 2 yrs prior to baseline with associated GTCS. No further seizures between baseline and repeat scan.</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-43.7cm³</td>
<td>(-3.5%)</td>
<td>Cryptogenic generalised epilepsy on no treatment. Single presenting seizure only.</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+27.7cm³</td>
<td>(+2.3%)</td>
<td>Cryptogenic TLE on CBZ. Pre-baseline, 4-year history of undiagnosed CPS, now well controlled on treatment. Only 1 CPS between baseline and repeat scans.</td>
</tr>
</tbody>
</table>

HV, combined hippocampal volume; HCT2, combined hippocampal T2; CBV, cerebellar volume; TBV, total brain volume; WMV, white matter volume; ICV, intracranial volume. All volumes have been corrected for baseline intracranial volume. TLE, temporal lobe epilepsy; FLE, frontal lobe epilepsy; NCSE, non-convulsive status epilepticus. VPA, sodium valproate; CBZ, carbamazepine; PHT, phenytoin. SPS, simple partial seizure; SGS, secondarily generalized seizure; GTCS, generalized tonic clonic seizure; CPS, complex partial seizure.
6.5 DISCUSSION

6.5.1 Individuals with significant volume changes

None of the patients developed HS over the follow-up period. The majority of individuals with significant volume change either had pre-existing cerebral lesions or a history of alcohol abuse that predisposed them to structural change. Once these individuals had been excluded, three patients showed hippocampal changes that fell outside the reference range established from the control subjects.

One patient (patient 1), suffered an episode of non-convulsive status epilepticus a fortnight before the baseline scan, whilst the second patient (patient 2) experienced clusters of simple partial seizures (SPS) several times a week and suffered a cluster the day prior to his repeat scan. In both cases, baseline and repeat hippocampal measures remained within normal limits. The change in patient 1 could best be explained by the development of hippocampal atrophy in the context of frequent partial seizures. In patient 2, the increase in HCT2 may be attributed to either the development of edema following frequent seizures before the repeat scan, gliotic changes secondary to frequent partial seizures, or an intrinsic progressive hippocampal disease process. In both cases, HV and HCT2 were not simultaneously affected, which is consistent with the view that a raised HCT2 has a different neuropathological basis to that of hippocampal volume loss (Van Paesschen et al., 1997c). The third case (patient 3), a patient with juvenile absence epilepsy, had a reduction in HV just outside the lower limits of the control reference range which could not be attributed to the resolution of edema. Although the volume loss may be the result of frequent seizures, the more likely explanation is that the significant reduction in measured ICV (-40cm³ or -2.7% baseline ICV) was due to an abnormally bright baseline scan in this patient and resulted in a miscalculation of the ICV-corrected hippocampal volume. The changes observed in patients 1 and 2 were comparable with the subtle one-year hippocampal changes observed in Van Paesschen's study (Van Paesschen et al., 1998).

Our findings favour an explanation of hippocampal change arising from oedematous change rather than structural damage from repeated seizures. Other significant individual findings were a reduction in WMV, a reduction in CBV, a reduction in TBV and an increase in TBV.
6.5.2 Group findings
The proportions of newly diagnosed patients and control subjects with quantitative changes exceeding the normative range were not statistically different. These findings, in conjunction with the lack of association between structural change and seizures on group analysis and the lack of difference in quantitative change for any of the MRI parameters between patient and control groups, suggests that generally patients with new onset seizures are not at increased risk of structural damage in the first 3.5 years following seizure diagnosis.

6.5.3 Incidence of hippocampal sclerosis in newly diagnosed epilepsy
As expected from previous studies (Van Paesschen et al., 1997b), our patient with bilateral HS went on to experience frequent secondarily generalized seizures. Hippocampal sclerosis is a rare finding in newly diagnosed patients. HS is reported in approximately 60% of surgical patients with intractable TLE (Margerison and Corsellis, 1966), yet only seen in 10% hospital-based patients (Van Paesschen et al., 1998) and three percent of community-based adult patients with newly diagnosed partial seizures (Everitt et al., 1997). Three explanations for the low incidence of HS in newly diagnosed seizures in this study are proposed. Firstly, patients with epilepsy resulting from HS commonly present before adulthood (Engel, 1996) and consequently below the lower age limit for inclusion in this prospectively acquired study group. Consequently the study subjects comprise a particularly good population for investigating the possibility that recurrent brief seizures cause secondary damage in the absence of pre-existing HS. Secondly, HS may be a progressive lesion that develops over the course of the disease, and thirdly, HS is inherently associated with a poor prognosis and tends to accumulate in prevalent populations. Patients presenting with habitual seizures due to HS in adulthood, may have a milder degree of hippocampal damage, since the dentate lamellar hypothesis (Sloviter, 1994) postulates that the length of the "silent period" is determined by the severity of the initial insult.

6.5.4 Prognostic outcome
Our study showed that 6% of patients with newly diagnosed seizures died over a 3.5-year period. Previous studies have reported an overall SMR of 2-2.3 in newly diagnosed patients, with the greatest risk of mortality in patients with remote symptomatic
epilepsy in the first few years of diagnosis. This suggests that the increased mortality is likely to reflect the underlying condition (Cockerell et al., 1997). The findings of our study reinforce the observation that patients with seizures generally have a good chance of achieving seizure freedom, although patients with symptomatic seizures are at an increased risk of premature death.

6.6 CONCLUSION

In comparison with longitudinal case studies reporting the rapid onset of hippocampal damage following SE, we did not detect comparable changes in patients with newly diagnosed seizures. Briellman et al. (Briellmann et al., 2001) described the development of HS over 33 months in a patient following seven secondarily generalised tonic clonic seizures and frequent simple partial temporal lobe seizures. In a separate case study, visual and quantitative progressive hippocampal changes following brief generalized seizures were detected only after 4.25 years of habitual seizures (O'Brien et al., 1999).

Although it is possible that changes may develop over a longer time-frame, we found no evidence for an increased risk of cerebral damage using our quantitative analysis in patients presenting with their first seizure.
CHAPTER VII  CEREBRAL DAMAGE IN EPILEPSY: A POPULATION-BASED QUANTITATIVE MRI STUDY

This chapter compares region-based volumetric data from the three cohorts and includes data on control subjects and newly diagnosed patients from chapters V and VI.

7.1 OBJECTIVE

This study reports on the structural effect of seizures on the hippocampus, cerebellum and neocortex in controls, patients with newly diagnosed seizures and patients with chronic active epilepsy. Risk factors for regional and global brain volume reduction and atrophy are addressed and possible mechanisms discussed.

7.2 INTRODUCTION

Chronic intractable epilepsy is associated with significant structural alterations both within and beyond the epileptogenic zone. Intractable TLE may be associated with extralesional volume deficits, including the ipsilateral and contralateral temporal lobe, contralateral hippocampus, ipsilateral amygdala, entorhinal and perirhinal cortices, thalamus and caudate, and cingulate gyrus (see section 1.6.4.2).

Despite the prevalence of structural abnormalities in patients with chronic epilepsy, the timing and pathogenesis of such changes remain obscure. The crucial question relates to whether the abnormalities are (1) progressive and the cumulative effect of years of epilepsy (Pringle et al., 1993), (2) the effect of an initial precipitating insult during a vulnerable phase of cerebral development (Annegers et al., 1987), (3) the presence of a pre-existing developmental abnormality predisposing to insults and further cerebral damage (Fernandez et al., 1998), or (4) a combination of these factors acting synergistically (Tasch et al., 1999).

Although case reports and small group studies exist of the evolution of HS and the worsening of HS in the absence of SE (Briellmann et al., 2001), (O'Brien et al., 1999), (Briellmann et al., 2002a), (Fuerst et al., 2003) evidence of progressive cerebral damage from prospective longitudinal studies with age-matched controls is lacking.
We aimed to investigate the effect of epileptic seizures on hippocampal, cerebral grey and white matter, and cerebellar volumes, by performing a population-based longitudinal study that incorporated image registration and automatic segmentation techniques. We sought to determine whether damage occurred at the early stages of the condition or only following years of repeated seizures and intractable epilepsy, and consequently performed serial MRI scans on patients with newly diagnosed seizures, patients with chronic epilepsy and control subjects, over a 3.5-year period. We also determined whether rates of cerebral damage varied according to epilepsy syndrome, by combining patients with newly diagnosed and chronic epilepsy. Clinical risk factors for the development of significant cerebral atrophy were investigated.

In contrast to hospital-based studies, seizures were largely well controlled and manageable with medication. Only a small proportion of patients proceeded to epilepsy surgery, therefore our findings pertain largely to non-surgical patients with epilepsy.

7.3 METHODS

7.3.1 Study population

All three cohorts, including the control and newly diagnosed groups referred to in Chapters 5 and 6 were included in this analysis. For further details, see sections 2.1 and 2.3.

7.3.2 Data acquisition and image processing

See sections 2.2 and 2.4.

7.3.3 Statistical analysis

Hippocampal sclerosis was defined as an ICV-corrected HV less than 2 standard deviations (SD) below the mean control volume and a HCT2 exceeding 2 SD of the mean control HCT2 value.

Two statistical approaches were used: analysis of group means and individual analysis. We determined that a minimum sample size of 35 would provide an 80% power ($\beta=0.2$) of detecting an effect size of 0.45 at $\alpha=0.05$, two-tailed.
7.3.3.1 Group analysis

Continuous variables across the three cohorts (e.g. baseline quantitative MRI data) and nominal variables were compared using analysis of variance (ANOVA) and $\chi^2$ tests respectively. Repeated-measures ANOVA assessed the effect of subject group, gender, initial precipitating injury (IPI), and status epilepticus on changes in MRI quantitation. The model integrated cross-sectional and longitudinal data into a single framework and determined the group-by-time interaction. Patients were classified as having an IPI if they had focal epilepsy and a clear history of an antecedent neurological insult e.g. febrile convulsion that was focal or >20 minutes in duration, a significant head injury causing loss of consciousness or skull fracture, previous meningitis or encephalitis, or a history of perinatal injury. Patients with electroclinical evidence of IGE were surrogate markers for “no insult” as they were less likely to have sustained a neurological insult, than those with cryptogenic focal epilepsy.

In a separate approach comparing volumetric change in the different epilepsy syndromes, patients with chronic and newly diagnosed epilepsy were amalgamated and stratified into the following syndromic categories: TLE with HS, TLE without HS, extratemporal focal epilepsy and generalized epilepsy. Volumetric changes were compared between the different syndromes.

The individual contributions of different variables were assessed using continuous variables as covariates. Clinical predictors of the change in each MRI parameter were assessed using multiple step-wise regression. Dependent variables included estimated seizure frequency, number of AEDs and alcohol intake between the two scans. The level of significance was set at $p<0.05$ for all statistical analyses.

7.3.3.2 Individual analyses

This approach compared the results of a single subject against the limits of agreement defined by the control group. The limits of agreement were defined as the mean change in ICV-corrected variable plus or minus 2.4SD of the difference (see section 5.3.3.2). Normative ranges were established for three age epochs (see sections 5.3.3.2 and 6.4.4).
7.4 RESULTS

7.4.1 Demographic data

Follow-up rates are shown in section 2.2. The demographic details of the patients and control group are summarised in Table 13. Additional information on the newly diagnosed patients is provided in section 6.3.1. There was no difference in age distribution or gender between the three groups. The median ISI was the same for each cohort. The patient groups were comparable in terms of seizure type, numbers of patients with episodes of SE between the two scans, history of birth injury, meningitis or encephalitis, and learning disability. However, patients with chronic epilepsy had received treatment with a greater number of AEDs (Mann Whitney U = 1522, p<0.0001) and were more likely to have experienced recurrent seizures between the two scans ($\chi^2$=26.14, p<0.0001). Patients with chronic epilepsy were also more likely to have suffered a febrile convulsion ($\chi^2$=11.04, p=0.004), which was not explained by the increased numbers of patients with HS in this group.

The syndrome classifications for the patients are shown in Table 14. Patients with newly diagnosed epilepsy were more likely to suffer from acute or remote symptomatic epilepsy compared with patients with chronic epilepsy who were more likely to have cryptogenic or idiopathic epilepsy ($\chi^2$= 8.32, p=0.004). The proportions of patients with TLE in the two patient groups were comparable.

Two patients with newly diagnosed seizures and four patients with chronic epilepsy had neurosurgery prior to the baseline scan, whilst four newly diagnosed patients and one patient with chronic epilepsy had neurosurgery between the two scans. The effect of surgical resection on volumetric measures was accounted for in the quantitative analyses (see below).
### Table 13  Demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>Newly diagnosed seizures</th>
<th>Chronic active epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>90</td>
<td>68</td>
<td>122</td>
</tr>
<tr>
<td>Median age in years (range)</td>
<td>35 (14-77)</td>
<td>31 (15-70)</td>
<td>35 (14-74)</td>
</tr>
<tr>
<td>Gender, males (%)</td>
<td>49 (54%)</td>
<td>37 (54%)</td>
<td>56 (46%)</td>
</tr>
<tr>
<td>female (%)</td>
<td>41 (46%)</td>
<td>31 (46%)</td>
<td>66 (54%)</td>
</tr>
<tr>
<td>Median ISI in mos (range)</td>
<td>42 (40-48)</td>
<td>42 (38-54)</td>
<td>42 (21-52)</td>
</tr>
<tr>
<td>Seizure type, partial (%)</td>
<td>-</td>
<td>42 (61.8%)</td>
<td>82 (67.2%)</td>
</tr>
<tr>
<td>generalised (%)</td>
<td>-</td>
<td>26 (38.2%)</td>
<td>36 (29.5%)</td>
</tr>
<tr>
<td>mixed (%)</td>
<td>-</td>
<td>-</td>
<td>4 (3.3%)</td>
</tr>
<tr>
<td>Seizure recurrence</td>
<td>-</td>
<td>38 (56%)</td>
<td>108 (89%)</td>
</tr>
<tr>
<td>Median no. of convulsive seizures (range)</td>
<td>0</td>
<td>1.5 (0-233)</td>
<td>3.0 (0-492)</td>
</tr>
<tr>
<td>Median no. of partial seizures (range)</td>
<td>0</td>
<td>21 (0-1820)</td>
<td>49 (0-6715)</td>
</tr>
<tr>
<td>Median no. of AEDs (range)</td>
<td>0 (0-1)*</td>
<td>1 (0-4)</td>
<td>2 (0-7)</td>
</tr>
<tr>
<td>Median no. of alcoholic units consumed per week (range)</td>
<td>7.5 (0-72)</td>
<td>6.5 (0-160)</td>
<td>1 (0-50)</td>
</tr>
<tr>
<td>No. of patients with a history of febrile convulsions (%)</td>
<td>-</td>
<td>1 (1.5%)</td>
<td>11 (9%)</td>
</tr>
<tr>
<td>No. of patients with episodes of status epilepticus between scans</td>
<td>-</td>
<td>1 (1.5%)</td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td>No. of patients with a history of meningitis / encephalitis</td>
<td>-</td>
<td>6 (8.8%)</td>
<td>8 (6.6%)</td>
</tr>
<tr>
<td>No. of patients with birth injury</td>
<td>-</td>
<td>3 (4.4%)</td>
<td>10 (8.2%)</td>
</tr>
<tr>
<td>No. of patients with a learning disability</td>
<td>-</td>
<td>6 (8.8%)</td>
<td>17 (13.9%)</td>
</tr>
</tbody>
</table>

* One control subject was taking carbamazepine following a cutaneous varicella zoster infection.

