Convulsive Status Epilepticus: Prolonged Childhood Seizures and their consequences

Michael Yoong MBBCh MRCPCH

2/21/2012

Thesis submitted for the degree of Doctor of Philosophy

Neurosciences Unit & Imaging and Biophysics Unit, UCL Institute of Child Health, London

Table of Contents

CONTENTS

LIST OF FIGURES

LIST OF TABLES

Declaration

I, Michael Yoong, confirm that, except where indicated otherwise, the work presented in this thesis is my own. Credit should be given for the original study design and patient recruitment protocol to Dr Rod Scott, Dr Richard Chin and Dr Chris Clarke. All analysis methods and data interpretation, including diffusion tensor imaging analyses were all my own work.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed.....

Convulsive Status Epilepticus: prolonged childhood seizures and their consequences

Abstract

Convulsive Status Epilepticus (CSE) is the most common medical neurological emergency in children. Prolonged seizures are associated with brain injury in both animal models and humans and there is concern about longer term outcomes. A qualitative difference between prolonged and shorter seizures is apparent, but information about the longer term prognosis of CSE remains scarce.

Retrospective studies have linked the commonest form of childhood CSE, prolonged febrile seizures (PFS), and temporal lobe epilepsy due to mesial temporal sclerosis (TLE-MTS). This study aimed to prospectively follow a population-representative cohort of children following CSE and look for signs of evolving MTS or other brain injury. Magnetic resonance imaging (MRI) was used to visualise underlying brain abnormalities and identify early signs of injury. To investigate the longitudinal evolution of any injury, MRI investigations were repeated at 6 and 12 months post-CSE and the child's clinical status monitored throughout.

31.2% of children had an abnormal MRI scan post-CSE. Most of these abnormalities pre-dated the episode of CSE and no clinically significant abnormalities were found in children with PFS.

Mean hippocampal volumes were only reduced in the group of children with symptomatic CSE; however 20-30% of all children showed loss of hippocampal volume during the year following CSE. This could represent a precursor to TLE-MTS.

Further abnormalities were shown on diffusion tensor imaging in children with PFS. Hippocampal mean diffusivity did not show the usual age dependency and widespread reductions in

fractional anisotropy were seen across major white matter tracts. These reductions were apparent at 1 and 6 months post-PFS, but resolved by 1 year.

These findings form important evidence that children with non-PFS CSE are also at risk of long-term hippocampal damage and that children with PFS appear to have extensive extra-hippocampal abnormalities. These will be further explored in the body of this thesis.

Acknowledgements

I would like to extend my sincere thanks to my PhD supervisors, Rod Scott, Richard Chin and Christopher Clark. They have provided invaluable support in all aspects of this project, from encouragement and assistance surmounting technical challenges to motivational criticism and deadline pressure. I look forwards to continuing to work with them in the future. Thanks are also owed to Suresh Pujar, Shekhar Patel and Tang Fosi in Neurosciences for assisting with patient recruitment and reviews, as well as providing a sounding board for discussion and complaints! Additional thanks must also go to my colleagues in Biophysics: Martin King, Kieran Seunarine, Jon Clayden and Michael Dayan for their invaluable assistance with statistics, processing the MRI scans and setting up the automated analyses contained in this thesis. On the clinical side of the project, I am indebted to the staff of the MRI department, especially the sedation nurses, Angie, Leo, Rachel and Amanda, as well as Brian Neville for providing clinical supervision and Mike Sury for providing anaesthetic support. Extra special thanks must go to Tina Banks, for her calm assistance persuading the children to co-operate with the scans. Naturally I am immensely grateful for the assistance of Marina Martinos, who worked with me on this project and successfully submitted her thesis covering the analysis of cognitive functions last year. I look forwards to a long and fruitful collaboration as we continue to explore the implications of our work.

We are grateful to the Wellcome Trust and Young Epilepsy for providing the financial support to enable us to carry out this project and complete the follow-up period.

Thanks should also go to my family and my wife, Monica, for their constant support and encouragement to complete this project.

Finally I would like to thank all our local collaborators (Dr. Simon Roth, Dr. Ruby Schwartz, Dr. Jacqueline Taylor, Dr. Edwin Abrahamson, Dr. Mark Kenny, Dr. Mark Peters, Dr. Shane Tibby, Dr. Adnan Manzur, Dr. Rajiv Sood, Dr. Elaine Hughes, Dr. Mohammed Ahmed, Dr. Satheesh Mathew, Dr. Arvind Shah, Dr. Warren Hyer, Dr. Michael Greenberg, Dr. Adelaida Martinez, Dr. Simon Nadel, Dr. Ian Maconochie, Dr. Adrian Goudie, Dr. John Jackman, Professor Mark Gardiner, Dr. Anthony Cohn, Dr. Corina O'Neill, and Dr. Andrew Robins) who were involved in helping us recruit our patients and the patients and families themselves, without whose contributions this work would not have been possible. I hope that the findings of this study will one day be of benefit to them.

Chapter 1: Background

1.1 Epileptic Seizures

Epilepsy is a paroxysmal disorder characterised by spontaneous and recurrent epileptic seizures(1). A seizure consists of a discrete episode of abnormal neuronal discharge combined with the clinical manifestation of that discharge, usually a change in behaviour.

It is the change in the electrical behaviour of the brain from normal background activity to epileptic discharges that has come to be the defining hallmark of an epileptic seizure. The work on this thesis concentrates on status epilepticus, but prior to discussing CSE I will provide some background on the electrical aspects of seizures.

1.1.1 The pathophysiology of a seizure

Normal neuronal activity as recorded on a typical scalp electroencephalogram (EEG) consists of asynchronous oscillations with a typical amplitude of around 10-100µV (Figure 1.1). The EEG measures the localised electrical potential at points on the surface of the scalp around each electrode. The aggregate signal received is thought to reflect the summated activity of large populations (10^5-10^7) of cortical neurons. Due to the complex nature of this activity it is difficult to separate out the relative contributions of different neuronal phenomena to the EEG signal, however experiments with simultaneous recording from scalp EEG and intracranial electrodes have shown that the best correlation is with the summation of multiple excitatory post-synaptic membrane potentials (EPSPs) across the dendritic spines of cortical neurons.

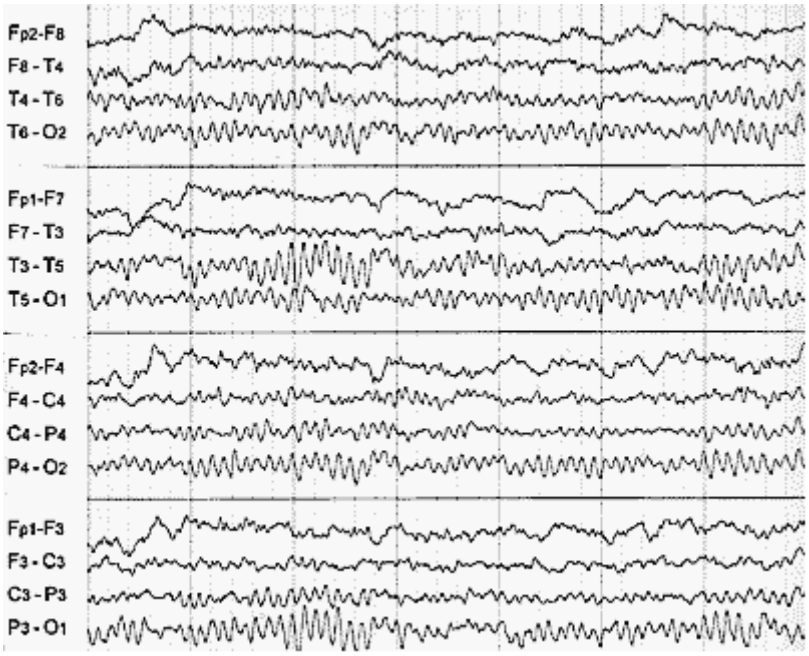


Figure 1-1: Normal EEG (eyes closed)

An EEG recorded during a seizure will show synchronised, rhythmic activity across a number of different electrodes (depending on seizure location and focality) as seen in Figure 1.2. This activity appears to be self-sustaining in the short term and capable of spreading from one brain region to another. Conceptually a seizure can be divided into three phases: *initiation*, *propagation* and *termination(2)*.

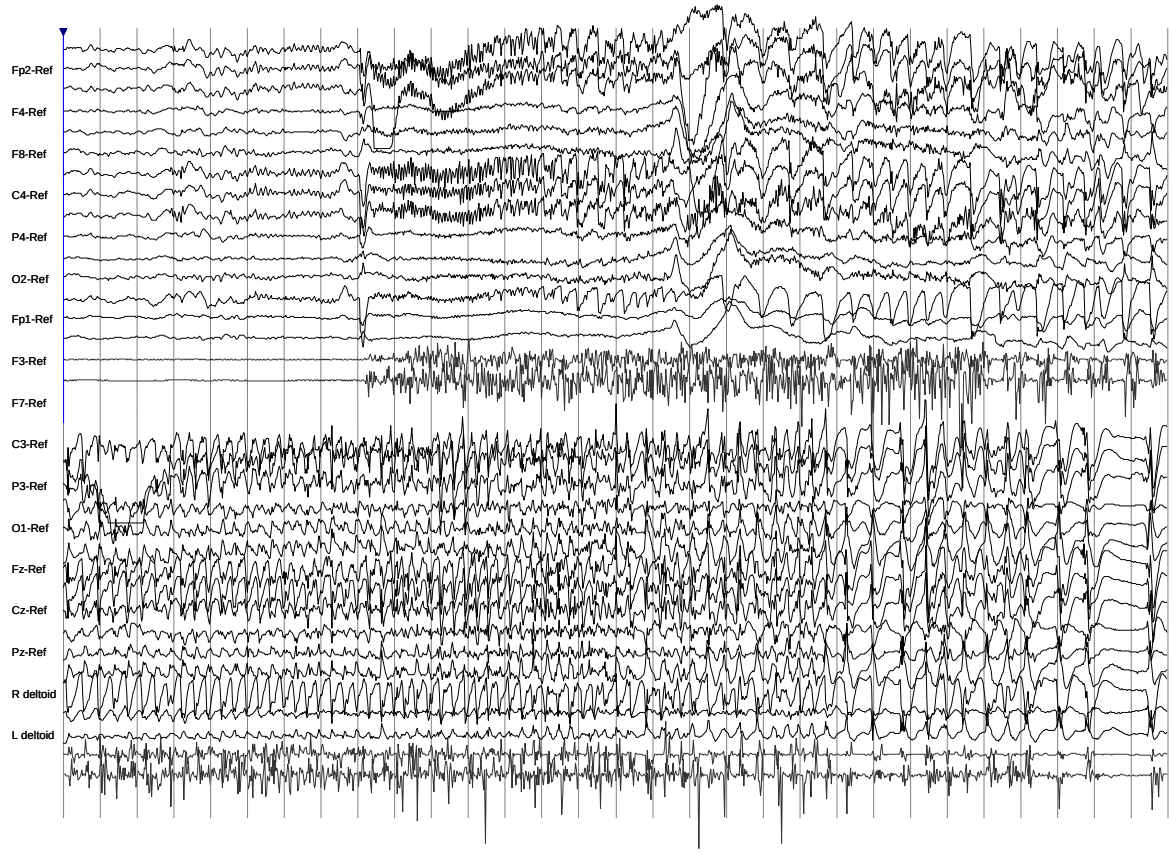


Figure 1-2: EEG showing seizure activity

1.1.2 Initiation

For a seizure to occur the activity in the brain must make a transition from the normal, low-amplitude unsynchronised activity (Figure 1-1), to the synchronised, rhythmic discharge typical of a seizure (Figure 1-2)(3). The initial phase of this process is known as *initiation.*

It is hypothesised that this initial transition usually takes place in a discrete, anatomically localised group of neurons(4). Burst-generating cells capable of high frequency discharges are thought to play an important role in this process. Pyramidal cells in the hippocampus and neocortex have been shown to be capable of this behaviour (5;6). Localised excitation of these cells leads to rapid recruitment of a larger population of neurons, synchronisation of neuronal firing and the beginning of a self-sustaining cycle of increasing excitatory activity.

Changes in the brain that make neurons more excitable tend to promote seizure initiation and changes that suppress brain activity inhibit it. These changes can be global, such as a global metabolic disturbance like hypocalcaemia; or local, such as a localised infarction or stroke. Genetic

mutations, especially those involving ion channels and structural brain abnormalities such as cortical dysplasias or areas of infarction are also known to lower the seizure threshold.

1.1.3 Propagation

Once a seizure has been initiated, the abnormal discharge needs to spread beyond the initial group of neurons to involve a wider area of the brain (should it fail to do this, it would not normally be classified as a clinical "seizure"). This is termed *propagation* and takes place along multiple routes, depending on the existing neuronal projections of the axons of the affected neurons. Focal seizures tend to involve predominantly local spread to adjacent brain regions(7), whereas it is thought that generalised seizures occur when sub-cortical structures are excited by the initial discharge(8) enabling the subsequent rapid recruitment of wide areas of cortex. As more brain structures are recruited, this causes the loss of consciousness associated with generalised seizures.

1.1.4 Termination

Thus far the seizure has been viewed in isolation: the abnormal burst of excitatory activity will tend to create more excitation in a self-sustaining loop. However, this process is counter-balanced by inhibitory mechanisms within the brain, which will act to try and *terminate* this activity. This *termination* step is considered to be the key to limiting seizure duration - with failure of termination being the basic cause of status epilepticus.

Normal activity in the brain is carefully balanced between excitation and inhibition. In the adult brain the main excitatory neurotransmitter is glutamate and the main inhibitory neurotransmitter is γ-aminobutyric acid (GABA). GABA inhibits action potential generation by binding to specific $GABA_A$ receptors on the surface of neurons, opening a chloride specific ion channel and increasing the chloride conductance of the cell membrane. Under normal physiological conditions this has the effect of hyperpolarising the neuron and inhibiting the generation of new action potentials. While this is an important mechanism of action for many antiepileptic drugs, it does not appear to be involved in physiological seizure termination(9).

Rather it is thought a combination of other mechanisms is primarily responsible for seizure termination in the majority of seizures. It has long been known that ongoing seizure activity causes a rapid local decrease in tissue pH (10;11) and recently pH sensitive ion channels have been shown to play an important role in seizure termination in a transgenic mouse model (12). Other changes that

have been hypothesised to contribute to seizure termination include energy failure(13), hypoxia(14), and adenosine release(15). Failure of these mechanisms leads to status epilepticus; the emphasis of the work presented in this thesis.

1.2 Convulsive Status Epilepticus

Status epilepticus is important as it is associated with mortality and important morbidity. Early observations suggested that longer seizure durations were associated with an increased risk of death: "A severe epileptic fit may kill the patient …. especially… if the disease extends into the second day" (Caelius Aurelianus, *Morb chron.*). Since then, numerous case studies have documented patients who are thought to have died from uncontrolled seizures (16-19). There are also other associations between extended seizure duration and adverse outcomes, such as brain injury and epilepsy, which will be discussed in more detail later.

The phrase "*status epilepticus"* to refer to such prolonged, unremitting seizures was first coined in 1868(20). Conceived as the "maximum expression" of epilepsy(21), *Status Epilepticus* came to be defined any seizure as "persists for a sufficient length of time or is repeated frequently enough to produce a fixed or enduring condition"(22). Before this the term was generally held to be restricted to overt convulsive seizures - convulsive status epilepticus (CSE) - and while it was extended to include electrical seizures without overt convulsive activity - non-convulsive status epilepticus (NCSE)(20) - the majority of research and debate has focused around CSE as it is both easier to define and observe; and has a greater association with adverse outcomes. In keeping with this, this thesis will focus mostly on CSE.

1.2.1 Definition

There have been many attempts to draw up a formal definition of CSE. Ultimately the definition used depends largely on the purpose to hand. The initial theoretical formulation of CSE as a seizure which will not terminate without outside intervention, while intellectually appealing, proves unwieldy in practice as there is no way to tell in a living organism when a seizure has crossed into this category. Most definitions therefore, use time based criteria to differentiate CSE from other seizures. The standard definition used for most scientific studies is a cut off of 30 minutes, based on ILAE guidelines(1). The reasoning behind this definition is that 30 minutes of seizure activity appears to be a cut-off in animal models, after which the risk of brain injury rises markedly(23).

While 30 minutes is typically the definition used for epidemiological studies, there has been a recent move towards a shorter definition for clinical use(24). If the risk of brain injury and benzodiazepine resistance increase with seizure duration then it is desirable to initiate interventions to terminate the seizure as soon as it becomes clear it will not stop on its own. Analysis of seizure duration has shown that most afebrile seizures are short and self-terminating, with a mean duration of 3.1 minutes(25). However there exists a sub-population that has a much longer mean duration of over 30 minutes. Models constructed from this data show that those seizures which last longer than 7 minutes are more likely to belong to this second group and become prolonged if intervention is not initiated(25). Recent analysis of children with febrile seizures reports a similar bi-modal distribution of durations(26). From a clinical point of view therefore, defining CSE as any seizure over 10 minutes will help ensure timely initiation of treatment while minimising the risk of unnecessary treatment(27).

Pragmatically then, there are two definitions in widespread use today, 30 minutes, in order to study the risk of brain injury and 10 minutes for the initiation of anti-convulsive medication. For the purposes of this thesis, the following definition of CSE will be used, unless otherwise stated:

"A convulsive seizure, or series of seizures without recovery of consciousness in between, lasting in total longer than 30 minutes"

1.2.2 Aetiology

There are as many causes of CSE as there are of convulsive seizures. Typically CSE is spilt into several aetiological groupings, each with their own subset of causes. A definition of some of the aetiological groups that were used in this study to classify CSE is given in Table 1-1 below.

Aetiological group	Definition	Possible causes of CSE
Prolonged Febrile Seizure	CSE occurring in a previously neurologically normal child with no history of central nervous system infection and associated with a temperature > 38.0C	Febrile Seizure
Acute Symptomatic	CSE occurring in a previously neurologically normal child with an acute neurological insult within the past 24 hours	Bacterial meningitis Viral encephalitis Hypoglycaemia Hypocalcaemia Hypo/hypernatraemia Head injury Cerebrovascular accident (CVA)
Remote Symptomatic	CSE occurring in a child with a previously known neurological disorder occurring over 24 hours previously. Also includes genetic causes for seizures.	Cortical dysplasia Tuberous sclerosis SCN1a mutation Previous Hypoxic Ischaemic Encephalopathy Previous CVA or head injury Mesial Temporal Sclerosis
Acute on Remote Symptomatic	As above with an additional acute neurological insult in the past 24 hours.	
Idiopathic Epilepsy	CSE in a child with idiopathic epilepsy and no other acute cause for the seizure	Idiopathic epilepsy
Cryptogenic Epilepsy	CSE in a child with cryptogenic epilepsy and no other acute cause for the seizure	Cryptogenic epilepsy
Unclassified	All other CSE	

Table 1-1: Aetiological classification of CSE (Adapted from Chin et al.(28))

1.2.3 Epidemiology

CSE is one of the most common medical neurological emergencies. Epidemiological studies have shown that it has an incidence of 6.8-61/100,000 persons/year and a bi-modal distribution, with peaks at the two extremes of the age spectrum: in children under 1 year and in the elderly (29;30) (Figure 1-3).

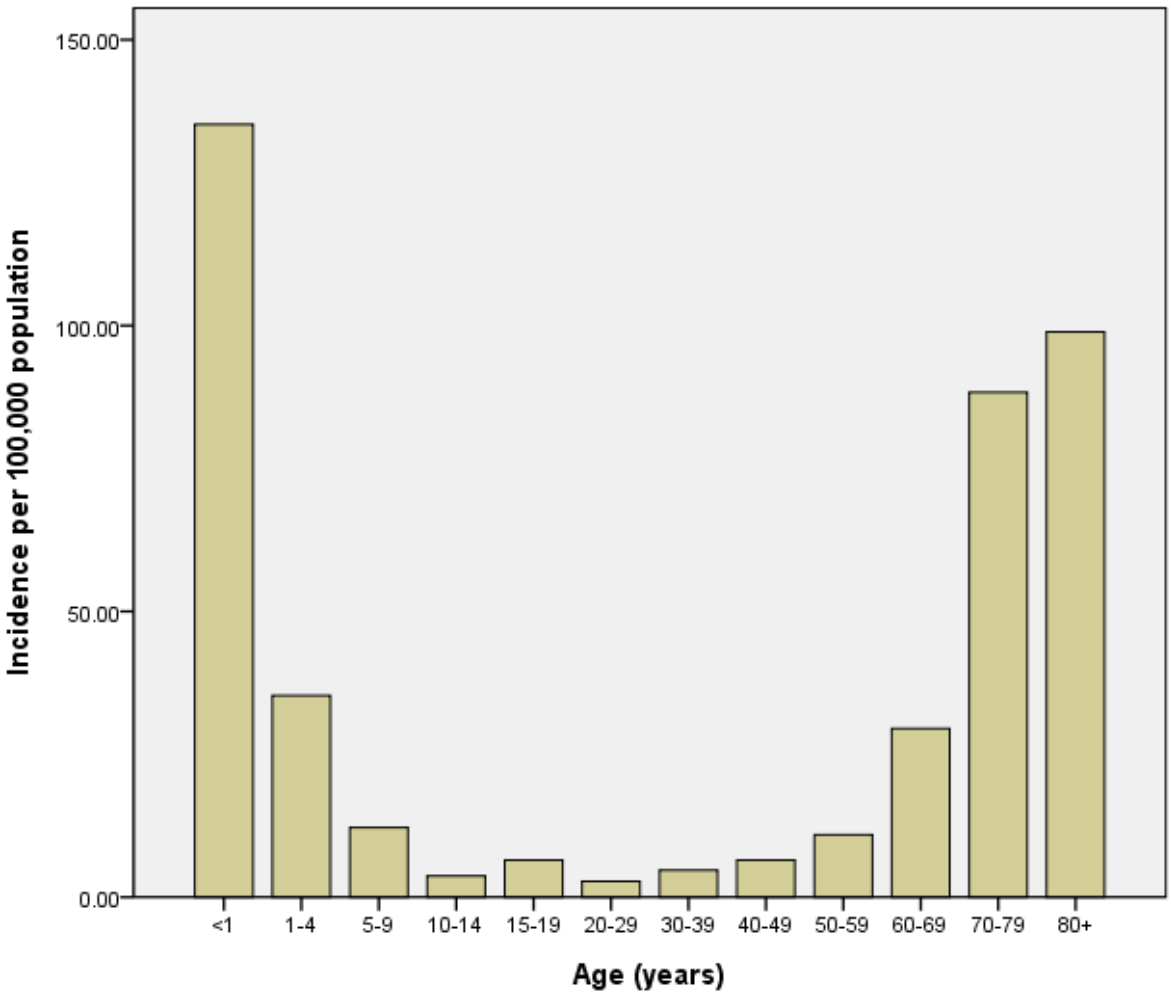


Figure 1-3: Age specific incidence of CSE (Adapted from Hesdorffer et al(30))

It is apparent that the main aetiologies and outcomes of CSE differ between children and adults and hence need to be considered separately. The findings of the main studies investigating the aetiology of CSE in children are summarised in table 1-2:

Authors	Year	Number of children	Incidence (per 100,000 children/yr)	Prolonged febrile seizure	Acute symptomatic	Remote symptomatic	Acute on remote	Idiopathic/ Cryptogenic epilepsy/Other
Chin et al.(28)	2006	176	17-23	32%	17%	16%	16%	**19%**
Coeytaux et al(31)	2000	64	21		66%	25%	-	**9%**
Hesdorffer et al(30)	1998	69	24	23%	46%	18%	-	**13%**
DeLorenzo et al.(29)	1996	29	38		52%	39%	-	**5%**

Table 1-2: The epidemiology of childhood CSE (adapted from Raspall-Chaure et al(32))

Due to methodological differences, and changes in classification systems, it is difficult to compare these studies directly. Overall an incidence of around 20-30/100,000 persons/year is reported, highest in children under 1 year and falling with age(33). There is also a low reported mortality rate, of the order of 1-3% (32;34). However the mixture of aetiologies appears to differ significantly between studies, which may represent a combination of the different approaches used to identify and classify cases of CSE, as well as true variability between the populations under study. In the two studies that reported it separately, the most common cause of CSE was prolonged febrile seizure (see table 1-1 for definitions), forming up to one third of cases. Many children who have an episode of CSE have underlying neurological abnormalities (remote/acute on remote symptomatic seizures) suggesting that CSE is more common in children with other neurological problems.

1.2.4 Pathophysiology

As previously mentioned, CSE is viewed as a failure of seizure termination. This implies that the ongoing seizure activity itself is similar to other epileptic seizures and that it is the failure of the normal inhibitory mechanisms that differentiates CSE. As there are many possible causes for seizures, there is unlikely to be a common mechanism for this failure of inhibition that is applicable to all cases of CSE. However if, as is commonly believed, seizures represent a final common pathway for the expression of multiple underlying pathologies, then there may be expected to be similarities in the physiological and pathological changes occurring during and after CSE.

As a seizure progresses, changes occur in neuronal receptors and the neuronal environment that appear to contribute to the establishment of a fixed and recurrent state of seizure activity. One change that is known to occur is a reduction in the number and efficacy of $GABA_A$ receptors on neurones with increasing seizure duration (Figure 1-4) (35-37). This leads to a reduction in interneuronal activity, which is predominantly $GABA_A$ mediated and may contribute towards the failure of inhibition. Other changes which occur during a seizure include increased cerebral blood flow and neuronal energy consumption(38;39) leading to lactic acidosis(40), cerebral pH changes(10) and increased extracellular glutamate concentrations(41). Intractable seizures lasting hours to days have been shown to lead to brain oedema and death with atrophy and demonstrable cell loss visible at post mortem in numerous case studies in both adults and children(17;42-44).

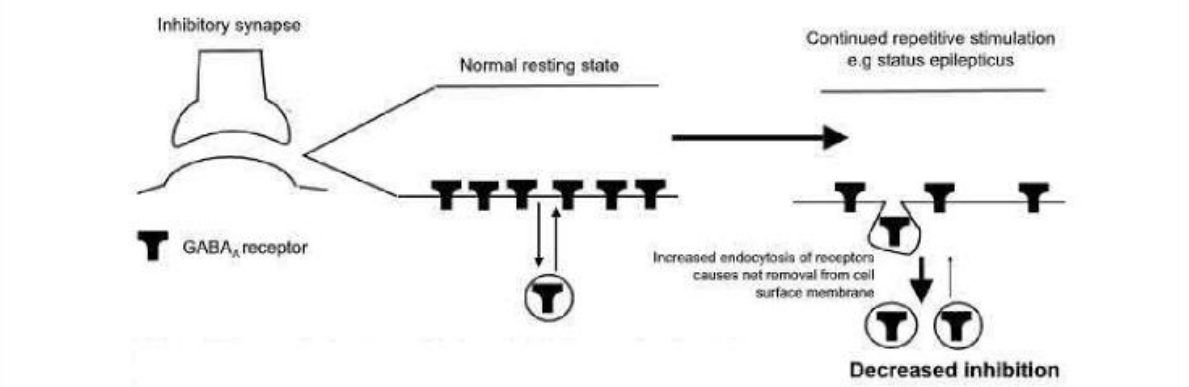


Figure 1-4: Changes during status epilepticus showing increased endocytosis of $GABA_A$ receptors resulting in lower membrane population and decreased inhibition

Evidence of the deleterious effect of less severe CSE is scarcer; magnetic resonance imaging (MRI) studies looking at children in the first few days after an episode of CSE have shown changes suggestive of hippocampal swelling and oedema (45-47) implying that some degree of acute injury occurs with even with shorter CSE, however the majority of our knowledge comes from animal models.

Initial work by Meldrum et al (39;48) showed that prolonged seizures in both baboons and rats were associated with neuronal loss and other evidence of brain damage following seizure induction by intracerebral injection of bicuculline. Similar findings have been found in other animal models of epilepsy (49-51), with cell loss and subsequent gliosis (52;53) being widely reported following the induction of CSE. It is thought that this damage is due to a combination of ischaemic damage from hypoxia during the seizure(54), followed by later glutamate-mediated excitotoxic damage(55). Seizure duration appears to be an important determinant of neuronal damage, with more severe and more extensive injury being reported with increasing seizure duration (23;56).

There is therefore strong evidence from animal models to suspect that CSE is harmful to brain tissue.

1.2.5 Treatment

Although the mechanism generating CSE may differ between individuals, the acute treatment of CSE is fairly well established. Guidelines on the treatment of paediatric CSE are widely available in several countries(57-61) and, while they differ in some particulars depending on the local availability of medication, they uniformly place an emphasis on early treatment with anticonvulsive medication(62). Neuronal inhibition is the mainstay of treatment, with benzodiazepines being the generally accepted first line drug of choice. Since the risk of injury appears to be related to seizure duration(56), and the fact that as a seizure progresses, benzodiazepines become less effective(63), early and, if possible, pre-hospital treatment is important.

Second line treatment with non-benzodiazepine agents such as phenytoin, phenobarbitone or sodium valproate is recommended if two doses of benzodiazepines do not achieve seizure termination(64) due to the increased risk of respiratory depression and reduced efficacy of further doses, although it is recognised that the evidence-base to support particular second or third line treatments is poor(65;66). After the failure of two different medications then CSE can be considered to be *"refractory"(67).* Opinions vary as to whether the optimal treatment in this case is for the trial of a further conventional anti-epileptic treatment (68;69), or for anaesthetic treatment with continuous infusions of agents such as thiopental, propofol and midazolam (70;71). Regardless, it is recognised that treatment of such seizures is difficult and the use of general anaesthesia to obtain seizure control should be considered in a timely fashion(72).

In the absence of any recognised neuroprotective agents suitable for use in humans, the treatment of CSE remains purely symptomatic. There are currently no recommendations for prophylactic or protective treatments besides what may be necessary to treat the underlying cause (73).

