CHILDHOOD STATUS EPILEPTICUS: STRUCTURAL CONSEQUENCES AND ASSESSMENT OF A NOVEL TREATMENT

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Submitted to the University of London in partial fulfilment for the degree of Ph.D.
Status epilepticus (SE) is the commonest medical neurological emergency in childhood. In animal models of SE, the hippocampus is frequently damaged. The histological features resemble mesial temporal sclerosis (MTS), the commonest pathology in temporal lobe resections in adult humans. The commonest association with MTS is prolonged febrile convulsion (PFC). Hippocampal damage only occurs if seizures persist for at least 30 minutes. Early termination of seizures may decrease the incidence of MTS. Treatment with rectal diazepam is not always acceptable. An effective, convenient and acceptable method of treating SE would be advantageous.

To address the question of whether MTS has different magnetic resonance (MR) characteristics dependent on antecedent, quantitative MR data from patients with histologically proven MTS was reviewed. Patients with a history of PFC have asymmetrical hippocampal volume (HCV) and T2 relaxation time (T2) when compared to patients with no history of PFC and controls. This may suggest that severity and extent of MTS may be, in part, determined by the cause.

The assessment of whether SE results in acute brain abnormalities was carried out by prospectively investigating children using MR techniques. Within 48 hours of PFC there is an increase in HCV and T2 relaxation time when compared to controls. Patients with SE and no fever have an increased T2 relaxation time but normal hippocampal volume. PFC appears to result in acute hippocampal swelling, consistent with animal model data. The effect of non-febrile SE on limbic structures is less certain.

Buccal midazolam was assessed as an effective, socially acceptable acute treatment for seizures. A pharmacokinetic/pharmacodynamic study confirmed rapid absorption into venous blood and brain. Buccal midazolam was shown to be an effective treatment for acute repetitive seizures and at least as effective as rectal diazepam in the treatment of seizures which have persisted for longer than 5 minutes.
I am indebted to many people who have helped me to complete this work. I have been superbly supervised by Professor Brian Neville, Dr Frank Besag and Dr Alan Connelly. They have all provided education, support and encouragement throughout my research time. I would also like to thank Dr Robert Surtees who was always available to offer advice. I am eternally grateful to the anonymous donor who provided me with the financial support required for this study.

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I am grateful to the children and their families who agreed to take part in all aspects of this trial. Without such families it would not be possible to carry out clinical research.

Finally I would like to thank Lisette who enjoyed the good times and endured the bad times.
PERSONAL CONTRIBUTIONS OF THE CANDIDATE

In addition to involvement in the design and implementation of the studies reported in this thesis, the specific personal contributions of the candidate to each chapter are detailed below.

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CHAPTER 1: INTRODUCTION

1.1. Aims

Convulsive status epilepticus (SE), defined as a seizure or series of seizures which last for 30 minutes or more without recovery of full consciousness between the seizures, is a common medical neurological emergency in childhood (DeLorenzo et al., 1995). Despite this, clinical research relating to SE is rare and tends to be directed towards SE in the developed brain. SE is more likely to occur in the developing brain which does not have the same physical or neurochemical characteristics as the developed brain (Holmes, 1997). This may mean that information about SE obtained in adults cannot easily be applied to childhood. Research in the paediatric population is essential for a complete understanding of SE pathophysiology, treatment and outcome. This thesis will address whether SE in childhood can result in brain injury and whether a novel early treatment for SE is effective.

The question of whether SE can cause brain damage, particularly hippocampal damage, remains a vexed one. The answer is of clinical importance as the commonest structural abnormality in patients undergoing temporal lobe resections for intractable epilepsy is mesial temporal sclerosis (MTS). If this lesion is acquired there will be scope for attempting to prevent the development of MTS following SE. Animal models of SE support the view that hippocampal damage may be a result of seizure activity and is not a pre-existing abnormality (Meldrum et al., 1973; Sperk et al., 1983; Cavalheiro, 1995; Meldrum, 1997). The outcome from surgery has been reported to be better in
patients with mesial temporal sclerosis (MTS) and a history of prolonged febrile convulsion (PFC), defined as SE associated with a fever not of central nervous system origin which occurs in children aged between 6 months and 5 years, than those without such a history (Abou-Khalil et al., 1993). This suggests that not all MTS is the same in terms of severity, type and extent. MTS may be acquired after PFC, but in patients without such a history it may be acquired via a separate mechanism to PFC. The prognosis of SE, and the incidence of subsequent neuronal damage, is determined by the underlying aetiology and overall seizure length (Scholtes et al., 1996). Prognosis worsens with increasing seizure length (Treiman, 1996). Early treatment of SE may result in decreased morbidity which is secondary to structural brain damage.

Most seizures begin in the community and so treatment which is initiated in the community is likely to have the greatest influence on outcome from SE (Lombroso, 1989; Alldredge et al., 1995). The usual acute pre-hospital treatment of seizures is rectal diazepam (Knudsen, 1979; Brown et al., 1991b) which is not always acceptable or convenient (O'Regan et al., 1996; Scott et al., 1998a). Physical similarities between the mouth and the rectum suggest that medications for the acute treatment of seizures could be absorbed across the buccal mucosa (De Boer et al., 1982). Pharmacological and some clinical data have suggested that midazolam may be a superior benzodiazepine to diazepam in the treatment of SE, but comparative trials had not yet been carried out.
This thesis will address three primary questions about SE and PFC in the paediatric population, with emphasis on cause and prevention of MTS. These are:

a) Does MTS associated with PFC have different magnetic resonance characteristics from MTS associated with other or no initial precipitating injury?

b) Does SE cause brain damage in children?

c) Is buccal administration of midazolam in solution an effective emergency treatment of seizures?

1.2. Historical Perspective

The probable first clinical description of status epilepticus (SE) can be found on the Sakkiku cuneiform which dates from 718-612 BC. That description concludes with a statement suggesting that SE may be fatal. At the time there was no suggestion that SE may originate in the brain. From then until the sixteenth century there is very little mention of SE in any medical literature, including that of Hippocrates and Galen. During the sixteenth century Gavessetti made the observation that the brain of cardinal Commendoni was swollen following SE which had lasted 24 hours before the cardinal demised but it was not until the descriptions of Thomas Willis in 1667 that the nervous system was identified as the origin of SE. More formal descriptions about SE cannot be found until the mid-18th century. The term “etat de mal” was first used in the thesis of Louis Calmeil from Salpetriere Hospital in Paris and was coined by the patients themselves. The latinised English version of this expression, status epilepticus, was first used in Bazires translation of
Trousseau’s lectures on clinical medicine (Shorvon, 1994b). Since these early descriptions it has become clear that SE is not a single condition and there are now many clinical descriptions of different types of SE.

1.3. Definition of status epilepticus (SE)

In pathophysiological terms, SE can be defined as a condition in which there is failure of the usual mechanisms which terminate seizures. Currently those mechanisms are not clearly identified and this definition is not clinically useful. SE has variously been described as “the maximum expression of epilepsy” (Shorvon, 1994b), “a condition characterised by epileptic seizures which are sufficiently prolonged or repeated at sufficiently brief intervals so as to produce an unvarying and enduring epileptic condition” (Gastaut, 1973) and, “a seizure or series of seizures which last for 30 minutes or more without full recovery of consciousness between the seizures”. None of these definitions is ideal. The first and second definitions do not attempt to clarify when a short seizure becomes SE and all assume that SE is always convulsive. All seizure types may be prolonged and a definition which does not recognise this is inappropriate. Shorvon has proposed the following definition; SE is a condition in which epileptic activity persists for 30 minutes or more, causing a wide spectrum of clinical symptoms and with a highly variable pathophysiological, anatomical and aetiological basis (Shorvon, 1994b). Although this definition attempts to solve some of the difficulties associated with previous definitions it is still not suitable for all situations. It is probable that different definitions of SE will be required in different settings.
1.3.1. **Definition for outcome purposes.**

Animal model data suggest that seizures need to persist for longer than 30 minutes before neuronal damage occurs (Lemos et al., 1995; Fujikawa, 1996). For pathophysiological, epidemiological and outcome purposes a definition of seizures which persist for at least 30 minutes is appropriate to identify those patients at risk of developing structural brain damage.

1.3.2. **Definition for treatment purposes.**

Many seizures which last for 5 minutes will continue for at least 30 minutes and therefore treatment is required for most seizures which last at least 5 minutes. It could be argued that most patients who have had a seizure for 5 minutes have already shown that the mechanisms responsible for seizure termination have failed and are in the early stages of SE. For treatment purposes a definition stating a time of 5 minutes is appropriate.

1.4. **Epidemiology**

Accurate estimates of incidence of SE are difficult to find and almost all available data relate to tonic-clonic SE. Figures differ according to the types of study i.e. hospital versus community based. In hospital based studies, SE accounts for approximately 0.01% of all admissions to a general hospital but 3.5% of all admissions to neurological intensive care units (Rowan et al., 1970; Goulon et al., 1985). In children, SE occurs in 13 to 16% of all children with epilepsy (Aicardi et al., 1970; Yager et al., 1988). Approximately 5% of all febrile convulsions will continue for at least 30 minutes (Aicardi, 1986). None of these data give information relating to the frequency of SE in the
community. Prospective epidemiological studies attempting to define the incidence and outcome from SE are difficult to perform, require a network between all hospitals in a delineated area and many man-hours work to ensure accurate data collection prior to patient discharge. In Richmond, Virginia, there has been a prospective epidemiological study in which only people living within the city limits were included (DeLorenzo et al., 1996). The hospital network went beyond the city limits and patients of all ages presenting to surrounding hospitals were identified. The success of this study depended on a SE research team being continuously on-call. Patients were reported to the team on admission, but the team also identified patients by reviewing notes which were identified using the ICD-9 codes for seizures. The notes of all patients were reviewed by the team. The incidence of SE was 41 episodes per 100 000 residents per year. If only the infant population (age 1 month to 1 year) are included in the analysis, the incidence was 156 per 100 000 infants per year. Further episodes of SE occurred in 35% of these children. Partial and secondarily generalised seizures accounted for the majority of episodes of SE in all age groups, with primary generalised SE occurring in 45% of paediatric cases. Great care was taken to identify all patients but the authors recognise that these figures are probably underestimates. Extrapolation of the above data to the United Kingdom, population 58 million, means that at least 25000 episodes of SE occur in the UK every year.

The Child Health and Education Survey (CHES) is a population based birth cohort study in which 14676 children born in a single week in April 1970 have been followed for ten years. Of these children, 37 had at least one episode of
SE by the time they were ten; 19 had prolonged febrile convulsion and 18 had non-febrile SE (Verity et al., 1993). Extrapolation of this data means that approximately 2000 of the children born every year will have an episode of SE before they are 10 years old. Thus SE is common, but is it harmful?

1.5. Outcomes

The possible outcomes from SE include death, permanent neurological or cognitive deficit and no sequelae. Mortality and morbidity associated with this condition remains common enough to cause concern.

The outcome of non-febrile SE is primarily dependant upon the underlying aetiology which in turn is dependant upon the age of the child (Maytal et al., 1993, Gross-Tsur et al., 1993). SE lasting more than 1 hour has a higher mortality than SE lasting less than 1 hour (Towne et al., 1994).

1.5.1. Mortality

There has been an apparent decrease in mortality since Aicardi and Chevrie published their review of 239 episodes of SE in 1970 in which they found a mortality of 11%. This study covered a huge catchment area and was biased toward more severe cases of SE (Aicardi et al., 1970). By 1989 the mortality was between 3.8 and 6% (Maytal et al., 1989; Phillips et al., 1989). Prospective epidemiological studies have revealed a mortality of 3-5.4% (Verity et al., 1993; DeLorenzo et al., 1996). It is tempting to attribute this apparent decrease to improved treatment, but that is unlikely to be the complete explanation. Mortality increases in proportion to seizure length and therefore
the introduction of benzodiazepines which reduce overall seizure length probably contributed to the decrease in mortality. However some of the apparent decrease is artifactual as definition also played a role; Aicardi required a seizure length of 1 hour to fulfil the criteria for SE, whereas the later series used 30 minutes as the cut off point. The patients in the earlier series would be predicted to have a worse outcome from their SE as it was more protracted. There are also inexplicable changes in disease incidences with time. These may result from improved socioeconomic status in large sections of the population. In a retrospective 10 year review of intensive care admissions for SE the mortality was 8% although a further 4% of children had died within 1 year (Lacroix et al., 1994). Mortality from SE lasting longer than 1 hour may not have altered much since 1970.

1.5.2. New neurological signs

In the Aicardi series, physical and cognitive morbidity occurred in 53% of patients. The hemiconvulsion, hemiplegia, epilepsy (HHE) syndrome seen in this series is now a rare complication of SE and only occurs in children in whom a seizure has lasted more than 1 hour (Aicardi et al., 1970). Neurological sequelae ranging from minor motor problems to persistent vegetative states occur in approximately 30% of patients treated in intensive care (Lacroix et al., 1994). All of these studies have a bias in their methodology as all patients were recruited from hospital based populations. These studies may overestimate the incidence of sequelae. In the CHES, new neurological signs were identified in only one child who had a very prolonged febrile convulsion (Verity et al., 1993). This effect of new neurological signs following PFC was
also identified in the National Encephalopathy Study which assessed whether pertussis vaccination was associated with an encephalopathy and subsequent neurological signs (Miller et al., 1981).

1.5.3. Subsequent epilepsy

1.5.3.1. Following prolonged febrile convulsions

Cavanagh and Meyer were the first to recognise the relationship between prolonged febrile convulsion (PFC) in childhood and subsequent temporal lobe epilepsy secondary to mesial temporal sclerosis (MTS) (Cavanagh et al., 1956). Since that time debate has raged as to whether PFC causes MTS and subsequent epilepsy or whether PFC is a result of pre-existing MTS. Approximately 50% of patients undergoing anterior temporal lobe resections for MTS and 80% of patients with anterior hippocampal atrophy have a history of PFC in childhood (Van Paesschen et al., 1997). All of these data were collected from hospital based studies and inevitably show a bias toward the more severely affected patients. Community based epidemiological studies reveal a much lower incidence of subsequent epilepsy although subsequent epilepsy was still more common than would be predicted. In the CHES, afebrile seizures were identified in 21% of patients with febrile SE compared to the expected risk of 0.5-1% (Verity et al., 1993).

The National Collaborative Perinatal Project is a multi-centre epidemiological study carried out in the USA. Following PFC, 5.4% of children developed non-febrile seizures by seven years of age. Previous developmental abnormality and family history were also significantly related to this outcome (Nelson et
al., 1978). The proportion of children developing non-febrile seizures following febrile SE increases up to the age of 25 years (Annegers et al., 1987). There is a lag period from the time of a PFC to the development of complex partial seizures and many studies probably underestimate the risk of subsequent epilepsy.

1.5.3.2. Following non-febrile SE

Approximately 10% of SE episodes are the first presentation of epilepsy and so all of these patients, by definition, must go on to have further afebrile seizures. In the CHES, 83% of the patients who had non-febrile SE went on to have further non-febrile seizures (Verity et al., 1993).

There is a relationship between SE and subsequent epilepsy but it remains unclear whether this relationship is causal or not.

1.6. Aetiology

As outcome is heavily influenced by aetiology it is important to identify the causes of SE. Children with acute neurological insults causing SE and those with progressive neurological disorders have the worst prognosis (Maytal, 1993; Gross-Tsur et al., 1993; Shorvon, 1994a; Towne et al., 1994). The aetiology of SE in children has a different profile to the aetiologies identified in adults. In children under the age of 5 years the causes can be divided into febrile convolution related (febrile) and non-febrile convolution related (non-febrile) as this has importance in terms of outcome. Febrile SE (status epilepticus associated with fever, not of CNS origin, in a neurologically normal
child between the ages of 6 months and 5 years) has been considered to have a good prognosis with the majority of children suffering no clinically obvious ill effects. The prognosis of non-febrile SE is primarily determined by the underlying aetiology. The aetiologies can be divided into three groups

1. Idiopathic – No cause identified, the first presentation of epilepsy or SE occurring in the context of idiopathic epilepsy.

2. Acute symptomatic – SE in the context of identifiable acute insults, e.g. meningitis, encephalitis, acute metabolic disorders.

3. Remote symptomatic – SE occurring in the context of pre-existing neurological abnormality, e.g. underlying acquired, developmental or congenital CNS disorder. This category also includes SE occurring in children with defined symptomatic / cryptogenic epilepsy syndromes (Phillips et al., 1989; Scholtes et al., 1996; DeLorenzo et al., 1996).

This classification is not entirely satisfactory. It may be difficult to determine which category an individual child will fall into at first presentation. This may make data collection in prospective epidemiological studies difficult i.e. a seven month old child presenting with an initial PFC may have idiopathic febrile convulsion and be expected to have a good outcome, or severe myoclonic epilepsy of infancy and be expected to have a poor cognitive, behavioural and neurological outcome. If children are not assigned appropriately at initial presentation, data may be difficult to interpret. Although outcome is primarily determined by the underlying aetiology, it remains unclear whether a prolonged seizure occurring as a further neurological insult worsens the prognosis of the underlying disorder, and
whether SE influences the later development of afebrile seizures (Shorvon, 1994a). The outcome from stroke associated with SE is worse than that identified in patients who had a stroke and no SE. This does not appear to be related to infarct volume and so it is possible that SE has worsened the prognosis of the stroke (Waterhouse et al., 1998).

1.7. Pathophysiology

1.7.1. Seizure initiation, prolongation and termination.

In order for SE to develop, a seizure must both be initiated and the putative mechanisms which terminate seizures must fail. These mechanisms remain uncertain. Understanding the underlying pathophysiology of SE could promote the development of better therapeutic options than those currently available. SE can be induced in animal models by creating an imbalance between excitatory and inhibitory neurotransmitter systems such that the ratio between the systems favours excitation (Wasterlain et al., 1993). The major excitatory neurotransmitter in the brain is glutamate and the major inhibitory neurotransmitter in gamma-aminobutyric acid (GABA) (Feldman et al., 1997). The effects of these systems may be modulated by the cholinergic and adenosinergic neurotransmitter systems (Stringer et al., 1990; Herberg et al., 1993; Malhotra et al., 1997). In the lithium-pilocarpine model of SE, the excitatory amino acid neurotransmitter glutamate increases just prior to seizure onset whilst aspartate decreases (Walton et al., 1990a). In the electrical stimulation amygdala-kindling model of epilepsy there is a 3-fold increase in glutamate and aspartate immediately prior to seizure onset. However, there is also a 3-fold increase in gamma-aminobutyric acid (GABA) which should
inhibit seizures (Zhang et al., 1991; Kanthan et al., 1995). Although the neurotransmitter changes appear to have both excitatory and inhibitory effects, it is hypothesised that the major effect favours increased excitatory neurotransmission.

Seizure onset in humans could also be a result of an endogenously-created imbalance between excitatory and inhibitory neurotransmitter systems (Wasterlain et al., 1993). Human studies assessing mechanisms of seizure onset have only been performed in patients with lesional epilepsy undergoing pre-surgical evaluation. The excitatory neurotransmitters glutamate and aspartate increase at the seizure focus in adults with temporal lobe epilepsy as measured by in-vivo intracerebral microdialysis (Ronne-Engstrom et al., 1992; During et al., 1993). Elevations of glycine and D-serine, which are both co-transmitters at the excitatory post-synaptic N-methyl-D-aspartate (NMDA) receptor, can also be identified (Carlson et al., 1992). Pre-ictal decreases in GABA concentrations occur at the site of seizure onset (During et al., 1993). There is, therefore, animal model and human evidence to support the view that excitatory / inhibitory imbalances initiate seizures.

It is possible that continued elevations of excitatory neurotransmitters would result in prolongation of seizures. However, animal models of SE universally show a decrease in extracellular or whole brain glutamate concentrations during SE (Chapman, 1985; Walton et al., 1990a; Cavalheiro et al., 1994) suggesting that the mechanisms of seizure prolongation are different to the
mechanisms of seizure initiation. Failure of the mechanisms which terminate seizures are likely to be responsible for seizure prolongation.

The mechanisms of seizure termination remain uncertain but correction of neurotransmitter imbalance may play a primary role. Inhibitory neurotransmitters such as GABA increase at the seizure focus subsequent to seizure onset and redress the balance between excitation and inhibition (During et al., 1993). GABA concentrations may continue to rise throughout the period of epileptic activity (Chapman, 1985; Walton et al., 1990a), an effect which is more easily maintained in the developing than the developed brain (Sankar et al., 1997). Subcortical grey matter nuclei including the substantia nigra pars reticulata (SNPR) have seizure modifying roles which are secondary to activation of GABA<sub>A</sub> receptors by endogenous GABA. This activates inhibitory neuronal projections to cortical regions and may suppress or terminate seizures (Wasterlain et al., 1993b).

Extracellular adenosine may also have seizure modifying or terminating effects (During et al., 1992). Injection of adenosine agonists or adenosine breakdown enzyme antagonists into rat prepiriform cortex protects against bicuculline (a GABA<sub>A</sub> antagonist) induced seizures (Murray et al., 1992). Adenosine receptor antagonism with aminophylline may account for the seizure prolonging effects of this drug (Dragunow, 1990). Adenosine agonists also block the SE inducing effects of pilocarpine in rats (George et al., 1997).
1.7.1.1. Seizures in the developing brain

Seizures are more likely to start and more likely to be prolonged in the developing brain than in the developed brain. This is well documented in animal models in which seizures are induced with global ischaemia, pilocarpine, kainate or electrical stimulation (Cavalheiro et al., 1987; Jensen et al., 1991; Stafstrom et al., 1992; McCown et al., 1992). There are several potential reasons for this age determined effect (Holmes, 1997).

1.7.1.1.1. Depolarising GABA<sub>A</sub> Receptors

Binding of GABA to GABA<sub>A</sub> receptors in developed brain results in opening of chloride ionophores, influx of chloride and hyperpolarisation of the post-synaptic membrane. In the neonatal rat brain, activation of the GABA<sub>A</sub> receptor results in chloride efflux and membrane depolarisation (Ben-Ari et al., 1997). The major inhibitory effect is negated in the immature brain predisposing it to hyperexcitability.

1.7.1.1.2. GABA<sub>A</sub> in substantia nigra

Seizure modifying circuits which involve the subcortical nuclei, especially the substantia nigra, can be identified in adult rats using deoxyglucose autoradiography. In immature rat pups this effect is not present. There are fewer high-affinity GABA<sub>A</sub> receptors in substantia nigra pars reticulata, which implies that the seizure modifying effects of this structure are impaired in the immature rat brain increasing the likelihood of a seizure becoming prolonged (Ackermann et al., 1989).
1.7.1.3. *Excitatory neurotransmission*

Glutamate exerts its excitatory effect by binding primarily to N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy methyl-4 isoxazole proprionic acid (AMPA) receptors on the post-synaptic membrane. NMDA receptors develop earlier than AMPA receptors which are silent in the immature brain (Ben-Ari et al., 1997). The NMDA receptor requires binding of glutamate and a co-transmitter (glycine or D-serine) as well as depolarisation of the post-synaptic membrane for the ionophore to open. In mature brain, initial depolarisation occurs as a result of AMPA activation. This receptor is non-functional in the neonatal brain. However, activation of the GABA\(_A\) receptor in this age group can depolarise the post-synaptic membrane sufficiently to allow opening of the NMDA channel (Ben-Ari et al., 1997). There is also decreased voltage dependency of the NMDA receptor in the immature brain. Opening of the ionophore results in larger excitatory post-synaptic potentials which are longer lasting than those identified in the developed brain (Morrisett et al., 1990). The net effect of all these features is hyperexcitability of the immature brain compared to the mature one.

On pathophysiological grounds it is not surprising that the incidence of SE in the paediatric population is higher than in the young adult population. Despite this, most of the basic science research attempting to clarify mechanisms of seizure genesis and termination is performed in adult models. There are, however, increasing numbers of animal models assessing epileptogenesis in the immature brain. These may help to clarify some of the mechanisms responsible for the vulnerability of the developing brain to SE (Scott et al., 1998b).
1.7.2. *Systemic and central pathophysiology.*

The early systemic effects of SE are initially dominated by apparent compensatory effects. Blood pressure and central venous pressure rise, blood glucose goes up and the patient becomes tachycardic. Cerebral blood flow, blood glucose and oxygen utilisation increase in experimental models and in humans during this compensatory phase of SE. These events attempt to ensure that metabolic demand is met by supply and that toxic metabolites are removed from the brain (Brown et al., 1991a; Shorvon, 1994b; Fountain et al., 1995).