\* Same subject group as in Chapter 6.

ISI, interscan interval. Median age, median age at baseline. Seizure recurrence, patients with further seizures between baseline and repeat scan. Median no. of convulsive seizures, median number of convulsions between scans in patients with recurrent seizures. Median no. of partial seizures, median number of partial seizures in patients with focal epilepsy and recurrent seizures. Median no. of AEDs, median number of antiepileptic drugs taken between the two scans.
Table 14 Comparison of epilepsy syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Newly diagnosed epilepsy</th>
<th>Chronic active epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Focal epilepsy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>18 (43%)</td>
<td>43 (52.4%)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>24 (57.1%)</td>
<td>39 (47.6%)</td>
</tr>
<tr>
<td>Temporal lobe epilepsy</td>
<td>20 (47.6%)</td>
<td>46 (56.8%)</td>
</tr>
<tr>
<td>Frontal lobe epilepsy</td>
<td>8 (19%)</td>
<td>13 (16.0%)</td>
</tr>
<tr>
<td>Parietal lobe epilepsy</td>
<td>2 (4.8%)</td>
<td>2 (2.5%)</td>
</tr>
<tr>
<td>Focal epilepsy, undefined</td>
<td>12 (28.6%)</td>
<td>20 (24.7%)</td>
</tr>
<tr>
<td><strong>Generalised epilepsy</strong></td>
<td>26 (38.2%)</td>
<td>36 (29.5%)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>6 (23.1%)</td>
<td>3 (8.3%)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>16 (61.5%)</td>
<td>5 (13.9%)</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>4 (15.4%)</td>
<td>28 (77.8%)</td>
</tr>
<tr>
<td><strong>Mixed epilepsy</strong></td>
<td>-</td>
<td>4 (3.3%)</td>
</tr>
</tbody>
</table>

Focal epilepsy undefined, focal epilepsy of unclear localisation.
7.4.2 Cross-sectional findings at baseline

Patients with a history of neurosurgery prior to the baseline scan were excluded from the cross-sectional analysis. Fourteen patients with chronic focal epilepsy (17.0%) and one patient with newly diagnosed focal epilepsy (2.4%) had HS at baseline. Patients with chronic active epilepsy had a significantly smaller mean ICV-corrected HV (6.8% smaller than that of controls) and ICV-corrected TBV (1.4% smaller than that of controls) compared with control subjects and newly diagnosed patients (F=8.76, p<0.0001 and F=5.00, p=0.007 for HV and TBV respectively), which persisted after exclusion of patients with HS. The difference in mean corrected HV between the three cohorts remained significant after covarying for baseline age and ICV-corrected TBV (p=0.003). Baseline HCT2 was lowest in the control group and highest in the chronic epilepsy group (p=0.001). There was no significant difference in ICV-corrected GMV, WMV or CBV between the three groups at baseline.

7.4.3 Longitudinal findings

Patients who underwent surgery before the baseline scan or between the baseline and repeat scan were excluded from the longitudinal analysis. Baseline and repeat quantitative measures and their percentage changes for each subject group are listed in Table 15.

7.4.3.1 Group analysis

7.4.3.1.1 Hippocampal volume (Figure 14a)

Repeated measures of ANOVA showed a significant reduction in HV over time (F=20.96, p<0.0001), a significant difference between the three cohorts (F=8.52, p<0.0001), but no time-by-group interaction. There were main effects of age at baseline (F=17.37, p<0.0001) and change in TBV (F=84.38, p<0.0001) on the rate of HV loss. In patients with chronic epilepsy, duration of epilepsy corrected for age, did not have a significant effect on either baseline HV nor change in HV.
Table 15 Quantitative data

<table>
<thead>
<tr>
<th>MRI parameter</th>
<th>Cohort</th>
<th>Number baseline cases</th>
<th>Mean (SD) baseline volume (cm$^3$)</th>
<th>Number repeat cases</th>
<th>Mean (SD) repeat volume (cm$^3$)</th>
<th>Number cases in difference calculation</th>
<th>Mean volume change between scans, cm$^3$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV</td>
<td>Control</td>
<td>90</td>
<td>2.657 (0.240)</td>
<td>90</td>
<td>2.645 (0.255)</td>
<td>90</td>
<td>-0.01 (-0.38)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed</td>
<td>63</td>
<td>2.578 (0.277)</td>
<td>60</td>
<td>2.553 (0.279)</td>
<td>60</td>
<td>-0.02 (-0.58)</td>
</tr>
<tr>
<td></td>
<td>Chronic epilepsy</td>
<td>111</td>
<td>2.473 (0.371)</td>
<td>110</td>
<td>2.460 (0.361)</td>
<td>110</td>
<td>-0.02 (-0.85)</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBV</td>
<td>Control</td>
<td>88</td>
<td>127.7 (10.44)</td>
<td>88</td>
<td>127.2 (10.69)</td>
<td>88</td>
<td>-0.51 (-0.40)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed</td>
<td>63</td>
<td>125.0 (14.93)</td>
<td>60</td>
<td>124.9 (15.70)</td>
<td>60</td>
<td>-0.94 (-0.75)</td>
</tr>
<tr>
<td></td>
<td>Chronic epilepsy</td>
<td>110</td>
<td>123.7 (15.80)</td>
<td>109</td>
<td>122.2 (16.54)</td>
<td>109</td>
<td>-1.41 (-1.14)</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBV</td>
<td>Control</td>
<td>90</td>
<td>1227 (38.17)</td>
<td>90</td>
<td>1221 (44.17)</td>
<td>90</td>
<td>-5.98 (-0.49)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed</td>
<td>62</td>
<td>1227 (41.68)</td>
<td>59</td>
<td>1217 (45.43)</td>
<td>59</td>
<td>-11.00 (-0.90)</td>
</tr>
<tr>
<td></td>
<td>Chronic epilepsy</td>
<td>113</td>
<td>1210 (48.67)</td>
<td>112</td>
<td>1198 (57.07)</td>
<td>112</td>
<td>-11.60 (-0.96)</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMV</td>
<td>Control</td>
<td>90</td>
<td>748.3 (53.40)</td>
<td>90</td>
<td>746.0 (55.23)</td>
<td>90</td>
<td>-2.33 (-0.31)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed</td>
<td>62</td>
<td>753.2 (66.98)</td>
<td>59</td>
<td>748.1 (62.88)</td>
<td>59</td>
<td>-7.82 (-1.04)</td>
</tr>
<tr>
<td></td>
<td>Chronic epilepsy</td>
<td>113</td>
<td>742.3 (62.65)</td>
<td>112</td>
<td>737.0 (69.78)</td>
<td>112</td>
<td>-5.28 (-0.71)</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WMV</td>
<td>Control</td>
<td>90</td>
<td>478.3 (52.40)</td>
<td>90</td>
<td>474.9 (51.25)</td>
<td>90</td>
<td>-3.41 (-0.71)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed</td>
<td>62</td>
<td>474.0 (60.35)</td>
<td>59</td>
<td>469.4 (54.09)</td>
<td>59</td>
<td>-3.21 (-0.68)</td>
</tr>
<tr>
<td></td>
<td>Chronic epilepsy</td>
<td>113</td>
<td>467.7 (56.34)</td>
<td>112</td>
<td>461.3 (58.63)</td>
<td>112</td>
<td>-6.40 (-1.37)</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT2</td>
<td>Control</td>
<td>90</td>
<td>86.09 (0.844)</td>
<td>90</td>
<td>85.83 (0.858)</td>
<td>90</td>
<td>-0.246 (-0.29)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed</td>
<td>64</td>
<td>86.46 (1.489)</td>
<td>61</td>
<td>86.22 (1.285)</td>
<td>61</td>
<td>-0.240 (-0.28)</td>
</tr>
<tr>
<td></td>
<td>Chronic epilepsy</td>
<td>108</td>
<td>86.82 (1.853)</td>
<td>112</td>
<td>86.81 (1.885)</td>
<td>107</td>
<td>0.050 (0.06)</td>
</tr>
</tbody>
</table>

HV, combined hippocampal volume; CBV, total cerebellar volume; TBV, total brain volume; GMV, grey matter volume; WMV, white matter volume. HCT2, combined hippocampal T2 relaxometry. All volumes were corrected for baseline intracranial volume. * significant differences between the three subject groups, p<0.05. Baseline analysis excludes individuals with surgery prior to baseline scan; analysis at repeat scan and difference calculation excludes individuals who had neurosurgery prior to baseline scan and individuals who had neurosurgery between scan. Variations in numbers of cases also reflect scans excluded due to movement and susceptibility artifact.
The mean baseline HV of the 38 patients with a history of IPIs was significantly reduced (13% smaller than the mean control HV) compared with the 90 control subjects and the 27 IGE patients without such a history (F=19.31, p<0.0001). The volume reduction remained significant after exclusion of patients with HS and correction for TBV (F=8.22, p<0.0001), suggesting that the impact of the insult on initial HV was independent of the effect on the whole brain. Subgroup analyses showed that in the absence of an IPI, newly diagnosed patients and patients with chronic epilepsy had comparable baseline HVs to control subjects (see Figure 15a). In contrast, patients with a history of an IPI had significantly smaller baseline HVs, and patients with chronic epilepsy had smaller HVs compared with patients with newly diagnosed seizures. A history of an antecedent neurological insult did not significantly affect the rate of HV loss (F=1.08, p=0.37).

The change in HV over 3.5 years was not significantly different between patients with TLE with HS, TLE without HS, extratemporal focal epilepsy and generalized epilepsy (F=0.54, p=0.65). HV losses were comparable in patients with TLE and extratemporal focal epilepsy (t=-0.14, p=0.89). Of the 33 patients with cryptogenic TLE, the epileptic focus was clearly lateralised in 19 cases. No correlation was observed between either the number of convulsive seizures or number of presumed partial seizures and change in ipsilateral HV loss in these 19 patients. The degree of HV loss was dependent on the number of convulsive seizures between scans only in patients with extratemporal focal epilepsy (F=6.96, p=0.012).

7.4.3.1.2 Hippocampal T2 relaxometry (Figure 14b)
Hippocampal T2 relaxation time was significantly different between the subject groups (F=9.78, p<0.0001). A significant group-by-time interaction was observed (F=3.76, p=0.025), with HCT2 relaxation times decreasing with age in control subjects and newly diagnosed patients, and increasing in patients with chronic epilepsy.

Exclusion of the 14 patients with HS at baseline resulted in a change in the group effect with a fall in overall HCT2 in patients with chronic epilepsy below that of newly diagnosed patients (F=3.46, p=0.033). Patients with a history of an IPI had a significantly higher baseline HCT2 compared with those without such a history.
Chapter VII

(F=20.54, p<0.0001). However seizure type, neurological insults, number of AEDs, and estimated number of convulsive and partial seizures did not influence the change in HCT2.

7.4.3.1.3 Cerebellar volume (Figure 14c)

Patients with chronic epilepsy had smaller baseline CBVs compared with patients with newly diagnosed seizures or control subjects, but the difference was not significant (F=2.48, p=0.086). A significant reduction in CBV was observed in all subject groups over 3.5 years (F=16.63, p<0.0001). The rate of cerebellar volume loss was not significantly different between the three groups (F=1.51, p=0.222). The main effect on the rate of cerebellar atrophy was change in TBV (F=86.18, p<0.0001), suggesting that a proportionate degree of generalised cerebral atrophy was usually present. The estimated number of convulsive seizures, use of phenytoin and alcohol consumption between scans, did not predict the degree of cerebellar volume loss over time.

A prior history of an IPI was associated with a significantly greater rate of cerebellar atrophy (-2.38% compared with -0.6% seen in control subjects over 3.5 years; F=9.42, p<0.0001). The increased rate of cerebellar atrophy persisted after accounting for possible confounding factors e.g. rate of cerebral atrophy, age, alcohol intake, recurrent head injuries and AED usage between the two scans.