1.2.6 Outcome

A variety of adverse outcomes have been associated with CSE, including increases in short and long-term mortality, subsequent epilepsy and delayed neurodevelopment. However the

reported risks are highly variable between different studies and populations(34). The immediate and short term mortality of CSE in children has been shown to be low, with death predominantly caused by the underlying aetiology rather than the seizure(74). Almost all immediate deaths occur in children with significant symptomatic causes for their CSE, whether an acute neurological insult or a progressive neurological condition(75). Children with prolonged febrile seizures have been shown to have minimal mortality from their CSE(76).

In adults it has been shown that the long term mortality rate of survivors of CSE is increased, with a cumulative 10 year-mortality of 43% (standardized mortality ratio 2.8)(77). However this appears to be predominantly due to increased mortality after symptomatic CSE, with the mortality rate after idiopathic/cryptogenic CSE or PFS not significantly different from the general population. Long term mortality for children with CSE is likewise increased, mainly as an effect of deaths in children with a symptomatic aetiology for their CSE, such as cerebral palsy or bacterial meningitis. By comparison, the mortality rate after idiopathic/cryptogenic CSE or PFS does not appear to be significantly different from the general population (78;79)

While some studies have reported a possible effect of age(75;80) and seizure duration(81) on outcome, this is difficult to disentangle from the fact that children with more serious causes for their CSE may have longer seizures which are more difficult to control(82) and that younger children are more likely to have a serious acute symptomatic cause for their CSE(83). An additional factor may be improvements to medical treatment, with older studies, prior to the widespread introduction of aggressive treatment of CSE reporting longer seizure durations and poorer outcomes.

Despite the lack of evidence for a specific effect of CSE itself on mortality, concerns remain that there may be significant associated brain injury attributable to the CSE itself. From a clinical context, there have been varying reports as to the long term risks of cognitive impairment, subsequent epilepsy or other neurological impairment following CSE(34). Partly this is due to the heterogeneous nature of the published literature and partly due to the difficulty of controlling for the influence of the underlying cause of the seizure.

There is a 37% risk of further unprovoked seizures within the first 2 years following a first unprovoked seizure(84;85) and prolonged duration does not appear to significantly alter this(76;82). This is not necessarily the case in children with acute or remote symptomatic CSE however, who have a greater than 50% risk of further seizures and subsequent epilepsy (76;86;87). For children with a pre-existing diagnosis of epilepsy, the occurrence of CSE does not appear to significantly alter

the natural course of the disease, as seizure frequency following an episode of CSE has not been shown to increase(88). Although an increased incidence of epilepsy in children with complex febrile convulsions has been reported (87;89;90), there is some controversy over these findings. A population-based study by Verity et al. found a significantly increased risk of further afebrile seizures in the group of children with febrile seizures over 30 min duration compared to those with shorter febrile seizures (21% vs. 3.4%)(87). Similarly a larger but earlier cohort study by Nelson et al. found an increased risk of 5.4%, but this did not attain statistical criteria for significance(91). In many studies, prolonged duration was only one of the possible criteria used to define complex febrile convulsions(89;90) and some multi-variable analyses have suggested that prolonged duration on its own is not associated with a higher risk of epilepsy(92;93).

While there is evidence that neurodevelopment can be impaired following CSE (86;94), very few studies report data from formalised neurocognitive assessments (34;95). Verity et al(87) found cognitive impairment on the British Ability Scales in 10/37 children with CSE, however 8 of these had evidence of a neurological or developmental condition prior to CSE and one had suspected previous hypoxic-ischaemic injury at birth. Only one child had cognitive impairment following PFS. Further evidence of the relatively benign impact of PFS comes from a study by Ellenberg et al(96), who showed that children with PFS did not have an IQ deficit compared to sibling pairs. In contrast Kolfen et al.(97) found significant deficits in non-verbal IQ in children with PFS compared to both healthy controls and children with short febrile seizures and a recent report from Hesdorffer et al.(26) suggests that children with PFS may have a degree of motor impairment and delayed motor milestones, although overall cognitive scores remain intact.

1.3 The Hippocampus

1.3.1 Anatomical location

The hippocampus is an archicortical structure located within the temporal lobe, sitting in the wall of the lateral ventricle. One of the most topologically complex structures in the brain, it appears as a slightly curved, “seahorse” shaped structure (Figure 1-5), which can be divided into three anatomical components: the anterior *head*; the middle *body*; and the posterior *tail*. The head is the widest part, with a transverse diameter of 1-2cm in an adult, the hippocampus then narrows to around

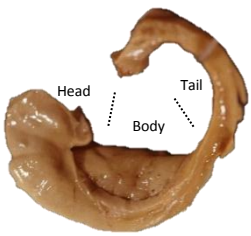


Figure 1-5: Human hippocampus preparation

1cm in the body and tail.

Anteriorly, the head of the hippocampus projects into the lateral ventricle before curving back on itself medially to form the *uncus*. The anterior limit of the hippocampus can be difficult to accurately delineate, as it is bounded by a thin section of the lateral ventricle. Therefore, particularly in the medial portions, it can be difficult to accurately separate from the ambient gyrus and amygdala anteriorly. Laterally the head of the hippocampus is bordered by the temporal horn of the lateral ventricle and superiorly, by the amygdala, with the *alveus* separating the two. Medially the relations of the hippocampal head are complex. It is bounded by the ambient gyrus, the parahippocampal gyrus and cerebrospinal fluid in the transverse fissure. The inferior border is also made up of several structures, including the parahippocampal gyrus and the uncal sulcus.

Moving posteriorly into the body of the hippocampus, here it takes up its classic oval appearance, with the lateral ventricle laterally and superiorly, parahippocampal gyrus and prosubiculum inferiorly, and transverse fissure medially.

Finally, the hippocampal tail begins to merge superiorly with the crus of the fornix. It continues to be bounded inferiorly by the parahippocampal gyrus. It forms part of the floor of the lateral ventricle and this forms the lateral border. Medially the border is the transverse fissure and posteriorly it merges with the subsplenial gyrus.

1.3.2 Internal structure

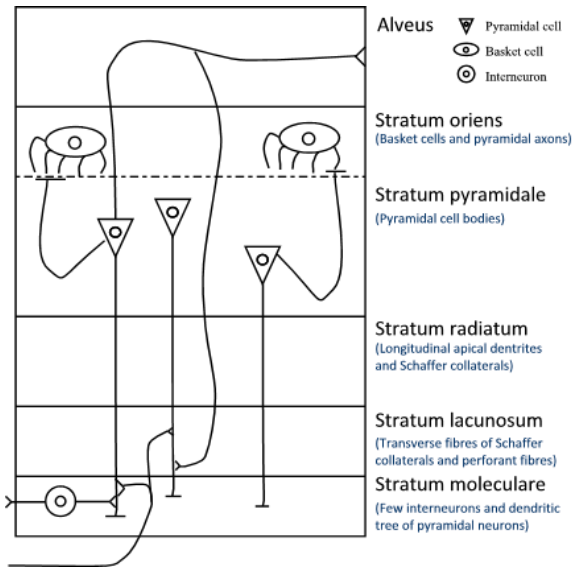


Figure 1-6: Hippocampal cell layers in the cornu ammonis

The internal structure of the hippocampus consists of two, interlocking and interconnected structures named the *Cornu Ammonis* and the *Gyrus Dentatus*. The *Cornu Ammonis* is the more complex and consists of 6 cell layers (Figure 1-6). It can be divided into four subregions - CA1, 2, 3 and 4, which are arranged around the radius of the hippocampus in coronal section (Figure 1-7). In the human CA1 is relatively large and contains an extensive pyramidal layer with triangular cell bodies. CA2 has a much smaller pyramidal layer,

with rounder, more densely packed cells. The unique feature of CA3 is that it contains unmyelinated mossy fibres that project from the dentate gyrus and synapse onto pyramidal cells. CA4 sits within the dentate gyrus and contains relatively few, large pyramidal cells with myelinated mossy fibres projecting from the dentate gyrus.

The *Gyrus Dentatus*, or dentate gyrus, curves around the end of CA4 and extends medially below the fimbria. It is a three layered structure made up mostly of granule cells (*stratum granulosum*), and their dendritic trees and axonal projections with few interneurons. Most axons project to CA3 and CA4 as mossy fibres.

There are thought to be two main neuronal systems within the hippocampus: the perforant pathway, an evolutionarily older polysynaptic pathway running through all fields of the hippocampus; and the direct pathway, running from entorhinal cortex straight to CA1.

The perforant pathway runs from layer 2 of the entorhinal cortex through the subiculum to synapse within the molecular layer of the dentate gyrus. These cells then project excitatory mossy fibres to pyramidal neurons in CA3 and CA4. Those neurons send axons into the alveus and fimbria, with back projections (Schaffer collaterals) to cells in CA1. From the alveus collaterals are sent to the subiculum and thence back into the alveus and fimbria, ultimately running back to the anterior thalamic nuclei. The direct pathway sends axons from layer 3 of entorhinal cortex directly to CA1

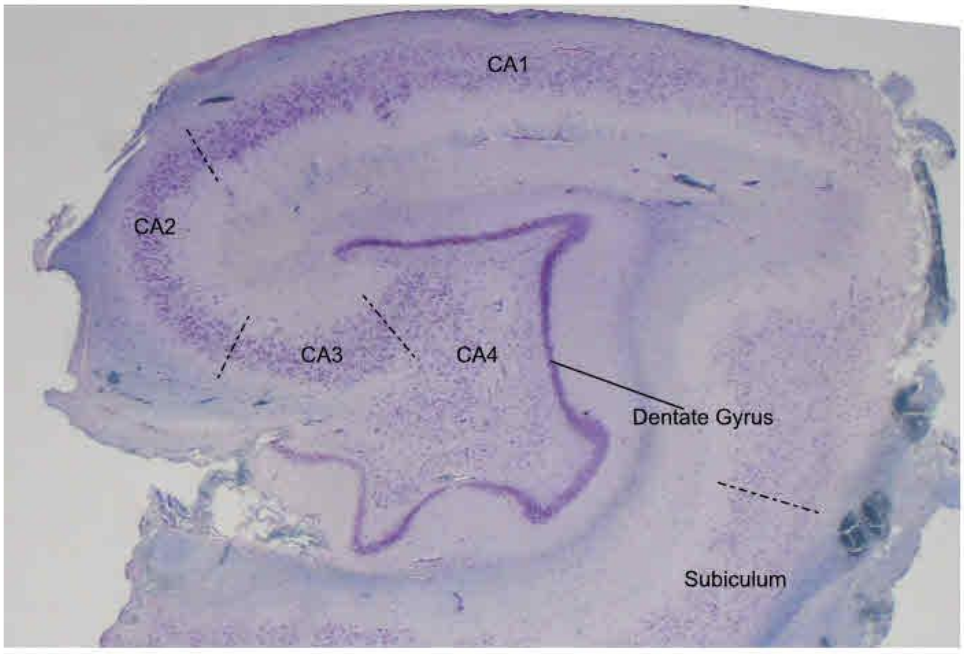


Figure 1-7: Histology of normal hippocampus showing hippocampal subfields (LFB staining)

pyramidal neurons. These then project into the subiculum and synapse there onto neurons that project directly back to entorhinal cortex.

1.3.3 From structure to function

The functions of the hippocampus have been the subject of much research and discussion for over 50 years. A full enumeration of the field is beyond the scope of this thesis, so what follows will be confined to a brief overview.

The earliest clinical evidence for the role of the hippocampus came from observational studies of patients with hippocampal lesions. Many patients (most famously H.M.) displayed profound short term memory loss and the inability to form new memories following surgical removal of both hippocampi(98). Further studies in humans with bilateral hippocampal damage have shown that in addition to this anterograde amnesia, a degree of retrograde memory loss, thought to be related to the extent of the associated extra-hippocampal damage can be found(99). A large body of data from both humans and animals, has now highlighted the important role of the hippocampus in the formation and retrieval of specific types of memory, including episodic memory(100), spatial learning (101) and contextual fear(102). Disruption of the hippocampus, whether temporary or permanent, impairs performance on many memory dependant tasks.

In addition to memory, there is also a link between the hippocampus and emotional processing. The hippocampus occupies a prominent position in the limbic circuitry thought to control emotion and affect. Connections exist between the hippocampus and the hypothalamic-pituitary-adrenal (HPA) axis that controls the stress response as well as other emotional processing areas such as the amygdala. Psychological disorders involving abnormalities of the stress response, such as post-traumatic stress disorder(103) (PTSD) and depression(104;105) have been linked to changes in hippocampal volume and altered hippocampal function. Furthermore, successful treatment of these conditions has been shown to reverse many of these changes suggesting that there is an intimate connection between the hippocampus and emotion.

The hippocampus therefore is involved in at least two distinct brain functions. Differential gene expression and differential connectivity between ventral and dorsal portions of the hippocampus may provide a basis for the segregation of these(106), but for practical purposes they remain closely linked, both anatomically and functionally for many activities.

1.3.4 Mesial Temporal Sclerosis

Of particular relevance to the study of epilepsy is a condition known as hippocampal sclerosis or mesial temporal sclerosis (MTS). This is an abnormality of the hippocampus that has a characteristic histological and radiological appearance. It is thought to be an acquired lesion, possibly linked to the result of epileptic seizures. Although it has occasionally been reported in people without clinical seizures(107;108), it is most frequently found in people with temporal lobe epilepsy (TLE) and is almost never found as an incidental finding in people having MRI scans for other reasons(109;110). It is generally considered, therefore, that MTS is a highly epileptogenic lesion and its occurrence likely to signify a very high risk of spontaneous seizures and TLE.

The first description of MTS was made by Sommer in 1880(111). He noticed a common pattern of cell loss within the hippocampi of patients with epilepsy at post-mortem. There was a loss of neurons in CA1 and CA4 sub-fields with a relative sparing of the cells in CA2. These changes were at first thought to be restricted to the hippocampus, but it soon became clear that there was a spectrum of changes, ranging from very subtle cell loss in CA1 to widespread sclerosis involving amygdala, parahippocampal gyrus and temporal neocortex(112). Other histological features of MTS that have been recognised include gliosis of the end folium and dispersion of the granule cells in the dentate gyrus. An example of hippocampal sclerosis can be seen in Figure 1-8.

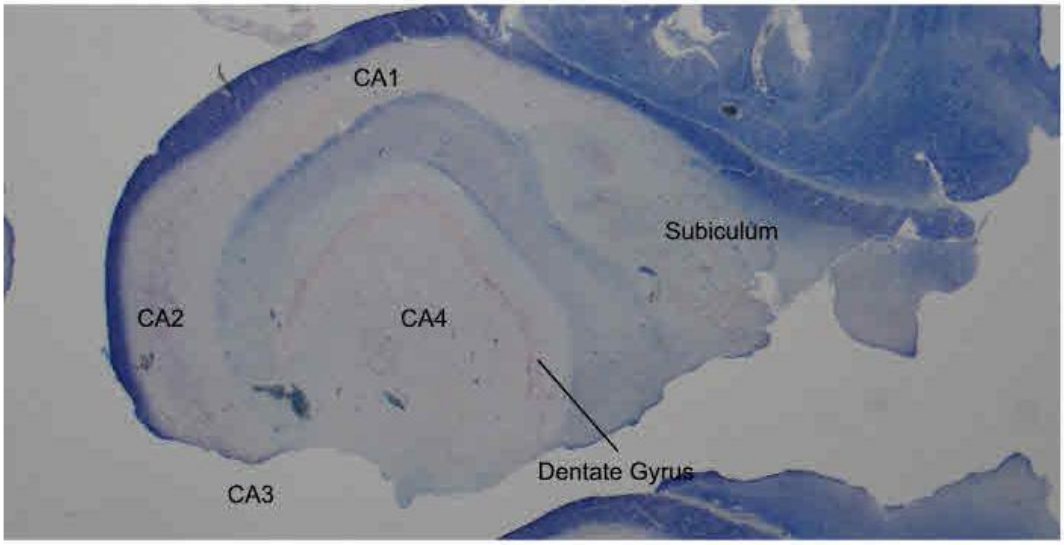
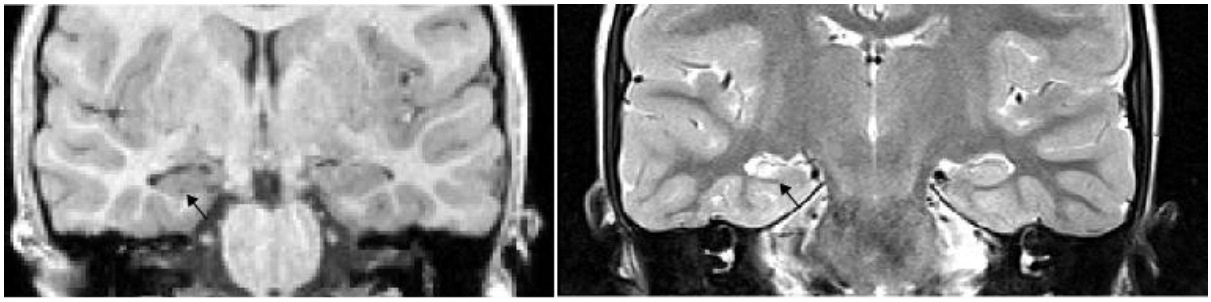


Figure 1-8: Histological specimen showing hippocampal sclerosis (LFB staining)

With the advent of Magnetic Resonance Imaging (MRI), non-invasive visualisation of brain structures became possible. The atrophy and cell loss associated with MTS can be visualised with MRI as a loss of volume in the hippocampus (Figure 1-9A), as well as increased signal intensity on T2 weighted scans (Figure 1-9B). Other techniques such as T2 relaxometry or hippocampal volumetry can also be used to increase sensitivity. Taken together, these defining features have been shown to correlate well with the eventual histological findings in patients who go on to have surgical removal of the affected hippocampus as well as predicting outcome (113;114).



A: 3D-FLASH MRI sequence showing volume loss of the right hippocampus (arrow)

B: T2-weighted coronal MRI sequence showing increased intensity of the right hippocampus (arrow)

Figure 1-9: MRI appearance of Mesial Temporal Sclerosis

Since hippocampal atrophy as shown on MRI is reliably detectable using an optimised scanning protocol(115), and this correlates very well with histological MTS, MRI has become the "gold-standard" method of pre-surgical diagnosis. This is usually done by visual inspection by a neuroradiologist, which has been shown to reliably detect a unilateral reduction in hippocampal volume of around 30%(116). Quantitative measurement of hippocampal volume may be preferred due to improved sensitivity where the atrophy is less than this, or if bilateral symmetrical atrophy is suspected(117).

1.4 Temporal Lobe Epilepsy

1.4.1 Overview

The clinical importance of MTS lies in its association with TLE. TLE is a form of epilepsy where the initial seizure focus is localised in one of the temporal lobes. Typically this presents with simple and complex partial seizures with and without secondary generalisation. The simple partial seizures often take the form of an aura, either sensory (classically olfactory or gustatory), psychic (such as déjà vu or jamais vu) or autonomic. This may then be followed by a complex partial seizure with

behavioural arrest, complex automatisms and post-ictal confusion. These seizures typically last 1-2 minutes and may progress to a secondarily generalised tonic-clonic seizure(118). TLE is arguably the most common single epilepsy syndrome and is often difficult to treat with medical management alone, with 70-80% eventually proving refractory to medical treatment (119). Chronic TLE also carries with it a recognised risk of cognitive impairment and psychological co-morbidity (120).

1.4.2 Natural History

The natural history of TLE is little understood(121). Since the diagnosis is mostly made in patients with intractable epilepsy being considered for surgical treatment, the true incidence of TLE is in the general population is unknown, as patients whose epilepsy is well controlled or not thought suitable for surgery may never receive a definitive diagnosis. Studies of patients receiving surgical treatment for TLE show that there is usually a long delay before the first seizure and the referral for surgery(122). This may be because it can be difficult to recognise temporal seizures in younger children(123) or because there is often a good initial response to anti-epileptic medication, with the epilepsy only becoming intractable in later childhood or adolescence(124).

The commonest cause of TLE is MTS, accounting for around 65% of cases overall(125), although this may be slightly reduced for childhood-onset TLE, as a greater proportion of cases with extra-hippocampal temporal lobe lesions, such as cortical dysplasia or other developmental abnormalities are reported in some studies(126-128). As extra-hippocampal TLE is a heterogeneous group of conditions, with prognosis and outcome dependent on the aetiology of the lesion(129), this thesis will focus almost exclusively on TLE-MTS.

It is difficult to accurately ascertain the age of onset of TLE-MTS, as most data is from retrospective, hospital-based case series, limiting the conclusions that can be drawn. It is possible that milder cases of TLE may not be diagnosed as such or may not be referred to tertiary centres, especially if they are following a benign course and hence hospital-based studies are likely to overestimate disease severity(130). Case series of patients referred for epilepsy surgery suggest that the mean age of onset of TLE-MTS in these patients is between 4-16 years(125). Studies of adult patients frequently report mean ages of onset of over 14 years(131;132), while those with a paediatric cohort usually report lower ages of onset of between 5-8 years(128;133;134). It has been suggested that TLE has multiple incidence peaks in childhood, adolescence and early adulthood(135), which may be one explanation for this discrepancy.

After onset of epilepsy the course of TLE-MTS is rarely smooth and often follows a relapsing and remitting course. Many children will have a good initial response to anti-epileptic medication (62% in a study by Dlugos et al.(136)), but subsequently seizures often become increasingly difficult to control. After 10 years of follow-up, Spooner et al. found 43/62 (69%) children with TLE continued to have seizures despite anti-epileptic medication with 21/62 (33%) progressing to epilepsy surgery (137). Despite this, many of the children who were later diagnosed with intractable seizures had had seizure-free periods of over 1 year during the course of their epilepsy. Similar findings were shown by Berg et al. in their study of 333 patients with intractable focal epilepsy (138): the median time to failure of the second anti-epileptic drug (and hence designation of intractability) for those patients with TLE-MTS was 8.0 years, and 51/178 (28.7%) had had seizure remissions of 1 year or more before they were seen for evaluation.

Although medical treatment frequently fails, the surgical treatment of TLE-MTS enjoys one of the highest success rates out of all the epilepsies(139). In patients with an identified hippocampal abnormality, congruent with the electrographic seizure focus, there is around a 60-70% chance of seizure freedom following temporal lobectomy (140). The relatively high success rate combined with the frequent medical intractability mean that TLE-MTS is the type of epilepsy most frequently referred for surgical management.

Along with difficulties with seizure control, there are also frequently reported co-morbidities in TLE. In their cohort of children with TLE, Spooner et al showed that there is a high rate of psychological co-morbidity in chronic TLE, with high rates of depression and anxiety disorders (141), although achieving seizure freedom appears to improve this. Cognitive impairment is also frequently reported, with memory and executive function being particularly affected in children(120) although an additional decrease in global IQ has been reported in adults(142).

TLE is frequently linked with an unfavourable prognosis. Although the seizures can be treated successfully in the majority of cases, this often requires surgical intervention, which is not without its consequences (143). As MTS is the most common cause of TLE and has some of the characteristics of an acquired lesion, it is worthwhile probing this association further in order to determine if this is the case and identify possible strategies for its prevention.

1.5 Linking TLE, MTS and PFS

The long-standing hypothesis proposing a causal link between TLE, MTS and PFS was first articulated by Sommer in 1880(111) but has yet to be definitively confirmed or refuted. That MTS can cause TLE is rarely disputed(144): MTS is the most common lesion found in TLE and, unless there is dual pathology, removal of the affected hippocampus and temporal lobe has a high chance of eliminating the seizures(113;114). Furthermore MTS is rarely found in people without seizures (110;145), suggesting that the presence of MTS can be used as a marker for TLE, even in the absence of reported clinical seizures.

One of the first observations about patients having surgery for TLE-MTS was that a high proportion of them had prolonged febrile seizures in childhood(146). In some series up to 50% of patients with TLE-MTS were shown to have a history of PFS (147;148). The incidence of febrile convulsions in the general population is around 2-5%(149), of which up to 5% will be prolonged(89). There is therefore a strong association between PFS and TLE-MTS and has led to extensive debate as to a possible causal relationship.

1.5.1 Experimental evidence

Supporting evidence for a causal link between PFS and TLE-MTS comes from both animal models and human data. As mentioned previously, several different animal models of CSE have now shown a causal relationship between induced status epilepticus and subsequent brain injury. The hippocampus appears to be particularly vulnerable to injury and is the most commonly affected brain structure. The changes which are seen in the hippocampus following seizure induction with lithium-pilocarpine or kainic acid resemble human MTS in many ways, with subsequent neuronal death, gliosis and volume loss (49;50). Serial MRI after CSE has shown early hippocampal changes suggestive of cytotoxic oedema that are predictive of the eventual degree of volume loss(150).

Models of febrile seizures, using hyperthermia-induced CSE in immature rats, have shown transient neuronal injury[52], particularly in CA1 and CA3, but no evidence of subsequent neuronal death (151;152). They have however shown that experimentally induced febrile seizures can lead to later spontaneous limbic seizures(152-154) and cognitive deficits(155).

A number of isolated case reports of children following PFS have shown that the pathological sequence leading from PFS to TLE-MTS does occur(156-158), but cannot provide information on how common this is or what factors are involved in this progression.

There have now been a number of studies that have addressed this more systematically. VanLandingham et al. and Scott et al. have studied children immediately following a PFS and consistently shown that in the first 2 days after PFS there is enlarged hippocampal volume and increased signal on T2 scans (45;47). This has been interpreted as evidence of acute hippocampal oedema and appears to have resolved by 5 days post-PFS.

Follow-up of 14 patients from their original study by Scott et al. showed that the initial changes resolve over the following months, but that an increased degree of hippocampal asymmetry was detectable at 4-8 months in 5/14 patients(46). None of these children met criteria for MTS and no children had received a diagnosis of TLE by the end of the follow-up period. Finally a more recent longitudinal study of 11 children by Provenzale et al. with an initial MRI scan within 72 hours of PFS and follow-up between 3-23 months later found unilateral hippocampal volume loss in 5 children and bilateral changes in 2 more children(159). They found evidence of MTS and TLE in 2 children and complex-partial seizures of uncertain origin in a further 4 children, however, the rate of epilepsy seems high when compared to other, population-based, studies of prolonged febrile seizures. Furthermore as the MRI sequences used in their study were not optimised for the accurate measurements of hippocampal volumes, the validity of the diagnostic criteria they used to define MTS (any reduction in hippocampal volume over the study period) can be called into question.

Both of these studies contrast with a 10 year follow-up study by Tarkka et al. who performed MRI scanning on 24 children with previous PFS 10-20 years after the initial event (160). They did not find any cases of clinical MTS and absolute left and right hippocampal volumes were not statistically different between patients with previous PFS and control subjects with simple febrile seizures only. They did find a significant reduction in right-left volume differences in patients with PFS compared to control subjects and in 3 patients the right-left volume difference was more than 2 standard deviations less than the mean of control subjects. They do not report on any other measures of asymmetry and were unable to comment on growth, so this is not necessarily in contradiction to the previous studies mentioned above; if progression to MTS only occurs in a minority of cases, increases in asymmetry or impairment of hippocampal growth may occur in these patients without significantly altering mean hippocampal volumes on a group level, especially with small study sizes. Alternatively there may be recovery after the initial injury, with resumed growth and a return to normality.

1.5.2 Epidemiological evidence

In contrast to the strong associations found in retrospective studies of surgical cohorts and the growing amount of mechanistic evidence from animal studies and prospective imaging studies of children with PFS, there is limited epidemiological evidence for a causal link between PFS and TLE-MTS. Some early case-control studies looking at risk factors for TLE-MTS found PFS to be a significant risk factor(161;162), but other, population-based studies of children with PFS, have not shown an increased risk of focal epilepsy such as TLE(163;164). While there is an increased rate of epilepsy in children with PFS, this does not appear to be specific to TLE-MTS, with PFS also being a risk factor for other types of epilepsy (165;166).

1.5.3 Advancing the hypothesis

In light of the existing literature, there are several plausible explanations for the association between TLE-MTS and PFS. Some of the most commonly advanced are:

1. PFS occur in a normal brain and produces an acute hippocampal injury, which over time develops into mesial temporal sclerosis and causes temporal lobe epilepsy

2. MTS is a developmental disorder or caused by an early neurological insult and pre-dates the PFS. PFS is caused by MTS and is merely the first clinical manifestation, later ones being temporal lobe seizures.

3. An underlying brain abnormality, possibly genetic or developmental in origin predisposes children to both PFS and later TLE-MTS. PFS may or may not hasten the ultimate development of MTS and TLE.

Since the peak ages for febrile seizures are 1-3 years (91;167) and TLE-MTS is only diagnosed after 5-8 years, if not later, this time lag of several years makes direct investigation difficult. In the study by Maher et al, epilepsy only developed a mean of 12 years after the initial febrile convulsion(162), therefore using TLE as an end-point would necessitate a study duration of over a decade. It is therefore more practical to use surrogate end points that can be studied over a shorter time period.

While alterations in hippocampal volumes have been shown following PFS, the long term evolution of these changes remains uncertain. Specifically whether they continue to progress to MTS or whether they normalise with time.

1.6 PFS and other forms of epilepsy

TLE is not the only form of epilepsy that is associated with a history of childhood prolonged febrile seizures. In particular, children with epilepsy syndromes such as Dravet syndrome (Severe Myoclonic Epilepsy of Infancy)(168) and Generalised Epilepsy with Febrile Seizures Plus (GEFS+)(169) may initially present with febrile seizures. Although these children may not be distinguishable at the initial presentation, continued febrile seizures beyond the age of 6 years(170), evolving developmental problems or the emergence of other seizure types would be reasons to suspect these diagnoses.

SMEI has been estimated to occur in around 1 per 40,000 children(171) and while the prevalence of GEFS+ is likely to be higher, the prevalence of febrile seizures is around 1,800 per 40,000(172). If 5% of these are prolonged(89), even if all children SMEI/GEFS+ presented with CSE, only a small minority of children (<1%) with PFS will have a diagnosis of SMEI or GEFS+. Even though, as previously alluded to in Section 1.5.2, PFS may be a risk factor for the subsequent development of epilepsy, it seems apparent that the majority of children with PFS will not develop epilepsy subsequently.