After approximately 30 minutes of continuous epileptic activity, the protective mechanisms described above begin to fail and subsequent events are potentially harmful to the brain. Cerebral blood flow declines during the course of SE as there is failure of cerebral vascular autoregulation i.e. the cerebral vasculature loses the ability to alter its diameter to ensure appropriate cerebral blood flow. Cerebral blood flow becomes dependant upon systemic blood pressure which also declines. Provision of oxygen and glucose for the brain becomes impaired. Respiratory and metabolic acidosis, electrolyte imbalance, hyperthermia and rhabdomyolosis may also occur and are potentially harmful. This is the phase of decompensation (Brown et al., 1991a; Shorvon 1994b; Fountain et al., 1995. Table 1.1). The decompensation phase could be worsened by treatment with drugs that have depressant cardiorespiratory effects (e.g. benzodiazepines and barbiturates) and perhaps these drugs should be used cautiously.
Table 1.1. Systemic and cerebral pathophysiological changes associated with seizures and convulsive SE

<table>
<thead>
<tr>
<th>Compensation</th>
<th>Decompensation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 minutes</td>
<td>&gt;30 minutes</td>
</tr>
<tr>
<td>Increased cerebral blood flow</td>
<td>Failure of cerebral vasculature autoregulation</td>
</tr>
<tr>
<td>Cerebral energy requirements matched by supply of oxygen and glucose</td>
<td>Hypoglycaemia</td>
</tr>
<tr>
<td>Increased glucose concentration in the brain</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Increased catecholamine release</td>
<td>Acidosis</td>
</tr>
<tr>
<td>Increased cardiac output</td>
<td>Hyponatraemia</td>
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<td></td>
<td>Hypo/hyperkalaemia</td>
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<td></td>
<td>DIC</td>
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<td></td>
<td>Leucocytosis</td>
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<tr>
<td></td>
<td>Falling blood pressure</td>
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<tr>
<td></td>
<td>Falling cardiac output</td>
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<tr>
<td></td>
<td>Rhabdomyolysis</td>
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</tbody>
</table>

1.7.3. Electrophysiology.

There is a predictable sequence of EEG changes associated with ongoing SE. These changes have been identified in at least 6 animal models and in adult humans. Secondarily generalised SE begins with localised epileptic activity followed by isolated generalised bursts of seizure activity with normal EEG activity between events. If the patient does not regain consciousness between these episodes then the patient will meet the clinical criteria for SE. The isolated ictal discharges merge and become a continuous discharge after about 30 minutes. Discharges then fragment and become interspersed with flat periods. Ultimately periodic epileptiform discharges (PEDs) which may reflect underlying metabolic failure will occur. The motor phenomena of convulsive SE follow a similar pattern to the EEG changes. Recurrent seizures merge into continuous motor activity followed by fragmentation of the motor activity and
myoclonus. If the seizure persists then electromechanical dissociation, defined as continuing epileptiform abnormalities in the EEG with no associated motor phenomena, will occur. The prognosis for a good outcome declines as the patient moves through the continuum (Treiman, 1995; Fountain et al., 1995).

1.8. Brain damage associated with status epilepticus.

The majority of work attempting to define brain damage associated with SE has been carried out in animal models. The seminal studies which identified that SE can result in widespread neuronal damage and neuronal death were carried out by Brierley and Meldrum using the GABA<sub>A</sub> antagonist, bicuculline, to induce SE in adolescent baboons (Meldrum et al., 1973). The experiments were repeated in paralysed ventilated animals in whom systemic decompensation was avoided. Similar areas of neuronal loss or damage were identified. This was the original evidence that SE itself might be a direct cause of neuronal damage (Meldrum et al., 1973; Wasterlain et al., 1994; Fountain et al., 1995; Meldrum, 1997).

Subsequent models of SE in which areas of neuronal damage occur manipulate other neurotransmitter systems. Glutamatergic drugs such as kainic acid, N-methyl-D-aspartate, quisqualate and domoic acid given to rats result in prolonged limbic seizures. Kainic acid can be given by the intraperitoneal, intraventricular or intra-amygdala routes and cause neuronal loss in similar areas to those damaged following bicuculline administration and subsequent SE (Sperk et al., 1983; Ben-Ari, 1985; Haglid et al., 1994; Golden et al., 1995; Cendes et al., 1995). Cholinergic drugs such as pilocarpine also cause limbic SE
and subsequent spontaneous recurrent seizures analogous to the situation in humans (Nagao et al., 1994; Liu et al., 1994; Cavalheiro, 1995; Priel et al., 1996; Fujikawa, 1996). It could be argued that it is the drug used to induce SE which is causing neuronal damage and not SE itself. Electrical models using continuous hippocampal stimulation protocols also result in unilateral or bilateral hippocampal damage similar to that identified in the other models (Lothman et al., 1989; Lothman et al., 1990). Many different experimental paradigms result in damage to similar areas of the brain which might suggest that SE itself is responsible for the damage.

1.8.1. Areas damaged.

Many brain regions are damaged by SE in animal models (Shorvon, 1994b). However, the relationship between hippocampal damage and intractable temporal lobe epilepsy has resulted in the hippocampus being the most studied brain structure in animal models of SE and in humans.
1.8.1.1. Hippocampus

1.8.1.1.1. Anatomy

Figure 1.1. Normal hippocampal anatomy. CA = cornu ammonis, DG = dentate gyrus, H = hilus

1.8.1.1.2. At risk neuronal populations

The histological abnormalities identified in patients with hippocampal sclerosis are very similar to the findings identified in animal models in which SE has been chemically or electrically induced (Wasterlain et al, 1993a). It is important that the animal models chosen are relevant to the human condition. The majority of pyramidal cell loss is in the CA1, CA3 and dentate gyrus regions with relative sparing of the CA2 region. The reasons for this selective vulnerability remain unclear but may be related to excitatory amino acid neurotransmitter receptor density or subtype.
1.8.1.1.3. Mossy fibre sprouting

It has been suggested that mossy fibre sprouting results in a re-entry circuit from CA3 to the dentate gyrus and is the epileptogenic lesion causing hippocampal seizures (Lemos et al., 1995; Mathern et al., 1996). However, prevention of mossy fibre sprouting with cyclohexamide does not prevent the occurrence of spontaneous recurrent seizures in the pilocarpine model of limbic epilepsy (Longo et al., 1997).

1.8.1.2. Other brain regions

Other brain regions which may be damaged by SE include the thalamus, cortex, amygdala, striate cortex and cerebellum. In all of these regions cell loss and gliosis have been identified (Shorvon, 1994b). Acute neuronal loss in these regions may be seen in children who have died during SE (Corsellis et al. 1983).

1.8.2. Mechanisms of damage
1.8.2.1. Hypoxia / Ischaemia

The pathological abnormalities seen in the hippocampus following convulsive SE are very similar to those seen after ischaemia. This led to the theory that the damage was of vascular origin (Wasterlain et al., 1993; Shorvon, 1994b). This theory fell into question following Meldrum’s work on SE in adolescent baboons in whom systemic decompensation was prevented. Subsequent research has suggested that excitotoxicity is the more likely mechanism of neuronal damage.
1.8.2.2. *Excitotoxicity*

The damaging effect of the excitatory amino acid neurotransmitter, glutamate, was first described by Lucas and Newhouse who identified that systemic administration of monosodium glutamate resulted in retinal damage in rat pups. They suggested that the damage was a direct effect of the chemical.

Subsequently there has been much interest in the role of glutamate in the development of neuronal damage both in stroke and in SE (Wasterlain et al., 1993; Shorvon, 1994b). Although there is direct evidence that glutamate results in delayed neuronal damage in patients with stroke (Davalos et al., 1997; Castillo et al., 1997), the evidence that glutamate is responsible for hippocampal damage following SE remains circumstantial. Glutamate, or glutamate receptor agonists, placed directly onto hippocampal cell cultures produces similar damage to the models discussed above (Vornov et al., 1995). These data, taken in combination with the animal model data, suggest that glutamate may be causing the damage. Animal model and human in-vivo microdialysis studies confirm that glutamate increases in the extra-cellular space immediately prior to seizure onset (Ronne-Engstrom et al., 1992; During et al., 1993) but there is no evidence that glutamate is increased late in the course of SE when damage would be expected to occur (Walton et al., 1990a; Cavalheiro et al., 1994). There are at least 3 possible reasons for this; either glutamate is not the cause of neuronal damage in SE, glutamate is rapidly metabolised, or glutamate elevations are localised to the synaptic cleft and cannot be measured.
1.8.2.2.1. Glutamate production, release and uptake

Glutamate is the major excitatory amino acid neurotransmitter in the brain. It is derived from glucose via the tricarboxylic acid cycle (Peng et al., 1993). It is taken up into pre-synaptic vesicles through excitatory neurotransmitter transporter systems, and stored (Maycox et al., 1990; Nicholls et al., 1990). On depolarisation of the pre-synaptic neurone, glutamate is released by exocytosis into the synaptic cleft (Nicholls et al., 1990). The timecourse of glutamate in the cleft is approximately 5 milliseconds as it is rapidly taken up into astrocytes and back into pre-synaptic neurones via excitatory amino acid transporter systems. In astrocytes glutamate is metabolised to glutamine, alanine and lactate. Glutamine may diffuse back into the pre-synaptic neurone where it is converted back to glutamate. Both glutamate from glutamine and that from the synaptic cleft are recycled into vesicles and stored (Feldman et al. 1997).

1.8.2.2.2. Glutamate receptors

There are 2 major groups of post-synaptic glutamate receptors; ionotropic and metabotropic. Ionotropic receptors allow influx of ions across the post-synaptic membranes whilst metabotropic receptors stimulate the production of intracellular second messengers via G-proteins although they can inhibit or activate ion channels.

1.8.2.2.2.1. AMPA

The α-amino 3-hydroxy 5-methyl-4-isoxazole propionic acid (AMPA) receptor is a ligand gated receptor which allows influx of sodium ions causing rapid neurotransmission following binding of glutamate. It is made up of four
subunits termed GluR1 – GluR4. Absence of the GluR2 subunit allows calcium to pass through the ionophore (Hollmann et al., 1991; Bochet et al., 1994). This may be important in the development of neuronal injury as increased intracellular calcium is found in neurones which die following SE (Griffiths et al., 1983; Sztriha et al., 1985; Sztriha et al., 1986).

1.8.2.2.2.2. NMDA
The N-methyl-D-aspartate receptor is both voltage and ligand gated. In order for NMDA receptors to open, 3 criteria need to be met; initial depolarisation, usually via AMPA receptors, binding of glutamate, and binding of a co-transmitter, either glycine or D-serine (Schoepp, 1994). Activation allows influx of sodium and calcium across the post-synaptic membrane. It is responsible for slower neurotransmission than the AMPA receptor (Schoepp, 1994). In the immature brain initial depolarisation can occur as a result of GABA_A receptor activation (Ben-Ari et al., 1997).

1.8.2.2.2.3. Metabotropic receptors
Metabotropic receptors may not be associated with an ion channel, but establish intracellular signalling by the stimulation of second messengers such as IP_3 and cAMP (Feldman et al., 1997). To date 7 metabotropic glutamate receptors (mGlu) have been identified (Schoepp, 1994). These receptors may have toxic and protective functions. The potential toxic effects of mGlu activation include potentiation of NMDA and other excitatory membrane currents, potentiation of intracellular calcium release, decrease in inhibitory membrane currents and decreased GABAergic inhibition. Conversely potential
protective effects include inhibition of synaptic glutamate release and decreased calcium influx (Ghauri et al., 1996; Akbar et al., 1996).

1.8.2.2.3. Downstream events

Excessive influx of calcium is neurotoxic (Griffiths et al., 1983; Sztriha et al., 1985; Sztriha et al., 1986) via several potential mechanisms. Intracellular calcium binds to calmodulin which is required for activation of several intracellular metabolic processes. There are potential neurotoxic effects from overactivity of the following enzymes:

- Protein kinase C. This enzyme is moved from the cytosol to the cell wall resulting in breakdown of cell wall proteins and destruction of the wall (Maiese et al., 1993).

- Nitric oxide (NO) and free radical formation. Calcium stimulates constitutive nitric oxide synthase (NOS) causing a rise in intracellular NO. NO can inhibit mitochondrial respiration directly or indirectly by forming peroxynitrite free radicals which are cytotoxic (Reif, 1993; Hewett et al., 1994; Maiese et al., 1994).

- Activation of phospholipase A$_2$. This enzyme breaks down membrane lipids with release of arachidonic acid and other fatty acids. A consequence of this membrane destruction can be cell death (Stella et al., 1995; Clapp et al., 1995).

- Activation of protease calpain I. The mechanism by which this enzyme causes cell death is unclear but calpain I inhibitors are partially neuroprotective. It may be a result of excessive spectrin breakdown causing instability of the cell wall (Widdowson et al., 1997).
Glutamate receptor stimulation also results in the formation of immediate early genes (IEGs) such as c-fos, fos-B, c-jun and jun-B which regulate the expression of a number of late effector genes. Some of the genes regulated are harmful and some are potentially neuroprotective (Mudo et al., 1996). IEGs may play a dual role; induction of gene determined cell death and activation of brain repair mechanisms.

The majority of the work described above was performed to provide insight into the pathophysiological basis of hippocampal damage secondary to SE and PFC. The classical association of brain damage and epilepsy is the association of MTS and PFC (Cavanagh et al., 1956). None of the animal models described have a genetic basis, as is the case with PFC, and therefore do not necessarily provide the basis of damage caused by PFC.

1.9. Treatment of Status Epilepticus

The previous sections provide data to support the view that prolonged seizures are potentially harmful. This implies that early termination of seizures is potentially beneficial and is universally accepted practice. Most anticonvulsant medications used in the treatment of SE are either GABA\(_A\) agonists or sodium channel blockers. The former may restore excitation / inhibition imbalance by increasing inhibition. The latter restore the balance by decreasing pre-synaptic excitatory amino acid release or by prevention of propagation of the action potential.
Acute treatment of seizures can be effectively carried out by parents, carers, teachers or paramedics in the pre-hospital setting (Martin et al., 1994). If pre-hospital treatment is unavailable or fails, emergency treatment needs to be carried out in the hospital setting. In this setting, management of SE should be considered to be analogous to management of cardiac arrest. A structured series of interventions is more likely to be more successful than a haphazard approach (Shepherd, 1994b). Guidelines on pre-hospital and hospital management are appropriate and should contain general and child specific points.

1.9.1. Pre-hospital treatment

Seizures treated early in their course are more likely to stop than those treated late, but most seizures begin some way from a hospital (Lombroso, 1989; Alldredge et al., 1995). As the systemic changes associated with SE are usually a direct result of the seizure, termination of the seizure will usually correct abnormalities. Most children have normal cardiorespiratory function and so pharmacological treatment of seizures without cardiorespiratory monitoring is justified. As parenteral administration of anticonvulsants is usually not feasible in the community setting, an effective, easy to administer treatment which could be given by parents or carers would be advantageous.

Rectal administration of diazepam (a GABA_A agonist) solution is effective in the treatment of acute repetitive seizures and SE (Knudsen, 1979; Dreifuss et al., 1998). There is rapid absorption through the haemorrhoidal veins and then across the blood brain barrier (De Boer et al., 1984). Therapeutic levels are
reached within 5 minutes and peak levels occur within 20 – 60 minutes of administration (Knudsen,1979; Lombroso,1989; Remy et al.,1992).

Approximately 80 % of seizures will stop if the treatment is administered within 15 minutes of the start of the seizure compared with approximately 60% if the drug is administered after this time (Knudsen,1979). There is also less chance of seizure recurrence if drug administration is early (Lombroso,1989). Although rectal diazepam has had a great impact on the acute management of seizures, it is not always convenient or acceptable for parents and carers to give rectal drugs and therefore alternative routes of administration which are more socially acceptable would be desirable. The buccal mucosa could provide an alternative to the rectal mucosa for rapid drug absorption.

The Advanced Paediatric Life Support (APLS) group suggest that rectal paraldehyde should be given following an intravenous bolus of diazepam. Rectal administration of drugs may result in unreliable serum and brain levels and it may be more appropriate to administer a further intravenous drug in the presence of intravenous access. Rectal paraldehyde should be given as an alternative to diazepam in children in whom it is already known to work, or when intravenous access has not been established and rectal benzodiazepines have failed. The only acceptable route is rectal. It should be administered emulsified with olive oil or arachis oil to reduce irritation of the rectal mucosa (Brown et al.,1991b; Tunik et al.,1992). The intramuscular route is no longer acceptable because of the high incidence of sterile abscesses (Shepherd,1994b).
1.9.2. Hospital treatment

When a child arrives in the emergency department it is important to ask whether they have had previous episodes of SE and if so which treatment was effective. This can potentially reduce the length of time it takes to stop a seizure. A rapid clinical assessment should be done prior to investigation and treatment. Termination of the seizure is the primary goal of initial hospital management of SE. The ideal acute anticonvulsant would have the following properties.

- Effective against all types of SE
- Reliable, fast and predictable absorption
- Lipophilic with rapid brain penetration
- Stable in solution
- Wide therapeutic index with low toxicity in the therapeutic range
- Long elimination half life
- No interaction with other drugs (Brown et al., 1991b)

Unfortunately no drug meets all of these requirements although the benzodiazepines have many of them and are justifiably the first-line agents in many countries.

1.9.2.1. Investigations

All children presenting in SE to the emergency department should have glucose, electrolytes (including calcium and magnesium) and a full blood count measured. Other investigations should be performed on the basis of the clinical situation. Less usual investigations that should be considered include toxicology, CT or MRI scanning and EEG. A lumbar puncture should be
considered for patients presenting in SE with pyrexia, other features suggesting meningitis and in whom meningitis should be considered (Tunik et al., 1992; Pellock, 1994). Correction of electrolyte abnormalities is essential as children may continue to seize despite drug therapy if the abnormalities persist. Hypernatraemic dehydration should be managed by gradual correction of the electrolyte abnormality so that cerebral oedema is not increased.

The possibility of raised intracranial pressure (ICP) should be borne in mind at the acute presentation of SE. The possible aetiologies of raised ICP include closed head injury, often non-accidental in babies, pyogenic meningitis or SE itself. Raised ICP may be difficult to assess during SE, especially if sedating drugs have been used. A persisting Glasgow Coma Score of less than 8 suggests a degree of raised ICP and the child should be managed accordingly. Treatment includes intravenous mannitol and/or artificial ventilation with the pCO₂ kept at least at a low normal level. If meningitis is suspected then intravenous antibiotics should be given and lumbar puncture should be delayed until the seizures are controlled and cardiorespiratory stability has been achieved.

1.9.2.2. First – line treatment

The first-line agents used in the treatment of SE tend to be benzodiazepines. All drugs in this class have sedative, anxiolytic and anticonvulsant properties (Feldman et al., 1997). Diazepam is the favoured benzodiazepine in the United Kingdom (UK) whereas the favoured benzodiazepine in the United States of America (USA) is lorazepam. Midazolam is a third benzodiazepine which may
be useful in the treatment of SE and has the advantage of water solubility and adverse events secondary to the diluent are less likely. It is effective in terminating seizures after intramuscular as well as intravenous administration, a property which should make it a favoured drug in the emergency department.

1.9.2.2.1. Pharmacology of benzodiazepines used in the treatment of SE

1.9.2.2.1.1. Routes of administration, uptake and distribution

Benzodiazepines can be administered orally, intravenously or rectally. For the treatment of acute seizures, oral administration is not appropriate as adequate brain levels for seizure control cannot be achieved rapidly enough. Oral benzodiazepines are useful in the treatment of acute repetitive seizures, a situation which is not life threatening and speed is not of the essence.

Intravenous benzodiazepines are widely used as first-line agents in the hospital setting (Treiman, 1990; Shepherd, 1994b; Shorvon, 1994b; Runge et al., 1996; Walker et al., 1996). Effective anticonvulsant concentrations of diazepam are reached within 3 minutes following a single intravenous bolus, following which there is rapid distribution from brain to peripheral compartments, particularly fat and muscle. An important consequence of this re-distribution is that non-effective cerebral concentrations are reached after approximately 15 minutes. Frequent boluses of diazepam for recurrent seizures lead to saturation of fat stores. Subsequent doses will result in high serum levels and increased incidence of adverse-events, especially respiratory depression (Walker et al., 1998). The apparent volume of distribution is 0.8-2.6 l/kg in adults but is higher in children (Abernethy et al., 1981a; Abernethy et al., 1981b).
Lorazepam is also effective in the acute management of seizures (Treiman, 1990; Walton et al., 1990b; Treiman, 1996). Lipid solubility allows effective cerebral concentrations to be reached within 3 minutes. However, it is less lipid soluble than diazepam so there is less re-distribution into peripheral compartments than with diazepam. Effective anticonvulsant cerebral concentrations are maintained for 12-48 hours (Walker et al., 1979; Giang et al., 1988; Walton et al., 1990b). Continued high brain concentrations result in longer periods of sedation than observed with diazepam. This may make clinical assessment of conscious level difficult, a problem which is less important when doctors become familiar with the use of this drug. Lorazepam has an apparent volume of distribution of 1-2 l / kg (Dundee et al., 1978; Walker et al., 1979).

Midazolam is a water soluble 1,4-benzodiazepine which has pharmacokinetic properties which lie between those of diazepam and lorazepam. Midazolam is effective when administered by either the intravenous or intramuscular route in SE and is currently the only benzodiazepine which is effective when administered intramuscularly (Mayhue, 1988). Recurrent boluses of midazolam, or midazolam infusions, can be safely administered as there is little accumulation of the drug in fat stores (Mayhue, 1988). The volume of distribution is 0.6-1.7 l / kg. The bioavailability after an intramuscular dose is 80-100 % and peak levels are reached within 25 minutes (Bell et al., 1991).

A single dose of rectal diazepam solution or gel, administered to healthy adult volunteers results in peak plasma concentrations approximately 20 - 60 minutes
after administration (Remy et al., 1992; Dreifuss et al., 1998). Many of these studies were performed in volunteers in whom the bowel had been prepared and the rectum was empty (Dreifuss et al., 1998). In the clinical situation, bioavailability and absorption are decreased by administration into faeces and emptying of the rectum soon after drug administration (Magnussen et al., 1979). Absorption data needs to be interpreted in the light of this. The bioavailability of rectal diazepam can be as low as 50% (Magnussen et al., 1979). The pharmacokinetic disadvantages of intravenous diazepam probably also apply to rectal administration.

1.9.2.2.1.2. Metabolism and excretion

All benzodiazepines used in the treatment of SE are metabolised in the liver by microsomal enzymes (Feldman et al., 1997). The major metabolite of diazepam is N-methyl diazepam which has limited intrinsic anticonvulsant properties, but does not reach effective cerebral concentrations following an intravenous bolus of diazepam. The elimination half-life of diazepam following a single intravenous bolus is approximately 20-40 hours but for much of that time the plasma concentration is insufficient for seizure control (Shorvon, 1994b). The elimination half-life of rectal diazepam gel is 45 +/- 18 hours (Dreifuss et al., 1998). It is unlikely that diazepam is effective in controlling seizures throughout that period. Lorazepam has no active metabolites. The elimination half-life is approximately 15 hours and during that time there is tight binding of the drug to receptors, suggesting that the anticonvulsant effect is present for much of the time (Dundee et al., 1978). Midazolam is metabolised to α-hydroxy midazolam which also has limited intrinsic anticonvulsant properties.
(Lehmann et al., 1995). The elimination half-life is between 1 and 4 hours (Heizmann et al., 1983).

1.9.2.2.2. Mechanism of action of benzodiazepines

The benzodiazepines are all agonists at the gamma amino butyric acid type A (GABA<sub>A</sub>) receptor (Feldman et al., 1997).