7.4.3.1.4 Total brain volume (Figure 14d)

Patients with chronic epilepsy had significantly smaller TBVs compared with newly diagnosed patients and control subjects (F=5.80, p=0.03). Total brain volume decreased significantly over the study period (F=45.43, p<0.0001). The loss in TBV was similar between the groups and epilepsy syndromes, and no time-by-group interaction was observed. The main effect on the loss of TBV with time was baseline age (F=11.35, p=0.001). The estimated number of convulsive seizures predicted TBV loss in patients with extratemporal focal epilepsy only (F=5.67, p=0.02). In patients with chronic epilepsy, duration of epilepsy corrected for age, had no effect on either baseline TBV or the rate of volume loss. The time-by-matter interaction was not significant (F=0.009, P=0.93), suggesting that both GMV and WMV loss were contributing similarly to the observed loss in TBV.
Patients with a history of IPIs had significantly smaller overall TBVs ($F=6.31$, $p=0.002$) and increased rates of cerebral atrophy (-1.45% over 3.5 years) compared with control subjects (-0.64% change) and patients without a history of insults (-0.49% change). The observed increase in cerebral atrophy was not influenced by recurrent head injuries, AED use or alcohol consumption between the two scans. Subgroup analysis of patients with a history of IPI showed that those with chronic epilepsy had smaller overall TBVs than patients with newly diagnosed seizures, but similar rates of atrophy (Figure 15b).

**7.4.3.1.5 Cerebral grey matter volume (Figure 14e)**
Total grey matter decreased significantly with time ($F=9.86$, $p=0.002$), but there was no difference between the cohorts and no time-by-group interaction. Once baseline age was accounted for, the change in GMV with time was no longer significant. Frequency of convulsive seizures predicted GMV loss in extratemporal focal epilepsy only ($p=0.037$).

**7.4.3.1.6 Cerebral white matter volume (Figure 14f)**
A highly significant reduction in WMV was observed with time ($F=32.87$, $p<0.0001$). There was no difference between the cohorts and no time-by-group interaction. Baseline age was the main determinant of the observed change in WMV ($F=15.66$, $p<0.0001$).
Figure 14 Longitudinal changes in MRI parameters for each subject group. The mean baseline and repeat values are shown for (a) combined hippocampal volume, (b) combined HCT2 relaxometry, (c) total cerebellar volume, (d) total brain volume, (e) grey matter volume, and (f) white matter volume. All brain volumes were corrected for baseline ICV.
Figure 15  The effect of antecedent neurological insults on longitudinal volume changes. Mean baseline and repeat volumes are shown for combined hippocampal volume (a) and total brain volume (b) and (c). Patients were classified as having a prior neurological insult if they had focal epilepsy and gave a clear history of an antecedent neurological insult. Patients with electroclinical evidence of idiopathic generalised epilepsy with no history of neurological insults were considered surrogate markers for “no insult” as they were less likely to have sustained an occult neurological insult, than those with focal epilepsy.
Gender, seizure type, epilepsy syndrome, duration of epilepsy and a history of SE did not have an effect on any of the quantitative changes measured.

### 7.4.3.2 Quantitative changes in individual subjects

Five control subjects and seven patients with newly diagnosed seizures had significant quantitative changes that could not be attributed to confounding factors (see sections 5.4.2.2 and 6.4.4).

One patient with chronic epilepsy and global atrophy involving the hippocampus, grey matter and whole brain was excluded from the individual analysis as the changes were considered the effect of alcohol abuse. There were 27 patients with chronic epilepsy with significant inexplicable changes in at least one MRI parameter. The numbers of individuals with significant volume loss were significantly higher in the chronic epilepsy group than the control or newly diagnosed groups ($\chi^2 = 9.59$, $p=0.008$). Details of individuals with inexplicable quantitative changes exceeding the normative range are given in Table 16. None of these significant volume changes was detected on visual assessment. Within the chronic epilepsy group, age at seizure onset and a prior history of an IPI was comparable between patients with and without the development of significant atrophy ($p=0.71$ and $p=0.58$ respectively).

Excluding the patient who underwent temporal lobe surgery, none of the remaining 14 patients with HS at baseline, had either a significant increase in HCT2 or a significant reduction in HV. Although there were 13 individuals with significant HV and HCT2 changes over the 3.5 years, none developed HS *de novo* since their follow-up volumes and HCT2 values remained within the control range defined in section 7.3.3.
Table 16  Individual patients with chronic epilepsy showing significant quantitative changes over 3.5 years

<table>
<thead>
<tr>
<th>Subject</th>
<th>HV</th>
<th>HCT2</th>
<th>CBV</th>
<th>TBV</th>
<th>GMV</th>
<th>WMV</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-3</td>
<td>mean ↓ 3.2SDD range (2.7-3.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All had IGE. Mean seizure frequency ranged from 0 to 210 GTCS. All taking VPA.</td>
</tr>
<tr>
<td>E4</td>
<td>↓ 2.4SDD</td>
<td>↓ 2.6SDD</td>
<td>↓ 4.5SDD</td>
<td>↓ 2.9SDD</td>
<td></td>
<td></td>
<td>JAE on VPA, ESM. No GTCS, &gt;1000 absences</td>
</tr>
<tr>
<td>E5</td>
<td>↓ 2.5SDD</td>
<td></td>
<td>↓ 4.4SDD</td>
<td>↓ 3.3SDD</td>
<td></td>
<td></td>
<td>IGE on CBZ. &gt;100 absences</td>
</tr>
<tr>
<td>E6</td>
<td>↓ 3.4SDD</td>
<td></td>
<td>↓ 2.5SDD</td>
<td></td>
<td></td>
<td></td>
<td>Cryptogenic generalised epilepsy on LTG, VPA. 4 GTCS between scans</td>
</tr>
<tr>
<td>E7-11</td>
<td>mean ↑ 2.8SDD range (2.5-3.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 TLE, 3 FLE. Seizure frequency ranged from 0-294 SGS. Number of AEDs ranged from 0-6.</td>
</tr>
<tr>
<td>E12-19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGE on CBZ. No history of overt seizures.</td>
</tr>
<tr>
<td>E21-22</td>
<td>mean ↑ 2.0SDD range (2.4-4.8)</td>
<td></td>
<td>↑ 2.4SDD</td>
<td>↑ 3.3SDD</td>
<td>↓ 2.6SDD</td>
<td></td>
<td>CAE treated with VPA. &gt;100 absence seizures TLE on CBZ, LTG, GBP. ~80 CPS</td>
</tr>
<tr>
<td>E23</td>
<td>↓ 2.8SDD</td>
<td></td>
<td>↓ 3.0SDD</td>
<td></td>
<td></td>
<td></td>
<td>Cryptogenic partial epilepsy. Treated with VPA, PHT, PB</td>
</tr>
<tr>
<td>E24-25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1) Cryptogenic generalised epilepsy, treated with PRM, VPA, LTG, GBP. 88 GTCS (2) Symptomatic TLE, on CBZ, GBP, CLB, TPM. 139 CPS, 6 SGS</td>
</tr>
<tr>
<td>E26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TLE, treated with CBZ, CLB, PHT, VPA. 82 CPS</td>
</tr>
<tr>
<td>E27</td>
<td></td>
<td></td>
<td>↓ 3.6SDD</td>
<td></td>
<td></td>
<td></td>
<td>TLE, treated with VPA, GBP, LTG, CBZ. 410 CPS</td>
</tr>
</tbody>
</table>

E, chronic active epilepsy; IGE, idiopathic generalised epilepsy; TLE, temporal lobe epilepsy; JAE, juvenile absence epilepsy; FLE, frontal lobe epilepsy; CAE, childhood absence epilepsy. CBZ, carbamazepine; VPA, sodium valproate; ESM, ethosuximide; LTG, lamotrigine; GBP, gabapentin; PHT, phenytoin; PB, phenobarbitone; PRM, primidone; CLB, clobazam; TPM, topiramate. NCSE, non-convulsive status epilepticus; GTCS, generalised tonic-clonic seizures; SPS, simple partial seizures; CPS, complex partial seizures; SGS, secondarily generalised seizures. AEDs, antiepileptic drugs.
Chapter VII

7.5 DISCUSSION
Two key questions are addressed in this chapter: (1) whether epilepsy is associated with MRI-detectable damage over 3.5 years and (2) whether particular clinical risk factors increase individual susceptibility to damage. The main findings were (i) there were significant differences between the three groups at baseline with patients with chronic epilepsy having smaller ICV-corrected baseline volumes and longer HCT2 times; (ii) the loss in hippocampal and neocortical volume over the study period was similar in the three subject groups; (iii) volume changes were not syndrome-dependent in that the rates of volume loss and signal change were comparable in patients with TLE with HS, TLE without HS, extratemporal focal epilepsy and generalized epilepsy; (iv) a history of an IPI was associated with an increased rate of cerebral and cerebellar volume loss; and (v) a significantly greater number of individuals with chronic epilepsy developed significant hippocampal and neocortical volume loss over 3.5 years.

7.5.1 Methodological issues and limitations of the study
As a population-based study, our patient groups were inevitably heterogeneous, and reflected a variety of syndromes and aetiologies with variable susceptibility to damage. Whilst this limited the power of our group observations, the range of epilepsy syndromes allowed us to explore a wider range of individual variation and is more relevant to the population developing epilepsy than a selected clinic population.

There were a number of important differences between the ‘chronic active epilepsy’ and ‘newly diagnosed’ group besides the duration of epilepsy. Patients with chronic active epilepsy were more likely to have refractory epilepsy – they had a significantly higher chance of seizure recurrence, a higher seizure frequency and had received treatment with a greater number of AEDs (see Table 13). Due to study design, all newly diagnosed patients were over the age of 14 years by the time of their presentation, whereas the chronic active epilepsy group included childhood-onset cases. The division into newly diagnosed and chronic active epilepsy groups was therefore useful in comparing two possibly distinct disease processes with different disease courses, and in establishing whether structural change occurred shortly after seizure diagnosis or only after years of established epilepsy.
Our individual analysis identified a significantly greater proportion of patients with chronic epilepsy with volume and signal changes exceeding the control reference range. However, no particular syndromes or aetiologies were over-represented in these relatively small numbers of patients.

Our study is based on the assumption that brain damage is detectable using a serial imaging approach. It is possible that damage induced by seizures may be subtle, and not readily picked up by current imaging techniques. Using our methodological approach we have previously demonstrated an 8% increase in HV, 11% increase in TBV and a 20% increase in CBV in an individual following abstention from alcohol (Liu et al., 2000). These observations combined with the fact that subtle age-related HV losses in the control subjects were similar to our automatically derived TBV losses, confirm that we have not sacrificed sensitivity for reproducibility.

All volumetric measurements were performed with the observer blinded to chronological scan order, subject status and all clinical information. This fully blinded approach may account for the comparatively small HV changes observed in our patients with cryptogenic TLE compared with volume changes reported in previous studies over a similar time-period (Briellmann et al., 2002a), (Fuerst et al., 2003). In their study, Briellmann and colleagues (Briellmann et al., 2002a) reported mean and maximum ipsilateral HV changes of - 9.0% and -30%, compared with our changes of -1.7% and -9.6% respectively.

7.5.2 Cerebral damage in epilepsy

There are histological and MRI studies that suggest that significant structural damage may precede the onset of spontaneous seizures. Traumatic head injuries (Bigler et al., 1997), prolonged complex febrile convulsions (VanLandingham et al., 1998), perinatal or postnatal trauma (Margerison and Corsellis, 1966) and SE following an encephalitic illness (Wieshmann et al., 1997) are examples of IPIs associated with HV reduction and epilepsy.

Some of the previous C-S studies noted significant reductions in ipsilateral HV (Salmenpera et al., 1998) and decreased neuronal densities in all hippocampal subfields (Mathern et al., 2002) in patients with frequent seizures and prolonged duration of TLE.
Our C-S findings of significantly reduced baseline HVs in patients with chronic epilepsy are consistent with these reports, which concluded that the volume reductions either reflected (1) the cumulative neurobiological effect of repeated seizures or (2) a bias towards the accumulation of refractory cases following a severe precipitating injury. Our longitudinal findings favour the latter supposition, that is, for the majority of patients, structural damage preceded seizure onset and subsequent volume loss was predominantly determined by age. This is supported by our observation (Fig 15a) that patients without an IPI have similar baseline HVs to control subjects.

Our observation of a reduced baseline CBV in newly diagnosed patients compared with control subjects suggests that these mechanisms cannot wholly explain the volume loss observed in patients with epilepsy, and that at least part of the CBV loss is likely to occur prior to the onset of seizures. This is consistent with suggestions that cerebellar damage may precede the manifestation of epilepsy (Dam, 1987), and that the loss of inhibitory function in patients with a structurally damaged cerebellum may worsen prognosis for good seizure control (Specht et al., 1997).

In our study, subject groups demonstrated differential changes in HCT2 relaxometry. Only patients with chronic epilepsy demonstrated an increase in HCT2, although this was not significant. In our patients with TLE, change in HCT2 was not correlated with seizure frequency, AED exposure or drug intoxication.

7.5.3 Initial precipitating insults
A major insult can cause ipsilateral hippocampal damage and damage to other structures, particularly on the side of the seizure focus (Gastaut et al., 1959), (VanLandingham et al., 1998). In their recent clinical-pathological study of 572 patients with TLE and 73 patients with extra-temporal lobe epilepsy, Mathern (Mathern et al., 2002) showed that IPIs were important in the pathogenesis of HS, and that HS only occurred in patients with extratemporal lobe epilepsy if there had been a prior IPI. In TLE patients, HS was strongly associated with IPIs involving seizures. Our results showed that a history of an IPI was associated with a significant reduction in baseline TBV and a reduction in HV that was independent of the change affecting the whole brain. This observation persisted after exclusion of patients with HS suggesting that the
reduced brain volumes observed in subjects with a history of IPI did not simply reflect the greater number of patients with HS in this group. A significant increase in the rate of cerebral and cerebellar atrophy was seen in patients with a history of IPIs which could not be attributed to other potential confounders e.g. alcohol consumption, age, AED use or a history of recurrent head injuries between the two scans. In our study, we selected patients with IGE as a population without neurological insult. We did not include patients with cryptogenic focal epilepsy as we felt that a remote cerebral insult could not always be reliably recalled by patients some time after the event.