1.7 Summary and hypothesis formulation

In this chapter the basic physiology behind a seizure has been reviewed as well as the factors that differentiate CSE. After discussion of the epidemiology, pathophysiology and prognosis of CSE, it is clear that CSE is associated with adverse outcomes, but it is also clear that the underlying cause and the child's previous neurological/developmental status are the predominant determinants of mortality and morbidity. None the less, it is difficult to rule out a harmful effect of CSE *per se* from the existing data.

In particular it would be useful to assess neurodevelopment after CSE using modern standardised tests, in order to rigorously assess the risks of developmental problems. There are conflicting reports about children with PFS, who are otherwise thought to be neurologically and developmentally normal; their risk of cognitive deficits following CSE and their risk of further afebrile

seizures/epilepsy need to be clarified. The postulated link between PFS and TLE-MTS also merits further investigation as this important hypothesis continues to resist either proof or falsification.

The aim of this doctoral project was to characterise the clinical, cognitive and structural outcomes after an episode of childhood CSE with a view to clarifying the short-medium term outcome. Since children at this age are growing and developing rapidly, longitudinal studies are essential to detect deviation from normal developmental trajectories. MRI investigations and formal cognitive testing were performed at multiple time points following CSE to capture the children's performance over time and track the evolution of any CSE associated changes in both brain structure and cognitive performance. In order to characterise the structural consequences of CSE, quantitative MRI techniques such as hippocampal volumetry and DTI, that have been shown to be abnormal in established MTS were used in addition to standard clinical reporting to investigate structural changes that may be precursors to clinical TLE-MTS. Results from the neuropsychological assessments undertaken during this project have already been reported by Dr Marina Martinos and form the basis of her PhD thesis *"The consequences of convulsive status epilepticus"*(173). They will therefore only be dealt with in summary in this thesis and the main part of this thesis will concentrate on presenting our findings related to the clinical and MRI investigations undertaken.

The overall theory underlying this project is that CSE can cause brain injury with long lasting consequences. In particular we hypothesise that the hippocampus is selectively vulnerable to CSE induced injury and a certain proportion of children will sustain sufficient hippocampal injury that develops over time into TLE-MTS. We further hypothesise that injury of this nature is related to PFS and not other forms of CSE. Clinical seizures and cognitive deficits will be associated with the degree of post-CSE brain injury. Specific hypotheses that will be advanced in this thesis are:

1) Clinical outcome and the immediate findings on structural MRI will largely be determined by the aetiology behind the episode of CSE. As previously demonstrated in other studies, children with symptomatic CSE (acute or remote) will have worse clinical, structural and cognitive outcomes than children with epilepsy-related CSE or children with PFS.
2) There is a specific effect of PFS on the hippocampus and a proportion of children with PFS will sustain hippocampal injury leading to the development of MTS. Disruption of normal hippocampal growth and development will be detectable on MRI within the follow-up period. The effect of other forms of CSE on hippocampal parameters will not be as profound or widespread.

3) Structural brain damage following CSE, in particular hippocampal damage will have a functional impact and children showing signs of hippocampal damage post-CSE will show reduced performance on cognitive testing.

CHAPTER 2: MAGNETIC RESONANCE IMAGING

Since its discovery in 1945, the principle of nuclear magnetic resonance (NMR) has led to the invention of a number of techniques of non-invasively imaging the internal structure of the human body that are collectively known as magnetic resonance imaging (MRI). This has revolutionised many areas of medicine, and led to many advances in the study of neurology and epilepsy in particular. The principles of NMR and MRI have been well described in a number of textbooks and will only be covered here in summary.

2.1 Basic MRI theory

Although NMR is at heart a quantum mechanical phenomenon, when considering the overall behaviour of large numbers of molecules, as when imaging the body in an MRI experiment, classical Newtonian mechanics provides a simpler and still adequate description. Therefore, in this description of NMR a combination of both the classical and quantum mechanical approaches will be used as appropriate.

All MRI techniques rely on measuring the magnetic energy of atomic nuclei within a magnetic field. This is possible because in quantum mechanics all atomic nuclei have a property known as "spin", which varies in half-integer levels according to the composition of each nucleus and is denoted by the spin quantum number *I*. The simplest and most commonly measured nucleus in human imaging is the hydrogen nucleus, which consists of a single proton and has a spin of +½.

A spinning nucleus possesses angular momentum (*p*), which is described by the following equation:

$p = \hbar \sqrt{(I(I+1)}$ ($\hbar$ = h (Planck's constant)/2π)

This is also quantised, both in magnitude and direction. Each nucleus also has a second quantum number associated with it, *m*, the magnetic quantum number, which can take the values from *+I to –I* in integer steps. Hence for hydrogen, *m* = +½ or –½. The z component of *p* along an arbitrarily defined z-axis is therefore:

$p_z = m\hbar$

As with any other moving charge, the spinning nucleus generates an apparent magnetic dipole field. This magnetic moment μ is related to the angular momentum by the gyromagnetic ratio, γ, which is a constant unique to each nucleus. Thus:

$\mu_z = \gamma p_z = m\gamma\hbar$

In the absence of any external forces, the energy states of each value of *m* are equal, so there will be an equal population of nuclei with *m* = +½ and *m* = -½, and hence no overall net magnetic moment. When placed in a fixed magnetic field B_0, however, this is no longer the case and the energy levels separate such that the difference between the low-energy state, aligned with the field ("spin-up") and the high-energy state, anti-aligned ("spin-down") is ΔE (Figure 2-1)

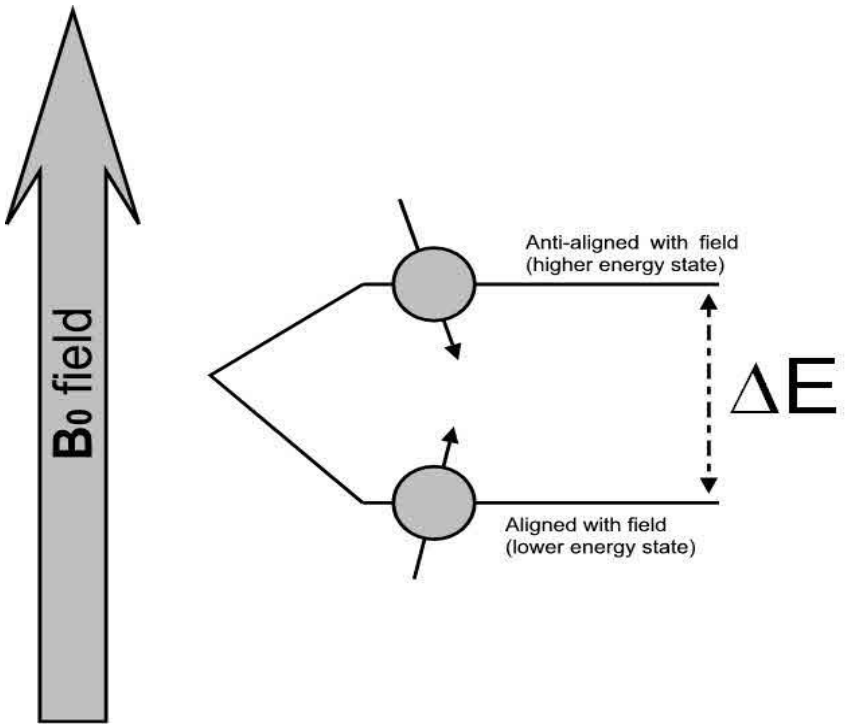


Figure 2-1: Energy levels in a fixed magnetic field

The energy level of each state is given by the equation:

$E = - B_0.\mu = - \mu_z B_0$ (taking the z-axis to be aligned with B_0)

$= - m\gamma\hbar B_0$

This implies that for hydrogen nuclei:

$\Delta E = \gamma \hbar B_0$ (as the two possible values for *m* are +/- ½)

This energy difference is what is used to create the NMR signal. The frequency associated with this energy difference is also related to ΔE by ħ and hence we can derive the following equation:

$\hbar\omega_0 = \gamma \hbar B_0$

$\omega_0 = \gamma B_0$

This is called the Larmor equation.

If we now consider each nucleus as a rotating particle within a static magnetic field, possessing a magnetic moment, μ and angular momentum, *p*, since μ is at an angle to the static field, the magnetic moment of any individual nucleus will experience a torque and hence tend to precess around the axis of the static field at the Larmor frequency.

Taking B_0 to be along the z-axis, at equilibrium these moments will tend to be equally spread around the x-y plane and so the net magnetic moment from all the nuclei at one position, M, will be parallel to B_0.

To generate the signal used for MRI, M is rotated towards the x-y plane by a radiofrequency (RF) pulse rotating around the z-axis at the Larmor frequency. Although this field is much weaker than B_0, because it resonates at the Larmor frequency M will move out of alignment with B_0 and into the x-y plane. Once the RF pulse has been turned off, then a process of relaxation will take place as M shifts back to its equilibrium position along B_0. The changes in M during this relaxation generate the NMR signal as a reciprocal current in a receiving coil which is then analysed to generate the MRI image.

2.2 T1 and T2 weighting

Once the NMR signal has been generated, in order for this to provide useful information, the signal needs to alter depending on properties of the tissue being probed. The most commonly used mechanisms that exist to generate this contrast in the NMR signal between different tissuesare called T_1 and T_2 after the two main processes at work during the relaxation phase.

T_1 relaxation is known as spin-lattice relaxation. After M has been rotated into the x-y plane by the RF pulse, M_z will be zero. Once the RF pulse is switched off, protons start to interact with the surrounding molecules and give up energy. When they do this they will move from the high energy state (anti-aligned) to the lower energy state (aligned with B_0) and hence M will shift back towards the z-axis. The time taken for M_z to recover by ~63% is known as T_1 (Figure 2-2).

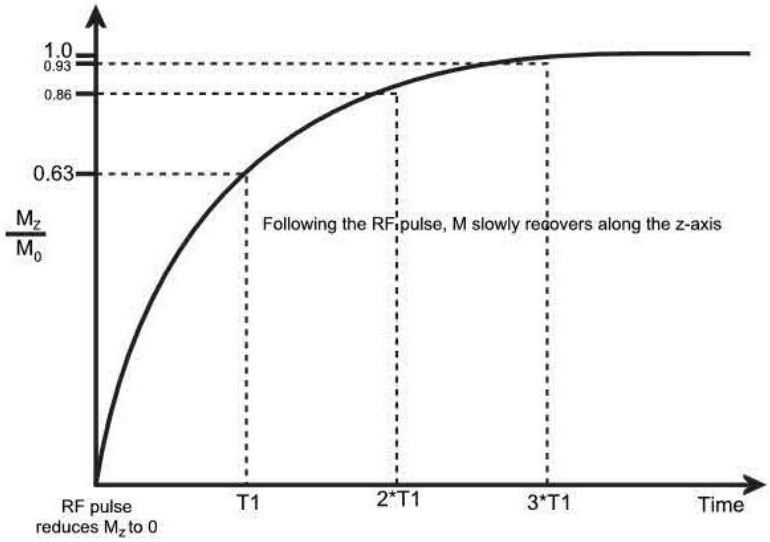


Figure 2-2: T1 relaxation as spins re-align with the z-axis

The other process at work is known as T_2 relaxation, or spin-spin relaxation. This occurs because once M has been rotated into the x-y plane, it will continue to precess (or rotate) around the z-axis in the x-y plane at the Larmor frequency. However, each proton generates its own magnetic field, which will add or subtract from the main B_0 field in its nearby vicinity. Therefore protons each experience a slightly different magnetic field, depending on their position and that of their neighbours. This means that each will have a slightly different Larmor frequency and, over time they will become out of phase with each other. As this happens, M_{xy}, which is the vector sum of their magnetic moments in the x-y plane, will decay exponentially (Figure 2-3). The time taken for the signal to decay by ~63% is known as T_2.

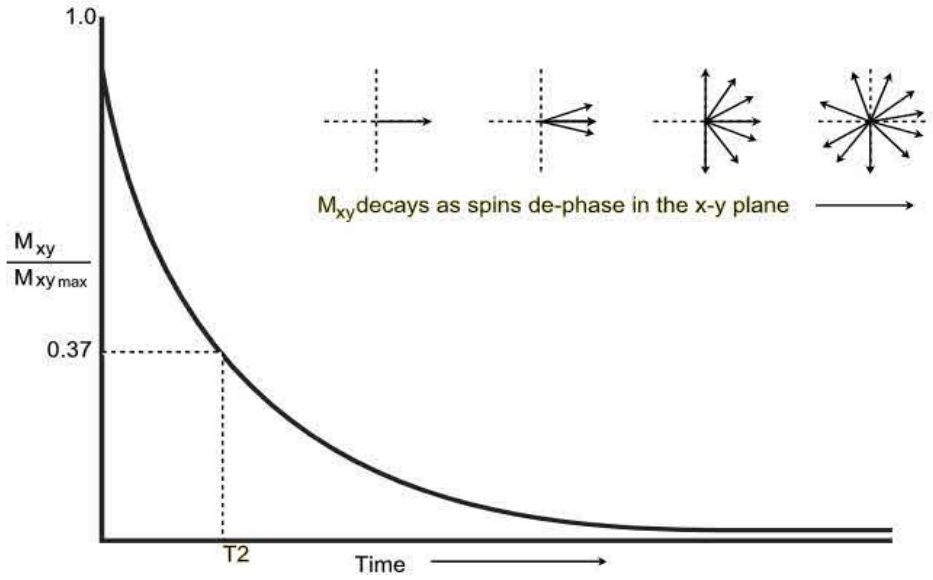


Figure 2-3: T2 relaxation from de-phasing of spins

In vivo T_1 and T_2 depend on the composition of surrounding tissues and occur simultaneously. Because they occur on different timescales and directions by varying the time intervals between pulses (TE and TR) the relative contribution to the NMR signal (weighting) can be adjusted and used to create contrast between different structures in the human body. The effects of this are summarised in Table 2-1 below. For example, tissues that are mostly free water, such as cerebrospinal fluid (CSF) have a long T_1 and a long T_2 and will be bright on a T_2 weighted scan and dark on a T_1 weighted scan; fat has a short T_1 but a longer T_2 and is bright on a T_1 weighted scan and grey on a T_2 weighted one.

	Short TR	**Long TR**
Short TE	T1-weighted image	Proton Density Image
Long TE	Noise	T2-weighted image

Table 2-1: The effects of varying TE and TR on image contrast

A typical MRI experiment will use additional pulses and different pulse sequences to generate spatial information, optimise signal to noise ratio, reduce scanning time and avoid artefacts but a full consideration of these is beyond the scope of this thesis.

2.3 Diffusion Tensor Imaging

One of the consequences of using hydrogen nuclei is that by far the largest component of the NMR signal comes from hydrogen bound in water molecules. In a free solution water molecules are constantly in motion. Biological systems however are rarely completely free solutions and so water diffusion is often restricted by other tissue structures. This means that the magnitude and direction of water diffusion can be used to give us important information about underlying tissue structure that may not be apparent on standard T_1/T_2 –weighted imaging(174;175). The process of making an MRI sensitive to diffusion information is known as diffusion-weighting.

In order to make a scan sensitive to diffusion, information from the NMR signal must be made sensitive to the small molecular movement from randomised diffusion. This is done by applying "diffusion gradients" in opposing directions along a plane after the initial depolarising pulse. The first diffusion pulse will tend to pull the spins out of phase with each other as nuclei at different relative positions on the plane experience different fields. Since the second diffusion pulse is equal and opposite to the first one, it should have the effect of refocusing the spins and reversing the effect of the first. However since diffusion is constantly taking place, in the short time interval between the pulses some water molecules will have moved within the plane and hence experience a slightly different field the second time. So molecules which have moved due to diffusion will not completely refocus and hence their signal will be attenuated. The effect of this is to sensitise the scan to incoherent molecular movement such as diffusion in the direction of the plane the diffusion gradients are applied along. Areas with a high amount of diffusion will give low signal intensity and those with little diffusion will give a stronger signal(176).

The underlying structure of the brain is not uniform. Axons are commonly bundled together into tracts and run in specific directions. Myelin, cell membranes and cytoskeletal structures all provide barriers to the free diffusion of water. Water diffuses more easily along rather than across white matter tracts, axonal filaments and cell membranes. This means that water diffusion within the brain is anisotropic and reflects the underlying brain structure. This being the case, it is not sufficient to measure diffusion in only one direction as the measurements will differ depending on whether the direction chosen is perpendicular or parallel to the main direction of water diffusion in that voxel(177).

In order to fully describe diffusion in an anisotropic system at least 6 measurements along different directions are required(178). Using these measurements, it is then possible to calculate the

diffusion tensor for any particular voxel. This 3x3 matrix describes the magnitude of the diffusion within that voxel along each of 3 orthogonal axes. The diffusion tensor itself is rarely analysed directly and instead scalar metrics such as mean diffusivity and fractional anisotropy are more commonly derived from the tensor for further comparisons(176;179;180).

Mean diffusivity is a directionally averaged measure of diffusion within a voxel. It is calculated by taking the magnitude of the three eigenvalues of the diffusion tensor and averaging them. MD is thought to reflect how free the water molecules within a voxel are to move around. It is increased by the presence of fluid, such as in oedema and inflammation and reduced by any barriers to diffusion, such as cell membranes and solid structures.

Fractional anisotropy is a measure of how isotropic diffusion within a voxel is.

It is calculated according to the following equation, where $\lambda_{1\text{-}3}$ are the 3 eigenvalues of the diffusion tensor matrix and λ is the mean diffusivity in that voxel.

$$FA = \frac{\sqrt{3}}{\sqrt{2}} \frac{\sqrt{(\lambda_1 - \lambda)^2 + (\lambda_2 - \lambda)^2 + (\lambda_3 - \lambda)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

FA ranges from 0, in a free solution where diffusion is equal in all directions, to 1, where diffusion is only possible in one direction. The average distance a water molecule travels during a diffusion-weighted scan is of the order of 4-15µm, so structures that alter FA must be smaller than this. In the brain these are thought to be cell membranes, myelin sheaths and protein filaments. Hence FA is thought to be a measure of the underlying structure present within a voxel, especially white matter tracts.

2.4 Quantitative MRI techniques

2.4.1 Hippocampal volumetry

Quantitative hippocampal volumetry is one method of improving the sensitivity to subtle volume changes over simple inspection. There have been a variety of automated techniques

developed for measuring the hippocampal volume from a suitable MRI scan(181;182), but to date the gold standard remains manual tracing of the hippocampus to create a region of interest.

Detailed descriptions of how to delineate the hippocampus have been given by a number of sources(183). While slight differences in anatomical landmarks and scanning sequences used between different studies(184), as well as idiosyncrasies of individual MRI scanners mean that the absolute value for the hippocampal volume may not be comparable between studies, it has been shown that relative values can be obtained with good consistency from one centre using a well-defined method(185).

2.4.2 Diffusion measures in the hippocampus

In order to study changes in water diffusion within the hippocampus, such as are hypothesised to occur following acute hippocampal injury and in MTS, the usual technique is to first generate MD and FA maps from diffusion weighted scans and then create a region of interest (ROI) within the hippocampus. Different methods of ROI placement have been used by different groups, ranging from small fixed size ROIs placed on the MD map(186), to full masks of the anterior hippocampus drawn on the MD(187;188) or the b_0 images(189).

In subjects with diagnosed TLE-MTS, there have been consistent findings of significantly increased MD in the abnormal hippocampus(186-189). When considering FA however, while some groups have found a significant decrease in the abnormal hippocampus(188;189), others have not(186;187).

This discrepancy is most likely due to methodological differences, both in terms of the degree of diffusion weighting and pulse sequence used and in the method used to place the ROI in the hippocampus. A summary of the results obtained and methods used are shown in Table 2-1 below:

	Subjects	No diffusion directions	ROI placement	Mean diffusivity	Fractional anisotropy
Wieshmann et al. (1999)(188)	20	3	ROI drawn on b_0 scan to cover entire hippocampus	Increased ipsilaterally	-
Assaf et al (2002)(186)	12	6	4x8mm ROI placed on MD map	Increased ipsilaterally	No significant change
Thivard et al (2005)(187)	35	23	ROI drawn on MD map to cover entire hippocampus	Increased ipsilaterally	No significant change
Salmenpera et al (2006)(189)	7	60	Elliptical ROI drawn on 4 consecutive slices on b_0 image	Increased ipsilaterally	Decreased ipsilaterally
Kimiwada et al (2006)(190)	14	6	ROI drawn on b_0 scan to cover entire hippocampus	Increased ipsilaterally	Decreased bilaterally
Knake et al (2009)(191)	12	6	4mm diameter spherical ROI placed on b_0 scan	-	Decreased compared to contralateral hippocampus

Table 2-2: Published studies of hippocampal MD/FA in TLE-MTS

As well as these chronic changes in established MTS, there have also been documented acute changes in MD and FA immediately following seizures in both animals(50;192) and humans(193;194). This suggests that diffusion parameters may offer another modality to track hippocampal changes following CSE in addition to hippocampal volumetry, however an optimal methodology for measuring hippocampal MD and FA needs to be established. This will be considered further in Chapter 7.

2.4.3 Automated methods of MRI analysis

In addition to manual measurements of hippocampal volume, MD and FA, there exist a number of complementary techniques that compare MRI scans between subjects on a voxel-by-voxel basis across wider areas of the brain. This avoids the subjective elements associated with

region selection and ROI placement. The most widely used of these automated techniques are voxel based morphometry (VBM)(195) and tract-based spatial statistics (TBSS)(196).

VBM compares grey matter concentrations and/or volume between groups of subjects. It relies firstly on the automated segmentation of each individuals MRI scan into grey matter, white matter and CSF containing voxels and secondly on the accurate co-registration of each individual to a common template, thereby ensuring that the voxel-by-voxel comparison is comparing the same area of brain in each subject. Because the technique was initially developed for use on adults, there are a number of difficulties that need to be considered when it is applied in younger subjects. Particularly in children under 1 year of age, the appearance of grey and white matter on a T1-weighted scan is different: whereas in adults white matter appears bright on a T1-weighted image, grey matter darker and CSF the darkest of all, in neonates, because of the unmyelinated nature of large portions of the brain and the relatively higher water content, grey matter appears brighter and white matter appears dark on T1-weighted images. As the brain matures then the relative intensities reverse and trend towards adult values, but during at least the first years of life this appearance is different enough to cause trouble with automated image analysis techniques. Co-registration of images relies to some extent on the contrast between the MRI signal from different areas of brain and therefore co-registration of infant MRI requires different parameters than that of older children. Furthermore, the automated segmentation of grey and white matter also requires specialised algorithms and hence it is very difficult to apply VBM to groups containing a mixture of ages.

TBSS is a separate technique for the automated voxel-by-voxel analysis of diffusion data along a white matter skeleton. It has a number of advantages to VBM, particularly as regards the robustness of co-registration and will be described in further detail in Chapter 8. The disadvantage to TBSS is that it can only detect differences along the major white matter tracts and does not compare areas of grey matter or smaller tracts.

TBSS will be preferred for the voxel-wise analysis presented in Chapter 8 because of the difficulties in applying VBM techniques to cohorts over this age range alluded to above.

Chapter 3: Materials and Methods

3.1 Definitions

For the purposes of this thesis, CSE was defined as:

"A single convulsive seizure lasting more than 30 minutes in total (continuous CSE), or a series of convulsive seizures without recovery of consciousness in between, lasting in total longer than 30 minutes (intermittent CSE)."

3.2 Patient recruitment and ethics

The aim of this study was to recruit a population representative cohort of children with CSE. To this end, a multi-tiered approach, successfully used in previous epidemiological studies of CSE and epilepsy in infancy (28;197) was used. Children aged between 1 month and 16 years were recruited from hospitals in North London (for a list of participating local hospitals, see Appendix A), following an episode of CSE. Recruitment began on 1st March 2007 and finished on 1st March 2010. Children were identified using a previously established clinical network, covering 18 hospitals with 24hr paediatric accident and emergency services, five paediatric intensive care units (PICU) and the regional centralised paediatric intensive care retrieval service (CATS). Eligible children with CSE were notified to a centralised research team by their admitting local paediatrician or CATS. Local hospitals, CATS and local PICU were also regularly contacted to see if any eligible children had been seen. Basic demographic details and contact information were recorded and each family was contacted by the research team and invited to take part in the Status Epilepticus Imaging and Neurocognitive study (STEPIN). Those that agreed to participate were invited to have a brain MRI scan and developmental/clinical assessment at Great Ormond Street Hospital (GOSH) within 12 weeks after the episode of CSE.

STEPIN was approved by the Local Research Ethics Committee at Great Ormond Street Hospital. Local Research and Development registration was obtained at all referring hospitals. Informed written consent to participate in the study and for each procedure involved was obtained from each patient and or family at their initial assessment.

3.3 Assessment timeline

Patients were invited to attend Great Ormond Street Hospital for their initial assessment 4-12 weeks following their episode of CSE. The initial assessment comprised of the collection of a clinical history from the child and his/her parents of the episode of CSE and any previous medical or developmental problems; neurological examination by a paediatric neurology trainee (Dr M Yoong) under the supervision of a consultant paediatric neurologist (Dr RC Scott/Dr RF Chin/Prof B Neville); MRI investigations as detailed below; and neurocognitive assessment as also detailed below. If possible all parts of the initial assessment were completed on the first visit. On occasion this proved impractical because of the sedation required for the MRI scan and in these cases a further appointment was required to complete the assessment as soon as possible after the initial visit.

Families were re-contacted 4 months after their initial assessment and invited to attend GOSH for further MRI investigations. Families were contacted again at 12 months after the initial assessment and invited for a final set of MRI investigations and repeat neurocognitive testing. The presence or absence of recurrent seizures and CSE, as well as what anti-epileptic medication the child was taking was recorded at each follow-up appointment.

3.4 Patient classification

Patients were assigned to one of 7 aetiological groups (as previously mentioned in Chapter 1.2.2) using information from the clinical history and neurological examination recorded at their initial assessment. This included details of any previous neuroimaging the child had had, but not the results of the MRIs performed during this project. Assignment was done independently by two paediatric neurologists (Dr RC Scott and Dr RF Chin) and disagreements resolved by consensus discussion.

For some analyses, in order to increase the statistical power, aetiological groups were combined into 3 categories: prolonged febrile seizures; symptomatic CSE (including acute, remote and acute on remote symptomatic CSE); and other CSE (including idiopathic/cryptogenic epilepsy and unclassified CSE).

3.5 MRI protocol

All children received MRI investigations on the same Siemens Avanto 1.5T whole-body MRI scanner based at Great Ormond Street Hospital. An imaging protocol used for the evaluation of children with epilepsy and optimised for the visualisation of mesial temporal structures was carried out. Additional diffusion weighted sequences were added to this for diffusion tensor imaging (DTI). A copy of the full MRI protocol in available in Appendix B. The same MRI investigations were performed at the initial assessment and at each follow-up.

MRI investigations were performed where possible with the child awake and lying still in the scanner or when they were in a natural sleep. This was successful for most children under 6 months and those over 6 years. Where this was judged not possible, or after an initial failed attempt, either due to the chronological age of the child or their developmental status, sedative medication was offered to increase the chances of the child staying still for the scan. Sedative medication was administered by the sedation nursing team at Great Ormond Street after assessment of the child for safety and with parental consent. A copy of the protocol used to assess children and prescribe medication is available in Appendix C.

3.6 MRI processing pipeline

Raw DICOM files were downloaded from the MRI scanner, anonymised, and archived to CD. Files were transferred to an encrypted hard disc and stored using a unique random number generated by the scanner. *TractoR* scripts (http://code.google.com/p/tractor/, Clayden, UCL) were used to sort the DICOM files into directories for each MRI sequence and *dicom2nii* (http://www.cabiatl.com/mricro/mricron/index.html, Rorden, GeorgiaTech Centre for Advanced Brain Imaging) used to convert the DICOM files for each sequence into NIfTI format.

Further processing of the diffusion datasets was performed using *TractoR* to generate the FA and MD maps according to a standard script.

3.7 Neurocognitive assessment

Standardised neurocognitive assessment was performed on each child by a psychologist (Dr M Martinos). 3 different neurocognitive batteries were used, depending on the chronological age of the child:

1. Children under the age of 42 months were assessed using the Bayley scales of infant and toddler development (3rd edition).
2. Children aged between 42 and 84 months were assessed using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-3rd UK edition).
3. Children over the age of 84 months were assessed using the Wechsler Intelligence Scale for Children-Revised (WISC-4th UK edition).

Each test was scored to provide an overall measure of full scale IQ (for the WPPSI and WISC) or overall cognitive development (for the Bayley) with mean 100 and standard deviation 15. Results from these composite scores were used to compare children for analysis.

In addition to these, children aged between 5 and 16 years were administered the Children's Memory Scale (CMS) and parents were given a number of questionnaires to complete: the Social-Emotional and Adaptive Behaviour questionnaire (children under 42 months age); the Communication and Symbolic Behaviour Scales Developmental profile (6 -24 months age); and the Strengths and Difficulties questionnaire (3-16 years age). Results from these have been previously reported on by Dr Martinos(173) and will not be presented in this thesis.

3.8 Recruitment of control subjects

Children were recruited from a number of different sources, including personal contacts, playgroups, hospital clinics and university e-mail lists, to act as control subjects for this project:

1) Volunteers with no history of seizures, developmental delay or neurological problems
    - These had to be able to complete an MRI scan without sedation, either during natural sleep or awake and watching a video
2) Children having an MRI scan under sedation for other clinical reasons with no history of seizures

- The main clinical indication for these scans was for further assessment of skin and soft tissue abnormalities such as haemangiomas or dermoid cysts requested by the Ophthalmology department and for children with sensorineural hearing loss undergoing pre-assessment for the cochlear implant program.
- All of these children had normal neurological examinations and normal developmental history except for isolated hearing loss with speech delay in some children.
- If the MRI scan was reported as showing any intracranial abnormalities then these children were not used as controls

Informed consent was obtained from parents by the research team before their participation in the project. Controls underwent the same set of neurocognitive and MRI investigations as patients; for the MRI the 3D-FLASH sequence was performed first, followed by the DTI sequences and then the T2 relaxometry. This was done in order to maximise the useful information obtained from the MRI, as some children did not tolerate the entire scan. All MRI scans performed were reviewed later by a neuroradiologist (KC/RC) who assessed them for any structural abnormalities. Children who had an intracranial structural abnormality present on their MRI scan were excluded from our group of control subjects. Any abnormalities found were notified either to the paediatrician responsible for the care of that child or the General Practitioner (GP) for those children who were not under a named paediatrician. The results of the neurocognitive tests were also notified to the child's GP to pass onto the parents.