1.9.2.2.2.1. Structure of the GABA<sub>A</sub> receptor

These pentameric receptors are made up of four different families of receptor subunits. The subunits are designated α, β, γ and δ, all of which have several isoforms. The commonest stoichiometric combination is α1β2γ2 although approximately 850 combinations are possible (Macdonald et al., 1995; Korpi et al., 1997). These subunits form a receptor complex associated with a chloride channel and are found on the post-synaptic membrane (Feldman et al., 1997).

1.9.2.2.2.2. Effect of benzodiazepines on GABA<sub>A</sub> receptors

Benzodiazepines are allosteric modulators at a unique binding site on the GABA<sub>A</sub> receptor complex (Braestrup et al., 1977; Mohler et al., 1988). The sites are saturable, stereospecific and unevenly distributed throughout the rat brain (Braestrup et al., 1977; Mohler et al., 1988). Activation of post-synaptic receptors allows influx of chloride ions into the post-synaptic neurone with consequent hyperpolarisation (Macdonald et al., 1995; Korpi et al., 1997). This makes subsequent depolarisation less likely to occur.
1.9.2.2.3. *GABA* A function during SE

Benzodiazepines are most effective in terminating seizures when administered early in the course of a seizure (Knudsen et al., 1979). This effect may be a result of rapid functional plasticity of the GABA A receptor and loss of benzodiazepine sensitivity. This has been identified in rat hippocampal neurones, but other brain areas may exhibit similar properties. There are no effects on GABA or barbiturate sensitivity, which suggests that it is only the subunit of the GABA A receptor responsible for benzodiazepine sensitivity which is altered (Kapur et al., 1997).

1.9.2.3. Clinical trials

Paediatric studies comparing diazepam to lorazepam showed that children treated with IV diazepam were more likely to require additional doses or an additional anticonvulsant drug than those treated with intravenous lorazepam (Giang et al., 1988; Appleton et al., 1995). In one of the studies, response to a single bolus of diazepam occurred in approximately 50% of patients compared to 76% of those treated with lorazepam. Fewer children in the lorazepam group required ventilatory support. Children were randomised according to which day they presented and many children were incorrectly treated. Nevertheless the study does support the pharmacokinetic impression that lorazepam may be the better drug (Appleton et al., 1995).

Clonazepam has been used both acutely and as an intravenous infusion in refractory SE. It has a half life of about 30 hours and is therefore unsuitable for infusion (DeVane et al., 1991). It probably has no advantages over the above benzodiazepines (Treiman, 1989). The major problem associated with
clonazepam infusions is extreme bronchorrhoea which may result in obstructive hypopnoea and aggravate the respiratory depression associated with the benzodiazepines (Brown et al., 1991b).

1.9.2.3. Second line treatment

Phenytoin was introduced into clinical practice for the treatment of SE in 1958. Since that time it has proven to be very useful (Wallis et al., 1968; Shepherd, 1994a; Roberts et al., 1995; Treiman, 1996). It is a sodium channel blocker and may inhibit propagation of the action potential. It must be given by the intravenous route as erratic plasma levels occur with rectal or intramuscular administration. It needs to be given as a slow bolus under ECG and blood pressure control as it has been associated with cardiac arrhythmias and hypotension (Wilder, 1983). Phenytoin is dissolved in propylene glycol and the cardiac effects are in part caused by the diluent (Brown et al., 1991b). Infusion rates should not exceed 1mg/kg/min, up to a maximum of 50mg/min. Despite this, effective levels are reached within 10 - 30 minutes (Brown et al., 1991b). Phenytoin has a half life of 50 - 70 hours (Cranford et al., 1979). Because of this property it has been suggested that phenytoin should be given following the first or second dose of diazepam to prevent recurrent seizures.

Fosphenytoin is a water soluble phosphate ester prodrug which is rapidly and completely metabolised to phenytoin and has no intrinsic antiepileptic properties. As no propylene glycol or alcohol is required for dilution, fosphenytoin can be administered three times as rapidly as phenytoin (Browne, 1997). Therapeutic levels can be reached within 10 minutes following
an intravenous bolus. Intramuscular administration of fosphenytoin is well tolerated and results in reliable venous blood concentrations of phenytoin (Uthman et al., 1996; Wilder et al., 1996; Browne, 1997). Clinical trials on the use of fosphenytoin are currently underway in the USA. There is little data available on pharmacology and clinical effects of fosphenytoin in the paediatric age group (Pellock, 1996).

1.9.2.4. Subsequent management

Once second line treatments have failed, further medications will potentially depress respiration and therefore respiratory monitoring is mandatory. This may be best achieved in intensive care units (ITU) where there is high level of nursing input and sophisticated technology which is used for continual monitoring of physiological parameters. It is important for all acute units to have access to ITU facilities as a small proportion of children will have

a) SE associated with a severe systemic or neurological disorder
b) SE refractory to first line anticonvulsant agents or
c) respiratory depression as a consequence of treatment.

Two drugs which may be used in the ward setting and may prevent intensive care admission are chlormethiazole and lignocaine. Chlormethiazole is a useful anticonvulsant used primarily in UK, the rest of Europe and Australia. It is almost never used in the USA. Its primary use is in the treatment of alcohol withdrawal and eclampsia (Braunwarth, 1990). However it may also be effective in many other situations. The clinical effect is short and therefore chlormethiazole needs to be given as an intravenous infusion (Brown et
al., 1991b). The dose can be titrated against seizure response in a minute to minute fashion during the initial stages. Accumulation in fat stores after approximately 12 hours negates this advantage (Robson et al., 1984). Unfortunately chlormethiazole needs to be given in large volumes of fluid and therefore it may be difficult to get adequate calories into a seriously ill child being treated with this drug. Nevertheless it can be useful in the neonatal intensive care unit (Miller et al., 1983). It is also important to be certain that the patient’s kidneys are functioning well before administering a large fluid load. Accepting these limitations, chlormethiazole has proved to be a very useful drug for SE by avoiding the significant problems associated with barbiturates. It can also be reliably used in the ward and could be useful in units without ITU facilities by stopping seizures long enough for the child to be safely transported to the nearest appropriate facilities.

Lignocaine is a common drug in the emergency department which is seldom considered in the treatment of SE (Teng et al., 1994; Walker et al., 1997). The major advantage is the absence of respiratory side-effects and can be given safely to patients with limited respiratory reserve (Shorvon, 1994b). Lignocaine has also been successful in patients who have been resistant to benzodiazepines and phenytoin (Brodtkorb et al., 1993). The initial dose needs to be followed up with an intravenous infusion as its half life is very short (Brown et al., 1991b; Shepherd, 1994b). If the infusion is only given for up to 12 hours, there is little risk of accumulation. Lignocaine has been used in neonates with some success. 15 of 24 neonates stopped fitting altogether whilst only 2 children in that cohort had no response at all (Maytal, 1993). Lignocaine should be
administered with ECG monitoring as it may cause cardiac arrhythmias despite its acceptance as a drug for treating these cardiac problems. In high doses lignocaine may be convulsant although this is not an expected side effect at the doses recommended.

The initial drugs used in intensive care are frequently barbiturates. Phenobarbitone has been widely used in clinical practice since 1912. A loading dose of 20 mg/kg followed by 10 mg/kg boluses every 30 minutes until the seizures stop has been suggested (Crawford et al., 1988). This regime will almost certainly result in the child needing ventilation and therefore should be used with caution. Phenobarbitone continues to be the drug of choice for seizures in the neonatal period. The elimination half life ranges from 50 - 150 hours and one can expect the patient to have side effects for several days after a single intravenous dose.

Thiopentone is the favoured barbiturate in the treatment of refractory SE in the UK, but needs to be used in the ITU and with particular care in patients with impaired myocardial function. Many intensive care units in Britain continue to use phenobarbitone as the barbiturate of choice in children (Walker et al., 1995). Pentobarbital tends to be the barbiturate of choice in the USA. The aim of treatment is to stop electrical and clinical epileptic activity for 12-24 hours followed by an attempt to wean the drug. Continuous EEG monitoring or cerebral function monitoring (CFM) in the ITU would be ideal, but few institutions have the facilities to carry this out. Intermittent EEGs provide an alternative to continuous monitoring in the majority of units. EEG monitoring
is also necessary to avoid the situation in which epileptic discharges persist, despite absence of abnormal motor activity. This worsens prognosis substantially (Treiman, 1996). For the same reason paralysing drugs should not be administered unless appropriate EEG monitoring is available.

The prognosis for patients who have required thiopentone is worse than that for the SE group as a whole (Yaffe et al., 1993). This is primarily because the underlying aetiology is likely to be more severe, but barbiturates themselves are hazardous particularly when cerebral perfusion is reduced by cerebral oedema and/or cardiac insufficiency. All physical signs used for the assessment of cerebral state are lost. The usual length of barbiturate coma is a few days but there is a report of an 18 year old male being maintained in barbiturate coma for 53 days. He had a good neurological outcome (Mirski et al., 1995). It is important to continue treatment unless the underlying aetiology suggests that outcome is going to be poor.

Several other drugs have been used in the treatment of SE. Propofol is a relatively new anaesthetic agent with probable GABAergic effects which is becoming widely used in the intensive care treatment of SE (Hantson et al., 1994; Stecker et al., 1998). Its advantages include a rapid onset of action, a short half-life and rapid elimination (Bansinath et al., 1995) There are concerns about prolonged infusions in childhood as it has been associated with severe metabolic acidosis and hypoxia of uncertain origin. Rhabdomyolysis has also been reported during maintenance infusion (Hanna et al., 1998). Seizure-like behaviours, myoclonus and opisthotonus have also been observed during
propofol infusions. The debate on whether propofol can be convulsant as well as anticonvulsant continues (Borgeat et al., 1994). Although propofol is a drug with potential it should be used with caution in childhood.

Other drugs including ketamine, etomidate and the inhalational anaesthetic, isoflurane have also been used to treat refractory SE (Tunik et al., 1992; Maytal, 1993; Shorvon, 1994b; Shepherd, 1994b). Isoflurane use is frequently associated with hypotension requiring fluid and dopamine support. Ketamine is an NMDA blocker which may be neuroprotective (Fujikawa, 1995) but human studies have not yet been carried out. There is limited data on the use of these drugs for SE but the need for further investigation is supported by these data. There remains a need for a simple regime such as that shown in Figure 1.2 to be widely available.

1.10. Hypotheses

The morbidity associated with SE is primarily due to a combination of the direct effects of SE and the drugs used in the treatment of SE. In order to reduce morbidity it is essential to determine the types of brain injury associated with SE, the length of SE required for brain damage to occur and to develop a socially acceptable treatment which can be used in the pre-hospital setting.

This thesis examines the following hypotheses related to SE;

- **Hippocampal and temporal lobe damage following PFC is different in terms of severity and extent to damage identified in patients with other or no underlying precipitating injury.** This is investigated in 16 children.
with histologically proven mesial temporal sclerosis using quantitative magnetic resonance techniques.

- **Status epilepticus causes hippocampal and other neuronal damage in humans.** This is investigated in 19 children collected prospectively following an episode of SE. Qualitative and quantitative magnetic resonance techniques were carried out within 48 hours and at follow-up.

- **Buccal administration of midazolam solution is an effective alternative to rectal diazepam in the acute treatment of seizures.** This was systematically investigated in three trials. An initial pharmacological study in healthy adult volunteers was followed by assessment of efficacy and adverse-effects in children having acute repetitive seizures. A randomised controlled trial comparing buccal midazolam and rectal diazepam in children having prolonged seizures was then carried out.
Treatment of Status Epilepticus in the Infant and Older Child

1. General supportive care. Airway, give oxygen and monitor cardiovascular and respiratory status. i.e. ECG and saturation monitors if available.

2. Pre-hospital treatment / Initial treatment in ward

   Rectal diazepam
   - 3-12 months: 2.5mg
   - 12m-5 yrs: 5mg
   - >5 yrs: 10mg

   Repeat once after 5 minutes if no effect (in hospital)

   OR Rectal paraldehyde
   - 3-12 months: 0.1ml/kg
   - 12m-5 yrs: 1ml/yr
   - >5 yrs max: 10ml

   Dilute 1:1 olive oil:paraldehyde

3. Hospital treatment

   Check electrolytes, glucose and correct abnormalities. LP if clinically indicated and seizure has stopped

   IV lorazepam 0.05 mg/kg bolus (may repeat once)

   ↓

   IV Phenytoin 20mg/kg slow bolus over 20 minutes
   (ECG control)

   ⇩

   Benzodiazepines
   Midazolam 0.05-0.4 mg/kg/hr

   Chlormethiazole
   infusion: 0.5-2ml/kg/hr

   ↓

   Thiopentone
   (EEG monitoring)

Figure 1.2. Algorithm for current treatment of SE in the infant and older child. The choice between chlormethiazole and benzodiazepines is not evidence based but is dependent upon clinical experience of the attending physician.
CHAPTER 2: MAGNETIC RESONANCE METHODOLOGY

2.1. Introduction

Nuclear magnetic resonance (NMR) techniques have revolutionised the non-invasive investigation of patients with central nervous system disorders. Both qualitative and quantitative data can be obtained. These techniques utilise magnetic fields, which interact with atomic nuclei, in order to acquire structural and functional information about the organism being investigated. Descriptions of NMR principles can be found in standard texts (see for example Gadian, 1995).

The principles of nuclear magnetic resonance can be described using two models, the classical and the quantum models. This description will confine itself to the classical model. In the classical model, nuclei which possess a magnetic moment can be considered to behave like a bar magnet. When placed in a magnetic field ($B_0$) oriented along the z-axis, the magnetic moments can only adopt a fixed number of orientations with respect to that magnetic field. In the case of the most frequently investigated nucleus, $^1$H (which forms the basis for most magnetic resonance studies in-vivo), there are only 2 allowed orientations, namely along or against the positive direction of the z-axis. There is a small excess of nuclear spins oriented in the direction of $B_0$. The vectors of nuclei oriented in opposite directions cancel each other out leaving a total magnetisation vector ($M_0$) oriented along the positive direction of the z-axis (Figure 2.1).
Figure 2.1. Schematic which represents protons precessing around the z-axis when placed in a magnetic field $B_0$. There are more protons in the direction of $B_0$ than against it. Opposite vectors cancel each other out leaving a total magnetisation vector $M_0$ in the positive direction of the z-axis, represented in the diagram by the heavy arrow.

The magnetic moments in the magnetic field precess around the z-axis, with the frequency of precession given by the Larmor equation ($\omega_0 = \gamma B_0$) where $\omega_0$ is the frequency of precession, $\gamma$ is the magnetogyric ratio and $B_0$ is the strength of the magnetic field in Tesla. The magnetogyric ratio is a constant that is characteristic of the type of nucleus being investigated (e.g. $^1$H, $^{31}$P, $^{13}$C etc). The frequency of the precession will therefore be constant in any given magnetic field. This is the state of equilibrium during which no signal can be acquired.
In order for a signal to be generated, the state of equilibrium must be disrupted. This is achieved by applying a radiofrequency (RF) magnetic field (B₁) along the x-axis causing the magnetic moments to rotate in the y-z plane. If the RF field is applied at the Larmor frequency along the x-axis for an appropriate time, the magnetisation vector will be moved to the y-axis. This is called a 90° pulse. The precession of the magnetisation in the x-y plane induces a voltage in a receiver coil, and it is this signal which is processed to give the final image or spectrum.

2.1.1. T1 relaxation and T1 weighting

Nuclei which have been subjected to a radiofrequency pulse will return to their equilibrium state, in the presence of B₀, after the RF field is switched off. T1 relaxation refers to the process by which nuclei return to alignment along the z-axis as in the equilibrium state. It is characterised by the exponential time constant T1. The physical environment of individual nuclei alters the T1 relaxation time e.g. cerebrospinal fluid has a long T1 relaxation time when compared to grey or white matter, which also have differing T1 relaxation times. If a second excitation RF field is applied after the equilibrium state has been reached by all nuclei then the same signal will be returned by all brain structures with similar proton density, and differentiation into CSF, grey or white matter may not be possible. If, however, the second field is applied prior to the equilibrium state being reached in some tissues, the signal returned from the tissues with a long T1 relaxation time will be less than the signal returned from the tissues with a short T1 relaxation time. This is referred to as T1 weighting. The time between the excitation pulses (usually 90° pulses) is called
the repetition time (TR). The amount of T1 weighting can be adjusted by
altering the TR. T1 weighted images are used primarily, but not exclusively,
for anatomical definition.

2.1.2. T2 relaxation and T2 weighting

T2 relaxation refers to the process by which nuclei adopt random positions
within the x-y plane, characterised by the time constant T2. Dephasing of spins
in the x-y plane is influenced by two processes; inhomogeneity of the magnet
and ‘true’ T2 relaxation. Loss of phase coherence as a result of magnet
inhomogeneity occurs because not all individual nuclei are experiencing the
same magnetic field strength and are therefore precessing around the z-axis at
different speeds. If a $180^\circ$ pulse is applied following a $90^\circ$ pulse, then the
nuclei which were moving away from the y axis move are flipped through
$180^\circ$, but continue to move in the same direction as prior to the $180^\circ$ pulse.
This results in rephasing of the nuclear spins along the y-axis prior to
dephasing again. This process, called a spin-echo, removes signal loss as a
consequence of inhomogeneity of the magnet and therefore only signal loss
which results from true T2 relaxation remains. The time between the $90^\circ$ pulse
and the peak of signal refocussing is called the echo-time (TE). It is possible to
create a series of echoes from a single excitation by means of multiple $180^\circ$
pulses. An image created from any of those single spin-echoes will be T2
weighted, with greater T2 weighting associated with increasing echo time. T2
weighted images are primarily used to detect pathology.
2.1.3. Chemical shift

Magnetic resonance spectroscopy is dependent upon the phenomenon known as chemical shift. If all nuclei experienced the same magnetic field in the presence of $B_0$, the NMR spectrum would consist of only a single spectral line i.e. the signal frequency from hydrogen in water and fat would be the same for both substances. In practice, this is not the case as the magnetic field which is experienced by individual nuclei is in part dependent upon its chemical environment. Individual nuclei within each chemical compound are shielded by the electrons in that compound and this alters the effective field experienced by the hydrogen nuclei. The Larmor equation is more accurately stated as

$$\omega_0 = \gamma B_0 (1-\sigma)$$

where $\sigma$ is the amount of shielding by electrons. A magnetic resonance spectrum separates into different resonance lines with different frequencies, corresponding to different chemical environments. The easiest resonance lines to identify in the proton spectrum of the brain are those from N-acetyl aspartate (NAA), creatine + phosphocreatine and choline containing compounds. From the Larmor equation, frequency is in part dependent upon $B_0$ and therefore chemical shifts produced in different strength magnets would not be directly comparable in units of frequency. The use of the dimensionless unit parts per million (ppm), $(\omega_x - \omega_{\text{ref}}) / \omega_{\text{ref}}$ where $\omega_x$ is the frequency of a individual chemical and $\omega_{\text{ref}}$ is the frequency of a reference substance, avoids this problem.

2.2. MR Methods

The following magnetic resonance investigations were carried out in the MR trials which will be reported in this thesis. The majority of patients were either
sedated or had a general anaesthetic for the magnetic resonance investigations. All of the studies were carried out on a 1.5-T Siemens SP4000 system (Erlangen, Germany)

2.2.1. Qualitative methods

2.2.1.1. 3D dataset

T1 weighted images were acquired using a three-dimensional magnetisation prepared rapid gradient echo (MPRAGE) sequence (TR 10ms/TE 4ms/TI 200ms/NEX 1), flip angle 12 degrees, matrix size of 256x256 and 128 sagittal partitions in the third dimension with partition thickness of 1.25mm.

2.2.1.2. Coronal and axial T2 images

In order to achieve T2 weighting a long TR and a long TE are used. The parameters are TE 90ms and TR 4600ms for coronal images and TE 90ms and TR 5700ms for axial images.

2.2.1.3. Analysis

All the films were reviewed by two consultant neuroradiologists (Dr Kling Chong and Dr T Cox) who were blinded to the clinical data, and the author. Particular attention was paid to the hippocampus and temporal lobe although abnormalities outside these regions were also sought.
2.2.2. Quantitative methods

2.2.2.1. T2 mapping

This technique allows quantification of hippocampal T2 relaxation times in milliseconds.

2.2.2.1.1. Acquisition and calculation of T2 maps

Hippocampal T2 maps were calculated from 16 images obtained with echo times ranging from 22-262 milliseconds using a modified Carr-Purcell-Meiboom-Gill sequence. The map is calculated by fitting a single exponential to the signal from each of the 16 spin-echoes. Logarithmic transformation of the curve produces a straight line from which an absolute T2 relaxation time can be calculated. The thickness of the selected plane was 8mm and its orientation was in a tilted coronal plane along the anterior border of the brainstem perpendicular to and at the level of the body of the hippocampus. The T2 relaxation time in milliseconds is displayed as pixel intensity on a map and the hippocampal T2 value was read using the largest possible region of interest placed in the hippocampus, avoiding boundaries where partial volume effects with cerebrospinal fluid might occur. Figure 2.2. shows the positioning of a typical region of interest within the hippocampus.
2.2.2. Control Data

Control data were obtained from 52 hippocampi in normal children with a median age of 135 months (range 2-216 months). The control data were acquired from siblings of patients with neurological conditions and children with possible neurocutaneous syndromes who have normal brains. Table 2.1. shows the raw control data. T2 relaxation time decreases during the early part of childhood, with the most rapid decline before the age of 2 years following which it plateaus. This is consistent with previous studies describing age-related changes in brain water content (Christiansen et al., 1994).
T2 relaxation time from control hippocampi was plotted against age at investigation. An inverse regression curve described by the equation $T2 = b_0 + \left(\frac{b_1}{\text{age} + 3.52}\right)$, and 95% confidence limits (95% CL) were fitted (Figure 2.3). From the regression analysis, $b_0 = 101.932$ and $b_1 = 232.489$. The constant 3.52 was calculated using non-linear regression. This curve significantly fitted the data, had a small scatter ($p < 0.001$, one-way ANOVA; $r^2 = 0.807$) and was biologically acceptable in that the T2 relaxation time was initially high and became constant within the first few years of life. The residuals are randomly distributed around 0 (Figure 2.4) and do not significantly differ from a normal distribution ($p=0.742$, Shapiro-Wilk). Values which lie above the 95% CL for age are considered abnormal. Although other models revealed a significant fit ($p < 0.001$) they did not fit the observed values as tightly as the inverse model, nor did they mimic the biological changes in T2 relaxation time and water content which have previously been described.
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<td>26</td>
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</table>

Table 2.1. T2 relaxation time in normal controls.
Figure 2.3. Scatterplot showing changes in T2 relaxation time with age. The red squares represent the predicted values for age calculated from an inverse regression model. The black squares represent the 95% confidence limits. Individual data points are shown as blue crosses.

Figure 2.4. Scatterplot of the residuals from the regression of T2 relaxation time with $1/(\text{age} + 3.52)$ confirming a random distribution.
2.2.2.2. Hippocampal volumetry

2.2.2.2.1 Calculation of hippocampal volumes

Images for hippocampal volumetry were obtained using a three dimensional magnetisation prepared rapid gradient echo (MPRAGE) sequence. The MPRAGE dataset was reformatted into 1.3mm thick contiguous slices in a tilted coronal plane which was perpendicular to the long axis of the hippocampus. Figure 2.5 shows three example sections of the hippocampus from the 3D MR dataset used to calculate the volume of the hippocampus. The reformatted images were transferred to a SUN workstation and analysed using Xdispim. The first slice of the hippocampus was defined as the one in which the fornix was first seen in its full profile. A one in two sampling strategy was used. The first slice was randomly selected, using a computer program, following which every second slice was systematically measured. The HCV was calculated by summing the hippocampal cross-sectional areas and multiplying this figure by the thickness of two slices. A previous study has assessed which sampling strategies provide reliable data but are not excessively time consuming (Van Paesschen et al., 1997). That study used a slice thickness of 1mm. A one in three sampling technique was reliable, but a one in four sampling technique was not. The reliable distance between slices was a maximum of 3mm. This study used 1.3mm slices and therefore a one in two sampling strategy, with an interslice distance of 2.6mm, was used.