Our observation that patients with chronic epilepsy and a history of IPIs had smaller HVs than patients with newly diagnosed seizures and a history of insults, is consistent with the dentate lamellar hypothesis (Sloviter, 1994). This suggests that the length of the "silent period" is determined by the severity of the initial insult. Due to study design, all patients with newly diagnosed seizures presented over the age of 14 and had relatively infrequent seizures. It is therefore possible that patients with chronic epilepsy were more likely to have sustained a more severe precipitating insult that resulted in a greater initial volume reduction, a more refractory form of epilepsy and an earlier age of presentation.

Our results corroborate previous suggestions that, in addition to causing measurable volume changes, an insult may prime the brain, making it more vulnerable to the effect of seizures (Schmid et al., 1999). Experimental studies in rats have demonstrated progressive cortical and subcortical neuronal loss for up to one year post traumatic brain injury (Smith et al., 1997) and suggested that a chronically progressive degenerative process my be initiated by the injury. Putative mechanisms for the progressive tissue loss observed after TBI include: the consequences of the primary insult i.e. Wallerian degeneration, and progressive secondary injury mechanisms including apoptotic cell death, inflammation and excitotoxicity in white matter tracts (Bramlett and Dietrich, 2002). Consistent with this hypothesis is the finding by Kim and colleagues (Kim et al., 1990) that temporal lobe CPS associated with an overt structural lesion show less neuronal loss than groups with a history of complicated febrile seizures or an IPI.

In contrast to a recent follow-up study of 12 patients with HS (Fuerst et al., 2003), none of the 14 patients with HS at baseline and without neurosurgical intervention developed
a significant loss in HV or increase in HCT2 relaxation time over the period studied. In this study only a relatively low number of patients had HS at baseline and this is likely to reflect both the broad spectrum of disease severity seen in population-based studies and the exclusion of children with HS presenting with seizures before the age of 14 years. However, the lack of detectable disease progression could be a manifestation of the “floor effect,” in that the initial insult was sufficiently severe enough to damage the hippocampus such that no further damage could be observed at least over 3.5 years (Briellmann et al., 2000). In contrast to Briellmann’s study in which one of the 24 patients developed HS (Briellmann et al., 2002a), we did not observe the development of HS in any patient with a normal scan at baseline.

7.5.4 Seizures
Regression analyses did not show a relationship between the number of convulsive seizures and partial seizures, considered independently, and the degree of volume or signal change. Analyses according to epilepsy syndrome showed that only patients with extratemporal focal epilepsy had a significant correlation between frequency of convulsive seizures and change in HV, TBV and GMV. This observation was not found in any of the other epilepsy syndromes including TLE, suggesting that regional volume loss seen in association with convulsive seizures is not necessarily localized to the site of seizure generation, but may be remote from the epileptic focus. Subgroup analyses of (a) all patients with TLE and (b) those with cryptogenic TLE, did not show a correlation between seizure frequency and ipsilateral HV loss. This would be consistent with the observation that only 50 - 75% of histological specimens taken from patients undergoing surgery for drug-refractory TLE show neuronal loss in the dentate gyrus and hippocampus proper (Margerison and Corsellis, 1966), (Honovar, 1997).

Methodological differences may explain the discrepancy between our findings and a recent study demonstrating an inverse correlation between GTCS number and ipsilateral HV loss (Briellmann et al., 2002a). These include differences in: scanner consistency, blinding to chronological order of scans, and use of appropriated age-matched controls (Liu et al., 2002a).
Despite the prospective documentation of seizures, an accurate seizure record of partial seizures was not always attainable, since patients were not always aware of their seizures. Nonetheless it would seem implausible that inaccuracy of seizure recall should impact significantly on our observations since the change in HV in the three subject groups was strikingly comparable (Figure 14). Patients with history of insults had accelerated rates of cerebral atrophy compared with control subjects and patients without history of insults (Figure 15b). Patients with chronic epilepsy without a history of neurological insults had baseline volumes and volume changes comparable to control subjects and patients with newly diagnosed seizures without insults (Figures 15a and 15c). This suggests that an IPI is more important in determining the degree of volume change than either seizure frequency or duration of epilepsy. Mathem and colleagues (Mathem et al., 2002) showed that longer durations of TLE were associated with decreased neuronal numbers in all hippocampal subfields, an observation independent of IPI-induced neuronal loss. However, the authors emphasized that a long time course (>30 years) was required to demonstrate the negative correlation, and thus the substantial hippocampal neuronal loss observed in HS was likely to be the result of an IPI rather than the effect of repeated limbic seizures. Their suggestion that limbic seizures slowly "damaged" the brain over several decades may contribute to the lack of correlation observed between seizures and hippocampal atrophy in our study.

None of the four patients with SE between the two scans experienced significant brain volume losses or HCT2 changes over the 3.5 years. Opinion regarding cerebral damage following SE is divided. Although a number of case reports have described the development of HS following an acute process (Wiesmann et al., 1997), Salmenperä and colleagues showed that progressive HV reduction was not an invariable consequence of promptly-treated SE (Salmenpera et al., 2000a).

7.5.5 Gender

A previous C-S study showed that men with TLE demonstrated greater brain atrophy compared with women with TLE. Since the number of convulsive seizures contributed significantly to these abnormalities in men but not women, the authors postulated that men were more vulnerable to seizure-induced brain volume loss although the initial damage was likely to be gender independent (Briellmann et al., 2000). In our study, we found no gender effect with regards to either initial volume loss or ongoing
susceptibility to brain damage. Changes were comparable in men and women in all MRI parameters studied.

7.5.6 Exposure to antiepileptic drugs

Our data did not provide evidence for increased cerebral damage with chronic exposure to antiepileptic drugs. Although patients with chronic epilepsy had been exposed to significantly more AEDs than patients with newly diagnosed seizures or control subjects, the rate of volume loss was comparable in the three groups.

The role of phenytoin in the pathogenesis of cerebellar atrophy has not been resolved in previous retrospective studies. Phenytoin treatment is frequently compounded by the cumulative effect of hypoxia in the context of repeated convulsive seizures. In the present study, we found no association between treatment with phenytoin – either chronic usage or acute intoxication - and whole cerebellar volume. It is possible that seizures or phenytoin usage might exert a selective regional effect, and a more detailed study of the cerebellar hemispheres and lobules of the vermis may be warranted.

7.6 CONCLUSION

In summary, our study of 190 patients showed that progressive regional or global cerebral damage is not an inevitable consequence of epileptic seizures. The significant difference in baseline hippocampal and cerebral volumes observed in control subjects and patients at different stages of the condition was largely attributed to an antecedent neurological insult. These findings suggest that a substantial amount of cerebral damage was incurred prior to or at the onset of seizures. We showed that the subsequent rate of hippocampal and cerebral atrophy over 3.5 years was primarily determined by age, and was in the majority of cases independent of a diagnosis of epilepsy. Individual cases showed significant volume losses that were independent of seizure frequency and this is likely to reflect patient heterogeneity. There is a suggestion that an early neurological insult may prime the brain, and increase vulnerability to subsequent cerebral damage. Further studies are required to characterise these neurological insults since measures, such as the prompt treatment of prolonged febrile convulsions, may modulate the impact of such insults on the brain and reduce subsequent susceptibility to seizure-induced damage.
CHAPTER VIII PROGRESSIVE NEOCORTICAL DAMAGE IN EPILEPSY

This chapter explores the development of focal and subtle changes in neocortical GM and WM using voxel-based analyses that would not be detected by the region-based neocortical volumes measured in Chapter VII. It also characterizes the patterns of neocortical damage seen in epilepsy and determines whether damage is distributed predominantly in the putative epileptogenic lobe.

8.1 OBJECTIVE
To perform a complementary voxel-based analysis investigating the pattern and extent of generalized and focal neocortical damage in epilepsy, and the factors associated with such secondary neocortical damage.

8.2 INTRODUCTION
Quantitative MRI studies investigating brain volume changes in epilepsy have generally used region-based methods, with manual and automated segmentation techniques (Chapters VI and VII). Such methods, however, restrict analyses to areas of a priori interest, are time-consuming, and subject to bias. Automated voxel-based methods to coregister, subtract and segment serial MRI scans avoid these problems (Freeborough and Fox, 1997), (Lemieux et al., 1998b), (Lemieux, 2001). Quantification of focal change of GM and WM can be applied using a region template that is applied to baseline and follow up scans (Hammers et al., 2002).

8.3 METHODS
8.3.1 Data acquisition
See common methodology (Chapter II).

Patients with conditions leading to explicable changes in brain volumes e.g. surgical intervention (6), progressive lesions (5), neurodegenerative conditions (3), chemotherapy (2), alcohol abuse (6) and steroid use (1) were excluded a priori. Thus, analysis was performed on 114 patients with chronic epilepsy (53 males, 61 females; median age at baseline, 34.5 years, range 14 to 74 years) and 53 patients with newly
diagnosed seizures (28 males, 25 females; median age at baseline, 30 years, range 15 to 56 years). All 90 control subjects were used to control for age-related changes and temporal variations in scanner performance.

8.3.2 Image processing
Image processing was performed on the T1-weighted inversion-recovery prepared volume acquisition sequence (see section 3.5).

8.3.3 Statistical analysis
Patterns of change between the three subject groups were compared using $\chi^2$. Factors associated with the pattern of signal change were investigated using $\chi^2$, Fisher's Exact test and the Mann Whitney U test. The level of significance was set at $p<0.05$. For the identification of clinical risk factors associated with neocortical damage, Bonferroni correction was used to account for multiple comparisons (four independent parameters) resulting in a significance level set at $p<0.013$.

8.4 RESULTS
8.4.1 Patterns of signal change
The main patterns of signal change are shown in Figure 16. Generalized changes were characterized by a red or green rim around the ventricular, cortical and brainstem surfaces (Bydder, 1995). Focal volume losses were most commonly observed in frontal or temporal lobes, either in isolation or in combination with generalized volume losses. Artifactual changes were characterized by a pattern of mixed signal change with a predilection for boundary regions. Despite the use of the structured noise map to minimize these, susceptibility or motion artifacts within the source images were responsible for the majority of the residual artifactual changes, although misregistration artifacts also contributed.

---

* Misregistration artifacts were geometric and mainly due to a varying degree of distortions associated with the different head positions in the head coil that could not be accounted by rigid-body registration.
Figure 16 Representative patterns of neocortical change. First column, baseline coronal T1-weighted volume scan; second column, matched repeat scan; third column, difference image; fourth column, map of changed voxels. (a) no change, (b) generalized volume loss, (c) focal volume loss showing bilateral temporal lobe atrophy, left greater than the right, (d) artifactual changes, showing biphasic appearance of signal change.
Patterns of signal change for all patients are shown for each of the three subject groups in Figures 17-19, and categorized according to epilepsy syndrome in Table 17.

Figure 17

- Temporal lobe volume loss (1)
- Temporal lobe + generalized volume loss (1)
- Frontal lobe + generalized volume loss (2)
- Cerebellar volume loss (1)
- Generalized volume loss (15)
- Generalized volume gain (1)
- No change (61)

Artifacts (8)
Problem with non-uniformity correction (5), pulsation artifact (2), metal artifact (1).
Total number of newly diagnosed cases excludes patients with conditions leading to explicable changes in brain volume.
Figure 19

Chronic active epilepsy (114)

- TLE (45)
  - HS (13)
  - No HS (32)
    - Temporal lobe volume loss (1)
    - Temporal & generalized volume loss (1)
    - Frontal & generalized volume loss (1)
    - Left hemisphere, cerebellar & generalized volume loss (1)
    - Generalized volume loss (6)
    - No change (2)
    - Artifact (1)
      Problem with non-uniformity correction (1)
    - Artifact (5)
      Movement artifact (5)

- FLE (10)
  - Frontal lobe volume loss (1)
  - Generalized volume loss (3)
  - Generalized volume gain (2)
  - No change (3)
  - Artifact (1)
  - Superior-inferior positional difference (1)

- Other focal epilepsies (21)
  - Frontal lobe volume loss (1)
  - Frontal & generalized volume loss (3)
  - Generalized volume loss (3)
  - No change (8)
  - Artifact (7)
    Movement artifact (3), no baseline volume scan (2), problem with non-uniformity correction (2)

- Generalized epilepsy (35)
  - (76% of generalized epilepsy were IGE)
  - Frontal lobe volume loss (1)
  - Temporal, cerebellar & generalized volume loss (2)
  - Generalized volume loss (10)
  - Generalized and cerebellar volume loss (1)
  - No change (16)
  - Artifact (4)
    No baseline volume scan (1), bright baseline scan (1), movement artifact (1), problem with non-uniformity (1)

- Mixed epilepsy (3)
  - Generalized volume loss (2)
  - No change (1)

Total number of cases with chronic active epilepsy excludes patients with conditions leading to explicable changes in brain volume
Table 17 Patterns of neocortical atrophy according to epilepsy syndrome

<table>
<thead>
<tr>
<th>Epilepsy syndrome</th>
<th>Focal volume loss (N=10) (%)</th>
<th>Focal + generalized volume loss (N=8) (%)</th>
<th>Generalized volume loss (N=51) (%)</th>
<th>No volume loss (N=68) (%)</th>
<th>Volume gain (N=3) (%)</th>
<th>Total (N=140) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLE</td>
<td>3 (5.6)</td>
<td>4 (7.4)</td>
<td>24 (44.4)</td>
<td>22 (40.7)</td>
<td>1 (1.9)</td>
<td>54</td>
</tr>
<tr>
<td>FLE</td>
<td>2 (20.0)</td>
<td>-</td>
<td>3 (30.0)</td>
<td>3 (30.0)</td>
<td>2 (20.0)</td>
<td>10</td>
</tr>
<tr>
<td>Other FOCAL</td>
<td>2 (8.0)</td>
<td>2 (8.0)</td>
<td>6 (24.0)</td>
<td>15 (60.0)</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Generalized</td>
<td>3 (6.3)</td>
<td>2 (4.2)</td>
<td>16 (33.3)</td>
<td>27 (56.3)</td>
<td>-</td>
<td>48</td>
</tr>
<tr>
<td>Mixed</td>
<td>-</td>
<td>-</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

Data includes newly diagnosed and chronic epilepsy patients. Percentages are expressed according to epilepsy syndrome and are shown in the parentheses. Total number excludes artifactual changes.