3.9 Statistical analysis

Except where otherwise stated, all statistical analysis was performed with the aid of SPSS for Windows version 17.0-20.0 (Chicago). $p < 0.05$ was taken as the cut-off for significance and appropriate corrections for multiple comparisons were made as stated in individual chapters.

Having presented the overall clinical and scientific methodology that was used for the studies set forth in this thesis, the following chapters will advance the findings and results of each study comprising this overall project, with further explanation of the methodology used for each individual portion as required.

Chapter 4: Characterisation of patient cohort

4.1: Patient cohort at enrolment

4.1.1 Demographics

During the period December 2006 - March 2010 225 children were notified to our study. Of these, a total of 86 children were recruited and underwent at least one assessment. A summary of the demographic details of the patients enrolled in the study is given in Table 4-1 and their age distribution shown in Figure 4-1.

	Not entered	Recruited
Median Age at CSE (years) (range)	2.43 (0.10 – 15.29)	1.98 (0.10 - 15.44)
Mean Gestational age (weeks) (s.d.)	36.94 (4.94)	37.90 (3.92)
Male:Female ratio	80:59	44:42

Table 4-1: Demographic details of initial patient cohort at enrolment

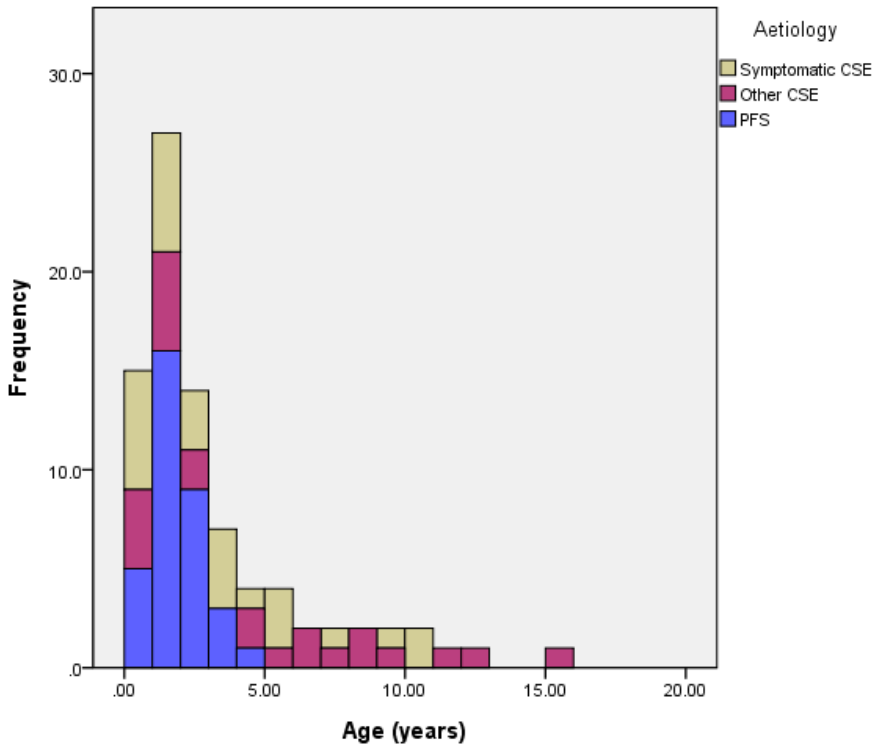


Figure 4-1 Histogram of CSE incidence by age and aetiology

Children were seen for assessment a mean of 31.78 days after their episode of CSE (Range 5 – 90). Amongst those that were not enrolled, 35 children were uncontactable due to missing or incorrect contact details; 32 children were unsuitable for MRI under sedation due to the instability of their clinical condition/co-morbidities; 48 children declined to take part in the study; 15 children lived far from the study area and were not willing to visit our centre; and 7 children died during their acute hospital admission. A further 2 children agreed to take part but did not attend their appointments.

4.1.2 Aetiology

As reported earlier, each child was assigned to an aetiological group (see Chapter 1), depending on the reported cause of their episode of CSE and their previous medical and developmental history. The most common cause of CSE in our cohort was PFC and a breakdown of our cohort by aetiology is given in Figure 4-2. As numbers in some groups were small, groups were merged into 3 categories - PFC, symptomatic CSE and other CSE - for the majority of our analyses. A summary of the demographic details of each group is given in Table 4-2.

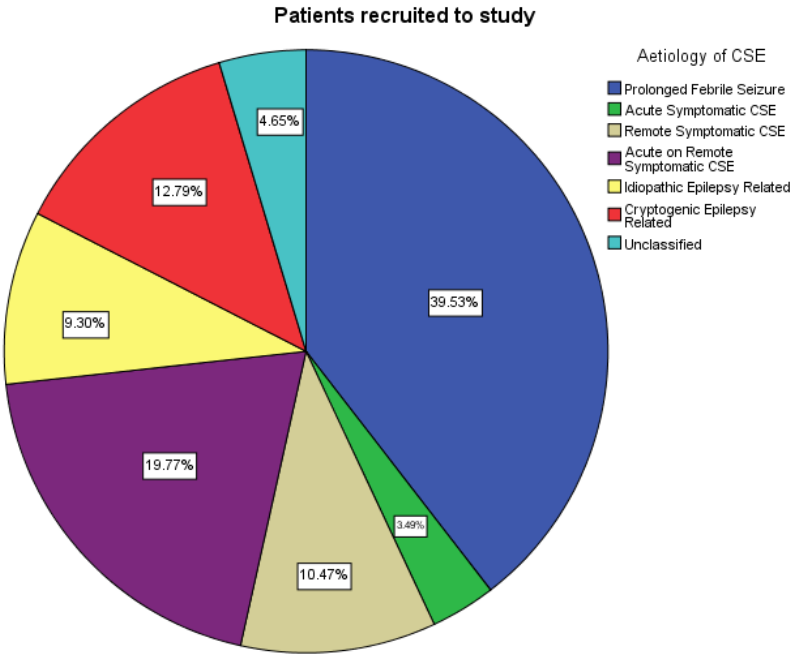


Figure 4-2: Aetiology of CSE within STEP IN

	PFC (n=34)	Symptomatic CSE (n=29)	Other CSE (n=23)
Age at CSE (s.d.)	1.92 (0.945)	3.35 (3.048)	4.99 (4.370)
Gestational age (weeks) (range)	38.44 (30 – 41)	36.21 (24 – 41)	39.22 (33 – 40)
Male:Female ratio	11:23	22:7	11:12

Table 4-2: Demographic details of patient cohort by aetiology

4.1.3 Semiology and medical history

The clinical characteristics of each episode of CSE are summarised in Table 4-3 and the previous medical history in Table 4-4.

		PFC (n=34)	Symptomatic CSE (n=29)	Other CSE (n=23)	Overall (n=86)
Focal onset		5(14.7%)	7(24.1%)	12(52.2%)	24 (27.9%)
Continuous seizure activity		21 (61.8%)	17 (58.6%)	13 (56.5%)	51 (59.3%)
Intermittent seizure activity		13 (38.2%)	12 (41.4%)	10 (43.5%)	35 (40.7%)
Semiology	Tonic	5 (14.7%)	2 (6.9%)	7 (30.4%)	14 (16.3%)
	Clonic	1 (2.9%)	3 (10.3%)	2 (8.7%)	6 (6.9%)
	Tonic-Clonic	28 (82.4%)	24 (82.8%)	14 (60.9%)	66 (76.7%)
Mean seizure duration in minutes (range)		72.21 (30 – 190)	73.03 (30 – 265)	69.17 (30 – 190)	71.69 (30 – 265)

Table 4-3: Seizure characteristics of CSE by aetiology

	PFC (n=34)	Symptomatic CSE (n=29)	Other CSE (n=23)	Overall (n=86)
Previous seizures	13 (38.2%)	19 (65.6%)	18 (78.3%)	50 (58.1%)
Previous episode CSE	2 (5.9%)	8 (27.4%)	11 (47.8%)	21 (24.4%)
Taking AED at time of CSE	0 (0%)	14 (52%)	16 (61%)	29 (33.7%)
Previous Hx developmental delay	5 (14.7%)	16 (55.2%)	6 (26.1%)	27 (31.3%)
FHx Epilepsy	7 (21%)	8 (30%)	8 (35%)	23 (27%)

AED: Anti-epileptic drugs
CSE: Convulsive Status Epilepticus
Hx: History
FHx: Family History

Table 4-4: Previous medical history of patient cohort

4.2: Patient cohort at follow-up

Children and their families were contacted after their initial episode of CSE and were seen an average of 31 days post-CSE for their initial set of investigations. Not all children received the full set of investigations, as some were unsuitable for MRI scans or had parents refuse consent. Inevitably there was some loss to follow-up, with families moving away or being unwilling to undergo further MRI investigations. An overview of the cohort is given in Table 4-5 and Figure 4-3 shows which children attended which assessments.

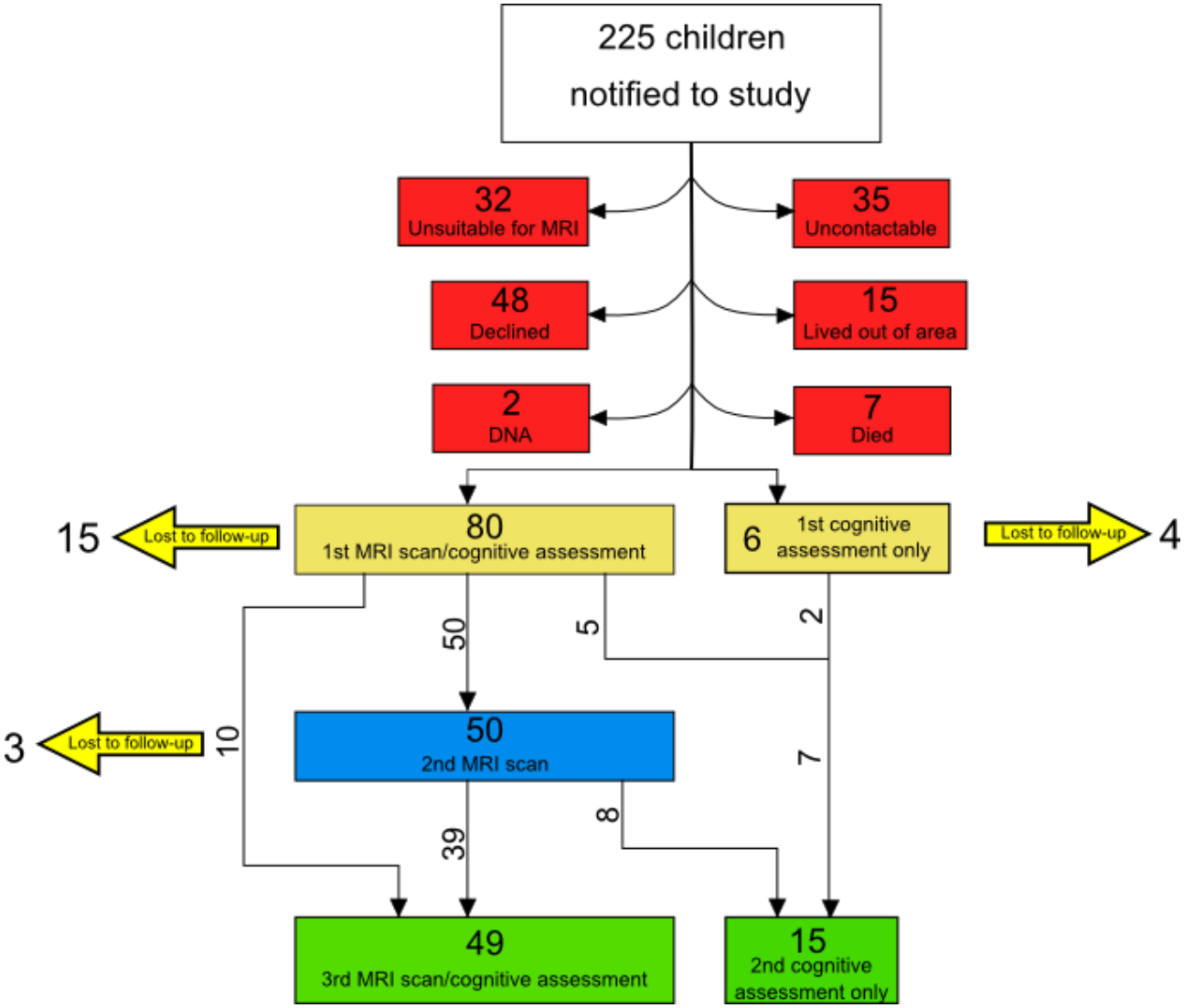


Figure 4-3: Flowchart of patient recruitment and follow-up

	PFS	Symptomatic CSE	Other CSE	Overall
1st MRI scan	33	24	23	80
Initial developmental test	33	25	20	78
2nd MRI scan	21	15	14	50
3rd MRI scan	21	15	13	46
Follow-up developmental test	26	18	13	57

Table 4-5: Patient numbers at each follow-up assessment

The median time after the initial episode of CSE to the first assessment was 31.6 days and this varied from between 5 and 90 days. A summary can be seen in Table 4-6.

	Median time after CSE in days (range)			
	PFS	**Symptomatic CSE**	**Other CSE**	**Overall**
1st MRI scan	37 (5 - 90)	33 (11 - 57)	19 (7 - 66)	29.5 (5 - 90)
Initial developmental test	37 (10 - 254)	30 (11 - 66)	19 (8 - 99)	29 (8 - 254)
2nd MRI scan	180 (124 - 280)	167 (116 - 221)	158.5 (123 - 210)	169.5 (116 - 280)
3rd MRI scan	427 (255 - 699)	394 (268 - 515)	386 (275 - 582)	391 (255 - 699)
Follow-up developmental test	427 (255 - 949)	402-5 (268 - 536)	386 (275 - 679)	398 (255 - 949)

Table 4-6: Timing of follow-up assessments

At each follow-up appointment patients were asked if they had had any further seizures (including both febrile and non-febrile seizures) since they were last seen. The results of this are given in Table 4-7.

	PFS (n=34)	Symptomatic CSE (n=29)	Other CSE (n=23)	Overall (n=86)
Further seizures by 4 months	8 (23.5%)	11 (37.9%)	12 (52.2%)	31 (36.0%)
Further seizures between 4 and 12 months	5 (14.7%)	12 (41.4%)	10 (43.5%)	27 (31.4%)
Cumulative incidence of further seizures by 1 year post CSE	10 (29.4%)	14 (48.3%)	14 (60.9%)	38 (44.2%)
Cumulative incidence of recurrent CSE by 1 year post CSE	1 (2.9%)	1 (3.4%)	4 (17.4%)	6 (7.0%)

Table 4-7: Incidence of recurrent seizures during first 12 months post-CSE

4.3 Analysis of Patient Cohort

Patients were recruited to our study in a prospective manner following an episode of CSE. This was intended to produce a population representative cohort for further study. In order to screen for potential biases in our patient population, basic demographic data on patients who were notified to our study, but declined or were unable to take part in the assessments was also collected anonymously. Data collected at notification by the local hospital or the Children's Acute Transport Service (CATS) was used to classify patients that were not seen by the research team to an aetiological group and to confirm that our cohort was representative of the overall population presenting with CSE. Sufficient detail was available to attempt this for 120/145 children who were not entered into the study. The index of multiple deprivation (IMD) score was also obtained for each child from their home postcode and this was used as a measure of socioeconomic status and compared between participants and non-participants.

		Children notified but not entered into study	Children entered into study
Number		139	86
Male:Female		80:59	44:42
Median age in years (range)		2.43 (0.10 – 15.29)	1.98 (0.10 – 15.44)
Mean Gestational age in weeks (range)		36.94 (22 – 41)	37.90 (24 – 41)
Mean seizure duration in mins (s.d.)		75.26 (73.71)	71.94 (42.73)
Median Index of Multiple Deprivation (range)		29.45 (3.38 - 62.51)	23.44 (5.28 - 61.38)
Aetiology of CSE (%)	Prolonged febrile seizure	34(24.5%)	34 (39.5%)
	Acute symptomatic CSE	22 (15.8%)	3 (3.5%)
	Remote symptomatic CSE	24 (17.3%)	9 (10.5%)
	Acute on Remote symptomatic CSE	22 (15.8%)	17 (19.8%)
	Idiopathic Epilepsy Related	4 (2.9%)	8 (9.3%)
	Cryptogenic epilepsy related	7 (5.0%)	11 (12.8%)
	Unclassified	7 (5.0%)	4 (4.7%)
	Unknown	19 (13.7%)	-

Table 4-8: Comparison of entered children with non-participating children

Independent sample t-tests were used to compare each group for age ($p = 0.175$), gestation ($p = 0.250$) and seizure duration ($p = 0.720$), there was no significant difference in any of these factors. A Chi-squared test was used to compare the proportion of children with each aetiology in both groups, this showed that the composition of the children entered into our study was significantly different from those referred ($p < 0.001$). A Mann-Whitney U test was used to compare IMD between each group and found no significant difference ($p = 0.130$).

One further difference between the patients initially notified to the study and those who were eventually recruited was the number of male patients with PFS. The initial PFS cohort consisted of 44.9% boys, but a disproportionate number of these families declined to take part in any further assessments, meaning that our eventual starting cohort had a female predominance, with only 32.4% boys.

By comparing our cohort to a previous epidemiological study of CSE done in the same geographic area (NLSTEPSS(28)), we were able to see how representative was of the general population of children with CSE (Figure 4-4). There were differences between the patients recruited

to this current study and those presenting to NLSTEPSS - in particular, less children with symptomatic CSE were recruited to this study than were found in NLSTEPSS. As the proportions of each aetiological group originally notified to our study were similar to NLSTEPSS, this appears to be mainly because a disproportionate number of patients with acute/remote symptomatic CSE declined consent for further investigations or were not able to take part despite initial parental consent as sedation for MRI was judged to be unsafe.

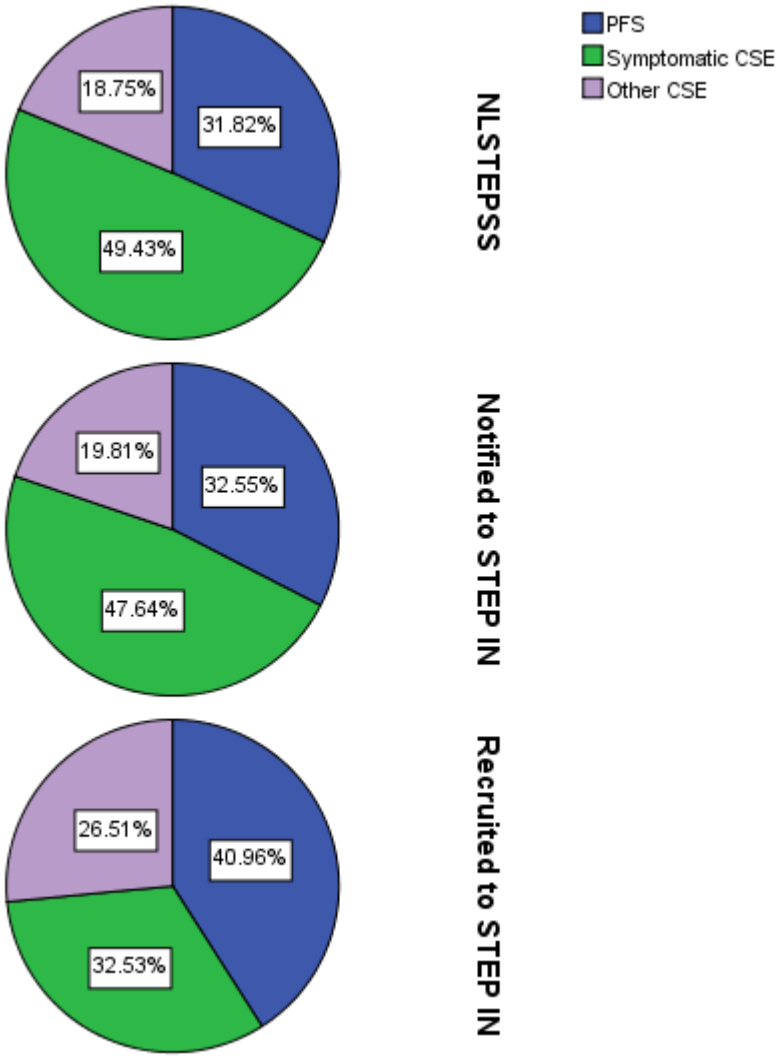


Figure 4-4: Comparison of STEP IN with NLSTEPSS

Other than these two differences, our cohort was broadly similar to the population of children notified to us and to those identified in NLSTEPSS.

Logistic regression was used to investigate how clinical factors and CSE semiology varied between the groups. Previous seizure history, seizure focality, history of developmental problems,

age at CSE and AED use were all significantly different between children with PFS and those with symptomatic or other CSE. Children with PFS were more likely to be younger at the time of CSE (p = 0.009), not have had previous CSE (p = 0.042), not be on any AED (p = 0.003) and have no history of pre-existing developmental problems (p = 0.025). The overall seizure semiology reported was similar to that seen in NLSTEPSS.

The age distribution of our cohort did not follow a normal distribution, with a skew towards younger children. This is similar to other reported studies of CSE, which show a bi-modal distribution with peaks in early infancy and old age (29).

Age to first scan was significantly associated with aetiology (ANOVA, p = 0.004), with children with PFS taking on average, 6 days longer to be seen for their initial MRI investigation and assessment. There was no difference between the times the groups were seen for any of the other assessments and there were similar drop-out rates between the 3 groups.

All groups had relatively high incidences of recurrent seizures during the first 12 months post-CSE. Seizure recurrence at 4 months did not differ between the groups (Chi-squared p = 0.058) however by 1 year, the recurrence rates were different (p = 0.032) with fewer recurrent seizures in the PFS group.

4.4 Demographics of control children

A total of 31 children were recruited as healthy control subjects. No control subjects had a major abnormality on their MRI scan. Demographic details are given in Table 4-9 and the age distribution is shown in Figure 4-5 below.

	Had any MRI		**Tolerated DTI**		**Tolerated T2 maps**	
	Age (range)	Number (Male: Female)	Age (range)	Number	Age (range)	Number
Children having scans for other clinical reasons	4.04 (0.21 – 12.69)	19 (7:12)	3.82 (0.62 – 11.70)	15 (6:9)	3.80 (0.62 – 12.69)	11 (5:6)
Normal volunteers	3.75 (0.26 – 9.23)	12 (5:7)	3.84 (0.26 – 9.23)	7 (3:4)	5.11 (2.28 – 9.23)	7 (2:5)
Total	**3.93 (0.21 – 12.69)**	**31 (12:19)**	**3.82 (0.26 – 11.70)**	**22 (9:13)**	**4.31 (0.62 – 12.69)**	**18 (7:11)**

Table 4-9: Demographics of control subjects

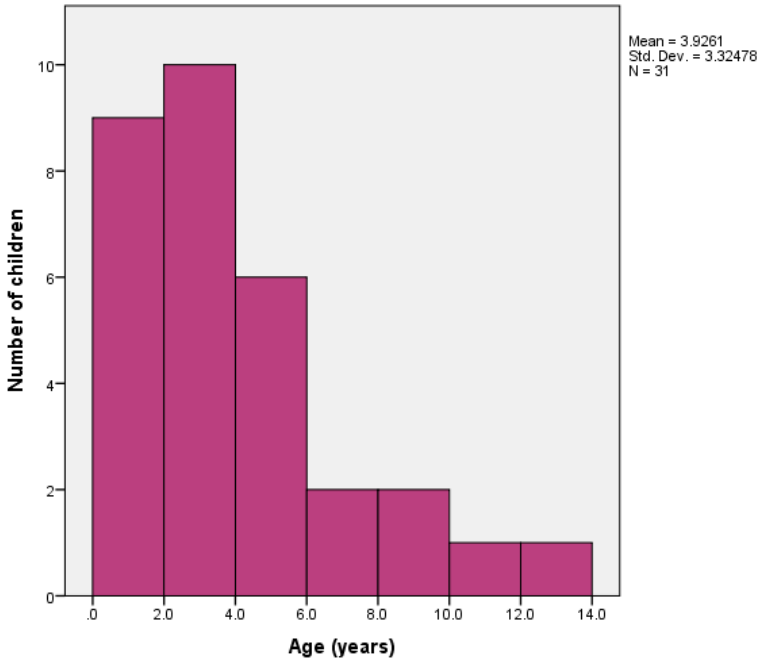


Figure 4-5: Histogram of ages of control subjects

19 of the children recruited as control subjects were having MRI scans for other clinical reasons, which are summarised in Table 4-10, and 12 were healthy volunteers. All control subjects had no previous history of seizures, neurological disease (other than that mentioned) or diagnosed developmental delay.

Indication for MRI scan	Number of patients
MRI scan for cochlear implant assessment	3
Abnormal visual function tests, MRI for assessment of optic nerve hypoplasia	3
Dermoid cyst	2
Facial haemangioma, assessment for Sturge-Weber syndrome	3
Non-intracranial tumour	2
Headache	2
Marcus-Gunn phenomena	1
Congenital nystagmus	1
Ptosis	1
Lymphangioma	1

Table 4-10: MRI indications for control subjects

4.5 Cognition

Standardised cognitive scores for each patient group are reported in Table 4-11. These data have been previously reported and analysed by Dr M Martinos in her PhD thesis(173) and are included here for illustrative purposes only.

	Controls	PFS	Symptomatic CSE	Other CSE
Mean (s.d.)	127.38 (11.55)	95.32 (13.97)	75.68 (22.09)	85.14 (21.15)
Range	110 - 144	65 – 120	55-123	41 - 130

Table 4-11: Standardised cognitive scores across each patient group at study entry

4.6 Discussion

When compared with previous epidemiological studies of CSE such the NLSTEPSS study, which covered a similar geographical area, children with symptomatic CSE are underrepresented in our sample. The reasons for this have been discussed and do have implications as to the power of our study to reach conclusions regarding children with symptomatic CSE, particularly those with more severe neurodisabilities, who were more likely to be unsuitable for sedation and to decline consent due to a high burden from existing multiple hospital appointments. Although there was some gender bias in our PFS group, there is no known gender association with PFS aetiology or prognosis, so this seems unlikely to significantly bias the results of this study.

As noted in previous studies, the aetiology of CSE changes with age. PFS is the commonest aetiology in the youngest age group, but declines after the age of 3 years. There is a lesser peak of symptomatic and other CSE in infancy, but they are then evenly distributed throughout childhood and adolescence.

Analysis of the clinical factors associated with the initial episode of CSE showed that children with PFS were less likely to have had a previous episode of CSE or developmental problems. This is in line with the aetiological definition and consistent with other studies. Likewise, studies have reported a rate of recurrent seizures within the first year of between 3-56%(34). Our overall recurrence rate of 46% falls at the upper end of this range. This risk is highest for those children with Other CSE or symptomatic CSE and lower for those with PFS, again in line with that found in previous studies(28). Of note, all recurrent seizures in children with PFS were febrile seizures and no children with PFS had had afebrile seizures by the end of the follow-up period.

In conclusion, other than those factors mentioned, our study population appears to be broadly similar to other reported studies of children with CSE in terms of demographics, medical history and seizure semiology. It therefore provides a reasonable foundation for investigating the outcome of CSE reaching conclusions applicable to the general population.

CHAPTER 5: CLINICAL MRI FINDINGS

5.1 Summary

The yield of brain MRI following CSE has not been fully investigated and uncertainty remains over the best use of neuroimaging after an episode of CSE. Using the data available from our cohort of children with CSE we found that around 30% had abnormalities on MRI. Regression analysis identified non-PFS aetiology, continuous CSE and persistent abnormal neurological examination as independent risk factors for an abnormal MRI scan.

5.2 Introduction

Following the successful termination of an acute episode of CSE, there is often a dilemma concerning the subsequent management and follow-up of the child. In particular, one investigation that is frequently considered is the need for neuroimaging and what type of neuroimaging this should be. Since outcome is largely dependent on aetiology(28;34), determining the correct underlying diagnosis is important both for treatment and prognosis and neuroimaging may be useful in this. Current guidelines on the acute management of CSE(62) focus on drug therapy(198) and the achievement of seizure termination, but guidance for the optimal use of follow-up investigations remains unclear(73) .

Based on historical data, a minimum yield of detectable lesions of 7.8% has been estimated if all children with CSE underwent neuroimaging(73). In practice this may be a conservative estimate,particularly if imaging can be restricted to children at particular risk of structural brain lesions. It would be beneficial to determine the yield in the general population of children with CSE and identify risk factors for the presence of structural lesions so that both unnecessary imaging can be avoided and those children who are likely to benefit are treated appropriately.

5.3 Methodology

5.3.1 Patient cohort and classification

The initial scans of the 80 patients within the STEP IN cohort who successfully underwent brain MRI were used for this part of the project. Their clinical details have been previously described in Chapter 4. All sequences were available to be used for the MRI classification and clinical factors were identified from the initial history and examination as described in Chapter 3.

5.3.2 MRI classification

Each MRI scan was evaluated by a paediatric radiology trainee (MC) and reviewed by a consultant paediatric neuroradiologist (W-KC), for abnormalities. Each scan was assigned to one of four diagnostic groups: normal (no abnormal features detected); normal-variant (some unusual feature detected, thought to be a variation of the normal range with no functional significance); minor abnormality (abnormal feature thought to be either unrelated to this episode of CSE or of no functional significance); or major abnormality (abnormal feature likely to have significant impact on the child and/or represent a cause for this episode of CSE.). Scans were also assessed for the presence of hippocampal malrotation (HIMAL)(199), which has been reported to be associated with seizures(200;201). This was performed blind to the clinical history of the child.