Intracranial volume (ICV) was measured on the sagittal unreformatted MPRAGE dataset using a one in ten sampling strategy. This has previously been shown to be a reliable method (Van Paesschen et al., 1997). Landmarks
for the ICV were the dura mater, or the brain surface if the dura was not visible.

Figure 2.5. Examples of three slices from a reformatted 3D dataset which were used in the calculation of hippocampal volumes. The top panel shows a posterior slice and the subsequent two examples are progressively more anterior. The slice numbers are marked from slice 1 in which the fornix is first seen in full profile.

2.2.2.2. Control data

Control data were obtained from 16 normal volunteers. Table 2.2. shows the raw data of the volunteers hippocampal and intracranial volumes. Hippocampal volume was assumed to be linearly related to intracranial volume. Each hippocampal and intracranial volume was measured twice, and the volume used was the mean of the 2 values. A linear regression model defined by the
equation \( HCV = b_0 + b_1 \times (ICV) \) was used to identify the slope of the regression line through the data. HCV was then corrected for ICV using the covariance method (Jack et al., 1995) which derives a HCV corrected for ICV using the following equation; \( HCV_{\text{corrected}} = HCV_{\text{measured}} - \text{gradient} \times (ICV_{\text{measured}} - ICV_{\text{mean}}) \).

HCV and ICV are expressed in mm\(^3\). Gradient is the slope of the regression line of control HCV versus ICV using a linear regression analysis. In this study the gradient was 0.002627. The mean intracranial volume was 1457470 mm\(^3\). Assessment of the candidate's intra-rater test-retest repeatability, with at least 2 weeks between measurements, revealed a coefficient of repeatability of 520 mm\(^3\) (13.2% of the mean hippocampal volume). The coefficient of variability is defined as 2 standard deviations away from the mean of the difference between the first and second measurements, divided by the mean hippocampal volume. The result means that 95% of repeat measurements were within 6.6%, in either direction, of the first measurement. The coefficient of repeatability for intracranial volume was 47350 mm\(^3\) (3.2% of mean ICV).

Mean corrected HCV was 3916 +/- 418 mm\(^3\). HCV of less than 3080 mm\(^3\) or greater than 4752 mm\(^3\) (2 SD away from the mean) are considered abnormal.
<table>
<thead>
<tr>
<th>Control Number</th>
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<th>HCV 2 (mm³)</th>
<th>ICV (mm³)</th>
<th>Mean HCV corrected (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>First</td>
<td>Second</td>
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<tr>
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<td>2974</td>
<td>2820</td>
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</table>

Table 2.2. Table showing first and second measurements of right and left hippocampal volumes, intracranial volumes and corrected hippocampal volumes in 16 normal controls.
2.2.2.3. Magnetic resonance spectroscopy

2.2.2.3.1. Acquisition of spectra

Single voxel $^1$H MR spectra were obtained at 1.5T from 2x2x2cm cubic volumes centred on each mesial temporal region. The region of interest was selected from coronal MR images. Figure 2.6 shows typical 2cm cube volumes of interest placed in the mesial temporal lobes for acquisition of $^1$H MRS data. The hippocampus only occupies a small proportion of the $^1$H MRS volume and any spectral abnormalities therefore reflect more widespread pathology within the temporal lobes. Spatial localisation was achieved using a 90-180-180 spin echo technique with three selective radiofrequency pulses applied in the presence of orthogonal gradients of 2mT/m. Water suppression was effected by pre-irradiation of the water resonance using a 90° Gaussian pulse with a 60 Hz bandwidth followed by a spoiler gradient. TR was 1600ms and TE was 135ms.
Figure 2.6. Coronal T1 weighted image showing the typical positioning of 8cm³ voxels in the temporal lobe from which were used for the acquisition of ¹H MR spectra

2.2.2.3.2. Analysis

Proton spectra from the brain show major contributions from N-acetylaspartate (NAA), choline-containing compounds (Cho) and creatine + phosphocreatine (Cr)(Figure 2.7). Signal return from NAA, Cr and Cho are calculated using peak integrals. In animal and cell culture studies NAA has been found to be located primarily in neurons, and any reduction in NAA signal is generally attributed to neuronal loss or dysfunction (Urenjak et al., 1992; Urenjak et al., 1993). Also the concentrations of Cr and Cho are higher in astrocyte and oligodendrocyte cell preparations than in cerebellar
granule neurons (Urenjak et al., 1992; Urenjak et al., 1993). It is possible that any increase in the Cho and Cr $^1$H MRS signals may reflect reactive astrocytosis. Reduction in NAA/Cho+Cr ratios can be used to help localise an epileptic focus in patients with temporal lobe epilepsy in whom neuronal loss and glial proliferation have occurred.

![MR spectrum](image)

Figure 2.7. $^1$H MR spectrum from a normal control showing major contributions from NAA, Cho and Cr

2.2.2.3.3. Control Data

Control data were collected from 45 temporal lobes in 26 normal controls with a median age of 137 (range 2-204) months. The control data were acquired from siblings of patients with neurological conditions and children with possible neurocutaneous syndromes who have normal brains. The raw data from the controls is shown in Table 2.3. NAA/(Cho+Cr) was plotted against age at investigation. A power regression model described by the equation $\text{NAA/(Cho+Cr)} = b_0 \text{(age)}^{b_1}$ was used to fit a line and 95% CL through the normal data ($p<0.001$, one-way ANOVA; $r^2=0.561$. Figure 2.8). From the regression analysis, $b_0 = 0.440$ and $b_1 = 0.103$. This is the curve
which fits the data most accurately and is biologically acceptable. The residuals are randomly distributed around 0 (Figure 2.9) and do not differ significantly from a normal distribution (p=0.466, Shapiro-Wilk). Values which lie below the 95% CL for age are considered abnormal. Although other models revealed a significant fit (p<0.001), they did not fit the observed values as tightly as the power model.
<table>
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<tr>
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<th>NAA/(Cho+Cr) Right</th>
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<td>0.59</td>
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Table 2.3. Raw data of NAA/(Cho+Cr) ratios and ¹⁵HMRS% in healthy controls.
Figure 2.8. Scatterplot showing changes in NAA/(Cho+Cr) with age. The red squares represent the predicted values for age calculated from a power regression model. The black squares represent the 95% confidence limits. Individual data points are shown as blue crosses.

Figure 2.9. Scatterplot of the residuals from the regression of NAA/(Cho+Cr) with loge(age) confirming a random distribution.
The above investigations were carried out in the magnetic resonance studies that will be reported in the following chapters. These quantitative investigations allow objectivity and increased sensitivity when compared to qualitative techniques.
CHAPTER 3. MESIAL TEMPORAL SCLEROSIS IN CHILDREN: THE INFLUENCE OF PROLONGED FEBRILE CONVULSION ON QUANTITATIVE MR FINDINGS

3.1. Introduction

Mesial temporal sclerosis (MTS), defined as hippocampal sclerosis with more widespread abnormality in the temporal lobe, is the commonest histopathological finding in patients who undergo temporal lobe resections for intractable epilepsy. Magnetic resonance (MR) technology has been used in the diagnosis of MTS during pre-surgical evaluation.

3.1.1. Use of MRI in the investigation of patients with epilepsy

Magnetic resonance imaging (MRI) has revolutionised the in-vivo investigation of patients with epilepsy. This technique became available in the mid 1980's and rapidly became recognised as a superior brain imaging technique to the standard imaging technique of the time, X-ray computed tomography (CT). CT offers less sensitivity and specificity in identifying the aetiology of epilepsy than MRI. Although CT may be useful in the identification of tumours or major malformations, it is poor for visualising the temporal lobe and the hippocampus and cannot be reliably used in the diagnosis of MTS. MRI has subsequently become the accepted imaging technique in the investigation of patients with epilepsy. The indications for MRI in patients with epilepsy include partial seizures, onset of seizures in the first year of life, evidence of a fixed deficit on neurological or
neuropsychological examination or a loss of seizure control (Jackson, 1994; Jackson, 1995; Duncan, 1997). Although MRI can be used to detect structural and chemical abnormalities anywhere in the brain, this chapter will only review the use of MRI in the investigation of MTS.

3.1.2. In-vivo imaging of the hippocampus and temporal lobe

Initial reports of MRI use in patients with MTS were conflicting such that it became widely held that MTS could not be reliably diagnosed in-vivo. As technology improved and studies became more systematic, it became increasingly clear that MRI would become a useful imaging modality in patients with partial epilepsy who were thought to have MTS. MRI primarily provides structural information about the hippocampus and temporal lobe, which if taken in conjunction with functional techniques such as EEG, positron emission tomography (PET) or single photon emission computed tomography (SPECT), provides adequate data for a decision on whether surgical treatment of epilepsy in an individual patient is justified. In order to achieve this reliably, special MR protocols are required. These protocols should include images which are oriented along, and at 90° to the long axis of the hippocampus (Jackson et al., 1993a; McBride et al., 1998). T1 and T2 weighted images are required. Both qualitative and quantitative MR techniques are useful in the context of pre-surgical evaluation of patients with temporal lobe epilepsy.
3.1.2.1. Qualitative investigations

The qualitative MRI characteristics of MTS include atrophy, decreased signal intensity and loss of the internal architecture on T1 weighted images with increased signal intensity within the hippocampus on T2 weighted images (Jackson et al., 1993a; Meiners et al., 1994). Optimal assessment of qualitative MRI of the hippocampus and temporal lobe requires a high degree of anatomical understanding on the part of the reviewer. An experienced neuroradiologist decides whether atrophy is present by comparing the hippocampi and temporal lobes from each side in individual patients and looks for the loss of internal structure on T1 weighted images. The alveus, pyramidal layer of the cornu ammonis and the molecular cell layer of the dentate gyrus can be observed on optimised scans (Jackson et al., 1993a). Loss of the internal architecture suggests that there has been neuronal loss and possibly gliosis in the hippocampus. The signal hypointensity on T1 weighted images and the hyperintensity on T2 weighted images may both reflect gliosis in the hippocampus. The presence of all four features is reliably correlated with histopathological evidence of MTS, with a sensitivity of approximately 90% and specificity of approximately 85% (Jackson et al., 1993a; Kuzniecky et al., 1993; Kuzniecky et al., 1997; Lee et al., 1998). Visual assessment is more difficult when patients have bilateral abnormalities. The diagnosis of MTS on visual analysis alone is, at least in part, dependent upon the skill and experience of the person reviewing the scans.
Hippocampal volume reductions of approximately 20% can be reliably identified on visual assessment. There appears to be a correlation between the degree of hippocampal atrophy identified with MRI and the degree of histological abnormality (Cascino et al., 1991; Bronen et al., 1991; Lee et al., 1998). By the nature of these studies, the correlation described is crude and quantitative analysis would be required to determine whether hippocampal volume loss reflects cell loss of a particular type.

3.1.2.2. Quantitative investigations

Quantitative MR techniques, including hippocampal volumetry (HCV) and hippocampal T2 relaxometry (T2) provide data which increase the sensitivity of identification of hippocampal abnormality above that of qualitatively assessed MRI (Van Paesschen et al., 1997b; Van Paesschen et al., 1997a; Quigg et al., 1997a; Namer et al., 1998). Single voxel proton magnetic resonance spectroscopy (1H MRS) has increased the sensitivity of identification of abnormalities within the temporal lobe. The techniques need to be objective, reliable and reproducible. These quantitative techniques have been shown to increase the sensitivity of the identification of bilateral or subtle abnormalities.

3.1.2.2.1. Hippocampal volumetry

Hippocampal volume assessment provides quantitative data about the whole length of the hippocampus (Jack, Jr. et al., 1992; Jack, Jr. 1994; Adam et al., 1994; Jack, Jr. et al., 1995; Watson et al., 1997b; Van Paesschen et al., 1997a). Hippocampal atrophy identified using MR techniques is
correlated with neuronal depletion and concomitant gliosis in CA1, CA2, CA3 and hilus regions of the hippocampus. Increased hippocampal T2 relaxation time is correlated with damage in CA1 and the hilus. The neuropathological basis of increased T2 relaxation time appears to be different to the neuropathological basis of hippocampal volume loss (Van Paesschen et al., 1997b). These investigations should be considered to be surrogate markers of hippocampal neuronal loss and cannot be considered as providing accurate and specific data about histopathological abnormalities.

Initial work on hippocampal volumes relied primarily on identification of hippocampal asymmetry for the diagnosis of MTS (Lencz et al., 1992; Jack, Jr. et al., 1995). This was because of the wide variability in the hippocampal volumes in normal controls. The majority of patients with MTS have asymmetrical hippocampi (Jackson et al., 1990; Berkovic et al., 1991; Van Paesschen et al., 1997a) but there are a proportion of patients with bilateral hippocampal volume loss. The use of hippocampal volume ratios does not allow diagnosis of symmetrically small hippocampi or asymmetric hippocampi, both of which are smaller than control hippocampal volumes. Methods which correct hippocampal volume for intracranial volume have decreased the wide normal range and has allowed absolute volumes to be compared between patients and normal controls more accurately (Free et al., 1995; Jack et al., 1995). These methods have also led to the identification of bilateral hippocampal volume loss in patients with suspected unilateral disease and may provide prognostic information for patients undergoing
temporal lobe resections for intractable temporal lobe epilepsy (King et al., 1995; Free et al., 1996; Quigg et al., 1997b; Barr et al., 1997). There is a correlation between side of hippocampal volume loss, side of seizure onset and good outcome from temporal lobe resection (Baulac et al., 1994; Watson et al., 1997b; Duncan, 1997; Watson et al., 1997a; Marsh et al., 1997; Lee et al., 1998). Although hippocampal volumetry has provided valuable insight into the diagnosis of MTS, it is time consuming. A comparison between the diagnostic accuracy of visual assessment and hippocampal volume measurement showed that the quantitative technique did not influence specificity, sensitivity, positive or negative predictive value when compared to visual assessment in patients with unilateral hippocampal sclerosis (Cheon et al., 1998). Hippocampal volumetry may continue to be useful in the identification of bilateral or subtle abnormalities.

3.1.2.2. Hippocampal T2 relaxometry

Increased T2 signal in the hippocampus is one of the diagnostic criteria for MTS. This variable can be quantified using T2 relaxometry. A visual increase in T2 signal return can be identified in up to 60% of patients with MTS. T2 relaxometry increases sensitivity above that of visual assessment alone (Jackson et al., 1993b). It is a precise, reproducible, reliable and stable measurement (Grünewald et al., 1994). There is a narrow normal range which allows identification of relatively subtle abnormalities (Jackson et al., 1993b). T2 relaxometry, when taken alone, correctly lateralises to the side of seizure origin in 70-79% of cases (Jackson, 1994; Kuzniecky et al., 1997; Van Paesschen et al., 1997a). Pathologically, hippocampal T2
relaxation time is inversely correlated with the ratio of the glial to neuronal density in the CA1 region of the hippocampus (Van Paesschen et al., 1997b). This is one of the histopathological abnormalities which would be expected in patients with MTS. In early childhood, T2 relaxation times are prolonged with respect to adults and older children. This change in T2 relaxation times with age resembles changes in total brain water over a similar age span (Christiansen et al., 1994). As T2 relaxation time is, in part, a reflection of hippocampal water mobility, increases in water content (e.g. oedema) in an older age range could also prolong T2 relaxation times.

3.1.2.2.3. Proton magnetic resonance spectroscopy

The quantitative data outlined above refers exclusively to investigation of the hippocampus. Patients with MTS may also have histological neuronal loss in a wider area of the temporal lobe than just the hippocampus (Cavanagh et al., 1956; Falconer et al., 1964). Quantitative MR investigation of the temporal lobe can be undertaken using proton magnetic resonance spectroscopy ($^1$H MRS). N-acetyl aspartate (NAA), creatine + phosphocreatine (Cr) and choline containing compounds (Cho) are most readily identified with $^1$H MRS (Connelly et al., 1994; Gadian, 1995; Novotny, Jr. 1995; Duncan, 1996; Laxer, 1997). The cell population location of these chemicals is discussed in section 2.2.2.3.2. The results in most studies are not reported as absolute signal intensities, but rather as ratios of NAA to either Cr, Cho or Cho+Cr.
Single-voxel $^1$H MRS with an 8cm$^3$ voxel placed in the mesial temporal lobe of patients with temporal lobe epilepsy reveals a reduction in NAA/(Cho+Cr) in approximately 85% of those patients, which suggests either neuronal loss or dysfunction, possibly associated with increases in the glial cell populations. Abnormalities ipsilateral to the seizure focus are frequently of greater magnitude than abnormalities contralateral to the seizure focus although bilateral abnormalities are common (40-45%) in both adults and children (Connelly et al., 1994; Breiter et al., 1994; Cross et al., 1996). Correct lateralisation of the seizure focus in over 80% of patients is possible (Connelly et al., 1994).

Chemical shift imaging (CSI), also known as MR spectroscopic imaging (MRSI), is a technique in which many voxels in a large region of the brain are investigated simultaneously. This can provide regional information which can be used to calculate a metabolic map providing regional information on the chemicals outlined above. Results from studies using CSI are similar to those using single-voxel $^1$MRS (See for example Cendes et al., 1994; Cendes et al., 1995).

The data reviewed above reveal that MR techniques are useful for the in-vivo diagnosis of MTS. The majority of the work assumes that MTS always has the same radiological characteristics irrespective of the cause. This may not be justified as MTS has many associations, the commonest of which is prolonged febrile convulsion (PFC) in childhood. Since the original descriptions of the association between PFC and MTS (Cavanagh et
al., 1956; Falconer et al., 1964), there has been an ongoing debate on whether MTS precedes or is caused by PFC. There is mounting evidence for the lesion being acquired following prolonged seizures in some cases. Animal studies in which SE is chemically or electrically induced have demonstrated hippocampal lesions which are very similar to the changes identified in humans with MTS (Lothman et al., 1989; Lothman et al., 1993; Cavalheiro, 1995). Although it is tempting to suggest that all MTS, if acquired, results from the same mechanism, the many associations make this unlikely. Therefore, different underlying pathogenetic mechanisms could result in different types of MTS. The outcome from temporal lobe resections is better in patients with MTS and a history of PFC than those with no such history (Abou-Khalil et al., 1993) but the reasons for this have been unclear. This also supports the view that different types and extents of MTS may exist.

There are some data which have attempted to separate MTS associated with PFC from MTS with other associations. Patients with PFC tend to have severe unilateral hippocampal volume loss, even in patients with bilateral hippocampal volume reductions, when scans are assessed both visually and with hippocampal volume measurements (Cendes et al., 1993; Barr et al., 1997; Van Paesschen et al., 1997a; Kanemoto et al., 1998). No studies that use several quantitative MR measures to try and differentiate MTS associated with PFC from MTS without such a preceding history have been reported.
3.2. Aims

Results of qualitative and quantitative magnetic resonance investigations, taken together, provide reliable and reproducible information regarding the human hippocampus in-vivo. The present study aimed to systematically investigate whether quantitative MR techniques could be used to identify radiological differences in the features of MTS in patients with a history of PFC compared to the findings in those patients without such a history. This may provide some insight into the pathogenesis of MTS in humans. No attempt was made to define histological characteristics of MTS during this study.

3.3. Patient Recruitment

Consecutive patients with a diagnosis of MTS were retrospectively identified from the Great Ormond Street Hospital epilepsy surgery program. Pre-operative investigations in that unit include video-EEG telemetry, MRI, $^1$H MRS, ictal and inter-ictal SPECT, neuropsychological and neuropsychiatric assessments. The decision to proceed to surgery was made at a multidisciplinary meeting. All of the patients included in this study underwent temporal lobe resections, eleven left sided, and were included only if MTS was confirmed histologically. No attempt was made to characterise different types of MTS on histological grounds as this would have entailed a separate study involving cell counting. Patients with extratemporal epilepsy and associated MTS were not included.
3.4. Methods

There were 16 patients identified whose notes were reviewed. They were divided into 2 groups: A summary of the clinical information can be found in Table 3.1.

- **Group 1**: Those patients with a history of PFC (8 patients; nos 1-8). All seizures lasted longer than 30 minutes. Two patients had focal seizures followed by a Todd's paresis (patients 2 and patient 4 in Table 3.1). The longest febrile convulsion, lasting one hour, occurred in patient 3.

- **Group 2**: Those with other or no initial precipitating injuries (8 patients; nos 9-16). These were; trauma (2 patients), febrile convulsion lasting less than 5 minutes (2), non-febrile SE (1) and no initial precipitating injury (3).

There were 2 patients who had head injuries as an initial precipitating event. One child was probably non-accidentally injured and was admitted with bilateral subdural haemorrhages and retinal haemorrhages at the age of 7 months (patient 14). The other was involved in a road traffic accident at the age of 3 years. She had a left parietal skull fracture. Following the accident she was ventilated for 2 days (patient 9). The patient with non-febrile status epilepticus had a 2 hour generalised tonic-clonic seizure with no identified precipitant. Pre-operatively, patients with PFC had between 2 and 90 seizures per month. Those with no history of PFC had between 3 and 500 seizures per month.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>IPI</th>
<th>Age at IPI</th>
<th>Age at MRI (years)</th>
<th>Electro-clinical focus</th>
<th>Seizure Frequency / month</th>
<th>Postoperative Follow-up</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>PFC</td>
<td>10 mths</td>
<td>18</td>
<td>LTLE</td>
<td>2-4</td>
<td>2 yrs</td>
<td>Sz free</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>PFC</td>
<td>2 yrs</td>
<td>5</td>
<td>LTLE</td>
<td>6-10</td>
<td>11 mth</td>
<td>Auras</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>PFC</td>
<td>6 mths</td>
<td>14</td>
<td>LTLE</td>
<td>70-90</td>
<td>1 yr</td>
<td>Sz free</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>PFC</td>
<td>10 mths</td>
<td>7</td>
<td>RTLE</td>
<td>2-5</td>
<td>26 mth</td>
<td>Sz free</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>PFC</td>
<td>10 mths</td>
<td>16</td>
<td>LTLE</td>
<td>2-3</td>
<td>2yr</td>
<td>Sz free</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>PFC</td>
<td>10 mths</td>
<td>11</td>
<td>LTLE</td>
<td>8-20</td>
<td>3</td>
<td>Sz free</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>PFC</td>
<td>9 mths</td>
<td>11</td>
<td>LTLE</td>
<td>4-8</td>
<td>2 yr</td>
<td>Cont Sz</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>PFC</td>
<td>24 mths</td>
<td>12</td>
<td>RTLE</td>
<td>2-10</td>
<td>14</td>
<td>Cont Sz</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>H/I</td>
<td>3 yrs</td>
<td>9</td>
<td>LTLE</td>
<td>5-6</td>
<td>11 mth</td>
<td>Auras</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>none</td>
<td>--</td>
<td>15</td>
<td>LTLE</td>
<td>3-4</td>
<td>2 yr</td>
<td>Sz free</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>none</td>
<td>--</td>
<td>12</td>
<td>LTLE</td>
<td>3-5</td>
<td>3 yr</td>
<td>Sz free</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>SFC</td>
<td>7 mths</td>
<td>7</td>
<td>RTLE</td>
<td>10-12</td>
<td>2 yr</td>
<td>Cont Sz</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>none</td>
<td>--</td>
<td>2</td>
<td>LTLE</td>
<td>Approx 500</td>
<td>2 yr</td>
<td>Cont Sz</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>H/I</td>
<td>7 mths</td>
<td>12</td>
<td>RTLE</td>
<td>12-14</td>
<td>14 mth</td>
<td>Sz free</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>SFC</td>
<td>8 mths</td>
<td>3</td>
<td>RTLE</td>
<td>10-15</td>
<td>4 mth</td>
<td>Cont Sz</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>SE</td>
<td>5 yrs</td>
<td>8</td>
<td>LTLE</td>
<td>4-5</td>
<td>15 mth</td>
<td>Sz free</td>
</tr>
</tbody>
</table>

Table 3.1. Clinical data of patients with histologically proven MTS

PFC = prolonged febrile convulsion, H/I = head injury, SFC = short febrile convulsion, SE = status epilepticus, IPI = initial precipitating injury, RTLE = right temporal lobe epilepsy, LTLE = left temporal lobe epilepsy, Sz = seizure, Cont = continuing
3.4.1. *Hippocampal investigations*

T2 relaxometry, as described in section 2.2.2.1., was performed in all patients. In addition to the absolute T2 values, T2 ratios (T2R) were calculated by dividing the ipsilateral T2 relaxation time by the contralateral value in individual patients. Hippocampal volume measurements, as described in section 2.2.2.2., were carried out in 7/8 patients in group 1 and 5/8 in group 2. The remaining patients did not have an appropriate dataset for volume measurement. As for T2 measurements, the corrected hippocampal volume measures were used to calculate hippocampal volume ratios by dividing ipsilateral volume by the contralateral volume in individual patients. The hippocampal volumes for this part of the study were measured by Dr S Wood (Dept of Cognitive Neurosciences, ICH) who used this period to teach the method to the candidate. Control data reveal that HCV of less than 3412mm^3 (i.e. mean-2SD) can be considered to be abnormal.