TLE, temporal lobe epilepsy; FLE, frontal lobe epilepsy; Other FOCAL, other focal epilepsies; Generalized, generalized epilepsy; Mixed, features of both focal and generalized epilepsy. Focal volume loss, voxel changes localized to discrete lobar regions; Generalized volume loss, symmetrical, multilobar voxel changes distributed at ventricular and cortical surfaces; focal + generalized, appearances consistent with both focal and generalized volume loss.
8.4.2 Individual analysis

8.4.2.1 Control subjects
After excluding eight cases marred by artifact, visual analysis identified 5 (6%) subjects with focal neocortical volume loss and 15 (18%) subjects with generalized volume loss (Figure 17). The observed changes were attributed to ageing in the absence of documented confounding factors. One subject (1%) had an inexplicable generalized gain in neocortical volume.

8.4.2.2 Newly diagnosed group
After excluding patients with artifactual changes, five patients (11%) showed a focal neocortical volume loss, 12 (27%) showed a generalized volume loss and one (2%) showed a generalized volume gain (Figure 18).

8.4.2.3 Chronic epilepsy group
Focal neocortical volume loss was identified in 13 patients (14%) with chronic epilepsy. Thirty-nine patients (41%) showed a generalized volume loss, and two (2%) showed a generalized gain in volume (Figure 19).

Quantification of the signal changes detected on visual assessment is shown in Tables 18-20. Mild generalized visual changes were associated with a 0.2% to 3.3% loss in TBV and moderate generalized visual changes with a 1.4% to 4.3% volume reduction. Temporal lobe volume losses ranged from a 3.6% to a 27.3% reduction in temporal lobe GM, and a 5.3% to a 29.6% reduction in WM. Frontal lobe GM losses in individuals with frontal lobe atrophy ranged from 0.3% to 7.4%, and WMV losses from 1.1% to 18.8%. GM and WM volume losses both contributed to the focal changes.

Based on the visual analysis, 1 of 27 (4%) patients with chronic TLE without HS and 2 of 12 (17%) patients with chronic TLE and HS developed extrahippocampal atrophy in the ipsilateral temporal lobe. None of the patients with newly diagnosed TLE and 2 of 82 (2%) control subjects developed temporal lobe atrophy. In chapter 7, we identified six patients (3.4%) with epilepsy with progressive hippocampal atrophy over 3.5 years. These patients did not develop temporal lobe atrophy over the same period.
Table 18  Changes in control subjects that were not attributable to confounding factors

<table>
<thead>
<tr>
<th>Visual assessment of</th>
<th>Quantification of change</th>
<th>Baseline Age / comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Change (number of cases)</strong></td>
<td><strong>Lobar</strong></td>
<td><strong>Global</strong></td>
</tr>
<tr>
<td>Bilateral temporal lobe volume loss (1)</td>
<td>6.0cm³ (15.4%) loss of WMV of right temporal lobe</td>
<td>57.3cm³ (5.3%) loss of TBV</td>
</tr>
<tr>
<td></td>
<td>6.1cm³ (18.3%) loss of WMV of left temporal lobe</td>
<td>46.8cm³ (7.8%) loss of GMV</td>
</tr>
<tr>
<td></td>
<td>No change in temporal lobe GMV</td>
<td>10.4cm³ (2.2%) loss of WMV</td>
</tr>
<tr>
<td>Right temporal lobe + moderate generalized volume loss (1)</td>
<td>3.9cm³ (13%) loss of WMV of right temporal lobe</td>
<td>35.5cm³ (2.8%) loss of TBV</td>
</tr>
<tr>
<td></td>
<td>1.8cm³ (3.9%) loss of GMV of right temporal lobe</td>
<td>42.3cm³ (4.9%) loss of GMV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.7cm³ (1.6%) gain in WMV</td>
</tr>
<tr>
<td>Marked right middle frontal + moderate generalized volume loss (1)</td>
<td>6.1cm³ (6.0%) loss of GMV of right frontal lobe</td>
<td>6.3cm³ (0.5%) loss of TBV</td>
</tr>
<tr>
<td></td>
<td>Minimal change in right frontal lobe WMV</td>
<td>No change in right frontal lobe GMV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No change in GMV</td>
</tr>
<tr>
<td>Moderate superior frontal + generalized volume loss (1)</td>
<td>3.2cm³ (4.2%) loss of WMV of right frontal lobe</td>
<td>6.3cm³ (0.5%) loss of TBV</td>
</tr>
<tr>
<td></td>
<td>No change in right frontal lobe GMV</td>
<td>No change in GMV</td>
</tr>
<tr>
<td></td>
<td>Minimal change in left frontal lobe WMV</td>
<td>6.3cm³ (0.5%) loss of WMV</td>
</tr>
<tr>
<td></td>
<td>1.2cm³ (1.2%) loss of GMV of left frontal lobe</td>
<td></td>
</tr>
<tr>
<td>Moderate cerebellar volume loss (1)</td>
<td>6.9cm³ (5.7%) loss of total cerebellar volume</td>
<td></td>
</tr>
<tr>
<td>Mild generalized volume loss (11)</td>
<td>Mean loss of TBV = 13.2cm³ (0.8%)</td>
<td>Median age for subjects with generalized volume losses only, 50 years; range 14-72 years.</td>
</tr>
<tr>
<td>Moderate generalized volume loss (4)</td>
<td>Mean loss of TBV = 16.9cm³ (1.4%)</td>
<td></td>
</tr>
<tr>
<td>Mild generalized volume gain (1)</td>
<td>26.2cm³ (2.1%) gain in TBV</td>
<td>28 yr</td>
</tr>
<tr>
<td></td>
<td>41.7cm³ (5.2%) gain in GMV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.8cm³ (3.4%) loss of WMV</td>
<td></td>
</tr>
</tbody>
</table>

TBV, total brain volume; WMV, white matter volume; GMV, grey matter volume. ‘Change’ refers to change in volume over 3.5 years.
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Visual assessment of change</th>
<th>Lobar quantification</th>
<th>Global quantification</th>
<th>Baseline Age / comment</th>
<th>AED</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLE (HS)</td>
<td>Mild generalized volume loss (1)</td>
<td></td>
<td>39.3 cm³ (3.3%) loss of TBV 34.5 cm³ (4.6%) loss of GMV</td>
<td>36 yr / meningitis at 18 months with complex FC. Bilateral HS. 88 SGS</td>
<td>CBZ</td>
</tr>
<tr>
<td>TLE (no HS)</td>
<td>Moderate superior frontal, parietal + generalized volume loss (1)</td>
<td>4.6 cm³ (5.9%) loss of frontal lobe WMV 2.9 cm³ (6.3%) loss of parietal lobe WMV Minimal change in frontal / parietal lobe GMV 6.7 cm³ (2.0%) loss of WMV</td>
<td>29.4 cm³ (2.5%) loss of TBV 22.6 cm³ (2.7%) loss of GMV</td>
<td>52 yr / cryptogenic TLE, ~20 CPS</td>
<td>VPA</td>
</tr>
<tr>
<td></td>
<td>Mild generalized volume loss (2)</td>
<td></td>
<td>Mean loss of TBV=10.8 cm³ (0.9%)</td>
<td>(1) 21 yr / 33 CPS, 1 SGS (2) 47 yr / only 1 CPS</td>
<td>CBZ, LTG, VPA, CLB</td>
</tr>
<tr>
<td></td>
<td>Moderate generalized volume loss (2)</td>
<td></td>
<td>Mean loss of TBV=26.2 cm³ (2.1%)</td>
<td>(1) 35 yr / 40 CPS, 1 SGS (2) 28 yr / &gt;150 SPS</td>
<td>CBZ, VPA, CBZ</td>
</tr>
<tr>
<td></td>
<td>Mild generalized volume gain (2)</td>
<td></td>
<td>4.0 cm³ (1.1%) gain in TBV 34.6 cm³ (4.2%) gain in GMV</td>
<td>27 yr / 1 SGS, 10 SPS</td>
<td>CBZ, LTG, CLB</td>
</tr>
<tr>
<td>FLE</td>
<td>Marked left superior middle frontal + cerebellar volume loss (1)</td>
<td>8.0 cm³ (7.7%) loss of cerebellar volume 11.7 cm³ (18.8%) loss of left frontal lobe WMV</td>
<td>31 yr / old infarct in left frontal lobe, 96 SGS, &gt;200 CPS</td>
<td></td>
<td>PHT, CBZ</td>
</tr>
<tr>
<td>Other focal</td>
<td>Mild bifrontal volume loss (1)</td>
<td>0.8 cm³ (1.4%) loss of frontal lobe WMV 2.8 cm³ (3.7%) loss of frontal lobe GMV</td>
<td></td>
<td>24 yr / no seizure recurrence</td>
<td>NIL</td>
</tr>
<tr>
<td></td>
<td>Marked left parietal + left lateral ventricular enlargement (1)</td>
<td>4.4 cm³ (11.1%) loss of WMV left parietal lobe No change in left parietal lobe GMV</td>
<td>5.2 cm³ (0.4%) loss of TBV 24.6 cm³ (5.4%) loss of WMV 19.1 cm³ (2.6%) gain in GMV</td>
<td>45 yr / no seizure recurrence</td>
<td>NIL</td>
</tr>
<tr>
<td></td>
<td>Mild generalized volume loss (1)</td>
<td></td>
<td>34.6 cm³ (5.7%) loss of WMV</td>
<td>31 yr / no seizure recurrence</td>
<td>VPA</td>
</tr>
<tr>
<td></td>
<td>Moderate generalized volume loss (1)</td>
<td></td>
<td>6.5 cm³ (1.7%) loss of WMV No change in TBV or GMV</td>
<td>30 yr / 5 CPS</td>
<td>CBZ, LTG</td>
</tr>
</tbody>
</table>

Table 19 Changes in newly diagnosed patients that were not attributable to confounding factors
Table 19 continued  Changes in newly diagnosed patients that were not attributable to confounding factors