Four factors were used to identify HIMAL:

- Incomplete inversion of the hippocampus
- Blurred internal structure
- Abnormal position of the collateral sulcus
- Downwards displacement of the fornix on one side

Patients with all 4 features were considered to meet full criteria for HIMAL and those with 3 features were considered to meet partial criteria.

5.3.3 Statistical analysis

All data were analysed using PASW Statistics 18.0.2 (Chicago, IL). Logistic regression was used to investigate candidate variables predictive of an abnormal MRI. As even minor abnormalities may have clinical relevance to the individual child and to maximise our model's predictive power,

major and minor abnormalities were combined for analysis as were normal and normal-variant categories. Based on previously reported clinical and demographic factors which may increase the risk for structural brain lesions (32;202), the following were investigated: age at CSE; seizure duration > 60 mins; seizure focality; continuous vs. intermittent CSE; seizure aetiology (PFS vs. non-PFS); previous history of seizures or CSE; abnormal neurological examination at assessment; history of developmental delay pre-CSE; and results of previous neuroimaging. These were entered into a multivariable logistic regression analysis. Bootstrapping techniques were used to assess internal validity (203).

Scaled cognitive scores were calculated for each patient who underwent neuropsychological testing at study entry and also at final follow-up, as described in Chapter 3 and t-tests were used to compare these scores between children with and without abnormal features on their MRI.

5.4 Results

5.4.1 MRI results

Overall, 25/80 (31.2% patient scans showed some abnormal features. These were evenly split between major and minor abnormalities, as defined earlier. All of the major abnormalities were found in children with acute, remote or acute on remote symptomatic CSE and only one child with PFS showed a minor abnormality. A full breakdown of findings by aetiological group is given in Table 5-1 and detailed description of specific abnormalities found is given in Table 5-2). One child with PFS and no children with other forms of CSE met criteria for unilateral HIMAL and a further 3 children with PFS and one child with an unclassified episode of CSE met partial criteria. Including these children within the diagnosis, there were a significantly higher proportion of children with HIMAL in the PFS group compared to the other groups (9.1% vs. 2.1%, p = 0.029, Chi-squared).

	Normal	Normal-variant	Minor abnormality	Major abnormality
PFS (n=33)	30 (90.9%)	2 (6.1%)	1 (3.0%)	0
Acute symptomatic CSE (n=3)	1 (33.3%)	0	1 (33.3%)	1 (33.3%)
Remote symptomatic CSE (n=7)	0	0	2 (28.6%)	5 (71.4%)
Acute on remote symptomatic CSE (n=14)	4 (28.6%)	0	3 (21.4%)	7 (50.0%)
Idiopathic epilepsy related CSE (n=8)	7 (87.5%)	0	1(12.5%)	0
Cryptogenic epilepsy related CSE (n=11)	7 (63.6%)	0	4 (36.4%)	0
Other CSE (n=4)	4 (100%)	0	0	0
Total (n=80)	53 (66.3%)	2 (2.5%)	12 (15.0%)	13 (16.2%)

Table 5-1: MRI findings by aetiology of CSE

Minor abnormalities		Major abnormalities	
Nonspecific focal white matter lesions	3	Previous hypoxic ischaemic injury at term	3
Delayed myelination and non-specific white matter lesions	1	Extensive malformation of cortical development	1
Labyrinthitis ossificans	1	Porencephalic cyst with VP shunt in situ	2
Lack of white matter bulk	4	Mesial temporal sclerosis	1
Nonspecific diffuse white matter signal change	2	Focal cortical dysplasia	1
Septo-optic dysplasia	1	Mature damage post meningitis	2
		Periventricular leucomalacia secondary to preterm hypoxic ischaemic injury	1
		Tuberous sclerosis	1
		Bilateral basal ganglia signal change with overlying cortical atrophy suggestive of neurometabolic disease	1

Table 5-2: Description of MRI abnormalities found

5.4.2 Risk Factors for an abnormal MRI

Since not all children had previous CT or MRI, previous neuroimaging was not included in the regression model. Regression analysis showed abnormal neurological examination, continuous CSE

and non-PFS aetiology as factors predictive of an abnormal MRI. 10 repeated bootstrap analyses using 1000 samples each consistently identified these 3 factors as significant and others were not. Odds Ratios, B-values and their 95% Confidence Intervals are reported in Table 5-3.

Predictive factor	Abnormal MRI (%)	p-value	B	95% confidence interval (B)	Odds ratio
Abnormal neurological examination at the time of MRI scan	12 (92.3%)	0.001	5.249	3.959 – 424.134	190.460
Non-PFS/Unclassified vs Prolonged febrile seizures	24 (51.1%)	0.001 – 0.002	4.345	2.194 – 316.923	77.108
Continuous vs. Intermittent CSE	19 (40.4%)	0.001 - 0.004	3.399	1.240 – 390.450	29.947
Developmental delay prior to CSE	15 (62.5%)	0.442 – 0.495	0.682	-40.391 – 47.439	1.978
Previous seizures	18 (37.5%)	0.716 – 0.786	-0.053	-50.912 – 97.662	0.949
Previous CSE	9 (42.9%)	0.114 – 0.140	-1.545	-145.027 – 16.245	0.248
Focal vs. Generalised onset	9 (39.1%)	0.854 – 0.904	-0.020	-52.063 – 45.551	0.980
Seizure duration > 60 mins vs. Duration < 60 mins	8 (22.2%)	0.032 – 0.053	-1.584	-162.625 – 1.134	0.205
Age	-	0.299 – 0.357	-0.135	-40.097 – 4.986	0.313

Table 5-3: Clinical factors predictive of an abnormal MRI after CSE

5.4.3 The implications of previous neuroimaging

30 of the 80 children (37.5%) had head CT scan during their acute admission with CSE. CT scan was abnormal in 7 (23.3%). 4 of these were also abnormal on follow-up MRI. Lesions which appeared to resolve were minor and included cerebral oedema, white matter asymmetry and wide subdural spaces. Of the remaining 23 children who had a normal acute CT scan, four (17.4%) had an abnormal MRI within 13 weeks of their CSE – 3 minor and one major abnormalities . 17/49 (34.7%) children who did not have a CT scan had an abnormal MRI scan (Figure 5-1).

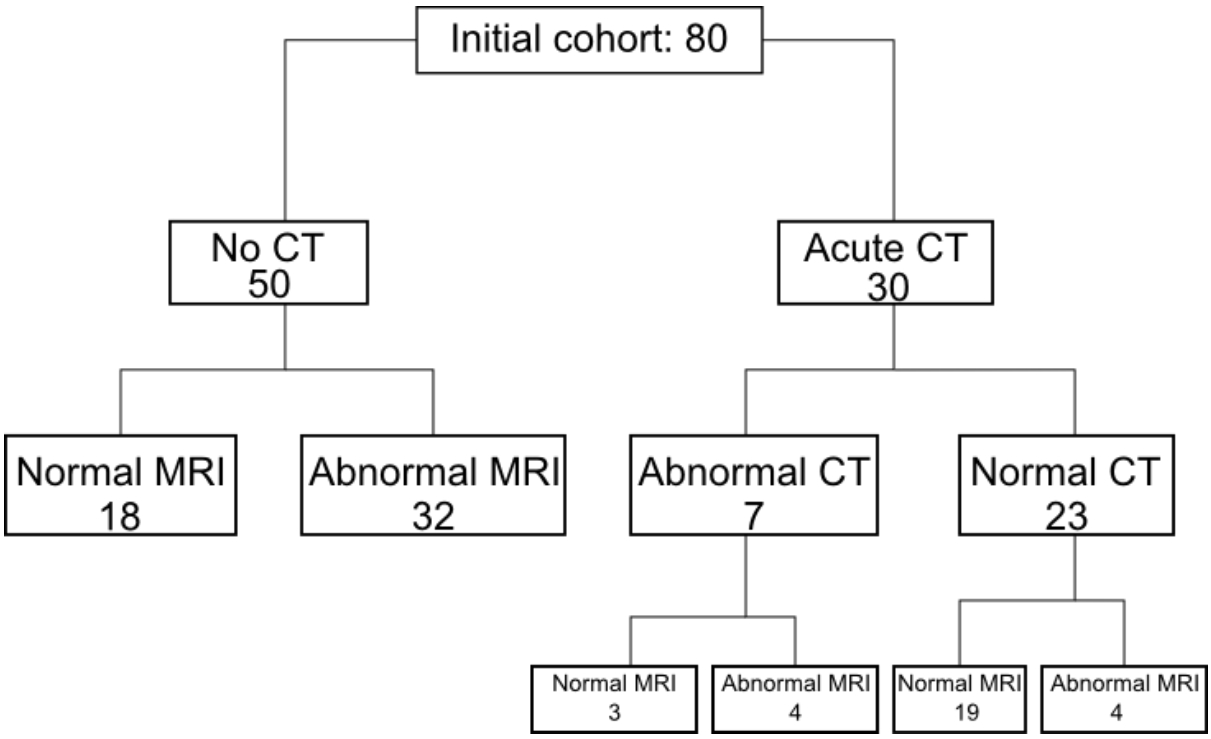


Figure 5-1: Flowchart of patients by previous CT result

27 of the children in our cohort had previous brain MRI before entry into our study. These had been performed for a variety of indications including investigation of developmental delay; previous unprovoked seizures; and acute meningitis. Repeat imaging after CSE allowed the detection of 5 minor abnormalities that had previously not been reported (Figure 5-2) although they were present on the original pre-CSE scans i.e. these were old lesions (consistent with a remote symptomatic aetiology) that did not represent new emergent findings and did not necessitate any change in treatment. No new major abnormalities were found in children with previous neuroimaging, although one patient who had previously been diagnosed as having cortical dysplasia was diagnosed as having tuberous sclerosis.

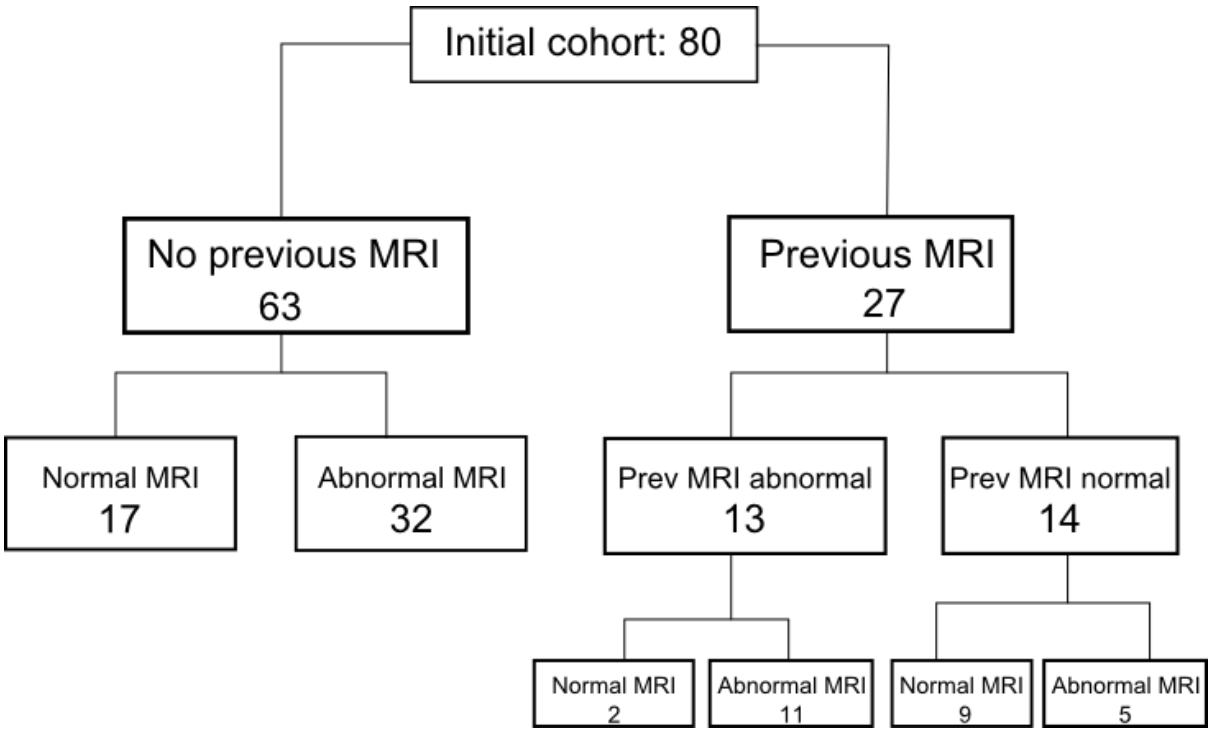


Figure 5-2: Flowchart of patients by previous MRI results

5.4.4 Neuroimaging and cognition

Children with either minor or major abnormalities reported on their MRI scan had lower mean cognitive scores at their initial assessment, although these did not reach statistical significance. By 1 year post-CSE however, their cognitive scores dropped further whilst children with a normal MRI scan maintained their cognitive ability and this difference was statistically significant. Full details are given in Table 5-4.

	Normal MRI	Abnormal MRI	p-value for difference
Mean cognitive score 1 month post-CSE	91.06 (20.32)	76.11 (20.33)	0.056
Mean cognitive score 1 year post-CSE	91.00 (18.17)	70.38 (17.65)	0.008*

Table 5-4: Cognitive scores for children with normal and abnormal brain MRI 1 month and 1 year post-CSE

5.5 Discussion

It is known from previous studies that have used a mixture of CT and MRI that a high proportion of children with CSE have abnormal neuroimaging. However, ours is the first study to

follow a cohort with complete MRI coverage and to systematically investigate clinical factors associated with an abnormal MRI. The main findings are: over 30% of children will have brain MRI abnormalities detectable a median of 1 month following CSE, and clinical features can predict those children who are most likely to have structural abnormalities on MRI if it is performed as part of their follow up investigations.

Our study found abnormalities on brain MRI in 31.3% (95% CI: 22.2 – 42.1%) of children with CSE. This is similar to the findings in a study looking at new onset CSE by Singh et al.(202), which reported a 30% yield of neuroimaging abnormalities based on combined CT/MRI in 144 children. Previous studies have reported that imaging children after a first unprovoked seizure has a yield of brain abnormalities of 13-32%(204-206), not accounting for seizure duration. As a high proportion of CSE is associated with an acute provoking factor(32), with the resultant possibility of associated structural brain injury, it is consistent that the yield of neuroimaging in CSE is at the upper end of this range.

A recent meta-analysis designed to estimate the prevalence of incidental MRI findings in healthy people found overall rates of intracranial abnormalities of 2.7% (95% CI 1.57 – 4.08%)(207). Thus, the yield of abnormal features in 31% of our cohort of children with CSE, is substantially higher than that expected in a normal population without CSE and unlikely to represent incidental findings.

The specific predictive factors for a MRI detected structural brain abnormality identified in the current study were having i) an abnormal neurological examination after CSE, ii) non-PFS CSE or iii) continuous CSE. Thus, in deciding whether a child should have MRI following status epilepticus, we would suggest that these findings be taken into account. We found no evidence that focality of the seizure, history of previous seizures or CSE, or duration of CSE were associated with abnormalities on MRI within 6 weeks after CSE and therefore suggest that these should be considered less important in the decision on whether an MRI is appropriate. Since the incidence of CSE is greatest in infants and younger children(32), the majority of children with CSE are unlikely to tolerate an MRI scan without some form of sedation or anaesthesia. These associated procedures do carry a risk(208), albeit a small one, (to the child) although the MRI itself is believed to carry minimal health risks. Therefore the risks and difficulties in obtaining a brain MRI in children following CSE need to be carefully weighed against the likely yield. We found only one minor abnormality and no major abnormalities in the 33 children with PFS at 13 weeks post-CSE, giving an estimated yield of major abnormalities of 0-10.3% (95%CI). Thus although children with PFS make up a substantial proportion of childhood CSE and there are concerns over their longer term risk of hippocampal damage(209), the majority of them will not have structural abnormalities on MRI during the

immediate follow-up period. Although previous reports have suggested an association between PFS and HIMAL (Lewis DV et al, FEBSTAT group, personal communication), and we found a greater number of children meeting HIMAL criteria in our PFS group the clinical implications of this remains uncertain(210).

Our study did not consider the emergency management of CSE, so we are unable to comment on the use of head CT in the emergency department. None of the children in our study required emergency neurosurgical intervention, although there may have been children where this was an important possibility to exclude. CT performed in the acute situation appears to detect a number of acute abnormalities that do not persist and while a normal CT scan reduces the likelihood of an MRI abnormality, the predictive power of either a normal or an abnormal CT scan in isolation is similar to the clinical factors described earlier. Therefore a normal CT scan does not offer complete reassurance that there would be no pathology found on MRI.

Although CT is more widely available in the acute situation, to detect acute causes of seizures (e.g. meningoencephalitis, venous sinus thrombosis), MRI is now the preferred imaging modality for epilepsy(73;211;212), due to its increased sensitivity in picking up remote symptomatic causes of seizures, such as cortical malformations(212). In addition, MRI does not utilise ionising radiation, to which children are more sensitive than adults(213;214). Although we found abnormalities in 5 children whose MRI scan were previously reported as normal elsewhere, their original pre-CSE MRI scans, on re-evaluation by the research team, were not normal and the abnormalities seen on repeat scans had already been present. This would suggest that, in a child with a known abnormality on MRI or with otherwise identified remote symptomatic CSE, an episode of CSE by itself would not be sufficient reason to repeat the MRI. It also suggests that CSE is unlikely to result in additional brain injury in children with pre-existing brain abnormalities.

One limitation of this study is that, as previously discussed, our patient cohort may not be completely representative of the complete population of children with CSE. Our cohort contained a lower proportion of children with acute symptomatic (3.8% v 17.1%; 95%CI for difference 4.8-19.6%) or remote symptomatic CSE (8.8% v 16.5%; 95%CI for difference 2-15%) than the previous epidemiological study of CSE in North London(28) and compared to epidemiological studies of CSE in other populations(29;31). The reasons for this have been previously discussed in Chapter 4, however one implication is that the patients included in our study do not include those with the most severe medical conditions. As these are among the children most likely to have structural abnormalities on neuroimaging this makes our estimate of the yield a conservative one and inclusion of these children would not be expected to alter the overall conclusion.

It is difficult to comment on how the MRI findings affected the clinical management of these children, as responsibility for this remained with the referring hospitals. We did however find that an abnormal MRI scan was significantly associated with poorer cognitive outcome at 1 year. This suggests that the MRI findings detected were of functional significance to the children. Reports from each scan were provided to the responsible clinician looking after each child and our classification system was defined that any major abnormalities found would have implications for further management and prognosis, if found in a previously imaging-naïve child. It is also important to remember however that a normal MRI scan may provide reassurance to families.

In this chapter we have discussed the clinical findings at MRI following CSE. Based on our findings, combined with those of previous studies, we suggest that MRI should be considered in the follow-up of children after an episode of CSE as a sizable proportion of children will have structural brain abnormalities. The findings have the potential to provide important diagnostic and prognostic information as well as directing therapy. The children most likely to have an abnormal scan are those with a persistent abnormal neurological examination and those who have had a continuous seizure lasting longer than 30 minutes, while children who can be identified as having had a PFS are less likely to benefit from routine neuroimaging. Whether CSE may lead to structural changes in the brain in the longer term, such as mesial temporal sclerosis will be addressed in the following chapters.

CHAPTER 6: HIPPOCAMPAL VOLUMES

6.1 Introduction

Although MTS can be diagnosed by a skilled observer on visual inspection of suitable MRI sequences(215), reliable detection in a clinical setting requires a unilateral reduction in hippocampal volume of over 30%(116). In the previous chapter we established that only one of the children in our cohort had MTS on visual inspection of their MRI scan, and in that child it pre-dated their episode of CSE. As our hypothesis proposes that MTS is an acquired lesion, developing in children with previously normal hippocampi as a result of CSE-related damage, more sensitive techniques are necessary to detect precursor stages to clinical MTS. Hippocampal volumetry is one method of improving the sensitivity to subtle volume changes over simple inspection and has the additional advantage of allowing the quantitative measurement of longitudinal changes. In the interests of clarity, the results and discussion sections of this chapter will be organised into 4 sections: the first will relate to the assessments of normative hippocampal growth; the second to the cross-sectional analyses of hippocampal volumes; the third to the analysis of hippocampal asymmetry; and the fourth will concentrate on a longitudinal analysis of hippocampal growth.

6.1.1 Technical aspects of volumetry

The sensitivity of hippocampal volumetry to true changes in hippocampal volume will be limited by the accuracy of these measurements. While a variety of automated techniques for measuring hippocampal volumes have been developed(181;182;216;217), the majority of these have been designed to process healthy, adult subjects and perform less well in the presence of brain pathology(218) or in younger children(219;220). Therefore to date the gold standard remains manual tracing of the hippocampus and measurement of the resulting region of interest (ROI).

Detailed descriptions of how to delineate the hippocampus have been given by a number of sources(183). While slight differences in anatomical landmarks and scanning sequences used between different studies(184), as well as idiosyncrasies of individual MRI scanners mean that absolute values for hippocampal volumes may not be comparable between studies, it has been shown that relative values can be obtained with good consistency from an individual centre using a well-defined method(185).

Although most of the widely accepted protocols predominantly use the coronal plane to define regions of interest, more recently simultaneous views of all 3 orthogonal planes has been reported to give increased accuracy and reproducibility(221). For the purposes of this study, a representative approach was adapted from the previously published literature (182;184;219;222-226). Particular emphasis was placed on those studies that featured a paediatric population and that included measurements of inter/intra-rater reliability.

6.1.2 Hippocampal growth

One complication of working with a paediatric population is allowing for growth. Over the age range included in our study (1 month – 18 years), brain size as well as hippocampal volume would be expected to increase and then plateau as children reach their full adult growth. In order to compare children who will, of necessity be of varying ages and sizes, this effect needs to be accounted for. Because of the technical and ethical difficulty in obtaining MRI scans in normal children there have been few studies of normal hippocampal development, and normalised growth curves are not available. However those studies that have been performed indicate that growth is rapid in the first few years of life and slower or static thereafter:

- Using a small sample of 6 children, aged between 14 and 60 months, who were having MRI for posterior fossa/spinal tumours, Obenaus et al(226) showed that hippocampal volume increases approximately linearly over this age range, and that intracranial volume is the most appropriate normalisation measure.
- Between that ages of 4 and 18 years, Gogtay et al(227) found no significant increase in hippocampal volume with age in a longitudinal study of 31 healthy children, suggesting that the majority of hippocampal growth occurs before 4 years age. They did show alterations in localised hippocampal morphology with age, implying that developmental maturation may still be occurring, without significant changes in absolute volume.
- Utsunomiya et al(224) studied 42 Japanese children aged from 3 weeks to 14 years with normal neurology who were having MRI scans for various clinical reasons but did not show any intracranial pathology. They showed an initial rapid growth phase in the first two years of life, followed by slower growth throughout the rest of childhood.
- Similarly Knickmeyer et al(228) found a 13% increase in hippocampal volumes between the ages of 1 and 2 years in their cohort of 13 children using automated segmentation techniques.

- Another study of 50 children between 1 month and 15 years having MRI for various clinical reasons but without epilepsy and without macroscopic structural brain lesions by Pfluger et al(229) used a sigmoidal function to model hippocampal growth, but found that the initial slow phase of growth predicted from this function occurred during the pre-natal period. They also demonstrated initial rapid growth in the first 2 years of post-natal life with plateauing of growth thereafter.

In order to assess the effect of CSE on hippocampal growth therefore, it will be necessary to construct a model of normal growth using our healthy control population.

6.1.3 Objectives

The objectives of this part of our project are: firstly, to establish normal hippocampal volumes in a healthy control population and use this to construct a model of normal hippocampal growth; secondly to longitudinally measure hippocampal volumes in a cohort of children following CSE in order to establish any deviation in the normal growth trajectory associated with the episode of CSE; thirdly to ascertain the impact aetiology has on these factors.

6.2 Methods

6.2.1 Hippocampal volumetry

Hippocampal volumetry was performed using the 3D-FLASH MRI sequence detailed previously. This sequence provided T1-weighted images with isometric voxels at a resolution of $1mm^3$. MRIcro (Rorden, GeorgiaTech Centre for Advanced Brain Imaging) was used to re-orient and re-slice the 3D-FLASH images perpendicular to the long axis of the hippocampus. Each hippocampus was then manually segmented with a region of interest using MRIcroN (Rorden, GeorgiaTech Centre for Advanced Brain Imaging).

The following anatomical rules were used to define the boundaries of the hippocampus (adapted from Hammers et al.(182)):

1. The hippocampus was visualised in 3 orthogonal planes using MRIcroN and then this used to guide placement of the ROI.

2. Initial definition of the ROI was done on successive coronal slices. Once the posterior extent of the hippocampus was reached then axial and sagittal views were used to further refine the ROI.
3. The anterior border of the hippocampus was defined as the most anterior coronal slice where the temporal horn starts to widen and comes to sit alongside the hippocampus
4. The posterior border was taken to be the last coronal slice on which the fornix is visible separate from the crus.
5. In the anterior section the amygdala was used to define the superior border of the hippocampus.
6. Where visible the alveus was identified and used to define this border. More posteriorly the hippocampus was defined by its border with the lateral ventricle.
7. The inferior border was taken to be variously the parahippocampal gyrus; uncal sulcus; interface of the prosubiculum and cornu ammonis; border between subiculum, and praesubiculum; or sulcus hippocampalis
8. Medially the hippocampus was taken to be bordered by the parahippocampal gyrus or cerebrospinal fluid and laterally by the lateral ventricle or overlying white matter

The volume of each ROI was measured and recorded separately using MRIcroN and each ROI file saved for use in further processing steps. An example ROI is shown in Figure 6-1.

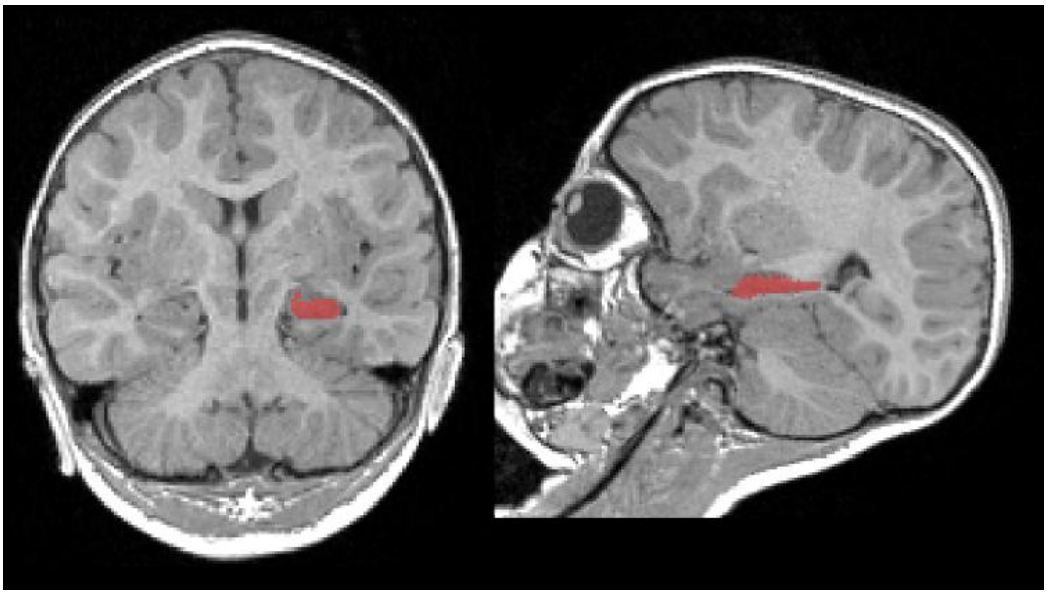


Figure 6-1: Example hippocampal region of interest

6.2.2 Intracranial volume

Intracranial volume was calculated using the Brain Extraction Tool (BET) in FSL to remove the skull and overlying tissues from the 3D-FLASH images. The initial parameters used were:

```
bet <input> <output> -f 0.6 -R -o
```

The output image was then reviewed for errors and if the segmentation was inadequate then settings were adjusted to achieve an optimal result. For some children who were either below the age of 4 months at time of scan or who had significant structural abnormalities, such as previous ventriculo-peritoneal shunt insertion, BET was unable to fully remove the skull without also excluding significant portions of brain. In these cases the best achievable result was taken from BET and then the mask was manually adjusted in *fslview*.

fslstats was then used to calculate intracranial volume (ICV) by summating all voxels in the segmented image using a threshold intensity of 30.

6.2.3 Validation

Hippocampal measurements were performed independently by 2 different observers (MM and MY) on separate occasions, blinded to the clinical status of the child. As scans were collected over time, volume measurements were performed at different times, therefore at each point some previously measured scans were included to check for any drift in measurements. Each hippocampus therefore had between 2 and 4 measurements recorded by 2 different observers. Some drift was seen with the final batch of hippocampi measured, with lower age-adjusted hippocampal volumes reported by one observer and therefore increased CoV. These scans were therefore all reviewed and compared to previous segmentations. All hippocampi with overall CoV greater than 12.5%, representing substantial disagreement between observers, were chosen for re-measurement and the initial ROIs discarded.

ROIs were used to calculate inter/intra-rater reliability measurements for each hippocampus. An overall, inter-rater and intra-rater coefficient of variation (CoV) was calculated for each hippocampus and averaged over all the scans. A random subsample of 200/420 hippocampi

also had inter-rater overlap ratios (OvR)(230) and similarity indices (SI)(231) calculated to provide additional metrics for comparison (Figure 6-2).