3.4.2. *Temporal lobe investigations*

$^1$H MRS, as described in section 2.2.2.3., was performed in all patients in Group 1 and 6/8 patients in Group 2. The remaining 2 patients did not have $^1$H MRS data collected. NAA/(Cho+Cr) ratios were calculated for each temporal lobe. Side-to-side $^1$H MRS ratios were calculated by dividing the ipsilateral value by the contralateral value in individual patients.
3.5. Statistical Analysis

Statistical analysis was performed using SPSS for Windows, release 8.0. T2 relaxation time and $^1$H MRS data were analysed using multiple linear regression. Group comparisons of corrected hippocampal volume and T2 relaxation times were carried out using the Mann – Whitney U test.

3.6. Results

The quantitative MR findings are summarised in Table 3.2. and Table 3.3.

3.6.1. Hippocampal findings

3.6.1.1. T2 Relaxometry

The T2 relaxation time was abnormal ipsilateral to the clinical / EEG seizure focus in all patients in Group 1 (PFC) and 7/8 patients in Group 2 (non-PFC). None of the 8 patients in Group 1 had abnormal contralateral T2 relaxation times compared to 3/8 in Group 2. Multiple linear regression analysis revealed that T2 relaxation time is significantly dependent upon whether patients fall into Group 1 (p<0.001), Group 2 (p<0.001) and to whether the hippocampus was ipsilateral or contralateral to the seizure focus (p<0.001), when compared to controls. T2 relaxation time was not dependent upon age over the age range of these patients and controls (p=0.15).

Characterisation of the group abnormalities was carried out by analysing T2 relaxation times and T2R. In both groups there were significant differences between ipsilateral and contralateral T2 relaxation times (Figure 3.1).
Group 1 $p<0.001$, Group 2 $p=0.003$). T2R revealed significantly more asymmetry in Group 1 than in Group 2 ($p=0.004$). Ipsilateral T2 relaxation time was significantly longer in Group 1 than in Group 2 ($p=0.03$). T2 ipsilateral to the seizure focus was significantly longer in both groups when compared to normal controls ($p<0.0001$). Contralateral to the seizure focus there was no difference between T2 relaxation time in Group 1 and controls ($p=0.35$) or between Group 1 and Group 2 ($p=0.15$). There was significant T2 prolongation contralateral to the seizure focus in Group 2 when compared to controls ($p=0.02$).

Figure 3.1. Box and whisker plots showing T2 relaxation time in patients with and without a history of prolonged febrile convulsion. The horizontal line represents the median, the box represents the interquartile range and the whiskers represent the complete range within 1.5 boxplots of the median. Outliers are shown as +. In the patient groups, ipsilateral findings are shown in the red boxes and contralateral findings are shown in the green boxes. In the controls, right sided data are shown in the red boxes and left sided data are shown in the green boxes.
3.6.1.2. Hippocampal volumetry

All of the patients in Group 1 had small hippocampi ipsilateral to the seizure focus compared to 3/5 in Group 2 (Figure 3.2). A small hippocampus was identified contralateral to the seizure focus in 3/7 patients in Group 1 and 1/5 patients in Group 2. There was a significant difference between the ipsilateral and contralateral volumes in Group 1 (p=0.01) but not in Group 2 (p=0.14). Comparison of the hippocampal volume ratio between the groups revealed more asymmetry in Group 1 than in Group 2 (p=0.02). Group 1 had significantly smaller hippocampi ipsilateral to the seizure focus than Group 2 (p=0.02). There was no significant difference between the groups in the size of the hippocampi contralateral to the seizure focus. The hippocampi in Group 1 were bilaterally smaller than those in controls (p<0.001 ipsilateral, p=0.03 contralateral). In Group 2, the hippocampal volume ipsilateral to the seizure focus was smaller than that in controls (p=0.008) but although there was a trend to abnormality the contralateral hippocampal volumes were not significantly smaller than contralateral control volumes (p=0.0693).
Figure 3.2. Box and whisker plots showing hippocampal volumes in patients with and without a history of prolonged febrile convulsion. The horizontal line represents the median, the box represents the interquartile range and the whiskers represent the complete range within 1.5 boxplots of the median. Outliers are shown as +. In the patient groups, contralateral findings are shown in the red boxes and ipsilateral findings are shown in the green boxes. In the controls, right sided data are shown in the red boxes and left sided data are shown in the green boxes.

3.6.2. Temporal lobe findings

3.6.2.1. \(^1\)H MRS

Abnormal NAA/(Cho+Cr) ipsilateral to the seizure focus was identified in 7/8 patients in Group 1 and 5/6 patients in Group 2. Contralateral to the seizure focus, 3/8 patients in Group 1 and 4/6 patients in group 2 had abnormal NAA/(Cho+Cr). Multiple linear regression revealed that NAA/(Cho+Cr) was significantly dependent upon age (p<0.001), or whether patients fall into Group 1 or Group 2 (p<0.001 for both groups).
NAA/(Cho+Cr) was not significantly dependant upon whether the data was collected from the side ipsilateral or contralateral to the seizure focus (p=0.109) (Figure 3.3). In Group 1 there was a mean reduction of NAA/(Cho+Cr) of 33% (95% CL: 20-45%) when compared to controls. In Group 2 there was a mean reduction in NAA/(Cho+Cr) of 41% (95% CL: 27-59%). The differences between Group 1 and Group 2 were not significant.

Figure 3.3. Scatterplot of mean predicted values for age in controls (blue dots), patients with a history of PFC (red dots) and patients with no history of PFC (green dots)
<table>
<thead>
<tr>
<th>Patient</th>
<th>T2 Relaxation Time (msec)</th>
<th>Hippocampal Volume (mm³)</th>
<th>NAA/(Cho+Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>128</td>
<td>106</td>
<td>1.21</td>
</tr>
<tr>
<td>2</td>
<td>128</td>
<td>108</td>
<td>1.19</td>
</tr>
<tr>
<td>3</td>
<td>133</td>
<td>107</td>
<td>1.24</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>103</td>
<td>1.21</td>
</tr>
<tr>
<td>5</td>
<td>111</td>
<td>99</td>
<td>1.12</td>
</tr>
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<td>6</td>
<td>125</td>
<td>102</td>
<td>1.23</td>
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<td>7</td>
<td>129</td>
<td>110</td>
<td>1.17</td>
</tr>
<tr>
<td>8</td>
<td>123</td>
<td>110</td>
<td>1.12</td>
</tr>
<tr>
<td>mean +/- SD</td>
<td>125.2 +/- 6.5</td>
<td>105.6 +/- 3.9</td>
<td>1.19 +/- 0.05</td>
</tr>
</tbody>
</table>

Table 3.2. Quantitative hippocampal and temporal lobe data in patients with a history of PFC. Abnormal values are shown in bold.

<table>
<thead>
<tr>
<th>Patient</th>
<th>T2 Relaxation Time (msec)</th>
<th>Hippocampal Volume (mm³)</th>
<th>NAA/(Cho+Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-PFC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>112</td>
<td>106</td>
<td>1.06</td>
</tr>
<tr>
<td>10</td>
<td>120</td>
<td>105</td>
<td>1.14</td>
</tr>
<tr>
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<td>110</td>
<td>107</td>
<td>1.03</td>
</tr>
<tr>
<td>12</td>
<td>121</td>
<td>105</td>
<td>1.15</td>
</tr>
<tr>
<td>13</td>
<td>116</td>
<td>112</td>
<td>1.04</td>
</tr>
<tr>
<td>14</td>
<td>126</td>
<td>105</td>
<td>1.20</td>
</tr>
<tr>
<td>15</td>
<td>119</td>
<td>116</td>
<td>1.03</td>
</tr>
<tr>
<td>16</td>
<td>121</td>
<td>116</td>
<td>1.04</td>
</tr>
<tr>
<td>mean +/- SD</td>
<td>118.1 +/- 5.2</td>
<td>109.0 +/- 4.9</td>
<td>1.09 +/- 0.07</td>
</tr>
</tbody>
</table>

Table 3.3. Quantitative hippocampal and temporal lobe data in patients without a history of PFC. Abnormal values are shown in bold.
3.7. Discussion

Quantitative MR techniques have previously been used to increase the sensitivity of qualitatively assessed MRI in the diagnosis of MTS, but not for classification of MTS. The aim of this study was to determine whether there are MR characteristics of MTS following PFC (Group 1) which separates it from MTS not associated with PFC (Group 2). The data presented in this chapter show that there are significant differences between the groups in terms of T2 relaxation time and hippocampal volume, but not in NAA/(Cho+Cr). The patients in this study were highly selected in that they had all undergone temporal lobe resections and were found to have MTS on post-operative histology. This study, therefore, is only able to define the magnetic resonance characteristics of patients with surgically treatable MTS. Nevertheless, given this selection criterion, the patients were then included consecutively, with no further selection on the basis of the presence or absence of PFC. Interpretation of these data should be cautious in view of the small numbers enrolled in each group and because the MR parameters provide only surrogate information about histological changes.

Hippocampal T2 relaxometry demonstrated abnormalities in all patients in Group 1 and 7/8 patients in Group 2, ipsilateral to the seizure focus, which is unsurprising as all the patients in this study were enrolled because they were shown to have MTS on post-operative histology. Ipsilateral to the seizure focus, the patients in Group 1 had significantly longer T2 relaxation times when compared to the patients in Group 2, as well as having significantly smaller hippocampi than those in Group 2. Prolongation of the
T2 relaxation time and reduction in hippocampal volume implies hippocampal damage with neuronal loss (Van Paesschen et al., 1997b). The findings suggest that patients with MTS and a history of PFC have more severe hippocampal damage with neuronal loss ipsilateral to the seizure focus than patients without such a history. Ipsilateral to the seizure focus, the patients with the highest T2 relaxation times were also those with the smallest hippocampal volumes. Most of these patients were in Group 1. These data are consistent with those previously reported (Cendes et al., 1993; Barr et al., 1997; Van Paesschen et al., 1997a; Kanemoto et al., 1998).

There were no significant differences between the groups contralateral to the seizure focus in terms of T2 relaxation time or HCV. There was, however, more asymmetry in hippocampal T2 relaxation time and HCV in patients with a history of PFC, as confirmed using ipsilateral to contralateral ratios. When compared to controls, T2 relaxation times contralateral to the seizure focus were not prolonged in patients in Group 1, but significantly prolonged in patients in Group 2. These data suggest that MTS is primarily a unilateral disorder when associated with PFC. However, patients in this study who have a history of PFC have bilaterally small hippocampi, with one hippocampus being much smaller than the other, consistent with previous reports (Barr et al., 1997). This appears to be inconsistent with the reported T2 relaxation time data in which the abnormality is unilateral. The confident radiological diagnosis of MTS requires both hippocampal atrophy and increased T2 signal (Jackson et al., 1993a; Van Paesschen et al., 1997b).
and therefore the contralateral hippocampus in patients with a history of PFC do not meet the diagnostic criteria for MTS. Potential explanations of a small hippocampal volume and normal hippocampal T2 relaxation time include hippocampal damage in which the damage does not cause prolongation of the T2 relaxation time, or that patients predisposed to PFC have pre-existing bilaterally small hippocampi. The patients with no history of PFC have bilaterally small hippocampal volumes and bilateral prolongation of the T2 which may suggest bilateral hippocampal sclerosis. Clarification will require a study specifically designed to determine whether patients predisposed to PFC have smaller hippocampi than controls.

Abnormalities in the temporal lobe were investigated with the use of $^1$H MRS. Low NAA/(Cho+Cr) ratios are consistent with neuronal loss or dysfunction. There were no differences in this temporal lobe finding in patients with and without a history of PFC although both groups had significantly abnormal NAA/(Cho+Cr) ipsilateral and contralateral to the seizure focus when compared to normal controls. Post-operative recovery of NAA/(Cho+Cr) in tissue remote from the seizure focus has been reported (Hugg et al., 1996; Cendes et al., 1997). This suggests that the reduction of NAA/(Cho+Cr) may reflect a reduction in neuronal function as a result of recurrent seizures rather than necessarily permanent neuronal loss resulting from the initial precipitating injury. There is no difference in pre-operative seizure frequency between the groups and so the similarity in NAA/(Cho+Cr) further supports the hypothesis that NAA/(Cho+Cr) ratios
may reflect an ongoing process rather than reflecting abnormality related to the initial precipitating injury.

The unilateral radiological characteristics of MTS associated with PFC may in part explain the reported better surgical outcome in this group of patients (Abou-Khalil et al., 1993). That observation was not confirmed in the present study although it is probable that there are insufficient numbers of patients in this study to identify such a difference. It is possible that patients with a unilateral abnormality could have all epileptogenic tissue removed and therefore become seizure free whereas those with bilateral abnormalities may develop seizures with the origin in the remaining hippocampus.

The methodology used in this study may have resulted in a selection bias toward patients with more severe disease as these are the patients who are more likely to be referred to Great Ormond Street Hospital for surgical management of their epilepsy. It is therefore possible that the MR findings described in this chapter could not be extrapolated to a less severely affected population i.e. children with medically controlled MTS. However, all of the patients enrolled in this trial had similarly severe disease and all had temporal lobe resections.

It is widely accepted that the pathogenetic mechanism which results in hippocampal injury is excitotoxic in nature (Wasterlain et al., 1993). If the same mechanism was responsible for all acquired hippocampal injury, irrespective of the initial precipitating injury, then one might expect that all
MTS would have similar radiological characteristics unless the degree of excitotoxic injury was dependent upon the severity of the event. It is possible that prolonged convulsions, whether they are febrile or non-febrile, are more damaging to the hippocampus than other initial events. Such an explanation would suggest that the findings in this study are simply a reflection of the severity of excitotoxic injury and would then not provide data on a specific pathogenetic sequence from PFC to MTS. However, this hypothesis would not explain the predominantly unilateral abnormality in the patients with PFC compared to a greater degree of bilaterality in those with other antecedents. The single patient who had non-febrile status epilepticus had a bilaterally elevated T2 relaxation times. Although that seizure lasted 2 hours, the ipsilateral T2 relaxation time was only higher than one of the T2 relaxation times measured in the patients with PFC.

An alternative hypothesis is that patients with MTS and a history of PFC have a more fundamental hippocampal abnormality prior to the episode of PFC. Febrile convulsions tend to run in families (Johnson et al., 1996, Maher et al., 1997). The genetic predisposition in patients with MTS and a history of PFC is one of the factors which separates them from patients with MTS and no history of PFC. It is possible that people with a predisposition to febrile convulsions have hippocampi which, if subject to a prolonged seizure, develop MTS with characteristic MR findings. This is supported by a study which assessed hippocampal volume in siblings of patients with MTS and a history of PFC showing that their hippocampi were smaller than controls although the siblings themselves had never experienced seizures.
(Wood, 1998). This may suggest that people with a predisposition to PFC and pre-existing small hippocampi may be more susceptible to the development of MTS than people with no such genetic predisposition. Excitatory amino acid receptor density or subtypes may be different in patients predisposed to PFC and those who are predisposed to PFC and develop subsequent MTS when compared to patients without such a genetic predisposition.

No well validated model of prolonged febrile convulsion, which has a genetic predisposition, has been reported. Therefore the current understanding of the pathogenesis of MTS relies on animal models in which SE is induced using chemical or electrical experimental paradigms, more akin to the patients in Group 2 in this study. The role of excitotoxic neuronal injury in the development of MTS cannot be fully explored until appropriate genetic animal models have been validated.

In animal models in which SE results in hippocampal neuronal injury, the seizures need to start in the limbic system (Meldrum, 1997). Patients with PFC may have seizure onset in one hippocampus (which may be predisposed to seizure onset) and it is that hippocampus which is primarily damaged. Further studies assessing the MR characteristics of MTS in patients with no history of PFC is required. These studies may help to explain why these patients have less severe and less asymmetrical hippocampal abnormalities than patients with a history of PFC. It is,
however, less likely that there is a genetic influence on the development of MTS in patients without a history of PFC.

Although the data presented in this chapter may suggest that MTS following PFC is different to MTS with other associations, the numbers are small and further data are required. It is not possible from this study to determine whether the relationship between PFC and MTS is causative. This requires a prospective study assessing magnetic resonance investigations in patients who have had a prolonged seizures in order to determine whether acute findings are different in patients with and without a history of PFC and whether acute abnormalities develop into MTS.
CHAPTER 4. MAGNETIC RESONANCE FINDINGS
WITHIN 48 HOURS OF AN EPISODE OF STATUS
EPILEPTICUS IN CHILDHOOD

4.1. Introduction

The data presented in the previous chapter provide some insight into possible mechanisms of neuronal injury following SE. In order for the concept of neuronal injury being related to initial precipitating injury to be explored further, a prospective trial looking for acute MR abnormalities within 48 hours of an episode of SE was carried out.

Status epilepticus in animal models may result in neuronal injury, especially in the hippocampus, although other brain regions may also be injured (see section 1.8). Many species and many experimental paradigms have been used. The hippocampal and mesial temporal lobe abnormalities following SE in these models is similar across species and methods (Sperk et al., 1983; Ben-Ari, 1985; Wasterlain et al., 1994; Haglid et al., 1994; Nagao et al., 1994; Liu et al., 1994; Fountain et al., 1995; Golden et al., 1995; Cendes et al., 1995; Cavalheiro, 1995a; Priel et al., 1996; Fujikawa, 1996; Meldrum, 1997). This suggests that it is SE which results in hippocampal and temporal lobe injury rather than something directly related to any particular method of inducing SE. The initial work was carried out in adolescent baboons in which bicuculline was administered (Meldrum, 1983). Subsequent work using chemical models was primarily carried out in rats. The drugs which are primarily used include kainic acid (a glutamatergic drug) (Ben-Ari, 1985)
and pilocarpine (a cholinergic drug) (Cavalheiro, 1995a). Electrical stimulation models in rats have also resulted in acute mesial temporal injury (Du et al., 1995). Each of these paradigms results in limbic SE which may be very prolonged. Animals which survive frequently develop spontaneous recurrent seizures resembling human temporal lobe epilepsy (Cavalheiro, 1995b).

The commonest association with MTS in humans is PFC, which probably has a genetic basis. The animal models of SE used to investigate the pathophysiological basis of acute hippocampal injury do not have a genetic basis and may not be relevant to the human condition. Therefore, patients with a genetic predisposition to SE and patients with a history of SE and no genetic predisposition need to be investigated to determine whether any acute injury identified is partly dependent upon aetiology. It is important to attempt to identify any causative relationship between SE and neuronal injury in humans as this will provide justification for attempting to define neuroprotective strategies.

It is not practical to obtain non-invasive structural or functional brain information in children prior to an episode of SE as it is not possible to predict which children will experience an episode of SE. The strategy adopted in this study is to obtain acute information and then to determine whether any abnormalities identified either return to normal or develop into typical findings associated with MTS.
Acute abnormalities of T2-weighted signal intensity, $^1$H MRS metabolite concentrations and ratios, and diffusion weighted imaging have been identified in animal models. T2-weighted signal intensity increases between 12 and 24 hours after SE induction (Ebisu et al., 1996). Increased lactate signal has been identified in 2 studies and this increase is correlated with degree of histological damage (Ebisu et al., 1996; Najm et al., 1998). This is suggestive of cellular acidosis and could be partially responsible for neuronal death as a result of SE. Studies using NAA as a marker of acute neuronal injury are less consistent. There may be a decrease in NAA signal between 12 hours and 3 days of SE induction with kainic acid (Ebisu et al., 1994; Ebisu et al., 1996). In another study, NAA/Cr ratios increased during ictal activity and returned to normal within 24 hours (Najm et al., 1998). The changes in this ratio may be a result of increased NAA signal or a decrease in Cr signal. An increase in NAA signal intensity is difficult to explain within the framework of current understanding of the cellular placement of NAA. NAA is located primarily within neurons and an increase in NAA signal intensity would suggest either an increase in the neuronal population, which appears extremely unlikely or increased production of NAA within existing neurons. An acute reduction in the Cr signal is also difficult to explain. Diffusion weighted imaging carried out acutely reveals a decrease in apparent diffusion coefficient (ADC) which may reflect underlying neuronal swelling (Zhong et al., 1993; Zhong et al., 1995; Ebisu et al., 1996). Termination of electrographic seizure activity results in a return of ADC to normal within minutes of seizure termination.
Early termination of seizures may reduce the likelihood of hippocampal oedema occurring following SE.

Some human MR data on brain injury following SE have been reported. Hippocampal hyperintensity on qualitatively assessed scans has been identified on T2-weighted images following SE, with subsequent MR identified MTS reported in 2/5 patients (Tien et al., 1995). Hippocampal swelling, identified using hippocampal volume measurements, has been identified in 4/15 children following a prolonged and lateralised febrile convulsion (VanLandingham et al., 1998). MR investigations in these studies were performed up to 4 weeks after the acute insult and do not provide data which could be considered to be acute. There is also some evidence that progression from normal hippocampus to MTS following SE can occur in children and adults (Nohria et al., 1994; Wieshmann et al., 1997).

The aim of this study was to provide acute qualitative and quantitative information, primarily about the hippocampus and temporal lobe, within 48 hours of an episode of SE in childhood and adolescence. Follow up MR investigations will assess whether any abnormalities identified acutely mature into MTS.

4.2. Methods

4.2.1. Patient recruitment

Patients were recruited from hospitals in the North Thames health region in London, and Great Ormond Street Hospital for Children NHS Trust. The
study was discussed with the medical and nursing staff working in the individual general paediatric units within the region to provide them with confidence to refer patients. Children with a recent history of SE were referred to the Neurology Unit at Great Ormond Street Hospital for further clinical evaluation and magnetic resonance investigations which were carried out within 48 hours of the episode of SE.

4.2.2. Clinical evaluation

On arrival at Great Ormond Street Hospital each child was assessed by the candidate. Clinical data relating to pre-morbid state, precipitating factors, seizure onset, seizure length and treatment were obtained.

4.2.3. Magnetic resonance investigations

Both qualitative and quantitative MR investigations were performed. Sedation or general anaesthesia was used if necessary. Repeat investigations were only carried out in patients with developmental delay, further seizures or in whom abnormalities in the acute scan required clarification. This restriction was in accordance with the protocol agreed with the Hospital Ethics Committee.

4.2.3.1. Qualitative Investigations

T1 and T2 weighted images were acquired. The T1-weighted MPRAGE 3D dataset, as described in section 2.2.1.1., was reformatted into contiguous axial and coronal images. T2 weighted images, in the coronal plane at $90^0$ to the long axis of the hippocampus and in the tilted axial plane orthogonal
to this, as described in section 2.2.1.2., were also acquired. The images were reviewed by two neuroradiologists (Dr WK Chong and Dr T Cox), who were blinded to the clinical details, and the candidate. A comment on the hippocampi and the temporal lobes was made about each scan. Abnormalities outside the hippocampi and temporal lobes were also sought. Lesions were considered to be present if identified by all three observers.

4.2.3.2. Quantitative investigations

Quantitative methods have been described in section 2.2.2.

4.2.3.2.1. Hippocampal investigations

Hippocampal T2 relaxation times and hippocampal volumes were measured. The T2 relaxation time provides information about the body of the hippocampus. Hippocampal volume assessment provides information about size of the entire hippocampus.