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Visual assessment of change (number)</th>
<th>Lobar quantification</th>
<th>Global quantification</th>
<th>Age / comment</th>
<th>AED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalized</td>
<td>Moderate cerebellar + left medial temporal lobe volume loss (1)</td>
<td>8.0 cm³ (6.6%) loss of cerebellar volume</td>
<td>1.5 cm³ (5.3%) loss of left temporal lobe WMV</td>
<td>4.9 cm³ (10.0%) loss of left temporal lobe GMV</td>
<td>56 yr / 1 GTCS</td>
</tr>
<tr>
<td></td>
<td>Mild generalized volume loss (2)</td>
<td>Mean loss of TBV=29.1 cm³ (2.4%)</td>
<td>(1) 33 yr / 2 GTCS</td>
<td>NIL</td>
<td>VPA</td>
</tr>
<tr>
<td></td>
<td>Moderate generalized volume loss (3)</td>
<td>Mean loss of TBV=22.4 cm³ (1.9%)</td>
<td>(1) 56 yr / 2 GTCS</td>
<td>VPA</td>
<td>CBZ, VPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2) 19 yr / 4 GTCS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(3) 15 yr / 23 GTCS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Seizure frequency refers to the numbers of seizures between the baseline and repeat scan.
AED=antiepileptic drugs taken at any stage between the two scans; TLE=temporal lobe epilepsy; FLE=frontal lobe epilepsy; FC=febrile convulsion; HS=hippocampal sclerosis; CPS=complex partial seizures; SGS=secondary generalized tonic-clonic seizures; SPS=simple partial seizures; PHT=phenytoin; CBZ=carbamazepine; VPA=sodium valproate; CLB=clobazam; LTG=lamotrigine; NIL=no AEDs were taken; TBV=total brain volume; GMV=grey matter volume; WMV=white matter volume.
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Visual assessment of change (no.)</th>
<th>Lobar quantification</th>
<th>Global quantification</th>
<th>Age / comment</th>
<th>AED</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLE (HS)</td>
<td>Moderate left temporal lobe volume loss (1)</td>
<td>1.6cm³ (7.7%) loss of left temporal lobe WMV&lt;br&gt;Minimal change in left temporal lobe GMV</td>
<td></td>
<td>71 yr / 21 SGS, 220 CPS</td>
<td>CBZ, VPA</td>
</tr>
<tr>
<td>Bilateral temporal lobe (right &gt;left) + generalized volume loss (1)</td>
<td>5.6cm³ (26.9%) loss of temporal lobe WMV&lt;br&gt;5.7cm³ (27.3%) loss of temporal lobe GMV</td>
<td>9.5cm³ (0.8%) loss of TBV&lt;br&gt;8.6cm³ (1.1%) loss of GMV&lt;br&gt;1.2cm³ (0.3%) loss of WMV</td>
<td>34 year old with bilateral HS following&lt;br&gt;status epilepticus due to encephalitis.&lt;br&gt;50 yr / measles encephalitis at 18 months. 5 SGS between scans.</td>
<td>PB, CBZ, TPM, CLB, LTG</td>
<td></td>
</tr>
<tr>
<td>Moderate left frontal lobe and mild generalized volume loss (1) cerebellar atrophy + moderate left hemispheric volume loss (1)</td>
<td>1.6cm³ (3.1%) loss of left frontal WMV&lt;br&gt;1.0cm³ (1.3%) loss of left frontal GMV&lt;br&gt;0.2cm³ (1.6%) loss of left temporal lobe WMV&lt;br&gt;0.6cm³ (3.1%) loss of left occipital lobe GMV&lt;br&gt;6.1cm³ (4.8%) loss of cerebellar volume</td>
<td>40.8 cm³ (3.4%) loss of TBV&lt;br&gt;42.7 cm³ (6.4%) loss of GMV&lt;br&gt;5.7 cm³ (0.5%) loss of TBV&lt;br&gt;17.6 cm³ (4.1%) loss of WMV&lt;br&gt;11.5 cm³ (1.6%) gain in GMV</td>
<td>51 yr / 3 SGS, 172 CPS.&lt;br&gt;Birth injury with left parietal infarct.&lt;br&gt;50 yr / measles encephalitis at&lt;br&gt;18 months. 5 SGS between scans.</td>
<td>CBZ, LGT, CLB, PB</td>
<td></td>
</tr>
<tr>
<td>Mild generalized volume loss (4)</td>
<td>Mean loss of TBV=13.6cm³ (1.1%)&lt;br&gt;Mean loss of GM=5.9cm³ (0.8%)&lt;br&gt;Mean loss of WMV=7.8cm³ (2.0%)&lt;br&gt;Mean loss of TBV=26.7cm³ (2.2%)</td>
<td>Mean loss of TBV=26.7cm³ (2.2%)&lt;br&gt;Mean loss of GM=16.4cm³ (2.3%)&lt;br&gt;Mean loss of WMV=10.6cm³ (2.2%)</td>
<td>(1) 43 yr / 9 SGS&lt;br&gt;(2) 57 yr / 38 CPS&lt;br&gt;(3) 44 yr / 80 CPS&lt;br&gt;(4) 18 yr / 80 CPS</td>
<td>CBZ, LGT, GBP&lt;br&gt;CBZ, PHT, VGB, PRM&lt;br&gt;PHT, PB, CBZ&lt;br&gt;CBZ, LGT, GBP</td>
<td></td>
</tr>
<tr>
<td>Moderate generalized volume loss (2)</td>
<td>Mean loss of TBV=26.7cm³ (2.2%)&lt;br&gt;Mean loss of GM=16.4cm³ (2.3%)&lt;br&gt;Mean loss of WMV=10.6cm³ (2.2%)</td>
<td>Mean loss of TBV=16.4cm³ (2.3%)&lt;br&gt;Mean loss of GM=10.6cm³ (2.2%)&lt;br&gt;Mean loss of WMV=5.9cm³ (0.8%)</td>
<td>(1) 53 yr / 8 CPS&lt;br&gt;(2) 33 yr / 34 SGS. Occasional alcoholic binges.</td>
<td>PHT&lt;br&gt;CBZ, LGT, GBP</td>
<td></td>
</tr>
</tbody>
</table>
Table 20 Changes in patients with chronic active epilepsy that were not attributable to confounding factors.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Visual assessment of change (no.)</th>
<th>Lobar quantification</th>
<th>Global quantification</th>
<th>Age / comment</th>
<th>AED</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLE (no HS)</td>
<td>Moderate left temporal lobe and cerebellar volume loss (1)</td>
<td>2.6cm³ (8.8%) loss of left temporal lobe WMV 3.9cm³ (6.3%) loss of left temporal lobe GMV 5.2cm³ (5.3%) loss of cerebellar volume</td>
<td>32 yr / 17 SGS, 67 CPS. Minimal alcohol intake.</td>
<td>CBZ, LTG, NZ</td>
<td></td>
</tr>
<tr>
<td>Marked cerebellar volume loss (1)</td>
<td></td>
<td>13.4cm³ (14.7%) loss of cerebellar volume</td>
<td>43 yr / 100 CPS. Cerebellar function intact</td>
<td>PB, PHT, CBZ</td>
<td></td>
</tr>
<tr>
<td>Mild generalized volume loss (7)</td>
<td></td>
<td>Mean loss of TBV=16.1cm³ (1.4%) Mean loss of GMV=12.0cm³ (1.7%) Mean loss of WMV=4.0cm³ (0.8%)</td>
<td>(1) 61 yr / 10 CPS (2) 28 yr / 126 SGS, &gt;200 CPS (3) 37 yr / 42 SGS (4) 34 yr / 42 SGS (5) 24 yr / 187 SGS (6) 62 yr / 1 SGS, 35 CPS (7) 38 yr / 210 CPS</td>
<td>CBZ, VPA, TPM</td>
<td></td>
</tr>
<tr>
<td>Moderate generalized volume loss (5)</td>
<td></td>
<td>Mean loss of TBV=41.0cm³ (3.5%) Mean loss of GMV=36.0cm³ (5.0%) Mean loss of WMV=3.1cm³ (1.2%)</td>
<td>(1) 54 yr / 6 SGS, 140 CPS (2) 55 yr / 27 SGS, ~120 CPS (3) 38 yr / 410 CPS (4) 64 yr / 46 SGS, ~160 CPS (5) 18 yr / ~410 CPS</td>
<td>CBZ, GBP, CLB, TPM LTG, CBZ, PIR GBP, VPA, CBZ, LTG LTG, PHT, VPA CBZ, VPA</td>
<td></td>
</tr>
<tr>
<td>Marked generalized volume loss (1)</td>
<td></td>
<td>38.3cm³ (3.4%) loss of TBV 33.0cm³ (4.3%) loss of GMV 5.6cm³ (1.5%) loss of WMV</td>
<td>68 yr / 3 SGS</td>
<td>VPA, PHT, VGB</td>
<td></td>
</tr>
<tr>
<td>FLE</td>
<td>Moderate left superior frontal volume loss (1)</td>
<td>6.4cm³ (6.7%) loss of left frontal lobe GMV</td>
<td>25 yr / 165 CPS. Some geometric distortions due to superior-inferior positional differences.</td>
<td>CBZ, VPA, CLB, GBP, TPM, OCZ</td>
<td></td>
</tr>
<tr>
<td>Mild generalized volume loss (3)</td>
<td></td>
<td>Mean loss of TBV=6.7cm³ (0.6%) Mean loss of GMV=3.9cm³ (0.9%) Mean loss of WMV=2.9cm³ (0.5%)</td>
<td>(1) 31 yr / 14 SGS (2) 56 yr / &gt;1200 CPS (3) 68 yr / 13 SPS</td>
<td>PHT, PB, PHT, CLB, CBZ GBP, PHT</td>
<td></td>
</tr>
</tbody>
</table>
### Table 20 Changes in patients with chronic active epilepsy that were not attributable to confounding factors.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Visual assessment of change (no.)</th>
<th>Lobar quantification</th>
<th>Global quantification</th>
<th>Age / comment</th>
<th>AED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild generalized volume gain (2)</td>
<td>Mean gain in TBV=17.3cm³ (1.5%)&lt;br&gt;Mean gain in GMV=27.1cm³ (3.8%)&lt;br&gt;Mean loss in WMV=9.9cm³ (2.0%)</td>
<td>(1) 16 yr / 80 SGS, 240 CPS&lt;br&gt;(2) 28 yr / 294 SGS</td>
<td>VGB, VPA, CLB, CBZ&lt;br&gt;TPM, GBP, LEV&lt;br&gt;CBZ, LTG, VPA, ACE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other focal</td>
<td>Marked right frontal volume loss (1)</td>
<td>Mean gain in TBV=17.3cm³ (1.5%)&lt;br&gt;Mean gain in GMV=27.1cm³ (3.8%)&lt;br&gt;Mean loss in WMV=9.9cm³ (2.0%)</td>
<td>(1) 16 yr / 80 SGS, 240 CPS&lt;br&gt;(2) 28 yr / 294 SGS</td>
<td>VGB, VPA, CLB, CBZ&lt;br&gt;TPM, GBP, LEV&lt;br&gt;CBZ, LTG, VPA, ACE</td>
<td></td>
</tr>
<tr>
<td>Other focal</td>
<td>Moderate superior frontal + mild generalized volume loss (1)</td>
<td>Mean gain in TBV=17.3cm³ (1.5%)&lt;br&gt;Mean gain in GMV=27.1cm³ (3.8%)&lt;br&gt;Mean loss in WMV=9.9cm³ (2.0%)</td>
<td>(1) 16 yr / 80 SGS, 240 CPS&lt;br&gt;(2) 28 yr / 294 SGS</td>
<td>VGB, VPA, CLB, CBZ&lt;br&gt;TPM, GBP, LEV&lt;br&gt;CBZ, LTG, VPA, ACE</td>
<td></td>
</tr>
<tr>
<td>Other focal</td>
<td>Moderate generalized + cerebellar volume loss (1)</td>
<td>Mean gain in TBV=17.3cm³ (1.5%)&lt;br&gt;Mean gain in GMV=27.1cm³ (3.8%)&lt;br&gt;Mean loss in WMV=9.9cm³ (2.0%)</td>
<td>(1) 16 yr / 80 SGS, 240 CPS&lt;br&gt;(2) 28 yr / 294 SGS</td>
<td>VGB, VPA, CLB, CBZ&lt;br&gt;TPM, GBP, LEV&lt;br&gt;CBZ, LTG, VPA, ACE</td>
<td></td>
</tr>
<tr>
<td>Other focal</td>
<td>Mild generalized volume loss (2)</td>
<td>Mean gain in TBV=17.3cm³ (1.5%)&lt;br&gt;Mean gain in GMV=27.1cm³ (3.8%)&lt;br&gt;Mean loss in WMV=9.9cm³ (2.0%)</td>
<td>(1) 16 yr / 80 SGS, 240 CPS&lt;br&gt;(2) 28 yr / 294 SGS</td>
<td>VGB, VPA, CLB, CBZ&lt;br&gt;TPM, GBP, LEV&lt;br&gt;CBZ, LTG, VPA, ACE</td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td>Mild bilateral superior frontal volume loss (1)</td>
<td>Mean gain in TBV=17.3cm³ (1.5%)&lt;br&gt;Mean gain in GMV=27.1cm³ (3.8%)&lt;br&gt;Mean loss in WMV=9.9cm³ (2.0%)</td>
<td>(1) 16 yr / 80 SGS, 240 CPS&lt;br&gt;(2) 28 yr / 294 SGS</td>
<td>VGB, VPA, CLB, CBZ&lt;br&gt;TPM, GBP, LEV&lt;br&gt;CBZ, LTG, VPA, ACE</td>
<td></td>
</tr>
</tbody>
</table>

---

185
Table 20 Changes in patients with chronic active epilepsy that were not attributable to confounding factors.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Visual assessment of change (no.)</th>
<th>Lobar quantification</th>
<th>Global quantification</th>
<th>Age / comment</th>
<th>AED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate cerebellar volume loss (1)</td>
<td>2.8 cm³ (2.2%) loss of cerebellar volume</td>
<td></td>
<td></td>
<td>25 yr / 1 GTCS. No symptomatic PHT intoxication. Cerebellar function intact.</td>
<td>PHT, CZP</td>
</tr>
<tr>
<td>Moderate generalized + cerebellar volume loss (1)</td>
<td>4.1 cm³ (3.7%) loss of cerebellar volume</td>
<td>53.0 cm³ (4.4%) loss of TBV</td>
<td>46 yr / 88 GTCS</td>
<td>PRM, VPA, LTG, GBP</td>
<td></td>
</tr>
<tr>
<td>Mild generalized volume loss (1)</td>
<td></td>
<td>11.4 cm³ (2.0%) loss of WMV</td>
<td>Normal cerebellar function.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate generalized volume loss (9)</td>
<td>Mean loss of TBV=34.6 cm³ (2.8%)</td>
<td></td>
<td>Mean loss of GMV=31.9 cm³ (4.2%)</td>
<td></td>
<td>CBZ, LTG, VPA, CLB</td>
</tr>
<tr>
<td></td>
<td>Mean loss of WMV=2.7 cm³ (1.4%)</td>
<td></td>
<td>(1) 19 yr / 54 GTCS</td>
<td>CBZ, LTG, VPA, CLB</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2) 21 yr / 1 GTCS</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(3) 33 yr / &gt;100 absences</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4) 17 yr / Learning disability. 4 GTCS.</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(5) 48 yr / 5 GTCS.</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6) 21 yr / No seizures. Hypoparathyroidism.</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(7) 74 yr / 3 GTCS</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(8) 31 yr / 22 GTCS</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(9) 29 yr / 12 GTCS, &gt;80 absences,</td>
<td>CBZ, VPA, ESM</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>Mild generalized volume loss (2)</td>
<td>Mean loss of TBV=0.48 cm³ (0.1%)</td>
<td>Mean loss of GMV=12.3 cm³ (2.3%)</td>
<td>Mean gain in GMV=11.8 cm³ (1.8%)</td>
<td>ACET, LTG, TPM, ESM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1) 16 yr / 8 CPS</td>
<td>(2) 57 yr / 25 SGS, 5 CPS</td>
<td></td>
<td>CBZ, DZP, VPA</td>
</tr>
</tbody>
</table>

GBP, gabapentin; VGB, vigabatrin; PHT, phenytoin; PRM, primidone; PB, phenobarbitone; NZ, nitrazepam; CLB, clobazam; TPM, topiramate; PIR, piracetam; DZP, diazepam; ESM, ethosuximide; TGB, tiagabine; ACE, acetazolamide; CZP, clonazepam; CBZ, carbamazepine; OCZ, oxcarbazepine. Antiepileptic drug refers to antiepileptic drug exposure at any stage during the follow-up period.
8.4.3 Group analysis

8.4.3.1 Comparison of signal change between subject groups

The proportions of subjects with focal change, generalized volume loss and no change were significantly different between the three cohorts ($\chi^2 = 17.1, \ p = 0.002$). Further analysis showed that significantly more patients with chronic epilepsy developed focal ($\chi^2 = 6.1, \ p = 0.013$) and generalized volume losses ($\chi^2 = 14.0, \ p = 0.0002$) compared with control subjects over the 3.5 years. There were no significant differences in the changes between controls and newly diagnosed patients or between newly diagnosed patients and those with chronic active epilepsy.