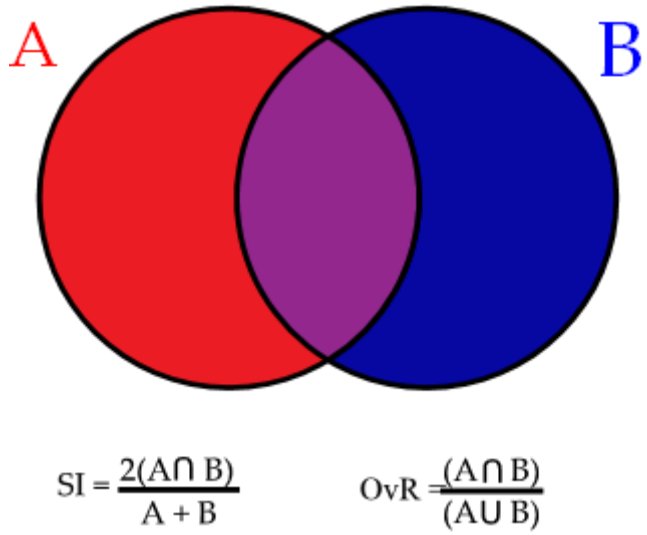


Figure 6-2: Similarity Index and Overlap Ratio

6.2.4 Statistical analysis

6.2.4.1 Cross-sectional analysis

All measurements for each hippocampus were averaged to provide mean left and right hippocampal volumes for each MRI scan, which were used for this and subsequent analyses. Control hippocampal volumes were then plotted against age and ICV and step-wise linear regression was used to test for possible relationships between hippocampal volume, age, ICV and gender. Pearson's correlation was used to test for a relationship between ICV and hippocampal volumes as well as ICV and ln(age). Cross-sectional analyses to compare each aetiological group (control children, children with PFC, children with symptomatic CSE and children with other CSE) at each time point were performed using univariate ANOVA with appropriate corrections for covariates. Post-hoc tests with Bonferroni corrections for multiple comparisons were used to compare volumes between each aetiological group-pair.

6.2.4.2 Hippocampal asymmetry

An asymmetry index (AI) was calculated for each scan according to the formula (where HV stands for hippocampal volume):

$$AI = \frac{2\ Left\ HV - Right\ HV}{Left\ HV + Right\ HV}$$

This was then compared between groups at each time point using a Kruskall-Wallis 1-way ANOVA and Mann-Whitney U to perform post-hoc comparison of individual pairs of groups. Moses extreme reactions tests were used to compare each patient group with controls to assess outliers. Bonferroni corrections for multiple comparisons were applied as appropriate.

6.2.4.3 Longitudinal analysis

To assess hippocampal growth, several models of normal growth were tested using linear/non-linear regression against control values. The model with the best fit was used to generate predicted hippocampal volumes for each patient. Predicted mean growth rates over the study period were also generated using the patient's mean age and differentiating the model equation. Linear regression was then used to estimate the actual hippocampal growth in each patient over the study period and then the predicted growth was subtracted from this to give an estimate of the difference from expected growth seen. All patients who appeared to show lower than expected hippocampal growth were reviewed and had their hippocampi re-measured in order to improve the precision of the growth estimate. These repeat measurements were done by the original observers (MM and MY), blinded to the time point of each scan. Patients who showed an estimated hippocampal growth with the upper bound of the 95% confidence interval below zero after this procedure were considered to have definite reductions in hippocampal volume over the study period. Linear and logistic regressions were used to explore the influence of baseline clinical factors on hippocampal growth.

6.2.4.4 Hippocampal volumes and cognition

The impact of hippocampal growth and volume on cognition was assessed using univariate linear regression to compare cognitive scores at baseline and 1 year to age-adjusted hippocampal size, ICV and hippocampal growth rates. Variables with $p < 0.10$ were then entered into a step-wise regression analysis, with criteria of $p < 0.05$ for entry and $p > 0.10$ for removal.

6.3 Validation studies and normative results

6.3.1 Results

6.3.1.1 Validation studies

A summary of the different validation measures that were used to estimate reliability is given in Table 6-1. Overall reliability of hippocampal volume measurements was acceptable, with both inter/intra-rater CoV falling within the middle of the range of reported values from a sample of published studies (Table 6-2). Given the heterogeneous nature and young age of our cohort, this was considered acceptable to proceed with further analysis.

Overall CoV	Inter-rater CoV	Intra-rater CoV (MY)	Intra-rater CoV (MM)	Similarity Index	Overlap Ratio
6.39%	6.27%	5.19%	7.88%	0.798	0.685

CoV = standard deviation of volume measurements/mean volume

Table 6-1: Overall, inter and intra-rater coefficients of variability for hippocampal volume measurements

Study	Number of scans	Age range	CoV	SI	OvR
Jack et al (1990)(185)	10	Adults	8.7%	-	-
Pfluger et al (1999)(229)	50	1mth – 15 yrs	4.9%	-	-
Pantel et al (2000)(221)	15	18-21 yrs	-	-	0.71 – 0.73
Obenaus et al (2001)(226)	5	1-5 yrs	13%	-	-
Hammers et al (2008)(219)	5	2-3yrs	9.0%	0.91	0.84

Table 6-2: Previously reported inter-rater reliability measures

6.3.1.2 Normal hippocampal and intracranial volumes

Left and right hippocampal volumes and ICV appeared to have an exponential relationship with age in controls (Figure 6-3).

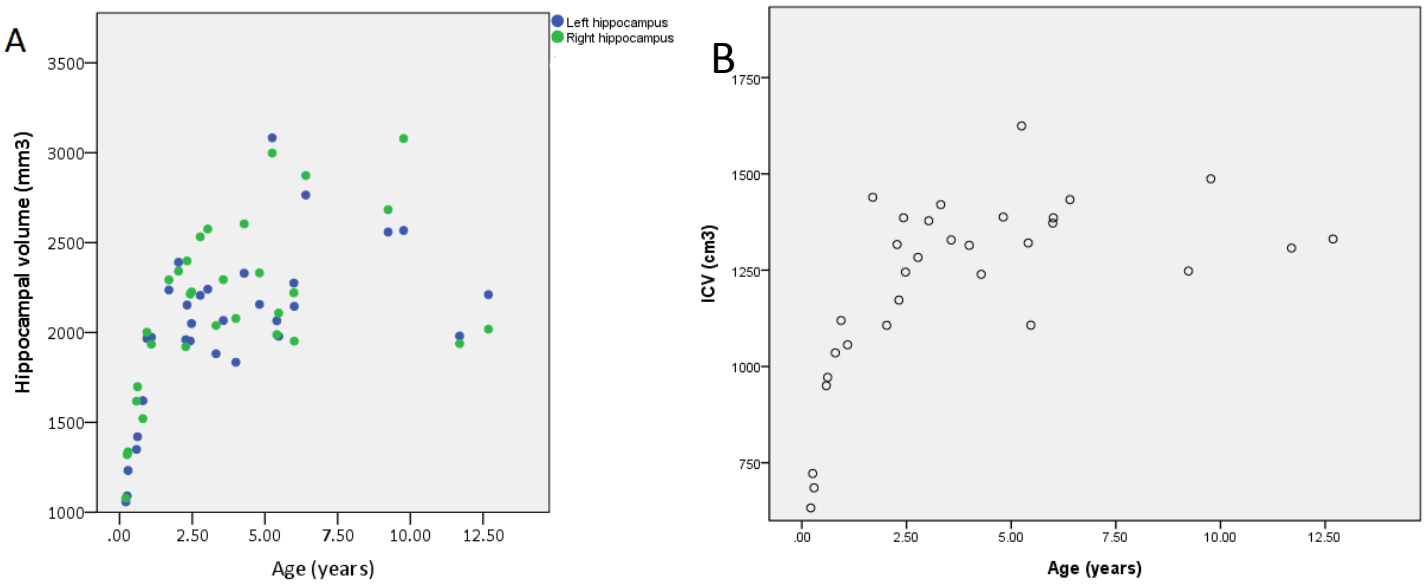


Figure 6-3: Left/right hippocampal volumes (A) and intracranial volume (B) in healthy controls with age

Both left and right hippocampal volumes were significantly correlated with ICV for controls ($p < 0.001$) and with ln(age) ($p < 0.001$), however only ICV emerged as an independent predictor on

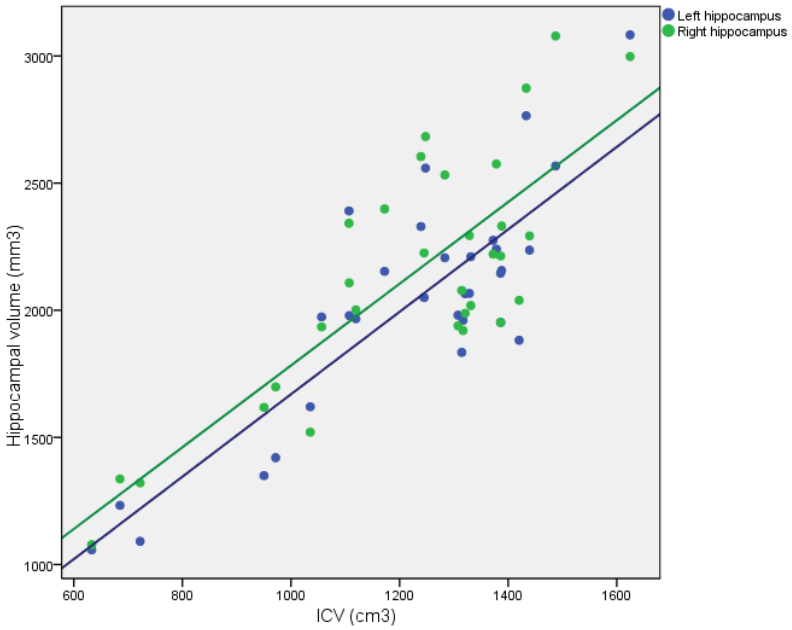


Figure 6-4: Left and right hippocampal volumes by ICV

multivariable linear regression. Figure 6-4 shows left and right hippocampal volumes plotted against ICV with regression lines. We did not find a significant relationship between gender and hippocampal volume.

There were significant differences in ICV at initial scan between each patient group (Figure 6-5) after adjusting for age (p = 0.001). Post-hoc tests with Bonferroni correction for multiple comparisons showed that children with symptomatic CSE had age-adjusted ICV approximately 110cm^3 lower than controls (p = 0.030) and approximately 152cm^3 lower than children with PFS (p = 0.001) but were not significantly different from children with other CSE (p = 0.057). There were no significant differences in ICV between the other 3 groups.

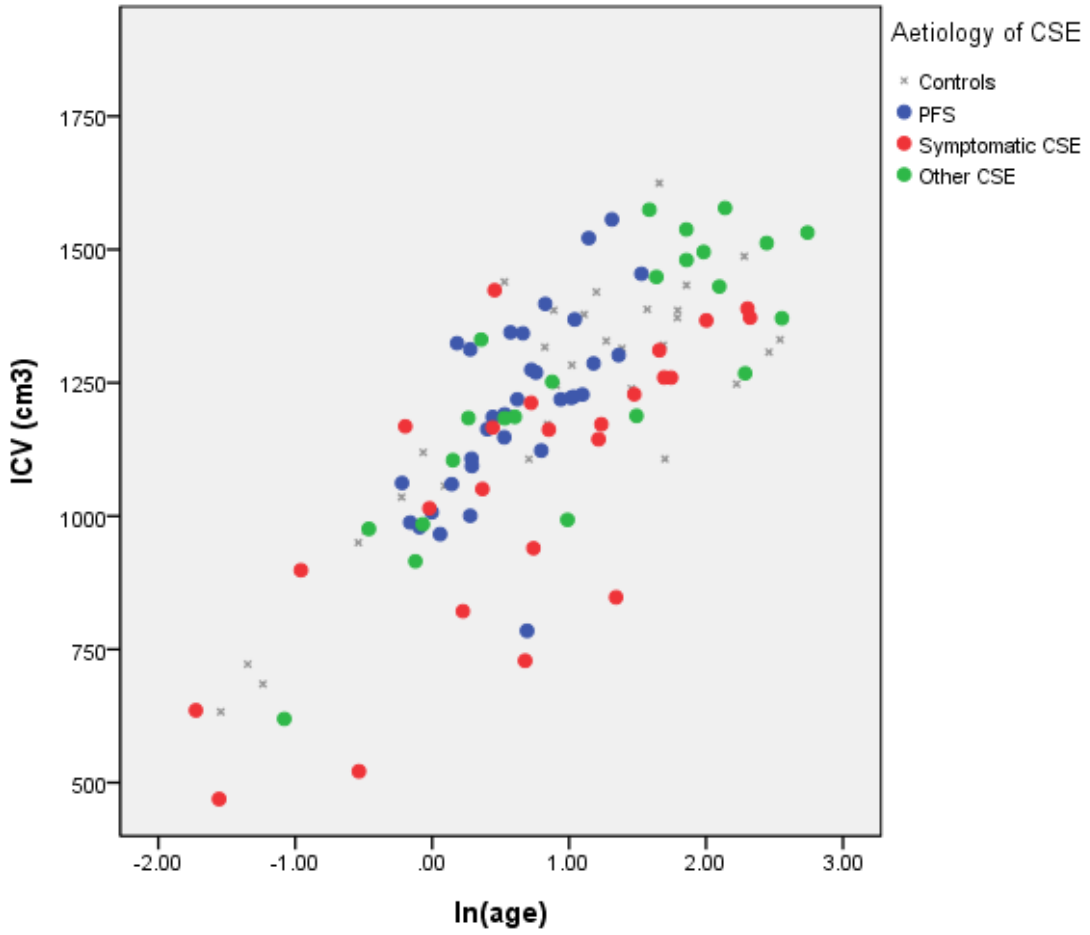


Figure 6-5: ICV by age for patients and controls at initial MRI scan

6.3.2 Discussion

Accurately modelling hippocampal volume and growth is necessary to fully assess hippocampal volume changes post-CSE. Because technical variations in methodology between

studies can result in different absolute measurements of hippocampal volume, it is necessary to create a unique model for any given set of experimental conditions. Measurement of hippocampal volume in our control cohort enabled the creation of such a model for this study, allowing comparisons with normal hippocampal volume and growth to be conducted in subsequent analyses.

Our findings that hippocampal volumes are proportional to intracranial volumes are similar to the conclusions of Obenaus et al(226). We also found that right hippocampal volumes were higher than left hippocampal volumes in the majority of children, again in line with earlier studies (229).

Both ln(age) and ICV were found to strongly correlate with hippocampal volume, but when both were entered into the same model, only ICV remained predictive. ICV was therefore chosen for use as a covariate in the subsequent analysis due to its stronger association with hippocampal volume. As children with symptomatic CSE had a higher incidence of previous brain injury and structural abnormality, it was not surprising that this was reflected in a reduced ICV compared to controls.

Normal hippocampal growth rates have been shown to approximate an exponential curve in childhood (229) so an exponential model was used to fit a growth curve to our control data and generate expected growth rates for later comparison. It was further assumed that hippocampal growth over this age group is always positive (i.e. the hippocampus does not shrink in healthy children under the age of 16), which was in accordance with the growth rates predicted by our model. This meant that any definite loss of hippocampal volume or negative growth in patients within our cohort was considered abnormal.

6.4 Comparison of mean hippocampal volumes

6.4.1 Results

6.4.1.1 Mean hippocampal volumes at 1 month

80 patients had MRI scans suitable for volumetry at their initial scan, a mean of 31.8 days post-CSE (median 29.5). These were compared with 31 healthy controls with no previous history of seizures. Figure 6-6 and Figure 6-7 show actual and predicted left and right hippocampal volumes for each aetiological group. Both left (p=0.023) and right (p=0.009) hippocampal volumes were significantly different between aetiological groups. Post-hoc analysis showed that children with symptomatic CSE had significantly smaller corrected-hippocampal volumes than controls bilaterally (Left p=0.023, mean difference 202 mm^3; Right p = 0.008, mean difference 243 mm^3) and smaller

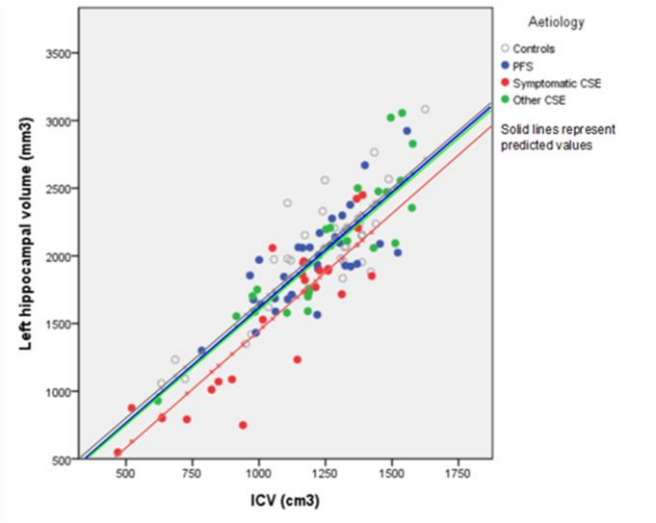


Figure 6-6: Left hippocampal volumes at 1 month post-CSE

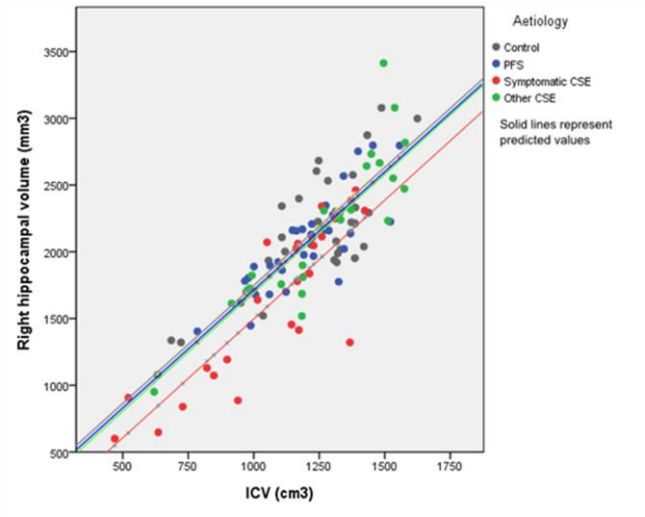


Figure 6-7: Right hippocampal volumes at 1 month post-CSE

right hippocampal volumes than children with PFS (p = 0.034, mean difference 206 mm^3). All other groups were not significantly different from each other. Results are summarised in Table 6-3 below.

Group	Mean left hippocampal volume (mm^3)	Mean right hippocampal volume (mm^3)
Controls	1977 (1890 - 2065)	2085 (1991 - 2180)
PFS	1944 (1859 - 2028) ★	2048 (1956 - 2140) ★ ★
Symptomatic CSE	1776 (1674 - 1878)	1842 (1731 - 1953)
Other CSE	1949 (1847 - 2051)	2044 (1933 - 2155)

All hippocampal volumes are given for an adjusted ICV of 1191 cm^3

★= **p < 0.05**

Table 6-3: Mean left and right hippocampal volumes at 1 month post-CSE

6.4.1.2 Mean hippocampal volumes at first follow-up scan

50 patients had MRI suitable for hippocampal volumetry at the first follow-up period. This occurred a mean of 174.8 days post-CSE (median 169.5). As it was not possible to repeat MRI on our control group, the same measurements are used for comparison.

Figures 6-8 and 6-9 show actual and predicted left and right hippocampal volumes for each aetiological group at first follow-up. Right hippocampal volumes were significantly different between groups (p= 0.005) whereas left hippocampal volumes were not (p=0.080). On post-hoc testing there was a trend for children with symptomatic CSE to have smaller left hippocampal volumes than

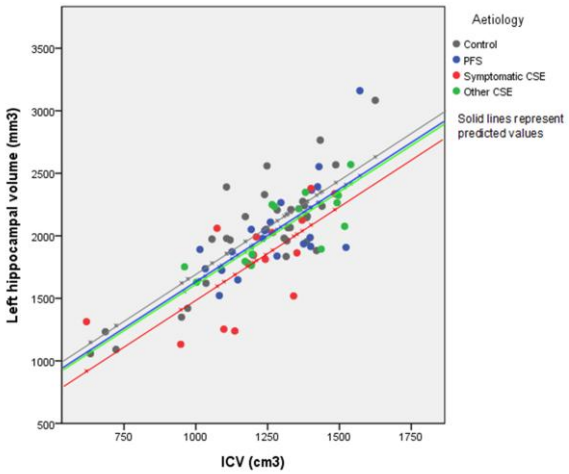


Figure 6-8 Left hippocampal volumes at 6 months post-CSE

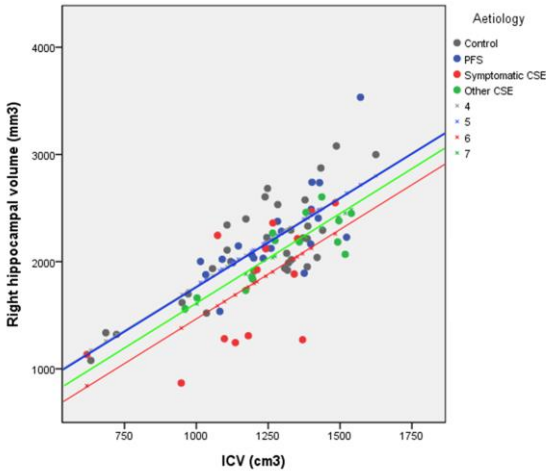


Figure 6-9: Right hippocampal volumes at 6 months post-CSE

controls (p=0.061, mean difference 210mm^3) but this did not reach statistical significance. Right hippocampal volumes were significantly smaller in children with symptomatic CSE compared to both controls (p=0.010, mean difference 309mm^3) and children with PFS (p=0.018, mean difference 316mm^3). There were no significant differences between the other groups. These results are summarised in Table 6-4 below.

Group	Mean left hippocampal volume (mm^3)	Mean right hippocampal volume (mm^3)
Controls	2062 (1970 - 2152)	2175 (2067 - 2284)
PFS	1992 (1882 - 2102)	2182 (2050 - 2313) ★
Symptomatic CSE	1851 (1720 - 1982)	1866 (1710 - 2022)
Other CSE	1975 (1839 - 2111)	2001 (1838 - 2163)

All hippocampal volumes are given for an adjusted ICV of 1244 cm ^{3}

★ = p < 0.05

Table 6-4: Mean left and right hippocampal volumes at 6 months post-CSE

6.4.1.3 Mean hippocampal volumes at final follow-up scan

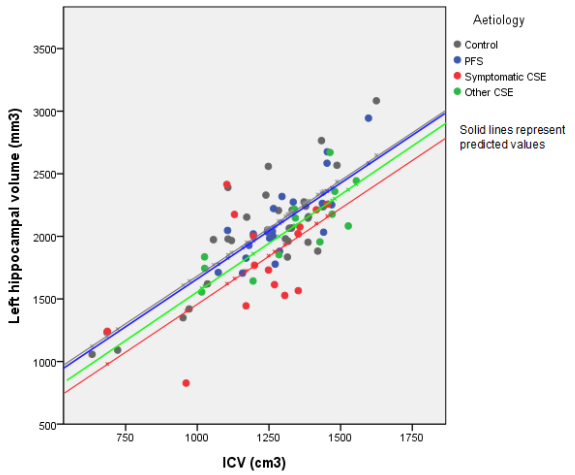


Figure 6-10: Left hippocampal volumes at 1 year post-CSE

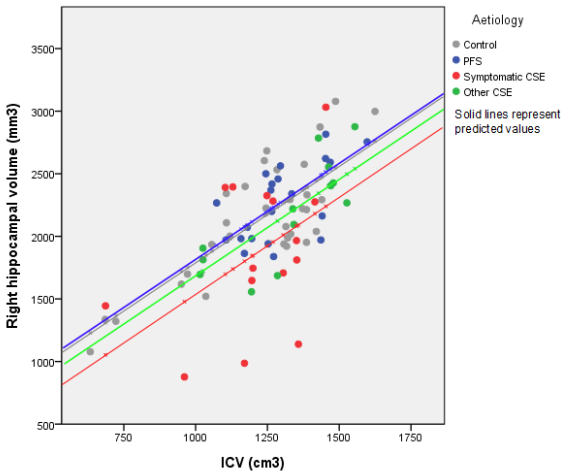


Figure 6-11: Right hippocampal volumes at 1 year post-CSE

49 patients had MRI suitable for hippocampal volumetry at the final follow-up period. This was performed a mean of 396.2 days post-CSE (median 391). Figures 6-10 and 6-11 show left and right hippocampal volumes at 1 year post-CSE. On the final follow-up MRI, Left hippocampal volumes showed a significant effect of group (p=0.036) whereas right hippocampal volumes did not (p=0.065). Post-hoc testing showed that children with symptomatic CSE continued to have smaller left hippocampi than control children (p=0.043, mean difference 224mm^3) but that the difference in right hippocampal volume was not significant (p=0.105, mean difference 258mm^3). These results are summarised in Table 6-5 below.

Group	Mean left hippocampal volume (mm^3)	Mean right hippocampal volume (mm^3)
Controls	2080 (1987 - 2172)	2190 (2068 - 2311)
PFS	2057 (1944 - 2169) ★	2207 (2059 - 2354)
Symptomatic CSE	1856 (1723 - 1989)	1932 (1757 - 2106)
Other CSE	1953 (1810 - 2097)	2076 (1888 - 2264)

All hippocampal volumes are given for an adjusted ICV OF 1255 cm^3

★= p < 0.05

Table 6-5: Mean left and right hippocampal volumes at 1 year post-CSE

6.4.2 Discussion

We found no evidence of group differences in either left or right corrected hippocampal volumes in children with PFS and healthy controls during the year following initial episode of CSE on cross-sectional analysis. This finding is in keeping with a previous cross-sectional follow-up study of children with PFS, which have also found no difference in mean left/right hippocampal volumes between children with PFS and controls (160). This implies that the population mean hippocampal volume on either side is not affected by PFS. Our original hypothesis was that long term hippocampal damage is sustained in a proportion of children with PFS. This is not necessarily contradicted by this finding, as it is possible that the subset of children affected is not great enough to significantly alter the population mean, whilst there still being clinically significant volume loss in a small proportion of children. Further investigation of growth rates in individual patients was therefore carried out.

Similar findings were apparent in children with other CSE, with no evidence of group differences in hippocampal volumes compared with controls.

Children with symptomatic CSE did have systematically smaller corrected hippocampal volumes on both left and right sides than healthy controls, although the statistical significance of the difference was borderline at some time points after Bonferroni correction for multiple comparisons. Given that these children also had significantly smaller ICV than controls and a high incidence of structural brain abnormalities, these findings need to be interpreted in context. Many of these children had severe neurological conditions and structural brain abnormalities both within and without the temporal lobe. It is difficult to conclude therefore that the reduction in mean hippocampal volume found was a direct result of CSE, as, apart from two patients with brain damage secondary to bacterial meningitis, the abnormalities were the result of insults that pre-dated the episode of CSE. Hence it is likely that the hippocampal changes found also pre-date the episode of CSE, and reflect an increased vulnerability to the preceding insult(s).

6.5 Hippocampal asymmetry

6.5.1 Results

Figure 6-12 shows the hippocampal asymmetry index at the initial scan and at each follow-up and group values are summarised in Table 6-6. Kruskal-Wallis testing showed that there were significant differences in AI between the groups at the initial MRI (p=0.048) and at first follow-up (p=0.040) but not at the final MRI (p=0.286). Post-hoc testing with Bonferroni correction showed that this was primarily because children with symptomatic CSE displayed significantly greater AI at

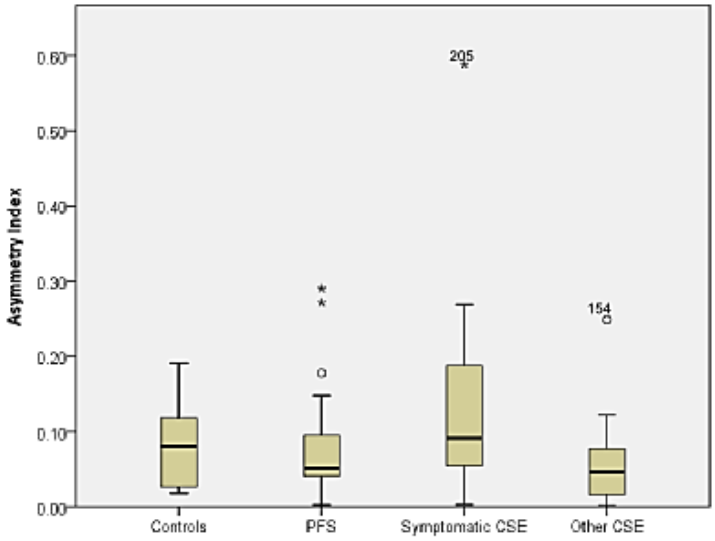
initial MRI (p=0.011) and first follow-up (p=0.021) than children with other CSE. No patient groups showed a systematic difference in AI from controls, although there appear to be a number of outliers from the histograms.

		Controls	PFS	Symptomatic CSE	Other CSE
Initial MRI	Mean	0.082	0.074	0.129	0.058
	Median	0.080	0.051	0.091	0.046
	Range	0.017 – 0.191	0.002 – 0.290	0.003 – 0.588	0.001 – 0.249
First follow-up MRI	Mean	-	0.092	0.148	0.053
	Median	-	0.069	0.147	0.031
	Range	-	0.006 – 0.263	0.005 – 0.503	0.004 – 0.316
Final follow-up MRI	Mean	-	0.097	0.182	0.085
	Median	-	0.072	0.166	0.054
	Range	-	0.014 – 0.279	0.010 – 0.583	0.001 – 0.350

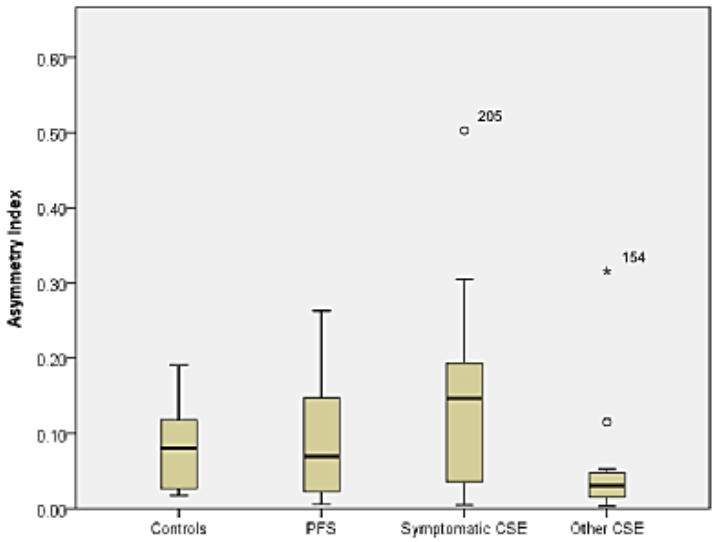
Figure 6-6: Summary statistics for hippocampal AI

Moses extreme reaction tests showed that extreme outliers were significantly more likely in children with PFS compared to controls at initial MRI (p=0.033) and first follow-up (p=0.002), but not at one year (p=0.079) and in children with symptomatic CSE at all 3 time points (p=0.001/0.010/<0.001). Excluding the one outlier described below, children with other CSE showed no increase in outliers at any time point. Histograms for each group at each time-point are shown in Figure 6-13, illustrating the differences in distribution of AI.