4.2.3.2.2. Temporal lobe investigation

Single voxel proton magnetic resonance spectroscopy (1H MRS) was carried out, with data obtained from 2x2x2 cm voxels centred in each mesial temporal lobe. The ratio of the signal from N-acetyl aspartate (NAA) to the sum of signals from creatine + phosphocreatine (Cr) and choline containing compounds (Cho) (i.e. NAA/Cho+Cr) was calculated.
4.2.4. Statistical analysis

T2 relaxation time and $^1$H MRS data were analysed using multiple linear regression. The mathematical models are described in Chapter 2. For analysis of hippocampal volume data, patients were divided into 2 groups; those with a history of PFC (Group 1) and those without such a history (Group 2). Corrected hippocampal volumes were used in the analysis. Comparisons between groups were carried out using the Mann-Whitney U test. Comparisons between initial and follow-up scans were carried out using the Wilcoxon paired sample test. All analyses were performed using SPSS for Windows version 8.0.

4.3. Results

4.3.1. Clinical data

There were 19 patients enrolled into the trial. 6 patients had a prolonged febrile convulsion and were defined as Group 1. There were 13 patients in Group 2; of these, 2 had acute symptomatic SE, 3 had idiopathic SE and 8 had remote symptomatic SE. See Table 4.1. for further details. The median age of this group was 18 months (range 1-185 months). Median seizure length was 62.5 minutes (range 30-1800 mins). The patient who had an episode of SE lasting for 1800 minutes developed the episode 2 weeks after a heart lung transplant for Eisenmengers syndrome. No cause for the episode of SE was identified. Within 24 hours after the episode of SE she died.
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<th>Age at second scan (months)</th>
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Table 4.1. Clinical data on patients with a history of SE. Patients 1-6 have a history of PFC. Patients 7-19 have a history of non-febrile SE.

PFC prolonged febrile convulsion
FC short febrile convulsion
AS acute symptomatic SE
RS remote symptomatic SE
GTCS generalised tonic clonic SE
CPS complex partial SE
Magnetic resonance investigations were performed in all 19 patients. 9/19 scans were reported as having an abnormality, with 1/9 of the abnormal scans being in a patient with a history of PFC. This patient had asymmetrical hippocampi. There was a large left hippocampus when compared to the right. The hippocampi were symmetrical on the follow up scan performed 7 months after the initial scan.

A wide range of abnormalities were identified in 8/13 of the patients with no history of PFC. Temporal lobe abnormalities were identified in one patient in whom the definition of grey and white matter was less obvious on the left than on the right. This patient had a history of frequent complex partial seizures but no previous history of SE. He had previously undergone MR investigations in the investigation of his seizure disorder. No change was identified in the acute scan when compared to the previous scan.

One patient with acute symptomatic SE had a widespread increase in T2-weighted signal intensity in the cortex (Figure 4.1) and the limbic system. She also had uncal herniation through the tentorium cerebelli and had herniation of the cerebellar tonsils through the foramen magnum and subsequently died. One patient with remote symptomatic SE had evidence of delayed myelination. She was developmentally delayed in all domains and had an episode of non-febrile SE. There was one patient with bilateral signal increases in T2-weighted imaging in both thalami in the acute scan, which disappeared in the follow-up scan 1 year later (Figure 4.2). She also
has profound developmental delay. She was considered to have bilateral shrinkage of the hippocampi on follow-up scanning. 2 patients had occipital lobe abnormalities; 1 had a posterior circulation infarct (Figure 4.3) and 1 had an area of cortical dysplasia. One patient with developmental delay had evidence of an old left hemisphere cerebellar infarct. The final abnormality was in a 1 month old baby with white matter abnormalities and a small immature brain suggestive of haemorrhage or congenital infection. No formal diagnosis was made. All other scans were reported as normal. 7 patients had follow-up scans. Changes from the acute scan were identified in 2 scans as described above.
Figure 4.1. Widespread increase in T2 signal in a patient with a history of SE lasting 1800 minutes and who subsequently died.
Figure 4.2. Axial T2-weighted images showing areas of increased signal in the thalamus bilaterally on the acute scan (arrows), with return to normal on the follow-up scan performed 1 year later.
4.3.3. Quantitative MR

The results of the quantitative MR investigations are summarised in Table 4.2.

4.3.3.1. T2 relaxometry

T2 maps were calculated in 16 children of whom 5 were in Group 1 and 11 patients were in Group 2. Reference ranges were calculated as described in
section 2.2.2.1.2. One patient in Group 1 had abnormal T2 relaxation times bilaterally. 5/11 patients in Group 2 had an elevated T2 relaxation time in at least one hippocampus.

Multiple linear regression revealed that T2 relaxation time was significantly related to age (p<0.001), a history of PFC (p=0.037) and a history of non-febrile SE (p<0.001) (Figure 4.4). T2 relaxation time increased by a mean of 4.9% (95% CL: 0.3-9.5%) in Group 1 and by a mean of 9.2% (95% CL: 5.9-12.1%) in group 2 when compared to controls. T2 relaxation time was not significantly dependent upon whether the right or the left hippocampus was affected (p=0.733). There was no correlation between degree of T2 elevation and seizure length (p=0.32) but the patient numbers are small.

Follow-up T2 maps have been acquired in 7 patients, 2 of whom were in Group 1. They were acquired after a median time of 8 months (range 7-12 months). There are no significant differences in T2 relaxation time between the initial and follow-up scans in either group (p=0.89 on the right and p=0.69 on the left.).
Table 4.2. Quantitative MR findings within 48 hours in patients with a history of SE. Values in bold are abnormal for age.
4.3.3.2. Hippocampal volumetry

Hippocampal volumes (HCV) were calculated in 15 patients, 5 in Group 1 and 10 in Group 2. Mean corrected HCV in 16 controls was 3916 +/- 418 mm$^3$ (see section 2.2.2.2.2). HCV of less than 3080mm$^3$ or greater than 4752mm$^3$ are considered abnormal. No patient in Group 1 had a small hippocampus but 2/5 had a large hippocampus. In Group 2, 1/10 patients had a small hippocampus and none had a large hippocampus.

Patients in Group 1 had a median (interquartile range) HCV of 4630mm$^3$ (4290-5117mm$^3$) on the right and 4499mm$^3$ (3920-4993mm$^3$) on the left.
Patients in Group 2 had a median (interquartile range) HCV of 4190 mm$^3$ (4046-4329 mm$^3$) on the right and 4044 mm$^3$ (3461-4273 mm$^3$) on the left. HCV in Group 1 was significantly larger on both left and right sides when compared to controls (p=0.03 on the right and p=0.01 on the left. Figure 4.5). There was no significant increase or decrease in hippocampal volumes in Group 2 (p=0.55 on the right and p=0.31 on the left). There were no significant side to side differences in either group.

Follow-up investigations have been carried out in 7 patients, 2 of whom were in Group 1. There were no significant changes in HCV between the first and the second scans (p=0.90 on the right and p=0.43 on the left).
Figure 4.5  Box and whisker plot of hippocampal volume data in patients with a history of PFC, patients with a history of non-febrile SE and controls showing elevation of hippocampal volume in the patients with a history of PFC. The horizontal line represents the median, the box represents the interquartile range and the whiskers represent the complete range within 1.5 boxplots of the median. Outliers are shown as +. Findings on the right are shown in red and findings on the left are shown in green.

4.3.3.3. Proton magnetic resonance spectroscopy

This investigation was carried out in 17 of the patients of whom 5 were in Group 1. Reference ranges were calculated as described in section 2.2.2.3.3.

No patient in Group 1 had an abnormal NAA/(Cho+Cr) ratio. Three patients in Group 2 had abnormal NAA/(Cho+Cr) ratios in at least one temporal lobe.
Multiple linear regression revealed that NAA/(Cho+Cr) was significantly related to age (p<0.001), but not significantly related to a history of PFC (p=0.982) or to a history of non-febrile SE (p=0.059). The latter does, however, approach significance (Figure 4.6). There was a mean reduction in NAA/(Cho+Cr) of 0.25% (95% CL: -21.25 – 21.7%) in Group 1 and a mean reduction of 15.8% (95% CL: -0.65 – 32%) in Group 2. In a single patient glutamine peaks were identified. This patient had a very prolonged acute symptomatic seizure (1800 minutes) and died within 24 hours of the MR investigations (Figure 4.7).

Follow-up investigations were carried out in 7 patients, 2 of whom were in Group 1. There was no significant difference in NAA/(Cho+Cr) between the first and repeat scans (p=0.11).
Figure 4.6. Predicted NAA/(Cho+Cr) ratios in normal controls (blue dots), patients with a history of PFC (red dots), and patients with a history of non-febrile SE (green dots).
Figure 4.7. $^1$H MR spectra from a normal control (top panel), from the patient who died in SE (middle panel) and from a phantom containing only creatine and glutamine. The patient has decreased NAA and glutamine is identified.
4.4. Discussion

This study was carried out to identify whether SE is associated with acute hippocampal abnormalities which subsequently develop into MTS. All of the patients in this study had experienced an episode of SE. They were divided into those with and without a history of PFC. There were 6 patients with a history of PFC, in line with the numbers expected from epidemiological studies (DeLorenzo et al., 1996). There was, however, an over-representation of patients with remote symptomatic SE. This is likely to be due to a referral bias of the more complex patients to Great Ormond Street Hospital.

Reliable in-vivo hippocampal data interpretation requires both qualitative and quantitative MR techniques analysed in conjunction. In the patients with a history of PFC, abnormality was identified in one patient using qualitative analysis. This abnormality was described as hippocampal asymmetry, with the larger hippocampus on the left. There was no signal hyperintensity on the T2 weighted images in either of the hippocampi in this patient and indeed, the T2 relaxation times were normal. There was no asymmetry of hippocampal volume on the follow-up scans. This qualitative assessment was not consistent with quantitative hippocampal volume measurements in which the right hippocampus was larger than the left. The most likely explanation for a finding of a unilaterally large hippocampus which subsequently returns to normal would be hippocampal oedema which did not result in permanent hippocampal abnormalities or MTS. This would be expected to be associated with an abnormality on T2-weighted images,
which was not observed in this case. As the correlation between subjective
and objective measurements in this patient is poor, the reliability of this
conclusion is in doubt. The scans of the other patients with a history of PFC
were all reported as normal on qualitative assessment.

Quantitative hippocampal investigations in the patients with a history of
PFC revealed that the hippocampi in this group were significantly larger,
and the T2 relaxation time was significantly prolonged when compared to a
control population. On an individual basis, only 2/5 patients had enlarged
hippocampal volumes and 1/5 patients had prolongation of the T2 relaxation
time. The patient with prolongation of the T2 relaxation time did not have
abnormally large hippocampi. Therefore, hippocampal oedema was not
definitively identified in this cohort of patients. Despite this, when the data
are analysed on a group basis there appears to have been a statistically
significant shift toward abnormality in T2 relaxation time and toward
increased hippocampal size. Such a combination of volume increase with
high T2 relaxation time is highly suggestive of hippocampal oedema. The
results in Chapter 3 suggest that patients with a history of PFC have a
unilateral very small hippocampus. The contralateral hippocampus is also
small, but to a much lesser extent, when compared to controls but the T2
relaxation time is only prolonged in the hippocampus ipsilateral to the
seizure focus. It was suggested that patients with a history of PFC may have
had pre-existing small hippocampi and that MTS may develop in the
hippocampus in which the seizure starts. The acute findings consistent with
bilateral hippocampal oedema suggests that generalisation of the seizure
causes bilateral abnormalities, but that one hippocampus may be predisposed to injury which may subsequently result in the development of MTS in that hippocampus.

Repeat scans in patients with hippocampal oedema would be expected to show a reduction in HCV and T2 relaxation time. However, only 2 patients with a history of PFC underwent repeat investigations at least 7 months after the first investigation. Neither of these patients showed evidence of hippocampal oedema on the initial scan and neither patient showed any change in T2 relaxation time between the first and the repeat scan. There are insufficient patient numbers to draw firm conclusions regarding how the acute MR findings are related to hippocampal oedema.

The data reported in the present work appear to be inconsistent with data from a previous study attempting to define acute hippocampal abnormalities following SE (VanLandingham et al., 1998). In that study, 4/15 patients with a history of prolonged lateralised febrile seizures, but 0/15 patients with prolonged generalised febrile seizures, had quantitatively defined hippocampal swelling, compared to 2/5 patients with prolonged generalised febrile convulsion in the present study. In the VanLandingham study, hippocampal volumes were not analysed as absolute volumes but as hippocampal volume ratios and T2 weighted MRI was visually analysed for increase in hippocampal T2 signal. The methodology, therefore, did not allow the identification of bilateral abnormalities. Bilaterally large hippocampi would not be identified using hippocampal volume ratios and
qualitative assessment of T2 weighted MRI is usually carried out by comparing sides. Although no age related normal hippocampal volumes, or hippocampal volumes corrected for intracranial volume are reported in the paper, several of the children with generalised convulsions appeared to have bilaterally large hippocampi when compared with hippocampal volumes considered to be abnormal in the population with lateralised seizures. It is therefore possible that that the data from the current study are not, in fact, inconsistent with those from VanLandingham’s study.

Abnormalities in the temporal lobe using $^1$H MRS were not identified in any patients with a history of PFC. This is unexpected when the results from Chapter 3 are considered. Patients in that study, with a history of PFC, had abnormal NAA/(Cho+Cr) when compared to controls. Acute findings in animal studies are inconsistent (Ebisu et al., 1994; Ebisu et al., 1996; Najm et al., 1997) but suggest NAA/Cr ratios are decreased. The data in this chapter, which do not reveal acute abnormalities, taken in conjunction with the chronic abnormalities identified in Chapter 3, support the hypothesis that the $^1$H MRS abnormalities identified in patients with a longstanding history of epilepsy may be a consequence of frequent repeated seizures and are not a consequence of the initial precipitating injury. Return of $^1$H MRS abnormalities, remote to the seizure focus, to normal after surgical treatment of epilepsy also supports this view (Hugg et al., 1996; Cendes et al., 1997). It is unlikely that patients who have no evidence of temporal lobe abnormality on acute imaging will develop an abnormality in the absence of further
seizures if the hypothesis that neuronal injury only occurs following prolonged seizures is correct.

There were no qualitative hippocampal abnormalities in the patients with no history of PFC, although abnormalities were identified in other brain regions in eight patients. Two of the patients may have had acute abnormalities which resulted from the prolonged seizure, one had an acute abnormality which may have caused the prolonged seizure and five were almost certainly pre-existing. Of the acute changes, one patient had T2 signal hyperintensity in the thalamus bilaterally. These abnormalities disappeared at the follow-up scan which was carried out 1 year after the acute scan. Thalamic damage has been identified in animal models of limbic SE, consistent with the findings identified in this patient (Meldrum, 1997). The other patient with acute abnormalities was the one who died. She had widespread increases in T2-weighted signal and evidence of coning. It remains uncertain whether the MR abnormalities identified in this patient were a result of SE, whether they were a result of the underlying aetiology or whether they represent acute ischaemic changes. Post-mortem did not reveal a cause of death and so the aetiology remains uncertain. It is known that some patients with SE develop raised intracranial pressure and so it is possible that this patient coned because of the SE irrespective of the underlying aetiology. One patient had an occipital stroke which may have caused SE.
Of the five patients with probable pre-existing abnormalities, one had poor
differentiation between the grey and white matter in the right temporal lobe.
This patient had complex partial seizures prior to his episode of SE. This
poor differentiation into grey and white matter can be seen in patients with a
long history of complex partial epilepsy and can be considered to be a soft
lateralising sign in patients undergoing presurgical investigation. One
patient had delay in myelination. She has severe learning disability of
unknown aetiology. It is probable that the finding in this patient does not
result from her episode of SE, but is part of the syndrome which is
associated with learning disability. She has not had a follow-up scan to
assess whether her myelination remains delayed. The patients with the old
cerebellar infarct, occipital cortical dysplasia and possible haemorrhage or
congenital infection all had clearly pre-existing abnormalities. The
relationship between the lesions and the episode of SE remains unclear.

Quantitative hippocampal assessment in the patients with non-PFC related
SE, indicated that as a group they had significantly prolonged T2 relaxation
times bilaterally when compared to controls, but their hippocampal volumes
were no different, as a group, when compared to controls. As only 1 patient
had a small hippocampus, the abnormality in the majority of these patients
cannot be described as hippocampal sclerosis. The patient with a small
hippocampus had previously experienced a PFC and the hippocampal
sclerosis in this case may be a result of this previous insult. The majority of
this group had remote symptomatic SE associated with learning disability. It
is therefore possible that prolongation of the T2 relaxation time, rather than
being an acute response to SE, primarily reflects a pre-existing brain
abnormality associated with the learning disability in this group. It is also
possible that some children with learning disabilities have immature brains
which have higher water content than their peers without learning disability.

The reduction in NAA/(Cho+Cr) identified in this group may also be
reflecting underlying brain abnormalities and may not be a consequence of
SE. Further studies assessing T2 relaxation times and ^1^H MRS findings in
children with learning disabilities compared to normal age-matched controls
would be required to confirm the hypothesis that the underlying neuronal
abnormality in patients with learning disability may affect these parameters
in the absence of an episode of SE.

The time to investigation of 48 hours was chosen because practical
difficulties make it unlikely that patients would undergo MR investigation
in much less than 48 hours. The timecourse of abnormalities which may be
identified with magnetic resonance techniques following SE are unknown
and so it remains unclear whether the results would have been different if
very early data or data acquired after 48 hours had been used.

This study supports, but does not confirm the view that PFC in childhood
may result in acute hippocampal, but not temporal lobe injury whilst non-
febrile SE may not. A major limitation of this study is the small numbers of
patients enrolled. A larger cohort of patients which confirms or refutes these
findings is required. This is consistent with epidemiological and pre-surgical
data which suggests that PFC is the commonest association with MTS. As the relationship between MTS and non-febrile SE is not as strong as the relationship between MTS and PFC, it is possible that the mechanisms of injury is different in patients with a history of PFC and those with non-febrile SE. The number of patients recruited to the study are small and follow-up data were only collected in very few patients. These findings need to be confirmed in a larger study in which all patients have at least one follow-up scan. The results reported in this chapter, taken in conjunction with the results from Chapter 3, suggest that the mechanisms of neuronal injury of the hippocampus are at least in part dependant upon the initial precipitating injury. These mechanisms need to be explored further. If the pathophysiological mechanisms of acquired hippocampal damage can be identified then appropriate neuroprotective strategies can be devised.

In animal models of SE, the seizure needs to continue for at least 30 minutes before hippocampal damage occurs. The results in this study suggest that the hippocampus may develop an acute abnormality in humans who have had a seizure which lasts at least 30 minutes. Termination of seizures as soon as possible after seizure onset may decrease overall seizure length and this may decrease the chances of hippocampal injury. Treatment in the community is usually with rectal diazepam which may not be acceptable or convenient. The following chapters will define work which assessed a novel treatment, buccal midazolam, that could potentially be used in the community setting, in the acute treatment of seizures.
CHAPTER 5. PHARMACOKINETIC /
PHARMACODYNAMIC EVALUATION OF BUCCAL
ADMINISTRATION OF MIDAZOLAM SOLUTION

5.1. Introduction

Data from animal models suggest that status epilepticus needs to last for at least 30 minutes before neuronal injury occurs (Lemos et al., 1995, Fujikawa, 1996). A major reason for wishing to terminate seizures quickly would be to prevent such neuronal injury. It can easily take more than 30 minutes from seizure onset to initiation of treatment in accident and emergency departments, in which time neuronal injury may already have occurred. Early treatment in the community could reduce the incidence of neuronal injury as a consequence of SE.

Benzodiazepines are the most frequently used first line treatments used to terminate seizures. Rectal diazepam is the usual medication used in the prehospital setting (Knudsen, 1979; Dreifuss et al., 1998) but is not always acceptable or convenient. An alternative medication which is safe, effective and can be administered in a socially acceptable way would be of great benefit in the emergency treatment of seizures. Midazolam has pharmacological advantages over the other benzodiazepines and was therefore chosen as the experimental drug for this study. The work carried out for this thesis assessed whether buccal midazolam is rapidly absorbed into venous blood and brain, whether it is effective in the treatment of acute
repetitive seizures and whether it is as effective as rectal diazepam in the acute treatment of seizures.

5.1.1. Pre-clinical pharmacology of midazolam

Midazolam (8-chloro-6-(2-flourophenyl)-1-methyl-4H-imidazo [1,5-a][1,4] benzodiazepine) is an imidazobenzodiazepine drug which is water soluble at acid pH, but becomes lipid soluble at physiological pH. Water solubility is beneficial as other benzodiazepines are dissolved in propylene glycol and alcohol which may increase the incidence of cardiovascular adverse-events. Midazolam has an overall potency which is similar to diazepam and its metabolite, α-hydroxy midazolam, has a negligible pharmacological action (Heizmann et al., 1983). The lethal dose in 50% (LD50) of rats or mice is 1600 mg/kg if the drug is administered orally. Midazolam has not been shown to be embryotoxic nor teratogenic (Pieri, 1983). The effect of midazolam on the normal EEG is similar to that observed with diazepam, with increases in the fast frequencies (Brown et al., 1979).

5.1.2. Possible alternative routes of administration

The introduction of rectal diazepam into clinical practice revolutionised the pre-hospital treatment of acute seizures. However, in the current climate of sexual abuse allegations parents, teachers and carers have become reluctant to administer medication by the rectal route. Older children and adults may find it embarrassing.
5.1.2.1. Nasal administration

Nasal administration of midazolam solution is an effective way of sedating children for dental procedures and is useful for pre-operative sedation (Fukuto et al., 1994; Karl et al., 1993). 50% of the dose is administered into each nostril. There is rapid absorption, with median times to maximum concentration being between 6 and 16 minutes (Malinovsky et al., 1993; Fukuta et al., 1994; Lejus et al., 1997). Absorption into arterial blood is quicker than absorption into venous blood. This is probably a consequence of rapid clearance of drug from capillaries such that it takes longer for the drug to be detected in venous blood. Adequate sedation is achieved between 20 and 60 minutes after administration (Fukuta et al., 1997). The elimination half-life is approximately 3 hours, the volume of distribution is 0.3-0.7 L/kg and bioavailability is approximately 50% (Burstein et al., 1997). Midazolam has also been administered as a nasal spray. Pharmacokinetic evaluation of this preparation reveals similar results to those seen with midazolam solutions except that there is superior bioavailability of approximately 80% (Bjorkman et al., 1997).

5.1.2.2. Buccal administration

Buccal administration of midazolam may also provide an alternative to rectal diazepam in the acute treatment of seizures. There are many physical similarities between the mouth and the rectum; they have a similar pH, similar surface area (200 cm^3), and they both allow absorption directly into the systemic circulation which avoids the problem of first-pass metabolism (De Boer et al., 1982; De Boer et al., 1984). Pre-medication with a solid
preparation of buccal midazolam results in more rapid and increased sedation scores when compared with oral administration (Lim et al., 1997). Direct comparisons between the nasal and buccal routes of administration tend to support the view that buccal administration has advantages over the nasal route. It is more readily acceptable, achieves higher plasma levels and has the least variable absorption (Karl et al., 1993; Geldner et al., 1997). There are no published data on half-life and volume of distribution following buccal administration of midazolam solutions.

The aim of this study was to establish whether buccal administration of midazolam in solution would result in rapid absorption into venous blood and cause rapidly detectable EEG changes.

5.2. Methods
This study consisted of an open-label phase followed by a double-blind phase. The double blind phase occurred 6-8 weeks after the open study.

5.2.1. Volunteer characteristics
Ten healthy adult volunteers were included in each phase of the study. Volunteer 4 was substituted with volunteer 5 in the double-blind phase of the study. Personal characteristics are shown in Table 5.1.

The median age of the volunteers was 30 years (range 23-47 years). Mean +/- 2SD weight was 68.9 +/- 10.6 kg and mean +/- 2SD height was 162.3 +/- 14 cm. Before being accepted onto the trial it was confirmed that each
volunteer had a normal clinical examination, full blood count, creatinine,
electrolytes and electrocardiogram. There were no restrictions on eating or
drinking on the morning of the investigation.