8.4.3.2 Risk factors for neocortical damage

Clinical characteristics of patients with focal neocortical volume loss, generalized change and no change are compared in Table 21. The pattern of change was significantly associated with baseline age (Kruskal-Wallis $\chi^2 = 15.9, \ p < 0.0001$) and AED exposure during the follow-up period (Kruskal-Wallis $\chi^2 = 12.8, \ p = 0.002$). Associations with seizure recurrence ($\chi^2 = 7.31, \ p = 0.026$) and epilepsy duration (Mann Whitney U 431, $\ p = 0.057$) were non-significant when multiple comparisons were accounted for. In the newly diagnosed group, patterns of atrophy were not significantly different between patients who continued to experience seizures and those for whom seizures were controlled with medication. Overall, there was no association with frequency of generalized seizures, gender, head injury, IPI, SE, or age of seizure onset. Furthermore, epilepsy syndrome did not influence the pattern of atrophy ($\chi^2 = 5.71, \ p = 0.22$). Since excessive alcohol consumption is known to influence brain volume (Liu et al., 2000), we excluded subjects with a history of alcohol abuse from our individual analyses. However, no statistical relationship was found between alcohol abuse and generalized volume loss on group analysis.

Post hoc analysis showed that individuals with focal neocortical volume losses were older (Mann-Whitney U 948, $\ p = 0.007$) and had been exposed to a greater number of AEDs (Mann-Whitney U 353, $\ p = 0.006$) than those with no change. Control subjects and patients with generalized atrophy were significantly older (Mann-Whitney U 2944, $\ p = 0.001$) and had taken more AEDs (Mann-Whitney U 1183, $\ p = 0.003$) than their counterparts without atrophy. The median baseline ages of the controls (50 years),...
newly diagnosed patients (32 years) and patients with chronic epilepsy (43 years) with generalized atrophy were not significantly different.

Table 21  Comparison of clinical characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Focal neocortical volume loss (N=23)</th>
<th>Generalized neocortical volume loss (N=68)</th>
<th>No change (N=127)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range) at baseline, yr *</td>
<td>43.0 (14-77)</td>
<td>39.5 (14-74)</td>
<td>31.0 (14-67)</td>
</tr>
<tr>
<td>Median duration of epilepsy, yr (range)</td>
<td>21.5 (0-66)</td>
<td>13.0 (0-68)</td>
<td>9.0 (0-47)</td>
</tr>
<tr>
<td>Median number (range) of AEDs*</td>
<td>2.5 (0-6)</td>
<td>2.0 (0-4)</td>
<td>1.0 (0-6)</td>
</tr>
<tr>
<td>Median number (range) of GTCS</td>
<td>2 (0-157)</td>
<td>2 (0-187)</td>
<td>0 (0-492)</td>
</tr>
<tr>
<td>Median age (range) at seizure onset</td>
<td>15.5 (1-56)</td>
<td>19.0 (0-62)</td>
<td>17.0 (1-61)</td>
</tr>
</tbody>
</table>

Median age (range) at baseline was calculated using data from control subjects and patients. The remaining variables were calculated from patient data only. Subjects with both focal and generalized volume loss showed a dominant effect of focal atrophy and therefore were included in the focal neocortical category.

AEDs = antiepileptic drugs taken between the two scans. GTCS = generalized tonic clonic seizures.

* Significant differences between the three groups (Kruskal-Wallis, p<0.013).
8.5 DISCUSSION

Cross-sectional and longitudinal quantitative MRI studies in epilepsy have mainly focussed on the relationship between seizures and hippocampal damage (Briellmann et al., 2002a), (Liu et al., 2001). The present study focuses on the development of global and focal neocortical damage in epilepsy using a voxel-based technique that interrogates the whole brain. Patients with chronic epilepsy were significantly more likely to develop generalized and focal cerebral atrophy over 3.5 years compared with control subjects. The patterns of volume loss were heterogeneous and associated with increased age and multiple AED exposure.

8.5.1 Methodological considerations

Biological changes in brain volume were identified using difference image analysis and removal of artifactual noise. The subtraction of registered images allowed the detection of subtle volume changes that could be missed on visual comparison or obscured by misregistration artifact. The voxel-based approach allowed evaluation of neocortical regions beyond areas of a priori interest. The study is based on a two-step approach: visual identification of genuine changes in the filtered difference images, followed by quantification of focal changes using a regional brain atlas applied to segmented source images and quantification of generalized changes using automatically derived measures of TBV, GMV and WMV (Liu et al., 2001).

For this study, the difference images used to construct the structured noise map were derived from scans performed 7 months apart rather than same-day repeat scans, to correct for physiological effects such as changes in nutrition, hydration, and menstrual phase. A limitation of the structured noise map is that a map constructed from control subjects may not be as noisy as one constructed from patients, who may move more during a scan than controls (Lemieux et al., 1998b). Therefore, although we assumed that persistent differences in the map of changed voxels in our study controls were due to biological changes, artifacts may also have contributed to the observed patterns of volume loss. We chose a threshold of 1 to 40 (0.025) because this would increase our ability to detect “genuine change” by minimizing the number of false-positives, even though this would lead to a generalized reduction in sensitivity to change in both patients and controls. Twelve percent of our patients were excluded from the analysis.
due to explicable changes such as progressive structural brain lesions, neurosurgical intervention and alcohol abuse. In addition, large hypo-intense lesions rendered the normalization process inaccurate in some cases, resulting in a mismatch around the lesion.

The proportions of patients with neocortical (generalized and focal) volume loss were far greater in this study (54% of chronic epilepsy patients and 40% of newly diagnosed patients) than identified in our region-based study (see chapter VII) which identified neocortical volume losses in 6.6% and 3.4% patients respectively (Liu et al., 2002c). Although both the region-based and voxel-based study use the same basic quantification (segmentation) process, they differ in the way change in individuals was detected. The discrepancy in the detection rates reflects the greater sensitivity provided by the difference image voxel-based approach, the additional identification of focal and diffuse changes not detectable using hemispheric measures of GM and WM, and our choice of subjects used to create the structured noise map which allowed the detection of physiological changes such as ageing in both patients and controls. Although the median age of the patients and controls with generalized volume loss were statistically comparable, the older age (median, 50 years) of the control subjects may have had biological implications that might have contributed to the relatively high proportion of control subjects with neocortical changes. Individuals in whom there were clear artifacts or changes that could be attributed to other factors were excluded from the analysis, and this may have contributed to an underestimate of focal and generalized volume losses. Our rates should therefore be regarded as minimum estimates.

Visual assessment of the maps of changed voxels was not sensitive at detecting hippocampal volume changes. This may be due to several reasons. The hippocampus is a small structure and therefore volume changes may be more susceptible to obscuration by pulsation artifacts within the temporal lobe (Lemieux et al., 1998b). Another explanation may be the lack of intensity differences between the hippocampus and neighbouring tissues along its inferomedial border.
8.5.2 Patterns of neocortical atrophy in epilepsy
The commonest pattern of cerebral damage observed in all epilepsy syndromes and throughout all subject groups was a generalized volume loss, seen in 30% of patients with chronic generalized epilepsy, 19% of patients with newly diagnosed generalized epilepsy and 17% of controls. The magnitude of the volume loss was comparable between the different subject groups. Patients with chronic epilepsy were significantly more likely to develop generalized volume loss than control subjects. Although a positive association was seen with age, the age distribution between the control, newly diagnosed, and chronic epilepsy group was similar, suggesting that factors other than age were instrumental in the development of generalized atrophy. Generalized atrophy was more commonly seen in patients with increased exposure to AEDs independent of seizure control, suggesting that antiepileptic medication may compound the effect of age-related atrophy. One control subject, one newly diagnosed patient, and two patients with chronic epilepsy had a generalized volume gain that was detected on both visual analysis and quantitative assessment. These volume increases could not be attributed to known obvious confounding factors e.g. alcohol abstention (Liu et al., 2000), but may have been explained by physiological variations between the two scans e.g. menstrual changes or covert alcohol histories.

8.5.3 Temporal lobe epilepsy and temporal lobe atrophy
The patterns of focal necortical damage were heterogeneous and did not appear to relate to a history of focal or generalized seizures. Temporal lobe atrophy developed in 17% patients with chronic TLE and HS, 4% patients with chronic TLE without HS, 2% controls, and 6% patients with generalized epilepsy. It has been suggested that HS is likely to be the result of an IPI rather than the consequence of repeated limbic seizures (Mathern et al., 2002). The relatively high incidence of temporal lobe atrophy in TLE patients with severe hippocampal damage, and the lack of further hippocampal volume loss in these patients, is consistent with the “floor effect” (Briellmann et al., 2000). This suggests that a severe neurological insult is capable of damaging the hippocampus to such an extent that end-stage hippocampal atrophy is reached. Proton MR spectroscopic imaging studies have demonstrated progressive neuronal dysfunction in the temporal lobes following an initial fixed brain injury (Tasch et al., 1999). Our findings are
consistent with these reports, in that they suggest that neuronal damage may extend beyond the hippocampus to involve the ipsilateral temporal lobe.

8.5.4 Putative risk factors
Although focal and generalized neocortical changes were more commonly seen in patients with chronic epilepsy, no association was seen with seizure frequency (convulsive or partial).

Although no association was observed between SE and neocortical volume loss, only 2% of patients suffered an episode of SE in the follow-up period, and any lack of association should be confirmed with larger numbers of patients. These findings suggest that the increased risk of neocortical damage shown by patients with chronic epilepsy is not related to a history of overt seizures. Putative factors for an increased risk of secondary cerebral damage include an underlying epileptic process such as a widespread developmental abnormality that leads to subclinical atrophy, longer exposure to multiple AEDs, and genetic susceptibility to brain insults (Kanemoto et al., 2000). The notion that genetic background may influence seizure-induced damage is demonstrated by pronounced strain-dependent differences in damage induced by SE and the modifying effect of genetic background in transgenic mutant mice with seizures (Schauwecker, 2002). The suggestion that the risk of developing cerebral damage is linked to the background process is consistent with theories that prognosis and responsiveness to AED therapy may be inherent and dependent on the epileptic process itself (Sander, 1993). It is possible that a combination of these factors and subclinical seizure activity may be superimposed on structural changes associated with normal ageing.

8.5.5 Comparison with region-based volumetric findings
On first appearances, these results may appear to conflict with the results of our region-based volumetric findings from the same population (see chapter VII). This showed that the reduction in hippocampal, cerebellar and cerebral volumes in patients with chronic epilepsy were primarily determined by a prior neurological insult and not by a diagnosis of epilepsy. We propose that damage to the hippocampus, cerebellum and whole brain is the result of an initial precipitating injury, whereas subsequent damage observed in
chronic epilepsy is subtle, diffuse, or focal and not evident using hemispheric measures of grey and white matter. Further, as noted previously the sensitivity of the voxel-based approach allowed the additional detection of subtle and focal changes. These hypotheses are consistent with suggestions by Mathern et al. that hippocampal neuronal loss correlates primarily with an initial precipitating injury, and additional effects are small and associated with a very prolonged duration of epilepsy (Mathern et al., 2002). Corroborating earlier positron-emission tomography (Koepp et al., 1997a) and single-photon-emission CT studies (Rabinowicz et al., 2002), we have demonstrated that cerebral damage was not restricted to the putative epileptic focus, but may be subtle and diffuse - possibly reflecting widespread cortical networks (Goldman-Rakic, 1988).

8.6 CONCLUSION
In conclusion, we identified focal or generalized neocortical atrophy in 54% patients with chronic epilepsy, 39% newly diagnosed patients and 24% control subjects using an objective approach that evaluates the entire brain. Factors associated with increased risk of neocortical damage were increased age and multiple AED exposure. Although neocortical atrophy was significantly more common in patients with chronic epilepsy, the increased risk was not related to the frequency of overt convulsive or partial seizures. Our findings favour the hypothesis that cerebral damage occurs in response to an underlying epileptic process and multiple AED exposure, rather than the direct consequence of overt seizures. The heterogeneity of our findings reflects the wide range of etiologies underlying epilepsy and suggests that multiple pathogenic mechanisms and individual genetic predisposition may be involved.
CHAPTER IX  CONCLUSIONS

9.1  INTRODUCTION

The theory that recurrent seizures lead to cumulative cerebral damage remains unsubstantiated despite extensive clinico-pathological and imaging studies. Epilepsy is the commonest chronic neurological condition affecting 1:150 to 1:200 individuals in the United Kingdom (MacDonald et al., 2000). Consequently, elucidation of the structural consequences of seizures is pertinent not only to epileptologists and general neurologists, but to all health professionals likely to come into contact with this condition.

The most enduring theory is that cerebral damage, particularly HS arises from a significant cerebral insult early in life. Although a prolonged or complex febrile convulsion (Berg et al., 1992) is considered a major risk factor, events such as an intracerebral infection, perinatal ischaemia, birth trauma and head injury have also been implicated (Rocca et al., 1987), (Gastaut et al., 1959). The suggestion that excitotoxic injury and structural damage, may arise from repeated seizures is derived from experimental studies, cross-sectional observational studies of patients with epilepsy, and longitudinal MRI studies involving single case reports and predominantly small, uncontrolled patient series (Worrell et al., 2002), (O'Brien et al., 1999), (Briellmann et al., 2002a), (Fuerst et al., 2003).

This thesis aimed to elucidate the relationship between cerebral damage and seizures using a large prospective community-based observational study that quantified morphological brain changes in 122 patients with chronic active epilepsy, 68 patients with newly diagnosed seizures and 90 control subjects over a 3.5-year period. Sensitive analytical tools allowed the detection of subtle structural change, whilst a combination of region- and voxel-based analyses allowed the quantification of anatomical regions of interest and interrogation of the whole brain.
9.2 SUMMARY OF THE MAIN FINDINGS

Volumetric approaches used in this study were capable of detecting serial hippocampal volume changes greater than 3.1%, cerebellar volume changes greater than 3.0% and automated brain volumes greater than 1%.

Significant cross-sectional relationships between age and reductions in intracranial volume, cerebellar volume and grey matter in healthy individuals were likely to reflect uniform rates of volume loss or secular changes.