A: Hippocampal asymmetry index at ~1 month post-CSE



B: Hippocampal asymmetry index at ~6 months post-CSE



C: Hippocampal asymmetry index at ~12 months post-CSE

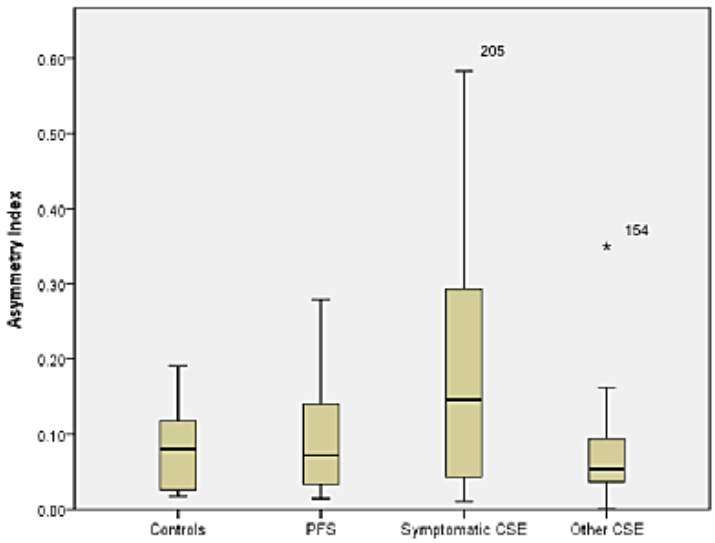
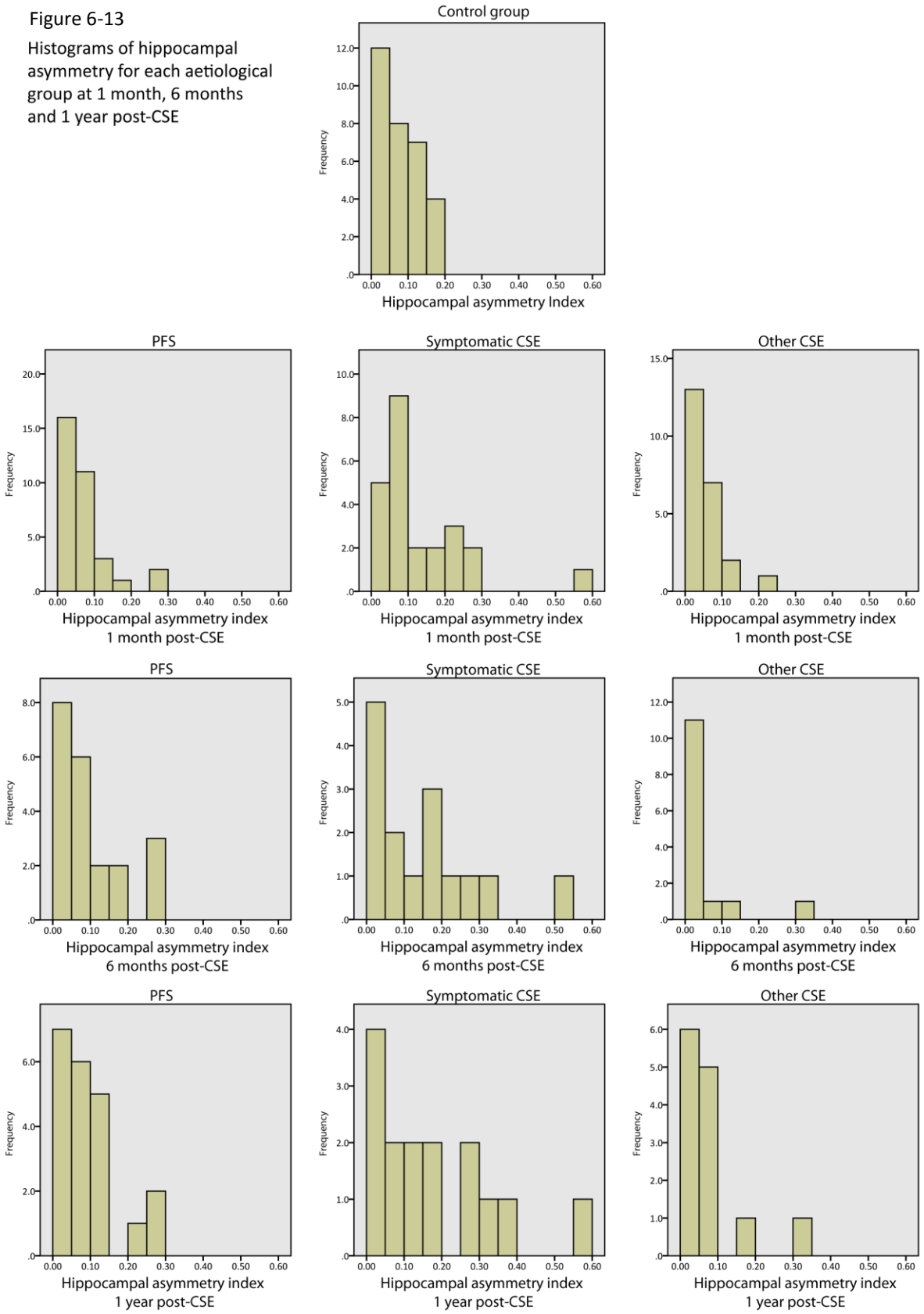


Figure 6-12: Hippocampal asymmetry index at A) 1 month B) 6 months and C) 1 year post-CSE

Figure 6-13

Histograms of hippocampal asymmetry for each aetiological group at 1 month, 6 months and 1 year post-CSE



Identified outliers with high AI on all 3 MRI scans included one patient with symptomatic CSE and a previous diagnosis of right MTS (AI at $1^{st}/2^{nd}/3^{rd}$ scan: 0.588/0.503/0.583); and a patient with other CSE who had a prolonged first afebrile seizure at 8 years but no subsequent seizures (AI at $1^{st}/2^{nd}/3^{rd}$ scan: 0.249/0.316/0.350). MRI was reported as normal at all points and the patient was attending mainstream school and performed above the expected norms on cognitive testing at the beginning and end of the study.

The 95th centile for AI in control children was 0.181, using this as a cut off, 2 children with PFS had a raised AI at their initial MRI scan, one child dropped out of the study after the initial scan and follow-up data was not available, the other child had a gradual reduction in AI to within the range for control children by the final follow-up. 3 children had an initially normal AI which then increased at the first follow-up point above 0.181. One of these was lost to follow-up at 1 year and the other two had subsequent reductions in AI at further follow-up, although one remained above the 95^{th} centile. Finally a further 2 children with PFS had increases above the 95^{th} centile at their final MRI scan, having previously had normal AI.

Raised AI at any time point was not significantly associated with any identified clinical factors on step-wise logistic regression.

6.5.2 Discussion

Asymmetry indices are a powerful and sensitive way to detect unilateral MTS. By choosing a measure that is independent of laterality, assumptions of which side is affected are avoided. As demonstrated by the patient in our sample, MTS presents with high hippocampal asymmetry, and therefore we would expect evolving MTS to be accompanied by a rising AI. However interpreting isolated changes in AI is complicated as there are multiple mechanisms that may increase hippocampal asymmetry: increased growth in one hippocampus; failure of growth on one side with continued growth in the contra-lateral hippocampus; as well as a unilateral reduction in hippocampal volume. Bilateral changes will not be detected with this measure and therefore changes in AI need to be interpreted carefully in the context of other clinical and radiological information.

There is a trend for increased hippocampal AI in children with symptomatic CSE compared to control children, but this does not reach statistical significance at any time point. Furthermore this disappears if the single patient with established MTS is excluded from the analysis. However there are significant increases in the tails of the distribution at all time points when compared to controls,

which remain even when this patient is removed. This suggests that any increase in mean AI is primarily driven by an increase in the number of patients at the extreme end of the distribution and not just by the occurrence of one case of MTS in our sample. Indeed 6/24 (25%) children with symptomatic CSE had an AI above the 95th centile for controls at 1 year.

A previous study of 14 children with PFS by our group found an increase in the extremes of distribution of hippocampal AI 4-8 months after PFS, with 5 children having AI above the 95th centile for controls (46). Similarly in the present study children with PFS were also found to have a significant increase in the tails of the distribution for hippocampal AI compared to controls at 1 month and 6 months post-PFS, however by 1 year this was no longer apparent, although 3/21 (14%) children continued to have hippocampal AI above the 95th centile for controls.

Reviewing the children with PFS who had raised AI at any point, it was apparent that in 3/7 (43%) cases the increase in AI was transient and normalised with time. Only one of these seven children displayed significant hippocampal volume loss (as discussed in Section 6.6) and they all had normal hippocampal volumes. Increases in AI were mostly due to either increased growth on one side or normal growth on one side with static/slower growth on the other. In the 3 cases with subsequent normalisation of AI, the affected side then caught up. Further follow-up was not available on the other 4 cases, so the subsequent trajectory of these patients is unknown; there were no patients with raised AI across 2 or more time points. Given the evidence that AI can normalise on subsequent re-measurement, this needs to be interpreted with caution and may not alone be sufficient evidence to diagnose evolving MTS; however given the normal growth rates of healthy control children at this age, even static growth of one hippocampus is potentially significant.

6.6 Hippocampal growth

6.6.1 Results

6.6.1.1 Normal hippocampal growth

Hippocampal growth was calculated separately for left and right hippocampi using control data. Different regression models were tested against the data and this is presented in Figure 6-14 and 6-15. An exponential model was chosen as the best fit for the data both from theoretical and observational criteria. Values for the constants in the model were derived using non-linear regression and are presented with their estimated standard errors (Table 6-7).

Model: HV = 1000*(a – (a-b)*exp(-c*age))						
	a	**S.E.**	**b**	**S.E.**	**c**	**S.E.**
Left	2.26562	0.06876	0.71187	0.29298	1.18510	0.39590
Right	2.37331	0.07888	0.84182	0.33414	1.17949	0.45688

HV = hippocampal volume; S.E. = Standard Error

Table 6-7: Regression models for left and right hippocampal volumes

The derivative of this model was then used to calculate expected growth rates for each patient, using their mean age over the study period. Overall, no aetiological group had statistically significant deviation from the predicted growth rate over the study period; however this does not exclude MTS being an uncommon outcome of CSE as originally hypothesised. Therefore the growth rates in individual patients were studied in more detail.

In order to minimise the standard errors (S.E.) around our estimates of hippocampal growth rates, especially those estimated from only 2 MRI scans, individual patients who showed large reductions in hippocampal volume were reviewed and their hippocampi re-measured, with the observers blinded to the child's clinical status or the time point the scan was taken at. The aim of this was to improve the precision of our measurements of hippocampal volumes, and hence our growth rate estimates. Those remaining patients who had an estimated rate of change of hippocampal volume with the upper limit of the 95% confidence interval below zero, were defined as showing definite hippocampal volume loss.

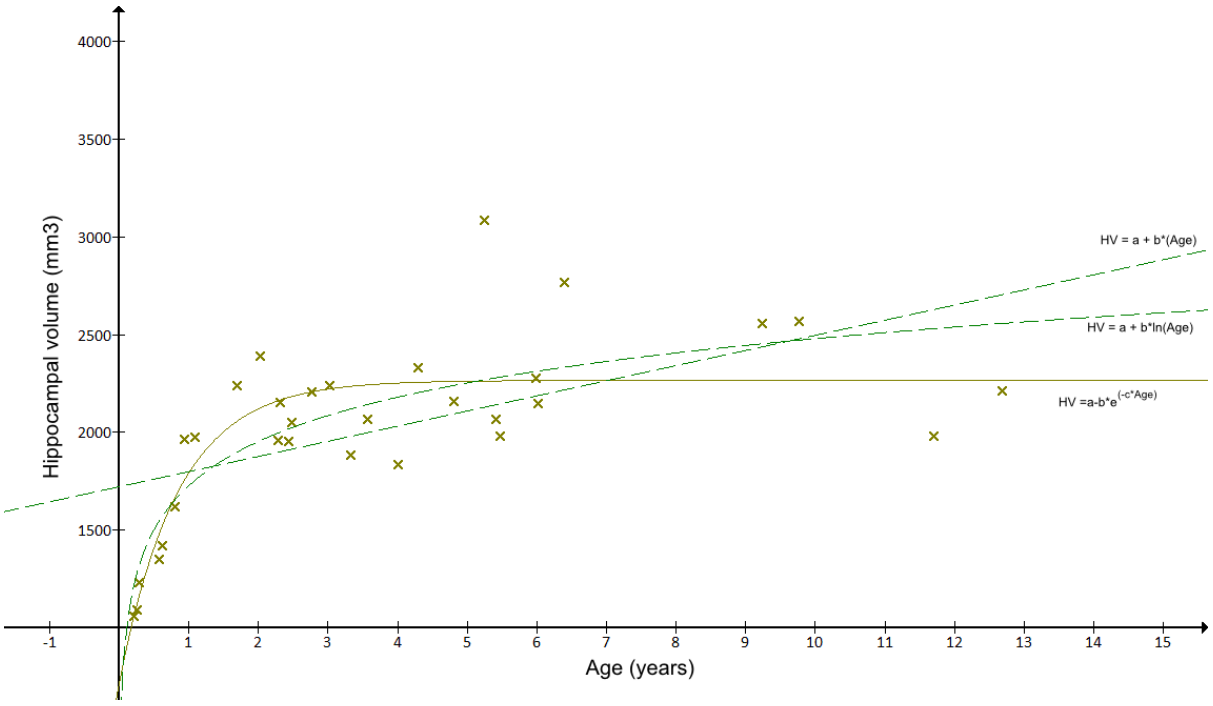


Figure 6-14: Left hippocampal volume by age in healthy controls

HV = hippocampal volume; a, b and c are the constants in the model equations

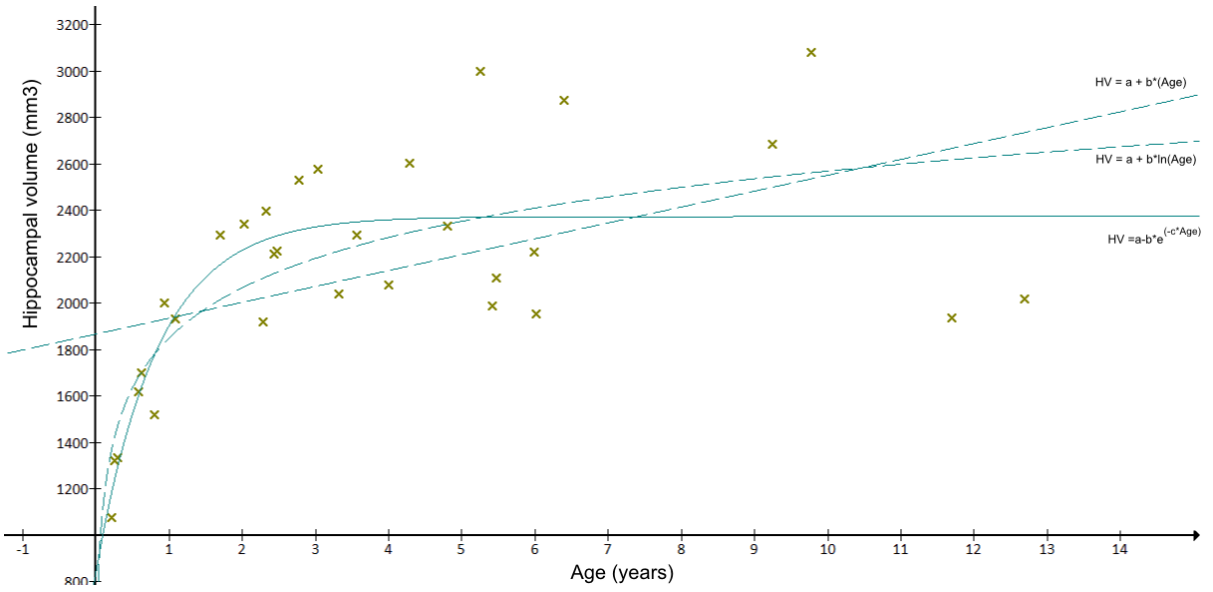


Figure 6-15: Right hippocampal volumes by age in healthy controls

6.6.1.2 Hippocampal growth in children with PFS

26 children with PFS had repeat imaging. Of these 10 successfully underwent MRI on two occasions and 16 attended and tolerated MRI at all 3 time-points. The remaining 7 children with an initial scan declined or failed to tolerate further imaging. There was no significant difference in age, seizure duration, seizure semiology or previous medical history between children who attended for 1, 2 or 3 scans. Hippocampal volumes over time of each patient are plotted in Figure 6-16 (left hippocampus) and 6-17 (right hippocampus) along with the predicted normal growth curve generated from control data and the estimated growth rates are shown in Figure 6-18. Overall the mean growth rate did not differ from that predicted from the control model for either hippocampus. Hippocampal growth did not correlate with any seizure-associated variables (seizure duration, focality, continuous vs. intermittent) on linear regression for either hippocampus.

3 children appeared to have a unilateral reduction in left hippocampal volume over the study period, one had a reduction in right hippocampal volume and one child had a bilateral reduction in hippocampal volumes (red lines on Figure 6-16/6-17). All had generalised tonic-clonic convulsions continuously for at least 30 minutes as part of their PFS. Further clinical details are summarised in Table 6-8.

Patient ID	Age at CSE (years)	Duration (min)	Previous seizure history	Seizure recurrence	Hippocampal changes	Number of scans
16	4.56	69	No previous seizures	No further seizures	Decrease in right hippocampal volume: 240mm^3/yr	3
17	2.96	45	2 previous febrile convulsions, 1 episode PFS	2 further PFS	Decrease in left hippocampal volume: 215mm^3/yr	2
119	1.21	60	2 previous febrile convulsions from 6 months age	1 further short febrile convulsion	Decrease in left hippocampal volume: 197mm^3/yr	2
151	1.61	105	No previous seizures	No further seizures	Small bilateral decreases in hippocampal volume: left 67 mm^3/yr; right 178 mm^3/yr	3
225	2.89	30	2 previous febrile convulsions	No further seizures	Decrease in left hippocampal volume: 660mm^3/yr	2

Table 6-8: Children with hippocampal volume loss following PFS

A further two children were found to have static or very slow hippocampal growth, at an age where our model predicted large increases in hippocampal volume. These are not included in Table 6-8 as they did not show absolute volume losses.

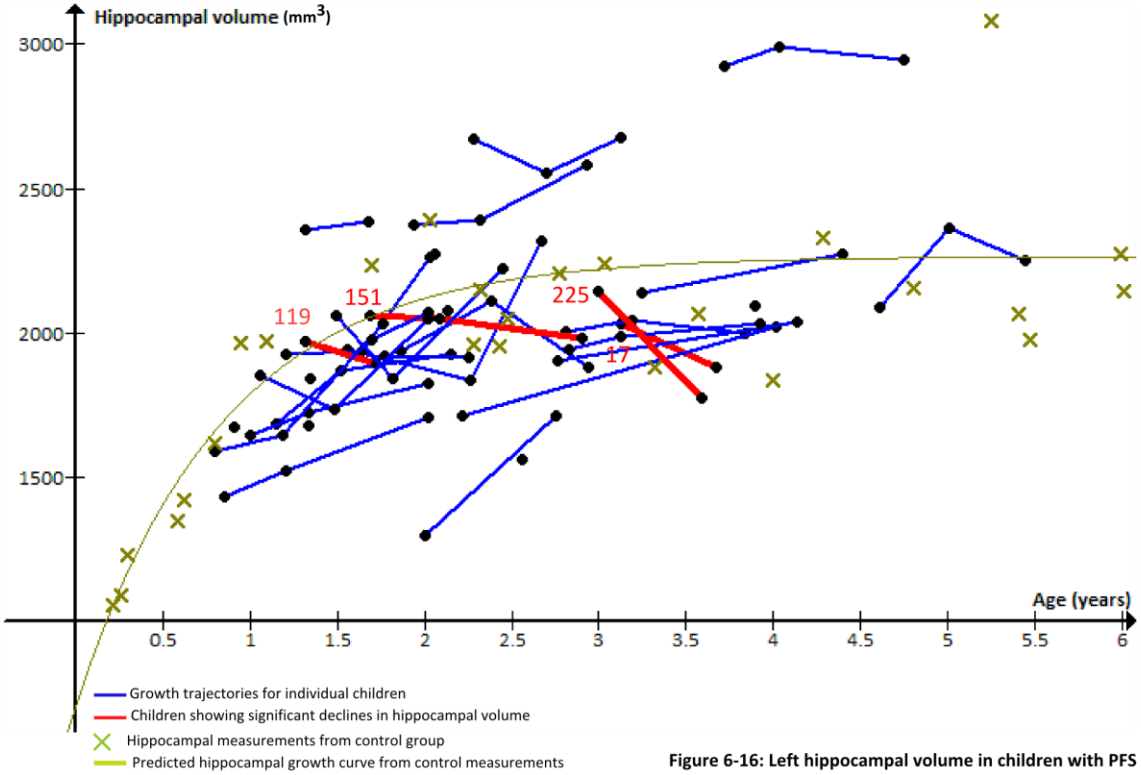


Figure 6-16: Left hippocampal volume in children with PFS

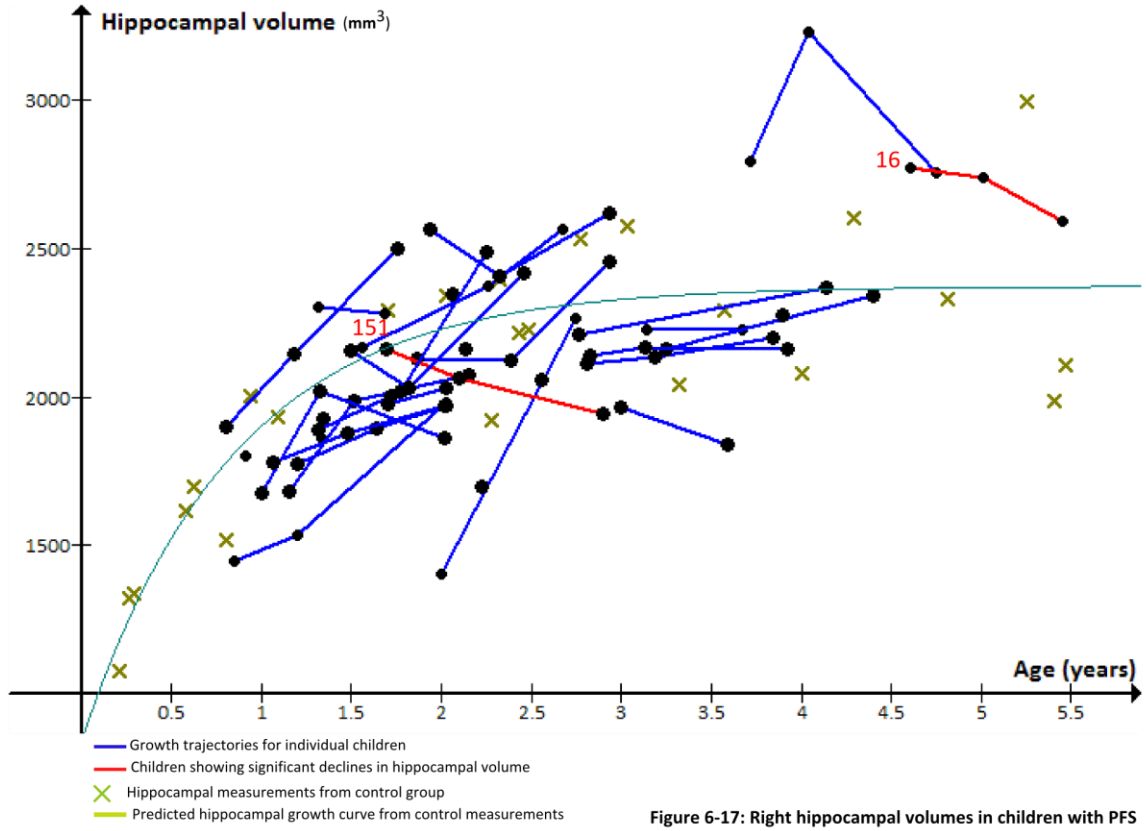
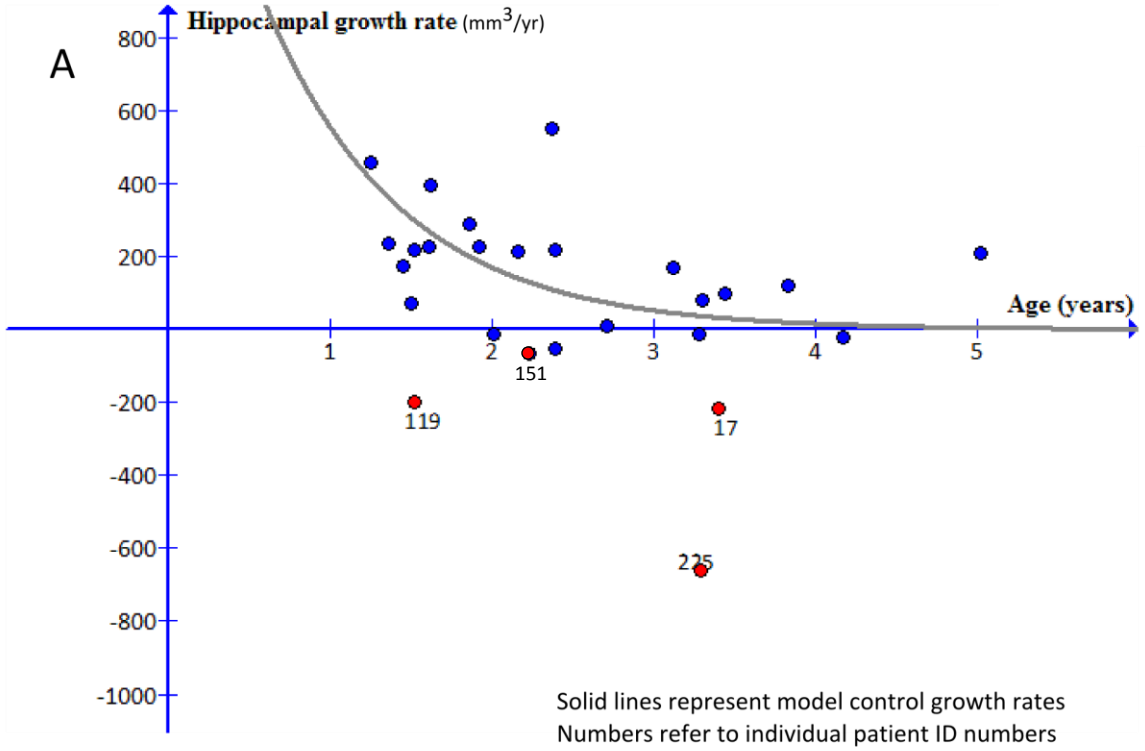


Figure 6-17: Right hippocampal volumes in children with PFS



Solid lines represent model control growth rates
Numbers refer to individual patient ID numbers
Red circles show patients with definite loss of hippocampal volume (growth rate over 1.96 S.E. below 0)

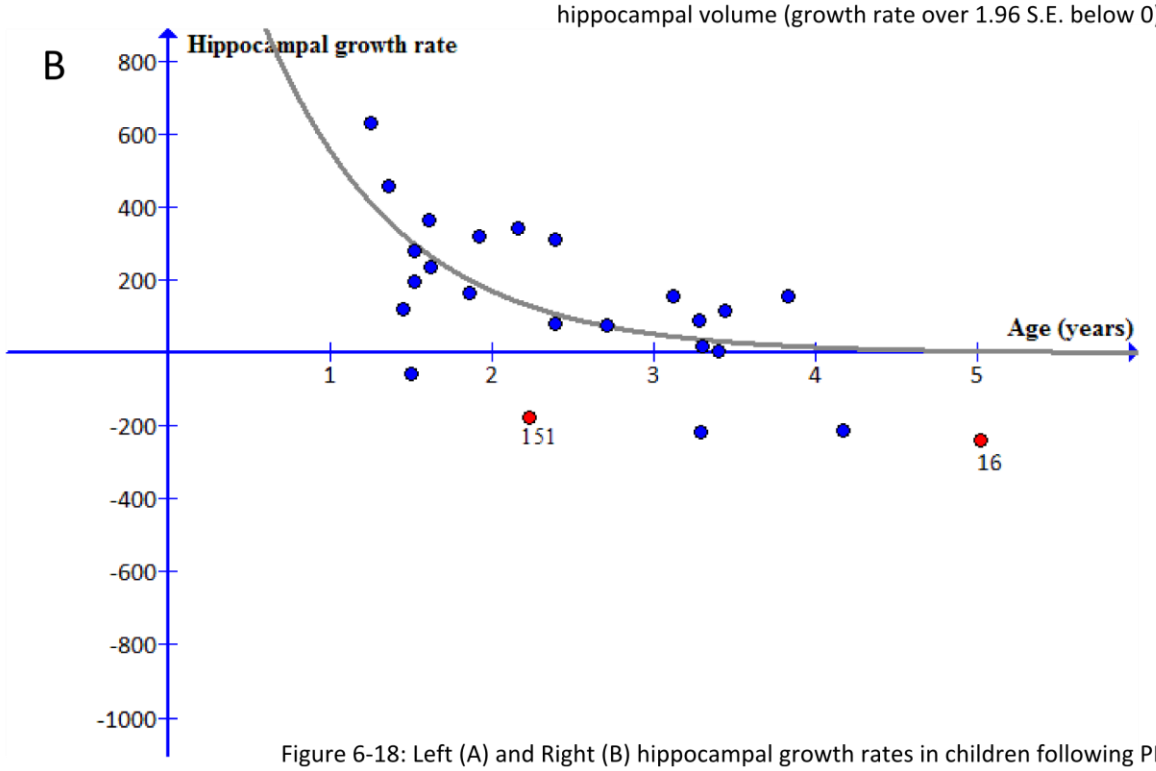


Figure 6-18: Left (A) and Right (B) hippocampal growth rates in children following PFS

6.6.1.3 Hippocampal growth in children with symptomatic CSE

17 children with symptomatic CSE were available for analysis, 13 children had 3 scans available and 4 had 2. The remaining 7 children with an initial scan declined or failed to tolerate further imaging. There was no significant difference in age, seizure duration, seizure semiology or previous medical history between children who attended for 1, 2 or 3 scans, although there was a tendency for more of the older children to re-attend. Hippocampal volumes over time of each patient are plotted in Figure 6-19 (left hippocampus) and 6-20 (right hippocampus) along with the predicted normal growth curve generated from control data and the estimated growth rates are shown in Figure 6-21. Hippocampal growth did not correlate with any seizure-associated variables (seizure duration, focality, continuous vs. intermittent) on linear regression.