<table>
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<td>Female</td>
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</tbody>
</table>

Table 5.1. Personal characteristics of volunteers enrolled into a
pharmacological trial assessing venous and brain absorption of buccal midazolam

5.2.2. *Equipment placement and drug administration*

On the morning of the study, each volunteer was attached to a multi-channel
recorder which continuously monitored ECG, thoracic and abdominal
respiratory excursion, nasal airflow, mean arterial blood pressure and 10
channels of EEG (Figure 5.1). Respiratory excursion was measured using
thoracic and abdominal respiratory bands. Nasal airflow was measured
using a nasal thermistor. An infrared light device was used for monitoring
oxygen saturation and non-invasive mean arterial blood pressure was
measured using a Dinamap. Silver - silver chloride EEG electrodes were
placed at selected positions using the 10-20 system of electrode placement.
Electrodes placed at F₃, F₄, F₇, F₈, T₅, T₆, C₃, C₄, P₃, P₄ and referred to a point between C₂ and P₂. A venous cannula was inserted into a peripheral vein to enable regular blood sampling. Because midazolam has a bitter taste it was flavoured with peppermint essence prior to administration. The volunteer held 2ml of the intravenous preparation of midazolam (5mg/ml) in the mouth for five minutes after which the remainder was spat out. In the double-blind phase of the study the volunteer was given either peppermint flavoured water or peppermint flavoured midazolam to hold in the mouth for five minutes. No blood samples were taken in the double-blind phase. The volunteer then lay on a bed, awake, with arousal maintained and eyes open, in a quiet room for the first 30 minutes of the testing period and was then allowed to sleep.

Figure 5.1. Typical setup of the volunteers. EEG electrodes, nasal airflow thermistor, respiratory bands, blood pressure cuff and venous cannula can be seen.
5.2.3. Pharmacokinetic evaluation

5.2.3.1. Venous blood sampling

In the first arm of the study venous blood samples (5ml) were drawn prior to buccal midazolam administration and then at 5, 10, 15, 20, 30, 45, 60, 120, 300 and 600 minutes following administration. The blood was put into lithium heparin tubes, centrifuged and the serum stored at -8°C until measurement of midazolam concentrations.

5.2.3.2. Measurement of midazolam

Midazolam was analysed with high performance liquid chromatography (HPLC) using a method similar to that described by Lehmann and Boulieu with a minor modification in that methyl t-butyl ether was used to extract
the drug from plasma using a liquid-liquid procedure (Lehmann et al., 1995).
The assay has a lower limit of quantification of 3 mcg/L and a coefficient of
variance within and between batches at the 20 mcg/L level of 6-9%. This
analysis was carried out by Dr David Berry at the National Toxicology Unit,
London.

5.2.4. EEG pharmacodynamic evaluation

5.2.4.2. Sampling

The same methods were used for both arms of the study. Digital data was
collected at 250 samples per second using a Compumedics
polysomnographic recorder and stored on optical disc.

5.2.4.3. Analysis

Data from the central channels bilaterally were combined. The EEG data
were analysed off-line. Filter settings were 100 Hz low pass, 0.4 Hz high
pass and 50 Hz notch. The low pass filter removes frequencies of greater
than 100 Hz, the high pass filter removes frequencies of less than 0.4 Hz
and the notch filter removes frequencies of 50 Hz. Spectral analysis was
carried out using an automatic analysis program. Thirty-second epochs from
5 minutes prior to 20 minutes after administration were analysed, allowing
determination of the relative power of the EEG at each frequency band (1-4,
4-8, 8-12, 12-16, and 16-30 Hz) during each epoch. The raw data was
assessed for artefact, especially EMG and sleep spindles. Use of central and
midline reference electrodes minimised muscle artefact.
Statistical comparisons were performed using SPSS for windows, release 8.0. One-way ANOVA was used to assess changes in the EEG and changes in venous drug concentration with respect to time. Two-way ANOVA was used to determine whether EEG changes over time were dependent upon the presence of midazolam. All data were logarithmically transformed prior to analysis in order to equalise variances.

5.3. Results

5.3.1. Pharmacokinetics

Maximum concentration (Cmax), time to maximum concentration (Tmax) and elimination half-life (T1/2) were calculated manually for each volunteer (Table 5.2). A mean maximum concentration of 32.73 ±6.4 mcg/L (Cmax±2SD) was achieved in 48±28 minutes (Tmax±2SD). Serum levels then declined and were either very low or undetectable by 10 hours (Figure 5.3). The mean elimination half life of midazolam was 202±113 minutes (T1/2±2SD). In two of the ten subjects the concentration rose to a level of 5mcg/L within 5 minutes, in seven subjects this level was reached by 10 minutes and in only one volunteer did it take fifteen minutes to reach this concentration. The venous levels continued to rise between 20 - 60 minutes after administration, but the increase in venous concentration over this period of time did not reach statistical significance. (One-way ANOVA from 20-60 minutes: p = 0.0912).
Table 5.2. Pharmacological data in volunteers following buccal midazolam. 
$C_{\text{max}}$ is maximum venous midazolam concentration, $T_{\text{max}}$ is time to maximum venous midazolam concentration and $T_{1/2}$ is elimination half-life of midazolam.

<table>
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<th>$T_{\text{max}}$ (mins)</th>
<th>$T_{1/2}$ (mins)</th>
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<td>42.6</td>
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<td>306</td>
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Figure 5.3. Box and whisker plot showing venous midazolam concentrations from the time of administration to 10 hours after administration. The horizontal line within the box represents the median, the box represents the interquartile range and the whiskers represent the complete range. Outliers (>1.5 boxplots from the median) are shown as +.

5.3.2 Pharmcodynamics

In the first arm of the study buccal administration of midazolam was associated with a reduction in 8-12 Hz activity for the first 5 minutes, followed by a progressive increase in the relative power of the EEG in the 8-12, 12-16 and 16-30 Hz frequency bands (One-way ANOVA. p<0.001 for all frequency bands). Statistically significant changes in the relative power of the above frequency bands were found within 10 minutes. The increase of relative power in the 8-12, 12-16 and 16-30 Hz frequency bands correlated
with plasma midazolam concentration. Spearman correlation coefficients are; 8-12 Hz, $r = 0.29$ and $p = 0.015$; 12-16 Hz, $r = 0.44$ and $p < 0.001$; 16-30 Hz, $r = 0.25$ and $p = 0.037$ (Figure 5.4). The 4-8 Hz band was not correlated with venous midazolam concentration ($r = 0.03$ and $p = 0.8180$).

Figure 5.4. Scatterplot showing the relationship between venous midazolam concentration and relative power in 8-12 (red diamonds, red line), 12-16 (green circles, green line) and 16-30 Hz (blue squares, blue line) frequency bands. Regression lines for each frequency band are shown.
Similar results to those in the open-label phase of the study were seen in the double-blind phase of the study although the initial reduction in relative power in the 8-12 Hz band was not clearly identified. There were significant increases over time in the relative power of the EEG in the 8-30 Hz frequency bands in those volunteers who received midazolam (One-way ANOVA for all 8-30 Hz frequency bands, p<0.001. Figure 5.5). There were no significant changes over time in the EEG of those volunteers who received peppermint-flavoured water (One-way ANOVA: 8-12 Hz, p=0.6951; 12-16 Hz, p=0.9862; 16-30 Hz, p=0.7365. Figure 5.6). In order to determine whether the effects of time upon the relative power of the 4-30 Hz frequency band were dependent upon the presence of midazolam, two-way ANOVA testing was performed. There was no significant interaction between time and drug in the relative power of the 4-8 Hz frequency band (p=0.6510). The interaction between drug and time on the relative power of the 8-30 Hz frequency bands was highly significant (p<0.001). No clinically important cardiorespiratory adverse events were noted. The lowest recorded oxygen saturation was 83% which lasted for 17 seconds before returning to normal. Seven of the 10 volunteers slept, with onset of sleep occurring between 30 and 45 minutes after drug administration.
Figure 5.5. Bar charts showing mean relative power at 8-12 (red), 12-16 (green) and 16-30 Hz (blue) frequency bands from 5 minutes prior to 20 minutes after water administration (top) and midazolam administration (bottom)
5.2. Discussion

The purpose of this study was to assess whether the pharmacology of buccal midazolam supported its use in the acute treatment of seizures. This requires the demonstration that buccal midazolam is rapidly absorbed into brain. The data presented in this chapter demonstrate that midazolam was undetectable in venous blood at 5 minutes in 8/10 volunteers. This would suggest that midazolam is slowly absorbed from buccal mucosa and would not be a clinically useful medication. However, increases in the fast frequencies in the EEG within 5-10 minutes suggests that there is rapid absorption into brain. If midazolam is administered intravenously to animal models of epilepsy, the changes in the 11.5-30 Hz frequency band are almost immediate (Cleton et al., 1998). In that study there appears to be a direct relationship between venous blood levels and EEG findings. These data are inconsistent with the findings in the present study in which EEG changes seemed to precede the identification of venous blood levels of midazolam. This apparent contradiction may be explained by rapid clearance from arterial circulation into brain and fat which may result in initial venous blood levels which are lower than arterial levels. This effect has been shown with at least 42 drugs including the benzodiazepines clonazepam and diazepam (Chiou, 1989). The effect is most marked in the early samples. Arterial sampling would theoretically have provided better data, but was considered to be too invasive for this volunteer study.

The time for maximum venous concentrations of midazolam to be reached was approximately 50 minutes. The majority of absorption was within the
first 20 minutes which is comparable with absorption characteristics of rectal diazepam in which therapeutic levels are reached within 5 minutes of administration and time to maximum concentration is 20-60 minutes (Remy et al., 1992; Dreifuss et al., 1998). No midazolam was detected in venous blood at 10 hours in 7 of the volunteers. The half-life of midazolam was 3-4 hours, dramatically shorter than the half-life of rectal diazepam, which is approximately 44 hours (Dreifuss et al., 1998), but comparable to the half-life of intramuscular and intravenous midazolam. The short half-life of midazolam suggests that patients are less likely to have prolonged periods of sedation without antiepileptic effect when compared with patients who have received diazepam.

Venous midazolam concentrations continued to rise from 20-60 minutes after administration. It is possible that this apparent continuing absorption is the result of some midazolam being swallowed by the volunteers. The increase in venous midazolam concentrations over this time did not reach statistical significance and therefore it is likely that the majority of the midazolam measured was absorbed through the buccal mucosa.

The major EEG changes in this study occurred in the 12-16 Hz frequency band. Spectral analysis of the EEG was confined to the waking state to avoid contamination of the 12-16 Hz band by sleep spindles which have a similar frequency. All volunteers were alert for the first 20 minutes of monitoring and therefore the acute changes identified during this 20 minute period are likely to be a direct result of drug absorption into the central
nervous system. In the double-blind arm there are significant differences between the volunteers who received midazolam and the those who received water, with marked increases occurring in the 8-30 Hz bands when active drug was administered.

Power spectrum and voltage changes in the EEG, similar to those seen in this study, have been used as evidence of pharmacodynamic effect of intravenous midazolam (Mandema et al., 1991; Mandema et al., 1992). These studies showed that level of sedation correlated with EEG changes. There are no published data which relate venous concentrations of midazolam to anticonvulsant effect, but initial EEG changes occurring within 5-10 minutes lend support to the hypothesis that midazolam had bound to GABA<sub>A</sub> receptors when the venous blood level had reached 5 mcg/L. Intravenous midazolam, via GABA<sub>A</sub> receptor agonism, has been shown to be effective in the treatment of SE. It is possible that the EEG changes identified in this study may be a surrogate marker of antiepileptic effect. Thus, processed EEG signals may be used to assess cerebral absorption in a situation in which venous samples may be misleading and arterial samples not feasible. In animal models (Cleton et al., 1998) the reduction in the power of the 11.5-30 Hz frequency band following a single intravenous bolus of midazolam correlated with falling blood levels. Therefore, this methodology could also be useful in determining how long buccal midazolam has a central nervous system effect following buccal administration.
This study, by demonstrating rapid effects on the brain when midazolam is administered by the buccal route, supports the view that buccal midazolam may offer an alternative to rectal diazepam in the emergency treatment of seizures. This supports a clinical study assessing whether acute repetitive seizures could be rapidly terminated by administration of buccal midazolam.
CHAPTER 6. TREATMENT OF ACUTE REPETITIVE SEIZURES WITH BUCCAL MIDAZOLAM

6.1. Introduction

The previous chapter provides pharmacological justification for a clinical trial assessing the effectiveness of buccal midazolam in terminating acute seizures. Acute repetitive seizures, defined as an increase in seizure frequency with return of consciousness between the events (Dreifuss et al., 1998), probably do not cause acute neurological injury but do require treatment in order to prevent progression to SE. Many patients with difficult to control epilepsy have periods during which seizure frequency increases. This may be distressing to the patient and most would like the seizures to be terminated. Oral benzodiazepines are widely used in this situation (Milligan et al., 1984; Shorvon, 1994b). It takes approximately 20 minutes for effective brain concentrations of a benzodiazepine to be reached following oral administration. Although this may be acceptable, it is likely that patients would prefer a treatment which has a faster onset of action. Rectal administration is frequently unacceptable to patients who are conscious and parenteral administration would require self-injection or hospital visits.

The aim of this study was to determine whether buccal administration of midazolam in solution is an effective treatment for acute repetitive seizures in childhood and adolescence.
6.2. Methods

6.2.1. Patient recruitment

Patients for this study were recruited from St Piers, a residential special school for children with epilepsy and other needs. It has a medical centre on-site which is staffed with nursing and medical personnel at all times during school terms. Consent was obtained from students who had previously required acute treatment with oral or rectal benzodiazepines for acute repetitive seizures. For children under 16 years of age consent was sought from the parents. In most cases, because of learning disability, consent could not be obtained from the subjects themselves. For students over 16 years of age consent was obtained from those able to give informed consent. In all cases written agreement from the parents was also obtained. The medical, nursing, care and teaching staff were given the names of students who had consented. Students who had 2 seizures within 30 minutes, whilst they were on-site, were brought to the medical centre by their carer.

6.2.2. Multi-channel recording

On arrival in the medical centre, the student was connected to the recording equipment used in the volunteer study. All of the monitors were placed in a standard way as described in section 5.2.2. Monitoring was continued for up to an hour after drug administration.
6.2.3. Drug administration

At the onset of the subsequent seizure in the cluster, buccal midazolam (0.5 mg/kg, maximum 10 mg) was administered. The solution was drawn up into a 2ml syringe via a hypodermic needle. The needle was removed, the nozzle of the syringe placed between the cheek and the teeth and the solution was squirted against the buccal mucosa, avoiding bolus administration. The child was encouraged to hold the medication in the mouth for 5 minutes following which they were allowed to swallow. If the seizures did not terminate within 15 minutes, an alternative treatment was used. The subsequent medication was chosen by the attending doctor.

6.2.4. Outcome measures

6.2.4.1. Clinical

Seizure termination was defined as cessation of clinical epileptic activity with return to the individuals background clinical state.

6.2.4.2. Electroencephalography

The patients had up to 10 channels of EEG monitored. The EEG was assessed by a neurophysiologist (Stewart Boyd) who was blinded to the clinical data, and by the candidate. The EEG was said to have improved if both assessors agreed that there was a reduction in epileptiform activity following buccal midazolam compared to the baseline recording. Transient improvement was defined as a reduction of epileptiform discharges in the EEG with return to pre-medication state prior to the end of the recording.
Unequivocal EEG improvement was defined as a reduction in epileptiform discharges for the entire length of the recording. Spectral analysis was attempted, but could not be used as most patients had markedly abnormal background rhythms.

6.2.4.3. Adverse events

The patients had cardiorespiratory monitoring throughout the test period. Adverse events were monitored both acutely and off-line. Automatic analysis of respiratory function was undertaken.

6.3. Results

There were 27 treatments in 18 students during the course of the trial. No student was treated more than 3 times.

6.3.1. Patient details

6.3.1.1. Personal details

See Table 6.1 for clinical information relating to patients treated in this arm of the trial.

6.3.1.2. Seizure types treated

The following seizure types were treated. Tonic (5 episodes), tonic-clonic (4), myoclonic (3), minor motor phenomena (6), complex partial seizures (1) and blank spells (8). Minor motor phenomena refers to multiple, small, distal myoclonic jerks. The term blank spells is preferred to absence
seizures as it was not possible to distinguish between typical absence, atypical absence and complex partial seizures on clinical observation alone.

6.3.2. Clinical outcome

Cessation of clinical epileptic activity occurred within 10 minutes in 19/27 (70%) episodes. Cessation within 15 minutes occurred in 21/27 (77%) episodes. Myoclonic jerks were the slowest to respond and were the only seizure type which persisted for longer than 10 minutes unless buccal midazolam had failed (Figure 6.1). No patient experienced any clinically significant adverse event.
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<td>M</td>
<td>LGS</td>
<td>TC, tonic, abs, atonic, CPS</td>
<td>NaV, CBZ, CMZ</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>M</td>
<td>Symptomatic generalised</td>
<td>TC, abs</td>
<td>NaV, LMT</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td>M</td>
<td>LGS</td>
<td>TC, tonic, abs</td>
<td>LMT, CBZ, TPM</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 6.1. Clinical details of patients with a history of acute repetitive seizures treated with buccal midazolam

LGS  Lennox-Gastaut Syndrome  NaV  sodium valproate  TPM  topiramate  PHB  phenobarbitone
TC   tonic clonic           LMT  lamotrigine  PHT  phenytoin  CMZ  chlormethiazole
abs  absence               GPN  gabapentin  Pred  prednisolone
myo  myoclonic             CLB  clobazam  Meth  methsuximide
CPS  complex partial seizures  CLN  clonazepam
CBZ  carbamazepine
Figure 6.1. Stacked bar chart showing time to seizure termination following buccal midazolam for the treatment of acute repetitive seizures. Each colour represents a seizure type. T/C = tonic clonic, MMP = minor motor phenomena, CPS = complex partial seizure.
6.3.3. *Electroencephalography*

EEG was assessed in 26/27 episodes treated. The other record was completely obscured by movement artefact and could not be interpreted. The background EEG was abnormal with epileptiform discharges in all records. Ictal changes were identified in most of these EEG recordings, but some of the background EEG was so abnormal that ictal events could not be identified. As it was difficult to determine ictal changes in some patients, primary assessment of improvement was on the grounds of reduction of background epileptiform abnormalities.

There were no identifiable changes in the 6 episodes in which there was no response to buccal midazolam. There were also no identifiable changes in 4/26 (15%) episodes in which there was a clear clinical response with clinical evidence of seizure cluster termination. There was a transient improvement in 4/26 (15%) episodes. There was an unequivocal EEG improvement in 12/26 (46%) episodes (See for example Figure 6.2). No EEG returned to normal following administration of buccal midazolam. The time from midazolam administration to EEG improvement was longer than the time from drug administration to clinical seizure termination in all cases. EEG changes became obvious between 10 and 20 minutes after administration.
Figure 6.2. EEG and physiological data from a single patient. The top panel shows the pre-midazolam recording, the middle panel is the recording 10 minutes after administration and the lower panel is the recording from 45 minutes after administration. There is a sustained improvement in the EEG for the period of the recording.
6.4. Discussion

This study shows that buccal administration of midazolam in solution results in seizure termination within 10 minutes in 70% of patients with acute repetitive seizures to whom it was administered. This is comparable to previous data on termination of seizures in patients following rectal administration of diazepam solutions in which approximately 70-80% of seizures are terminated (Milligan et al., 1984; Knudsen, 1979, Dreifuss et al., 1998). Midazolam has a relatively short half-life and its therapeutic effectiveness may not last long enough for buccal midazolam to prevent a recrudescence of acute repetitive seizures within a relatively short period. No attempt was made to assess seizure recurrence as the majority of the children enrolled had very severe epilepsy and many had daily seizures. The students who responded did return to their baseline clinical state, although two students required 2 doses of buccal midazolam within 24 hours because of a second increase in seizure frequency. The second dose was given approximately 1 hour after of the first and was effective on both occasions.

As prolongation of all seizure types can occur it is important to determine whether buccal midazolam is effective for all, or whether its usefulness will be limited to only a few seizure types. In this study several seizure types were treated. Patients, irrespective of seizure type, responded quickly to BMDL except for those with axial major myoclonic seizures who seemed to be slow to respond. Only 3 myoclonic episodes in 2 patients were treated so it is difficult to be confident about this effect.
An attempt at using EEG as a marker of seizure improvement was carried out in this study. EEG monitoring was commenced as soon as possible after the onset of acute repetitive seizures. Prior to buccal midazolam administration, the majority of the patients had very frequent interictal discharges between the seizures. The majority of patients also had identifiable changes during the clinical ictal events. However, only 60% of patients who had a clinical response to medication showed an unequivocal improvement in their EEG. The EEG response was always slower than the clinical response, usually taking between 10 and 20 minutes to become obvious. This may be because we were primarily assessing background inter-ictal EEG. It is unclear whether there was a cessation of ictal EEG abnormalities which was temporally related to the termination of clinical seizures. The delay in, or lack of, inter-ictal EEG response identified in this group of patients may be related to the severity of the underlying neural abnormality. Most of the patients do not appear to be able to produce normal rhythms. This may be because the neural networks required to produce normal rhythms have been interrupted by the underlying process. If normal rhythms cannot be generated then improvement in the EEG in which there are epileptiform abnormalities may be inhibited. Using EEG to confirm clinical efficacy of midazolam in stopping seizures was therefore unhelpful in many of the patients in this study. The method of EEG monitoring described in this study could be useful in patients without such severe inter-ictal abnormalities as those seen in the patients enrolled in this study, and in those in whom there are clear ictal events identifiable in the EEG.
Cardiorespiratory monitoring did not reveal any clinically significant adverse events in any student. However, since a case of cardiorespiratory collapse 20 minutes after intramuscular administration of midazolam has been reported (Shorvon, 1994b), further data relating to adverse events will need to be collected before buccal midazolam can be considered safe for the treatment of acute repetitive seizures.

The pharmacological and treatment data reported thus far are comparable to historical data on rectal diazepam. As rectal diazepam is effective in the acute treatment of seizures, it is possible that buccal midazolam would also be effective in the same situation. In order to confirm this hypothesis, a prospective randomised trial comparing these two drugs was carried out.
CHAPTER 7. BUCCAL MIDAZOLAM AND RECTAL
DIAZEPAM IN THE ACUTE TREATMENT OF
SEIZURES

7.1. Introduction
The two previous chapters in this thesis provide evidence that buccal midazolam is rapidly absorbed into the brain and is effective in terminating acute repetitive seizures in children with difficult to control epilepsy. In the current era of evidence based medicine it is important to show that a new medication has advantages over previously used medications prior to the drug being introduced into clinical practice. A randomised, placebo controlled trial is the standard way of confirming efficacy of a new medication. The aim of this trial was to determine whether there are differences in efficacy and adverse events between buccal midazolam and rectal diazepam in the acute treatment of seizures.

7.2. Methods
7.2.1. Patient recruitment
Patients for this trial were students at St Piers. There are 224 students at St Piers with an age range of 5-22 years. The majority of these students have severe epilepsy and learning disabilities. Students who had previously required acute treatment for prolonged seizures were identified. One of the major difficulties in performing clinical therapeutic research into SE is acquiring consent. It is clearly impossible to obtain consent from a patient
during an episode of SE, and parents or carers would not be given sufficient
time to understand the trial and give consent. St Piers offers a unique
environment in which it may be possible to predict which students will have
prolonged seizures and therefore obtain consent prior to an event. The
strategy adopted in this study was to identify students who had previously
required rectal diazepam as an emergency treatment for seizures. Consent
was obtained as in described in section 6.2.1. Forty-two students were
enrolled onto the trial.

7.2.2. Randomisation and drug administration

Randomisation to treatment occurred at the time of an acute event. In order
to achieve this, randomisation envelopes were produced. Sealed, unmarked,
identical envelopes naming which medication was to be administered were
randomised by simple shuffling. A box containing both buccal midazolam
and rectal diazepam, together with the randomisation envelopes, was
prepared and kept readily available. A list of the names of students with
consent was placed in a readily accessible place next to the most frequently
used telephones in the medical centre. The same list was also sent to all the
residential houses and the school.