Ageing in healthy individuals was associated with increased cerebral atrophy from the age of 35 to 54 years onwards and increased rates of hippocampal atrophy from the age of 54.

Baseline hippocampal, neocortical and cerebellar volumes were significantly different between the three groups, being lowest in the chronic epilepsy group, highest in the controls and intermediate in the newly diagnosed patients. The differences were attributed to antecedent neurological insults rather than duration of epilepsy.

Rates of hippocampal, cerebellar and cerebral atrophy were comparable in control subjects, patients with newly diagnosed seizures and patients with chronic epilepsy; and primarily determined by age. A history of a prior neurological insult was associated with an increased rate of cerebral and cerebellar atrophy over 3.5 years.

Significant atrophy of the hippocampus, neocortex or cerebellum occurred in significantly more patients with chronic epilepsy (20/121, 16.5%), than patients with newly diagnosed seizures (5/58, 8.6%) or control subjects (3/90, 3.3%).

Overt seizures, duration of epilepsy, antiepileptic drug use, status epilepticus, and gender had no effect on the change in brain volume or hippocampal T2-relaxation time.

Rates of hippocampal, cerebellar and cerebral atrophy were not syndrome-specific and comparable in patients with IGE, TLE with HS, TLE without HS and extratemporal focal epilepsy.

Focal and generalized neocortical atrophy was significantly more likely to develop in patients with chronic epilepsy than control subjects. Voxel-based analyses identified
neocortical volume losses in 54% of patients with chronic epilepsy, 39% of newly diagnosed patients and 24% of control subjects. A significant proportion of these changes were due to physiological changes such as ageing.

Consistent with findings from our region-based approach, neocortical damage was associated with age but not with a history of overt seizures. Additional risk factors for neocortical atrophy included multiple antiepileptic drug exposure and chronic epilepsy.

9.3 NEUROBIOLOGICAL IMPLICATIONS
The epilepsies are a heterogeneous range of conditions covering a wide range of aetiologies and syndromes. It was therefore important to identify individuals showing significant changes in imaging parameters as well as analyses of group means. It was evident from the individual analyses of this and other studies, that MRI-detectable hippocampal and neocortical damage can develop in some patients with epilepsy over several years and even months (Liu et al., 2002a), (Briellmann et al., 2002a), (Worrell et al., 2002), (Wiesmann et al., 1997), (Fuerst et al., 2003). Identification of these individuals is important in identifying the biological factors that places them at greatest risk from cerebral damage, and may distinguish those at increased risk of cognitive impairment. This study showed that although the risk of continuing damage that confounds age-related atrophy may be increased by underlying epileptic processes such as widespread developmental abnormalities, AED exposure and individual genetic susceptibility; most of the damage occurs following an initial precipitating injury. This injury may also predispose individuals to increased rates of cerebral and cerebellar atrophy. Thus, secondary cerebral damage may occur with epilepsy, but was not shown to be a direct consequence of overt seizures in our study. Neocortical damage was, in general, widespread and remote from the putative epileptic focus and may reflect extensive networks and interconnections between cortical regions.

Therapies to prevent cerebral damage in epilepsy should therefore aim to identify and ameliorate the effects of initial pathological processes, including avoidance and prompt treatment of febrile convulsions, and neuroprotective agents to minimize damage from perinatal ischaemia and traumatic head injury. Compared with experimental models,
the study showed that cerebral damage is not an inevitable consequence of overt seizures and therefore antiepileptic agents that merely control seizures are unlikely to reduce the risk of structural damage. Conversely, antiepileptogenic compounds with neuroprotective properties targeted at specific individuals are more likely to prevent or limit the extent of neuronal damage.

A requisite for the implementation of neuroprotective strategies is an effective means of assessing the impact of interventions. This work shows that serial volumetry and difference image analysis can act as useful surrogate markers when evaluating and comparing the relative effectiveness of neuroprotective agents.

9.4 LIMITATIONS OF THE STUDY

Whilst the community-based design had the advantage of being a more accurate representation of the typical case-mix seen in patients with epilepsy, allowed investigation into a wide range of individual variation, and permitted the rapid identification of patients following their first seizure; the resultant cohorts were inevitably heterogeneous. Although the substantial numbers of patients re-scanned allowed sub-group analyses according to epilepsy syndrome, follow-up of more homogeneous groups might identify more subtle syndromic-related evidence of structural damage. It is also likely that documentation of seizures particularly complex partial seizures over a prolonged period may be more accurate in a hospital-based series where patients are more regularly monitored and recorded.

Although longitudinal studies over a longer time frame would be of considerable interest, the need for reproducible techniques necessitates that post-processing techniques are limited by the image acquisition techniques that were available at the time of the initial scan. Further, a balance needs to be met between allowing a sufficiently long period of follow-up to allow the identification of biological differences between control and patient subjects, whilst offsetting reduced longitudinal integrity through increased attrition rates, scanner inconsistency and scanner upgrades.

It is evident from this and other longitudinal studies that the magnitude of volumetric change in the brain remains small. In this regard, new volumetric methods of the
neocortex based on difference-image analysis e.g. using the brain boundary shift integral, might produce more objective methods of directly quantifying signal change.

9.5 FUTURE WORK

This study focussed on structural changes over 3.5 years. Follow-up of well-characterised patient groups over longer time-frames might identify individuals with particular features that increase the risk of epilepsy-related damage. The inclusion of serial neuropsychological data could provide a functional correlate to quantifiable volume loss. Attention needs to be invested into the investigation of cerebral insults and the mechanisms through which they may influence subsequent cerebral and cerebellar atrophy.

The heterogeneity of the study findings likely reflects not only the range of aetiologies observed in a community-based population, but pathogenic mechanisms such as genetic vulnerability. A situation akin to patients with drug-refractory epilepsy and drug-resistance proteins may exist where patients may be genetically predisposed to the development of cerebral damage. Genetic analysis looking at polymorphisms and susceptibility factors in animals (Lere et al., 2002) and humans either resistant or susceptible to the development of cerebral damage would be of considerable interest.

This work does not exclude the possibility that hippocampal changes may occur that are beyond the resolution of MRI. Hippocampal neuronal loss has been observed on resected specimens from MRI negative patients, and the advent of 3-Tesla scanners may help resolve this issue. This would, however, require stringent calibration techniques to ensure the integrity of longitudinal studies spanning the use of 1.5- and 3-Tesla scanners. Current efforts to increase reproducibility and reduce operator intervention by automation, for example through automatic propagation of manually-drawn baseline segmentation to co-registered follow-up scans (Schnabel et al., 1999), may provide more sensitive measures in the future, though their current performance does not surpass the current study method. Further MR contrasts e.g. diffusion transfer and magnetic transfer ratio imaging may detect more abnormalities than standard T1- and T2-weighted imaging and may be more sensitive to subtle changes over time. Serial MR spectroscopic studies may also be of value in assessing functional changes that are
not detected on structural imaging, however, changes are non-specific and test-retest reliability is comparatively poor. Concerns over the reproducibility of single photon emission computed tomography and positron emission tomography studies have similarly limited their use in longitudinal studies.
Bibliography


Hagemann, G., Lemieux, L., Free, S.L., Krakow, K., Everitt, A.D., Kendall, B.E.,
Stevens, J.M., and Shorvon, S.D. Cerebellar volumes in newly diagnosed and chronic

Hajnal, J.V., Saeed, N., Oatridge, A., Williams, E.J., Young, I.R., and Bydder, G.M.
(1995a). Detection of subtle brain changes using subvoxel registration and subtraction

Hajnal, J.V., Saeed, N., Soar, E.J., Oatridge, A., Young, I.R., and Bydder, G.M.

Haller, J.W., Banerjee, A., Christensen, G.E., Gado, M., Joshi, S., Miller, M.I.,
hippocampal MR morphometry with high-dimensional transformation of a

Hammers, A., Koepp, M.J., Free, S.L., Brett, M., Richardson, M.P., Labbe, C.,
Cunningham, V.J., Brooks, D.J., and Duncan, J.S. (2002). Implementation and
application of a brain template for multiple volumes of interest. Hum. Brain Mapp; 15:
165-174.

Hammers, A., Allom, R., Koepp, M.J., Free, S.L., Myers, R., Lemieux, L., Mitchell,
atlas of the human brain, with particular reference to the temporal lobe. Hum. Brain

binding site for phenytoin in the cerebellum. Epilepsia; 24: 269-274.

Hand, K.S., Baird, V.H., Van Paesschen, W., Koepp, M.J., Revesz, T., Thom, M.,


tolerance' for investigating neuroprotection, epileptic susceptibility and gene

Li, L.M., Fish, D.R., Sisodiya, S.M., Shorvon, S.D., Alsanjari, N., and Stevens, J.M.
(1995). High resolution magnetic resonance imaging in adults with partial or secondary
generalised epilepsy attending a tertiary referral unit. J.Neurol.Neurosurg.Psychiatry;
59: 384-387.

Li, L.M., Cendes, F., Watson, C., Andermann, F., Fish, D.R., Dubeau, F., Free, S.,
Olivier, A., Harkness, W., Thomas, D.G., Duncan, J.S., Sander, J.W., Shorvon, S.D.,
pathology: relevance of lesion and of hippocampal atrophy to seizure outcome.
Neurology; 48: 437-444.

Li, L.M., Cendes, F., Andermann, F., Watson, C., Fish, D.R., Cook, M.J., Dubeau, F.,
Duncan, J.S., Shorvon, S.D., Berkovic, S.F., Free, S., Olivier, A., Harkness, W., and
Brain; 122: 799-805.

World; 5: 31-36.


Liu, R.S., Lemieux, L., Bell, G.S., Bartlett, P.A., Sander, J.W., Sisodiya, S.M.,
Shorvon, S.D., and Duncan, J.S. (2001). A longitudinal quantitative MRI study of
community-based patients with chronic epilepsy and newly diagnosed seizures:
methodology and preliminary findings. Neuroimage; 14: 231-243.

Liu, R.S., Lemieux, L., Bell, G.S., Bartlett, P.A., Sander, J.W., Sisodiya, S.M.,
Shorvon, S.D., and Duncan, J.S. (2002a). The structural consequences of newly


APPENDIX

SCRIPTS AND PROGRAMS

List of locally developed scripts and programs used in this PhD. Functionality of each item is described specifically as for this work.

**User-called scripts and programs**

**Name: mrw**

*Description:* Main processing script for registration and segmentation of scan pairs in batches

*Version used in project for this purpose:* mrwv1.6

*Input:* pairs of scans (Analyze format) grouped in subject-specific sub-directories

*Output:* Scripts and programs called: nuc (N3), Exbrain, MRreg

**Name: w2b**

*Description:* For blinding registered and segmented scan pairs (mrw output) with regards to subject name and scan order.

*Version used in project for this purpose:* w2b

*Input:* Registered and segmented pairs of scans (Analyze format) grouped in subject-specific sub-directories in a batch-specific directory

*Output:* new directories, with names from 1 to #subjects in batch, each containing anonymous links to the input data (registered & segmented scans).

*Scripts and programs called:* none

**Name: mreg (MRreg)**

*Description:* Registration; Hippocampal and cerebellar volumetry; Structured difference image analysis

*Version used in project:* mreg_1.5.5 (registration and volumetry)

*Input:* For registration and volumetry: pair of scans; For SDI analysis: normalised baseline and SDI, and SNM.

*Output:* For registration: registration parameters, resampled image; For volumetry: ROI and volume files.
**Name:** exbrain (Exbrain 2)

**Description:** Brain segmentation: GM, WM, CSF

**Version used in project for this purpose:** exbrain_2.4.2

**Input:** T1 volume (Analyze format)

**Output:** brain and IC images; GM, WM, CSF, TBV and IC volumes

**Scripts and programs called:** none

---

**Name:** wsdi

**Description:** create structured difference image

**Version used in project for this purpose:** wsdi

**Input:** co-registered and subtracted scan pair

**Output:** structured difference image

**Scripts and programs called:** mreg, fliph

---

**Name:** sdi2b

**Description:** to blind SDI

**Version used in project for this purpose:** sdi2b

**Input:** blinded batch number

**Output:** blinded links to normalised base and SDL

**Scripts and programs called:** none

---

**Name:** wdiff

**Description:** To view difference images, blinded

**Version used in project for this purpose:** wdiff

**Input:** blinded batch number

**Output:** blinded visual display of correctly chronologically ordered registered scan pairs and difference

**Scripts and programs called:** mreg
SPM99: Used for normalizing base scans and SDI for visual analysis; used for normalising AH atlas to patient scans.

Name: AHat_report

Description: to do ROI tissue analyses

Version used in project for this purpose: AHat_report v1.1

Input: Volume analysis: Tissue (GM, WM) map (from Exbrain)

Output: .rois file with tissue volumes for each ROI

Scripts and programs called: none
Script-called scripts and programs

Name: **nuc**

*Description:* Perform N3 non-uniformity correction, with brain mask

*Version used in project for this purpose:* nuc_1.0

*Input:* Volume scan (Analyze format)

*Output:* Basic brain-segmented image volume, non-uniformity corrected volume

*Scripts and programs called:* anh, exbrain, irspgr2minc, minc2anal; nu_correct, mincmath, mincreshape.

---

Name: **isrpgr2minc**

*Description:* Convert Chalfont volume Analyze image to MINC format

*Version used in project for this purpose:* isrpgr2minc_1.0

*Input:* Chalfont IRSPGR volume scan in analyze format

*Output:* MINC format

*Scripts and programs called:* anhr, range

---

Name: **minc2anal**

*Description:* Convert MINC image to analyze format

*Version used in project for this purpose:* minc2anal_1.0

*Input:* MINC format image

*Output:* Analyze format

*Scripts and programs called:* minctoraw

---

Name: **range**

*Description:* Program to calculate the intensity range in an Analyze format image

*Version used in project for this purpose:* range_1.0

*Input:* Analyze image

*Output:* minimum and maximum intensity (standard output)

*Scripts and programs called:* none