When a consented student had been having a seizure which had lasted for at
least 3 minutes, the medical centre was contacted. The on-duty nurse then
took the pre-prepared box to the student using the on-site ambulance. Since
the student could be anywhere on the 200 acre site, it typically took the
nurse 3-5 minutes to reach the student. If the seizure was continuing when
the nurse arrived, an envelope was opened and the named medication was administered. Buccal midazolam was administered in the same way as previously described (section 6.2.3.). Rectal diazepam was administered from a commercially available pre-packed rectal tube. The package was opened, the student placed in the left lateral position with the legs bent, the anus was exposed, the tube inserted to the hilt and the tube pressed until all the liquid had been administered. The buttocks were held together for 2 minutes after withdrawal of the administration device in an attempt to prevent expulsion of the diazepam. The dose of both buccal midazolam and rectal diazepam in all events was 10 mg. If the seizure did not terminate within 10 minutes a second medication, usually intravenous benzodiazepine, was administered.

7.2.3. Monitoring for adverse events

Following drug administration the student was taken back to the medical centre where oxygen saturation and blood pressure was continuously monitored for 30 minutes using a Criticare monitor. Oxygen saturation was measured from a finger probe using infra-red technology. Blood pressure was monitored non-invasively from the arm with sampling set at 5 minutes. The student remained in the medical centre until well enough to return to daily activities.

7.2.4. Outcome measures

The following data were collected

Time from arrival of the nurse to administration of medication
Seizure description

Time from drug administration to seizure termination

Total seizure duration

Continuous oxygen saturation

Blood pressure every 5 minutes for a total of 30 minutes

Seizure termination was defined as cessation of all clinical epileptic phenomena and the return of purposeful response to external stimuli.

7.2.5. Statistical analysis

The purpose of this study was to determine whether there are differences in efficacy and adverse events between buccal administration of midazolam solution and rectal administration of diazepam solution in the acute treatment of seizures. In order to show a 5% difference between the groups in terms of drug efficacy, with a power of 90%, a total sample size of 54 episodes was required. Statistical analysis was carried out using SPSS for Windows, release 8.0. There were three arms to the statistical analysis of efficacy;

1. Comparison by episode. All the episodes were assessed as independent variables. Times are reported as median and interquartile range (IQR). Comparisons using the Chi-square test for binary data and the Mann-Whitney U test for continuous data were performed.

2. Comparisons by student. Paired analysis using the first treatment with BMDL and the first treatment with PR DZP in each student treated at least once with each drug. Nine pairs were identified. Additional analysis was carried out using consecutive pairs of BMDL and PRDZP treatments in
individual patients. McNemar’s test was used for binary data and
Wilcoxon’s signed rank sum test was used for continuous data. Drug
efficacy in the first seizure treated in all patients was analysed using the
Chi-square test.

3. Comparisons in individual students. The two students who made up 39 of
the episodes were analysed separately. Analysis was carried out using the
Chi-square test for binary data and the Mann-Whitney U test for
continuous data.

Further statistical analysis assessing time to drug administration and effect of
drug on blood pressure and oxygen saturation was carried out using the Mann-
Whitney U test.

7.3. Clinical Data

7.3.1. Age and sex

There were 18 patients treated on the trial, some several times (Table 7.1).
The median age was 14.5 years (range 5-19 years). There were 9 males and
9 females.

7.3.2. Epilepsy syndrome diagnosis

The patients were classified according to the ILAE Classification of
Epilepsy Syndromes (1989). Symptomatic epilepsy was identified in 16/18
of the students; 7 have Lennox-Gastaut syndrome, 5 have symptomatic
partial epilepsy, 2 have polymorphic epilepsy, 2 have symptomatic
generalised epilepsy and 2 have unclassifiable epilepsy. All of the patients
have multiple seizure types.
7.3.3. *Antiepileptic drugs*

All of the patients were on at least 2 maintenance antiepileptic drugs, including 2 on maintenance benzodiazepines. See Table 7.1 for details.
<table>
<thead>
<tr>
<th>Number</th>
<th>Age</th>
<th>Sex</th>
<th>Epilepsy Diagnosis</th>
<th>Seizure Types</th>
<th>Antiepileptic Drugs</th>
<th>Episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>M</td>
<td>LGS</td>
<td>TC, abs, tonic, myo, CPS</td>
<td>LMT, PHT, CBZ, CMZ</td>
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<tr>
<td>2</td>
<td>16</td>
<td>F</td>
<td>Polymorphic epilepsy</td>
<td>TC, tonic, myo, abs</td>
<td>GPN, NaV, PHB, LMT, CMZ</td>
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</tr>
<tr>
<td>3</td>
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<td>M</td>
<td>Symptomatic partial</td>
<td>TC, tonic, myo, CPS</td>
<td>CBZ, NaV</td>
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<tr>
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<td>12</td>
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<td>Cryptogenic generalised</td>
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<tr>
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<td>F</td>
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</tr>
<tr>
<td>6</td>
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<td>10</td>
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<td>NaV, LMT</td>
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<td>19</td>
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<td>CBZ, LMT</td>
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<td>LGS</td>
<td>TC, atonic, tonic, myo, abs</td>
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<td>NaV, CBZ, Rem</td>
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<td>19</td>
<td>F</td>
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<td>NaV, CBZ, acetazolamide</td>
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</tbody>
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Table 7.1. Clinical characteristics of the patients treated in a randomised trial comparing buccal midazolam to rectal diazepam in the acute treatment of seizures

LGS = Lennox Gastaut Syndrome, TC = tonic clonic, abs = absence, myo = myoclonic, CPS = complex partial seizure, CBZ = carbamazepine, NaV = sodium valproate, LMT = lamotrigine, PHT = phenytoin, GPN = gabapentin, CLB = clobazam, CLN = clonazepam, TPM = topiramate, PHB = phenobarbitone, CMZ = chlormethiazole, Rem = remacemide, Pred = prednisolone, FBM = felbamate
7.4. Results

7.4.1. Seizure types treated

The seizure types treated with each medication are shown in Table 7.2. The majority of episodes were tonic-clonic in nature but it is unclear which were primary generalised and which were secondarily generalised as the nurses only saw the patients well into the seizure. Descriptions from care staff were not detailed enough to derive this information.

<table>
<thead>
<tr>
<th>Seizure Type</th>
<th>BMDL</th>
<th>PRDZP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalised tonic-clonic</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Complex Partial</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Myoclonic</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Tonic</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>39</td>
</tr>
</tbody>
</table>

Table 7.2. Number of episodes of specific seizure types treated with each drug

7.4.2. Efficacy of medications

7.4.2.1. Comparison by episode

Buccal midazolam was used to treat 40 episodes in 14 patients and rectal diazepam was used for 39 episodes in 14 patients. Seizure termination following buccal midazolam occurred in 30/40 (75%) of the episodes. Seizure termination following rectal diazepam occurred in 23/39 (59%) of the episodes. Although these results suggest that buccal midazolam is superior to rectal diazepam there was no statistical difference between the groups (p=0.13. Figure 7.1)
Figure 7.1. Bar chart showing efficacy of each medication in terminating seizures, each episode treated as an independent variable. The red bars represent the seizures which terminated and the green bars represent the seizures which did not terminate. BMDL = buccal midazolam PRDZP = rectal diazepam

7.4.2.2. *Comparison by student*

Analysis of the 9 pairs of treatments (each pair consists of first BMDL treatment and first PRDZP treatment) did not reveal any statistical difference in efficacy between the drugs (p=0.38). The same seizure type was treated in each pair. Analysis of all pairs from consecutive treatments also revealed no differences in terms of drug efficacy between the drugs (p=0.18, McNemar),
nor did it reveal a statistical difference in efficacy when comparing first
episode treated in each patient (p=1.00).

7.4.2.3. **Comparisons in individual students**

Analysis of the two students who were treated 39 times revealed the following.
Student 1 was treated 24 times, 12 times with each medication. 8/12 episodes
treated with BMDL responded within 10 minutes compared to 4/12 episodes
treated with PRDZP (p=0.10). Analysis of only generalised tonic-clonic
seizures shows that BMDL was more effective than PRDZP in terminating
seizures in this patient (p=0.03). Patient 2 was treated 15 times; 6 times with
BMDL and 9 times with PRDZP. BMDL was effective in 5/6 episodes and
PRDZP was effective in 8/9 episodes (p=0.75).

7.4.3. **Time to clinical effect**

7.4.3.1. **Comparison by episode**

The time from drug administration to seizure termination was recorded in
each student. The median time from administration of medication to seizure
termination was 6 minutes (IQR 4-10 minutes) for midazolam and for
diazepam it was 8 minutes (IQR 4-12 minutes), (p=0.31. Figure 7.2). If only
patients who responded to the medication within 10 minutes are included in
the analysis, the median time to clinical response was 4-5 minutes in both
groups. Median total seizure duration was 17 minutes (IQR 12-20 minutes) in
the midazolam group and 15 minutes (IQR 13-20 minutes) in the diazepam
group (p=0.61).
Figure 7.2. Box and whisker plots showing the time from drug administration to seizure termination. The horizontal line represents the median, the box represents the interquartile range and the whiskers represent the complete range.

7.4.3.2. *Comparison by student*

There was no statistical difference between the drugs in terms of time from drug administration to seizure termination when paired analysis was carried out (p=0.28). The same seizure type was treated in each pair. Analysis of all pairs from consecutive treatments also revealed no differences in time from administration to seizure termination between the drugs (p=0.32).
7.4.3.3. *Comparison in individual students*

This analysis in patient 1 and patient 2 revealed no statistical difference between the drugs in terms of time from drug administration to seizure termination (p=0.11, patient 1; p=1.00, patient 2).

7.4.4. *Ease of drug administration*

Time from arrival of the nurse to administration of the medication was recorded and used as a measure of ease of administration. The median time between arrival and administration was 2 minutes (IQR 1-4 minutes) in the group treated with BMDL and 2 minutes (IQR 1-3 minutes) in the group treated with PRDZP (p=0.81. Figure 7.3).
Figure 7.3. Box and whisker plot showing time from arrival of the nurse to drug administration. The horizontal line represents the median, the box represents the interquartile range and the whiskers represent the complete range. Outliers (>1.5 boxplots from the median) are shown as +.

7.4.5. Adverse events

No clinically significant adverse events were identified in either group and no systemic support was required in any patient. Median reduction in systolic blood pressure was 11 mmHg (IQR 2.5-28 mmHg) in the group treated with BMDL and 6 mmHg (IQR -1.5-14 mmHg) in the group treated with PRDZP (p=0.15). Median reduction in diastolic blood pressure was 10 mmHg (-2-19 mmHg) in the group treated with BMDL and 8.5 mmHg (3-20 mmHg) in the group treated with PRDZP (p=0.74). The lowest recorded oxygen saturation was 93% and occurred in a patient who received BMDL. This lasted approximately 2 minutes before returning to 98%. All patients were monitored after every treatment.
7.5. Discussion

This study took place in a residential centre for children with severe epilepsy. Consequently all of the subjects enrolled into the study had severe, difficult-to-treat epilepsy. However, the setting and facilities, with on-site medical and nursing staff, made this study possible. Due to difficulties with consent, randomised controlled trials assessing treatments for SE are rare. This is the first trial comparing possible pre-hospital treatments for acute treatment of seizures. In terms of the basic aim of the study, namely to determine differences between BMDL and PR DZP the null hypothesis was not disproved. Overall, there were no significant differences between the drugs in terms of time from arrival at the patient to drug administration, time to seizure termination or total seizure length. Both drugs were effective in stopping seizures within 10 minutes in a large proportion of the cases: 75 % for BMDL and 59% for PR DZP. No clinically significant adverse cardiorespiratory events were identified in either group, suggesting that both drugs were safe in the subgroups tested. These data rely on the assumption that both of the medications are effective in treating seizures and are superior to placebo. It would not be ethical to carry out such a placebo controlled trial in patients requiring emergency treatment of seizures. The longer seizures last, the more difficult they are to treat (Alldredge et al., 1995), potentially placing patients at risk of neuronal injury. It has recently been shown that rectal diazepam is superior to placebo in the treatment of acute repetitive seizures (Dreifuss et al., 1998). A randomised controlled trial comparing the novel treatment, buccal midazolam, to the standard treatment, rectal diazepam, was therefore considered to be ethical and safe.
There are clear practical and social advantages of BMDL over PR DZP. The oral mucosa provides a route into the systemic circulation (De Boer et al., 1982; De Boer et al., 1984) which is easier to access in the acute situation than either the rectum or the nasal mucosa. Seizures often occur in public places and carers do not like administering rectal medication in public view. Consequently treatment may be delayed. Most older patients are embarrassed when told that they had required rectal DZP. Whatever view is taken of the public attitude to people with epilepsy, it is clear that placing medication into the mouth is unlikely to carry much social stigma whereas the need to use the rectal route is potentially highly stigmatising. Thus carers are more likely to treat seizures early when a socially acceptable route is available.

The patients in this study all had severe epilepsy which was difficult to control with antiepileptic medication. The fact that the emergency treatment used in this group of patients was effective in the majority suggests that it might be effective in children who do not have pre-existing severe epilepsy. This, in turn, suggests that BMDL might be very effective in the wider group of children who present to accident and emergency departments with SE. The youngest patient treated in this trial was 5 years of age. Many episodes of SE occur in children under this age and so further studies assessing the effect of BMDL should be carried out in young children. Studies in a normal community setting should help to confirm which type of treatment is most effective in practice.
This study shows that buccal midazolam is at least as effective as rectal diazepam in the emergency treatment of seizures. Taken together, the studies reported in the last 3 chapters provide convincing evidence that buccal midazolam is effective in the treatment of patients who develop acute repetitive seizures or SE in the context of severe epilepsy. There is no apparent reason why buccal midazolam should not be effective in terminating seizures in other populations in which rectal diazepam is effective. These populations would include children having febrile convulsions and children receiving initial treatment in accident and emergency. Because the buccal route of administration offers distinct advantages in terms of convenience and social acceptability over the rectal route, it should become the preferred treatment in patients with SE in the context of severe epilepsy.
8.1. Conclusions

The overall aim of this study was to determine whether SE in childhood can be harmful to brain and whether a socially acceptable pre-hospital treatment which reduces seizure length could be developed. The brain structure which was primarily investigated was the hippocampus as animal model and human surgical data suggest that this is the structure which is most likely to be damaged (Shorvon, 1994b). Qualitative and quantitative magnetic resonance techniques were used because data from these techniques provide reliable information about human hippocampal and temporal lobe abnormalities in-vivo. Midazolam is an effective benzodiazepine used in the treatment of SE, but it has only been administered parenterally (Lahat et al., 1992; Orebaugh et al., 1994; Lal Koul et al., 1997). Parents and carers are not usually comfortable about injecting children, especially if an alternative is available. Buccal administration of midazolam in solution was assessed as a potential acute treatment for seizures which could be widely used in the community. Prolonged seizures which result in MTS could therefore potentially be terminated early, in the community, and ultimately reduce the incidence of MTS.

There is an ongoing debate on whether MTS may be acquired following SE. Previous studies have not attempted to address this question in humans, by separating the commonest association with MTS, prolonged febrile convulsion (PFC), from other non-PFC related associations with MTS. The
study described in Chapter 3 attempted to separate MTS associated with PFC from MTS with other associations, and use this as evidence that there may be more than one mechanism of injury to the hippocampus. The results show that MTS in patients with a history of PFC in childhood tends to primarily affect one hippocampus and this unilateral damage tends to be more severe than that identified in patients without such a history. This suggests that the mechanism through which the PFC and MTS are associated may be different to the mechanisms which relate MTS and other initial precipitating injuries. These data do not address the question of whether MTS may be acquired following a prolonged seizure, but provides justification for grouping patients with a history of SE into those with and without a history of PFC when assessing whether MTS is an acquired disorder. A prospective magnetic resonance study which determines whether there are hippocampal abnormalities which can be identified soon after an episode of SE and which develop into typical features of MTS was carried out.

Chapter 4 describes the study which attempted to define hippocampal abnormalities following SE. The results show that PFC is associated with hippocampi that were large and had prolongation of the T2 relaxation time when compared to controls. These data are consistent with oedematous swelling of the hippocampi. No abnormalities of the temporal lobe were identified using $^1$H MRS in this group. Patients with non-febrile SE also have prolongation of the T2 relaxation time but no evidence of increased hippocampal volume. There were reductions in NAA/(Cho+Cr) which
approached statistical significance. It remains unclear whether the findings in this group of patients are a consequence of SE or whether they reflect an underlying brain disorder. This separation is particularly difficult to establish in patients with remote symptomatic aetiologies. The data support the view that acute MR abnormalities following SE are part of the sequence of events that lead to MTS. Only one patient had magnetic resonance evidence of mesial temporal sclerosis (MTS) on the acute scan. This patient had a previous history of PFC and does not contribute to the understanding of the pathogenesis of acquired hippocampal damage in humans. On the basis of epidemiological studies, approximately 10% of patients with a history of SE would be expected to develop partial seizures (Verity et al., 1993). If MTS was present prior to an episode of SE then MTS should have been identified in at least one patient enrolled into the study. This was not the case and supports the view that hippocampal damage may be acquired following SE.

If the results from chapters 3 and 4 are considered in conjunction, then it is possible that PFC results in acute hippocampal swelling, and that a proportion of the patients will go on to develop severe unilateral MTS. A much larger prospective study is required to establish that relationship in individual patients. The mechanisms through which PFC and MTS are related remain unclear. Animal models which show hippocampal injury following SE all have seizures which begin in the limbic structures (Meldrum, 1997). As PFC are associated with primarily unilateral, severe MTS, it is possible that PFC are of limbic origin. Both hippocampi may...
swell during the phase of secondary generalisation, but the hippocampus in which the seizure began may be more prone to injury than the hippocampus which swelled because of secondary generalisation. However, no patient was found to have MTS on follow-up scans but the data was limited by the small number of patients who had follow-up scans. Nevertheless, the hypothesis that PFC originate in one hippocampus could explain why children with a history of PFC who have acute hippocampal swelling may develop unilateral severe MTS.

This study supports, but does not confirm, the hypothesis that MTS may be acquired following PFC in childhood. MTS is an uncommon outcome of a common event. However, it is an important outcome as the social and economic burden of MTS is large because there are associated cognitive and behavioural abnormalities and there is frequently a necessity for surgical treatment. Any acquired condition may lend itself to preventative measures, especially if the cause and mechanism is understood. Work in animal models suggests that neuronal injury only occurs following a seizure which lasts at least 30 minutes (Lemos et al., 1995, Fujikawa, 1996). Prevention of prolonged seizures may therefore decrease the incidence of MTS. This will account for approximately 50% of potential cases of MTS as there are other causes of this structural abnormality.

Most seizures, including PFC, begin in the community (Alldredge et al., 1995). The time required for a carer to recognise that a child is having a seizure, contact the ambulance services, await the arrival of the ambulance,
have the child transported to the nearest accident and emergency department and receive initial treatment, is probably at least 30 minutes in the majority of cases. An effective, socially acceptable treatment which can be administered in the community by parents, carers, teachers or paramedics is likely to reduce the overall length of seizures. Rectal diazepam in solution has gone some way to meeting that need, but the social unacceptability of the route of administration has meant that many carers and professionals are reluctant to use this medication. The work described in Chapters 5-7 systematically assessed whether midazolam in solution administered against the buccal mucosa would provide an effective and socially acceptable acute treatment for seizures.

The initial study was carried out in adult volunteers and confirmed that buccal midazolam (BMDL) is rapidly absorbed into venous blood and brain, as evidenced by venous blood levels and increases in the relative power of the faster frequencies in the EEG. The minimum effective venous concentration of midazolam required for termination of seizures is unknown, but the EEG methodology used in this phase of the study suggested that there had been brain absorption of midazolam when the venous concentration was 5 mg/dL. The EEG changes were identified within 5-10 minutes of midazolam administration. These data taken in combination suggest that buccal midazolam could result in sufficient brain concentrations to terminate seizures within 10 minutes of administration. The next phase of the trial, carried out at St Piers, confirmed that acute repetitive seizures could be terminated within 10 minutes in children with
severe epilepsy and learning disability. An attempt to confirm seizure termination with EEG failed as there appeared to be no direct correlation between clinical termination of seizures and electrographic improvement. This may have reflected the severity of epilepsy and learning disability in the population which took part in the trial. The last phase of the trial compared rectal diazepam to buccal midazolam in the acute treatment of seizures. The children enrolled into this phase of the trial were also from St Piers. There were no differences in terms of efficacy, incidence of cardiorespiratory adverse events or ease of administration. Concerns about aspiration or placing things into the mouth of children having seizures appear to be unfounded. There was no resistance amongst the nursing staff in using buccal midazolam.

This study, therefore supports the view that PFC may cause MTS, but the relationship between non-febrile SE and MTS remains uncertain. Buccal midazolam is an effective treatment for SE which could become widely used in the community as well as in the accident and emergency settings.

8.2. Future Directions

The causative relationship between PFC or SE and MTS, in humans, has still not been confirmed. A similar magnetic resonance study to that already carried out, but with larger numbers, is still required. It is not clear which magnetic resonance investigations are likely to be the most informative in this group of patients. In addition to the MR measures described in the present work, parameters such as the apparent diffusion coefficient of water
(ADC) may be a marker of metabolic failure and cell swelling with later neuronal death (Minematsu et al., 1992; Shimizu et al., 1993; Righini et al., 1994; Verheul et al., 1994). Perfusion imaging provides information of regional blood flow and may give insight into the role of ischaemia in the development of acquired hippocampal damage. If these parameters were added to those already used the time required for a single investigation profile would be approximately 2.5 hours. Although this is possible, it would be better if the investigations could be more directed and therefore take less time.

Several animal models of SE have been validated (Wasterlain et al., 1994; Meldrum, 1997). The animals have an initial prolonged limbic seizure followed by spontaneous recurrent seizures resembling human temporal lobe seizures (Cavalheiro, 1995). These models could be used to determine which MR parameters are most useful as surrogate markers of later MTS and at which time point the investigations should be carried out. Once spontaneous recurrent seizures have developed, the animals would be sacrificed to confirm hippocampal abnormalities histologically.

The proposed mechanism of brain injury during SE is release of cytotoxic concentrations of glutamate. In-vitro excessive concentrations of glutamate, in whole brain preparations, have not been identified in animal models at the time of neuronal injury (Walton et al., 1990; Cavalheiro et al., 1994). Potential explanations for the failure to identify excessive glutamate include incorrect hypothesis or rapid metabolism of glutamate to glutamine, lactate
and alanine. The role of glutamate could be examined in animal models using $^1$H MRS in conjunction with in-vivo microdialysis. $^1$H MRS could also be used to detect increased glutamate, glutamine or lactate signal immediately following SE. Extracellular fluid from in-vivo microdialysis could be analysed for excitatory amino acids, glutamine, alanine and lactate. Longitudinal data can be acquired in single animals. Excessive concentrations of these substances and timescale of changes would give insight into the role of glutamate in neuronal injury of the hippocampus and temporal lobe.

Buccal midazolam is effective in terminating seizures in children with severe epilepsy and learning disabilities but it is unclear whether it will be effective in the community or accident and emergency settings. Further drug trials which compare rectal diazepam to buccal midazolam in these settings will be required before the drug could be marketed. The youngest child treated in this trial was 5 years. Many seizures occur in children under this age and therefore any future trials should include children of all age groups.

The effect of terminating seizures early, on the development of MTS, can also be investigated using the magnetic resonance methodology. If acute MR surrogate markers of MTS can be identified, then early termination of seizures or potential neuroprotective strategies could be assessed by determining whether the surrogate marker is altered by the intervention. If successful in animal models then this methodology could also be useful in humans.
This study has attempted to advance understanding of the treatment and pathological sequelae of status epilepticus. The MR studies reported provide pilot data which would support larger MR studies designed to assess the pathogenesis of acute hippocampal injury in humans. Buccal midazolam has been shown to be effective in a select population but further studies assessing efficacy and side-effects in young children, adults and patients who were previously healthy are required.
CHAPTER 9. REFERENCES

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