One patient, with known right sided TLE-MTS had decreases in both left and right hippocampal volumes and a further 4 children had unilateral decreases in either left or right hippocampi (Blue lines on Figure 6-19/6-20). Further clinical details are given in Table 6-9.

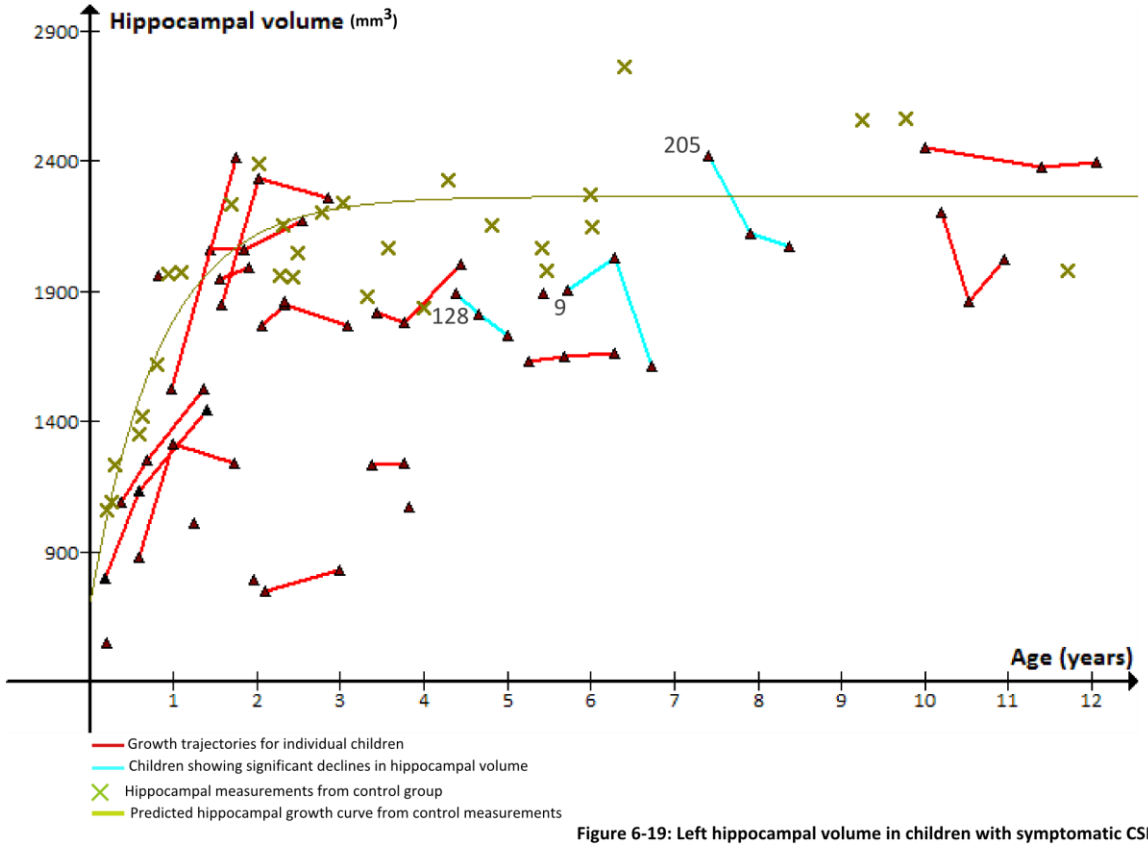


Figure 6-19: Left hippocampal volume in children with symptomatic CSE

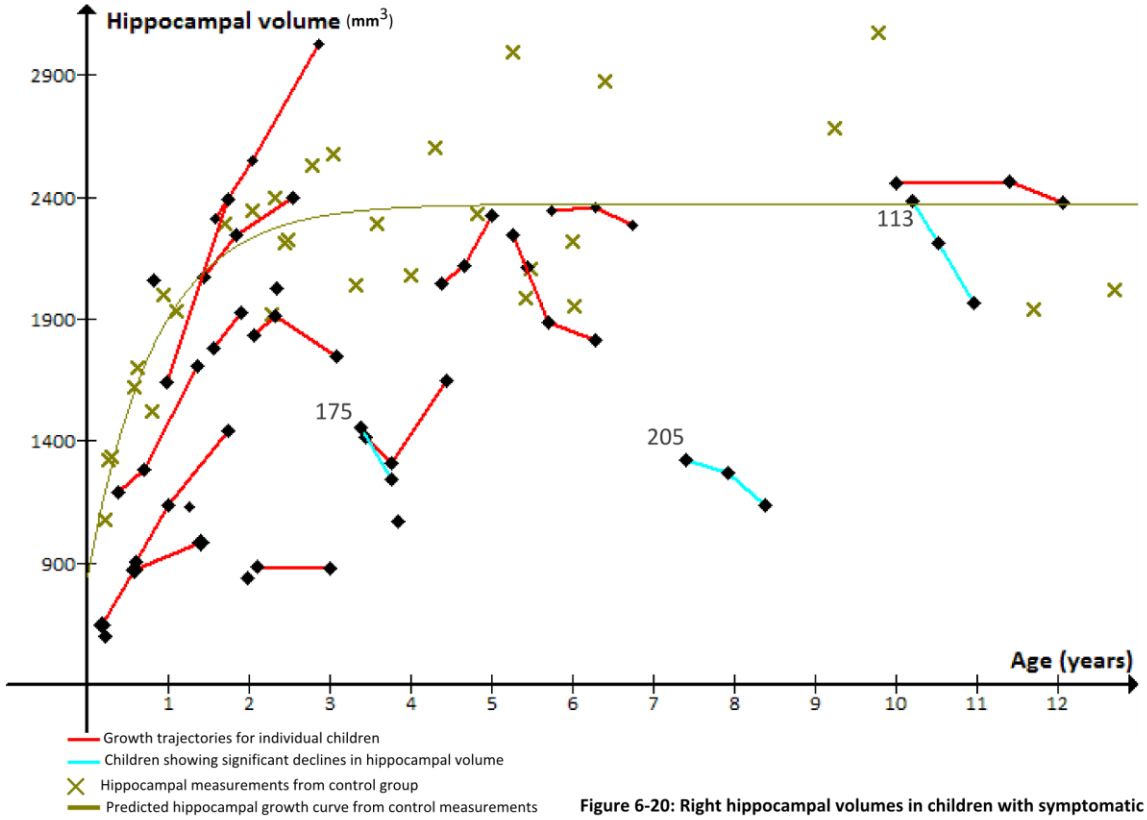


Figure 6-20: Right hippocampal volumes in children with symptomatic CSE

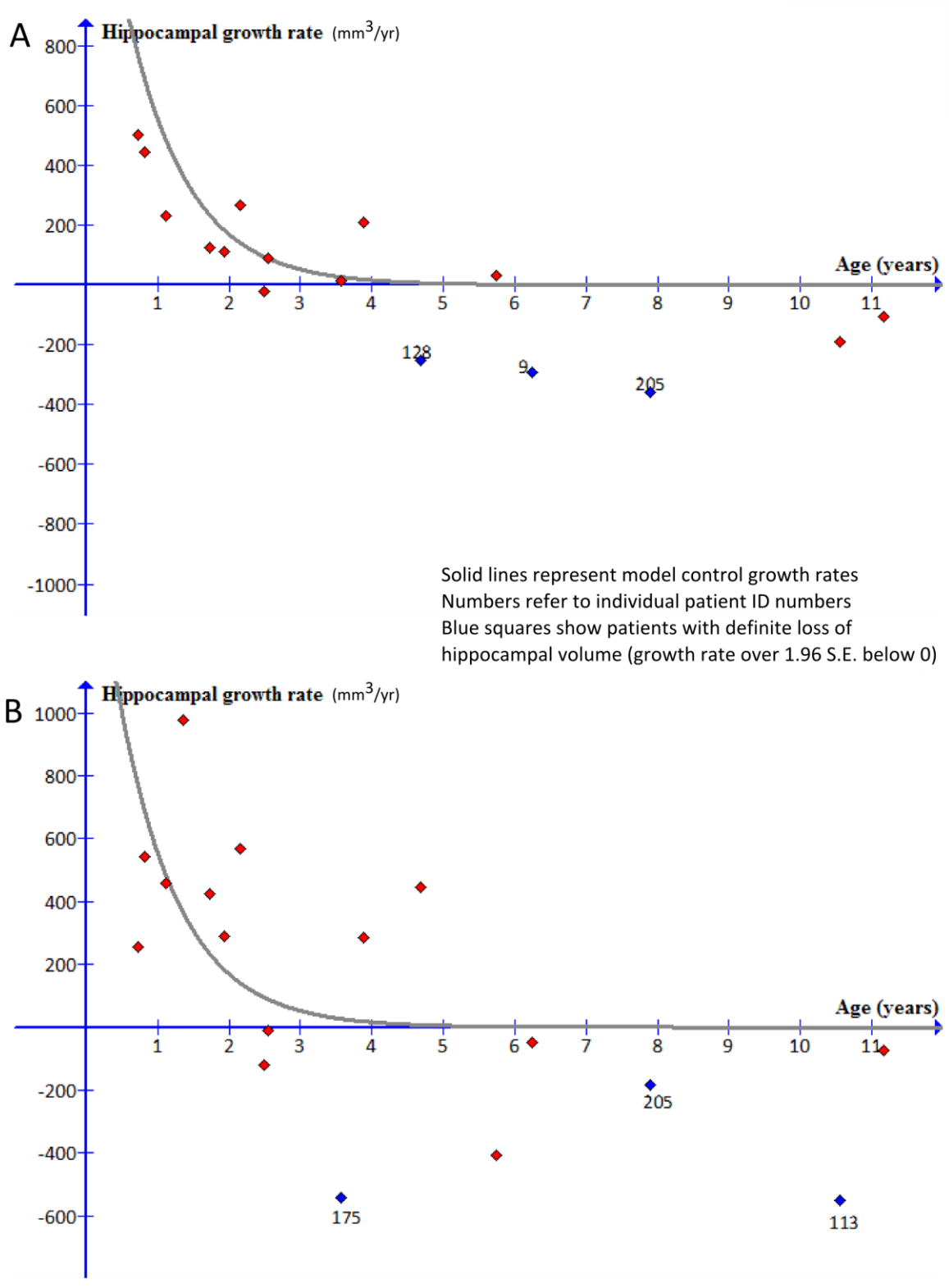


Figure 6-21: Left (A) and Right (B) hippocampal growth rates in children following symptomatic CSE

Patient ID	Age at CSE (years)	Duration (min)	Previous seizure history	Focal	Continuous	Clinical information	Medication	Seizure recurrence	Hippocampal changes	No of scans
9	5.68	30	6 previous short febrile seizures	No	No	Ex-prem 26/40 Previous IVH Developmental delay	None	No further seizures	Decrease in left hippocampal volume 292mm^3/yr	3
113	10.10	215	3 neonatal seizures	No	Yes	Ex-prem, 24/40 Previous IVH Developmental delay	Carbamazepine	Started on medication, no further seizures	Decrease in right hippocampal volume 550mm^3/yr	3
128	4.27	110	Absence seizures since 6 months age, 10 prior episodes of CSE	No	No	Known developmental delay with acute febrile illness	Topiramate	5 absence seizures	Decrease in left hippocampal volume 252mm^3/yr	3
175	3.31	45	Neonatal seizures and then 5 seizures over past 3 months	No	No	Left occipital infraction from neonatal sepsis and haemorrhage	Phenytoin, Sodium Valproate	Weekly short clonic seizures	Decrease in right hippocampal volume 544mm^3/yr	2
205	7.36	30	8 previous CPS	Yes	Yes	Right MTS	None	Monthly CPS	Bilateral decreases in hippocampal volume: left 360mm^3/yr; right 185mm^3/yr	3

CPS: Complex partial seizures
IVH: Intraventricular haemorrhage
MTS: Mesial Temporal Sclerosis

Table 6-9: Children with hippocampal volume loss following symptomatic CSE

6.6.1.4 Hippocampal growth in children with other CSE

17 children with other CSE had repeat imaging. 10 attended for all 3 MRI scans and 7 were imaged twice. The remaining 6 children with an initial scan declined or failed to tolerate further imaging. There was no significant difference in age, seizure duration, seizure semiology or previous medical history between children who attended for 1, 2 or 3 scans, although there was a tendency for more of the older children to re-attend. Hippocampal volumes over time of each patient are plotted in Figure 6-22 (left hippocampus) and 6-23 (right hippocampus) along with the predicted normal growth curve generated from control data and the estimated growth rates are shown in Figure 6-24. Hippocampal growth did not correlate with any seizure-associated variables (seizure duration, focality, continuous vs. intermittent) on linear regression for either hippocampus.

1 child had a bilateral decrease in hippocampal volume over the study period and one child had a unilateral decrease in right hippocampal volume. 2 children had a large decrease in right hippocampal volume and a smaller decrease in left hippocampal volumes, although the confidence intervals surrounding the growth rate of the left hippocampus in both children contained both positive and negative values. A further child showed bilateral decreases in hippocampal volumes, but the confidence intervals again contained both positive and negative values. Children showing decreases are highlighted in red in Figure 6-22/6-23. Further clinical details are given in Table 6-10. There was also one child under 1 year of age, who presented with CSE due to cryptogenic epilepsy and recurrent febrile and afebrile seizures who showed low growth of both hippocampi compared to that predicted by our control model at that age. This child was later found to have a SCN1a mutation and continues to have difficult to control seizures and an increasing cognitive deficit.

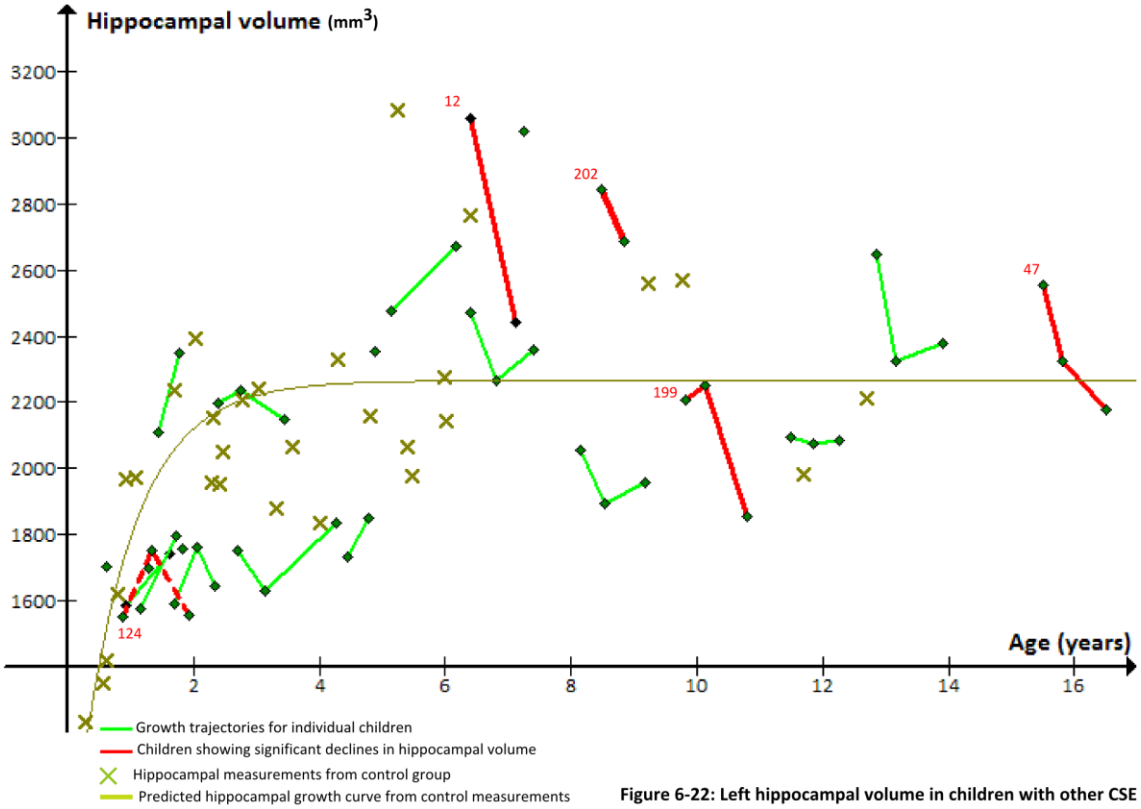


Figure 6-22: Left hippocampal volume in children with other CSE

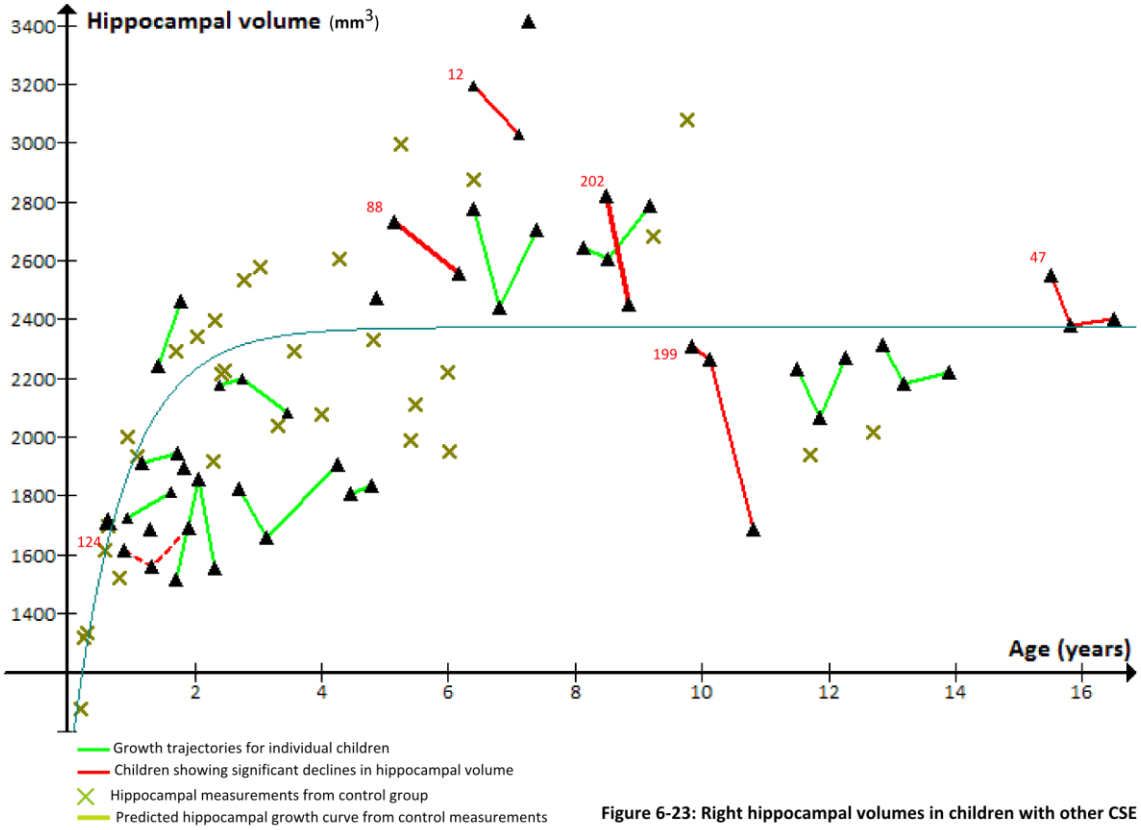


Figure 6-23: Right hippocampal volumes in children with other CSE

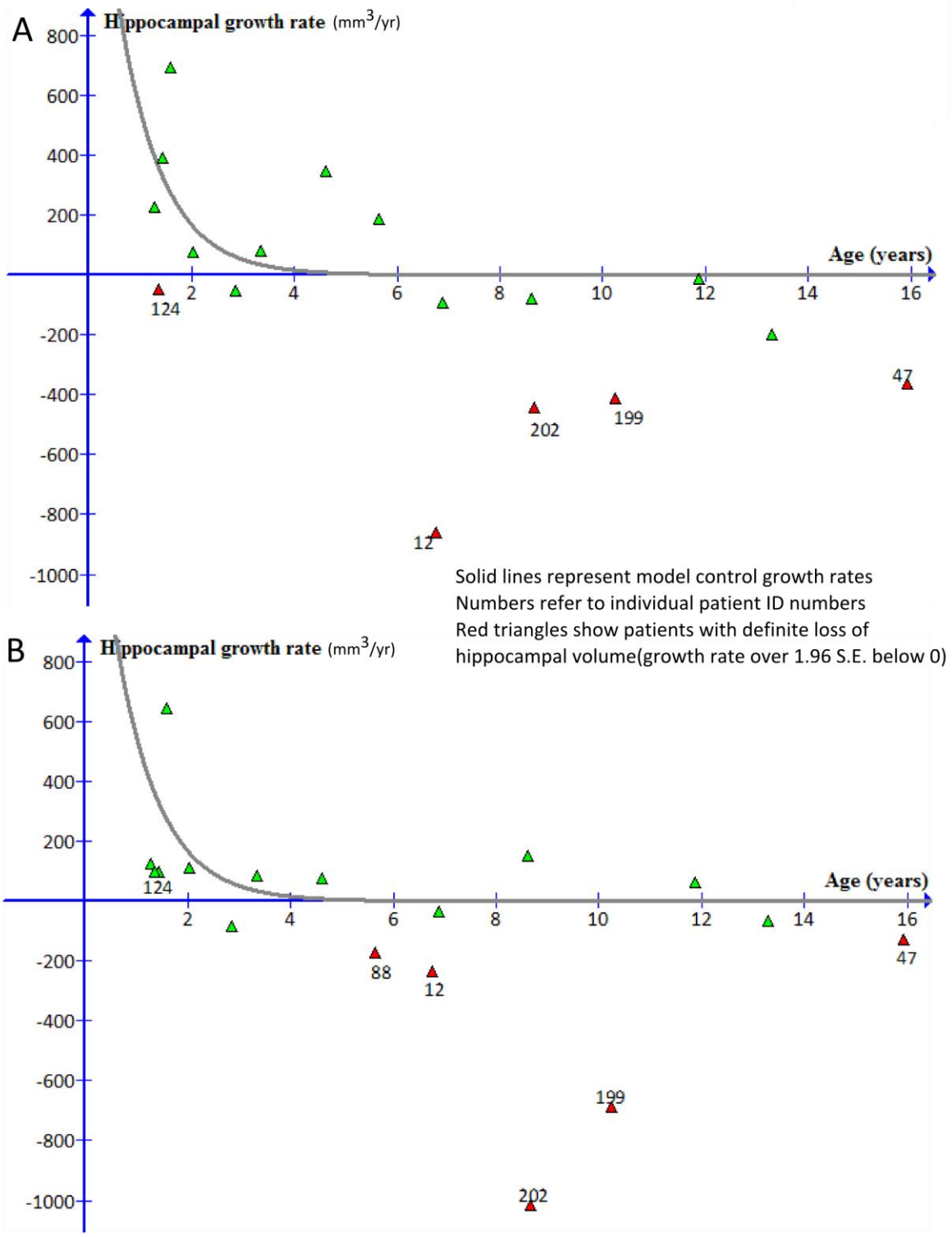


Figure 6-24: Left (A) and Right (B) hippocampal growth rates in children following other CSE

Patient ID	Age at CSE (years)	Duration (min)	Previous seizure history	Focal	Continuous	Clinical information	Medication	Seizure recurrence	Hippocampal changes	No of scans
12	6.34	45	1 previous unprovoked focal seizure	Yes	No	Idiopathic epilepsy	None	Recurrent episode of CSE	Decrease in left hippocampal volume 860mm^3/yr, right 286mm^3/yr	2
47	15.44	103	Daily seizures for past 1 ½ years	No	No	Cryptogenic epilepsy	Sodium valproate Topiramate	Daily recurrent seizures, myoclonic and generalised tonic-clonic	Decrease in left hippocampal volume 360mm^3/yr, right 130mm^3/yr	3
88	5.10	30	Previous PFS followed by 2 subsequent simple febrile convulsions	No	Yes	Idiopathic epilepsy	Phenytoin Carbamazepine	No further seizures	Decrease in right hippocampal volume 174mm^3/yr	2
124	0.86	80	3 previous febrile and afebrile seizures	No	No	Cryptogenic epilepsy SCN1a mutation	Sodium valproate Topiramate	Breakthrough seizures when unwell	Reduced growth of both hippocampi	3
199	9.83	47	None	Yes	Yes	Unprovoked new onset CSE	None	1 further possible seizure	Bilateral decrease in hippocampal volume: left 410mm^3/yr; right 685mm^3/yr	3
202	8	90	1 previous febrile convulsion, previous cluster of clonic seizures	Yes	No	Cryptogenic epilepsy	Sodium valproate	None	Bilateral decreases in hippocampal volume: left 442mm^3/yr; right 1017mm^3/yr	2

Table 6-10: Children showing hippocampal volume loss following other CSE

6.6.1.5 Predictive factors for hippocampal volume loss

Linear regression did not show any significant association between hippocampal growth (left/right) and a number of clinical factors, including: seizure duration (p = 0.150/0.425); focal vs. generalised CSE (p = 0.253/0.124); continuous vs. intermittent status epilepticus (p = 0.941/0.742); previous history of CSE (p=0.150/0.096);and number of previous seizures (p=0.948/0.541). Each factor was entered individually into the regression model (p-values quoted) and in all 2-factor combinations, with no significant associations being found. Logistic regression was also performed using a significant decrease in hippocampal volume as the outcome measure and the same factors as predictors, and similarly failed to show any significant associations.

Regression analysis was repeated on the PFS subgroup alone and showed that the number of previous febrile seizures had a significant association with left hippocampal growth ($p < 0.001$; B = -76.68; 95%CI: -111.16 - -42.20) and that children who showed a definite decrease in hippocampal volume were also more likely to have previous febrile seizures (p = 0.051, OR 1.75; 95%CI: 1.00 – 3.06). No associations were found with other clinical factors. Subgroup analysis of the other groups did not reveal any significant associations (p-values all > 0.1 on univariate linear and logistic regression).

Seizure recurrence during the follow-up periods was also not associated with a definite decrease in hippocampal volume, but there was a borderline association between hippocampal volume loss and repeated CSE (p=0.079, OR: 4.61 95%CI 0.84 – 25.5).

6.6.2 Discussion

Many attempts to evaluate hippocampal volume changes following CSE have relied on single-time point cross-sectional data (45;47;160). This presents inherent limitations when considering a quantity that is changing over time. Analysis of data at any single time point is unable to detect progressive changes in hippocampal volume over time and may cause misleading extrapolations from a single outlying measurement. Since our initial hypothesis linking CSE and TLE-MTS presupposes that the changes occurring in the hippocampus are progressive in nature, longitudinal measurements and analysis of hippocampal volumes are essential to detect volume changes over time.

Only two studies to date have reported longitudinal measurements of hippocampal volumes after CSE in children (46;159). In both these studies, which only considered children with PFS, initial hippocampal volumes were measured within 3-5 days of the initial episode of CSE, with follow-up measurements a number of months-years later. It is known that patients imaged within this acute period show increased hippocampal volumes, thought to be attributable to hippocampal oedema (232). Because of this our group previously interpreted a fall of 203mm^3 in mean corrected hippocampal volumes in children with PFS between initial MRI scan and follow-up at 4-8 months post-CSE as the result of the resolution of this initial oedema, as hippocampal volumes at follow-up were not significantly different from control values(46). Provenzale et al(159), despite taking initial measurements at a similar time point, had a longer period of follow-up spanning 2-23 months post-CSE and reported absolute falls of 715-1217mm^3 in uncorrected hippocampal volumes in 3/11 children along with persistent abnormal T_2 signals, and smaller reductions in a further 2 children. 4/5 of these children had recurrent unprovoked seizures and 2 were diagnosed with TLE. All 5 of these children were interpreted as having MTS. The relatively high drop-out rate from their original cohort of 38 children along with generous diagnostic criteria may explain the relatively high rate of MTS (45.5%) reported in their study.

6.6.2.1 Hippocampal growth following PFS

In this current study, we found 5/26 (19.2%; 95%CI: 8.5-37.9%) patients with PFS, to have definite hippocampal volume loss, defined as a rate of change of hippocampal volume with the upper bound of the 95% confidence interval below zero, in the 6-12 months following CSE. Growth rates for two of our patients are based on 3 serial hippocampal measurements, and 3 patients only attended for the initial scan and one follow-up. Only one of these 5 patients (ID: 16) was amongst those identified as having increased hippocampal AI in Section 6.5. The others had symmetrical hippocampi on their initial MRI and AI remained within normal limits at all times. The reason for AI remaining normal, despite volume loss was that in the 4 children with unilateral volume loss, it was the larger hippocampus at initial scan that lost volume and then became the smaller hippocampus. Since AI is independent of laterality, this did not cause an increase in AI. This may also be reflective of the fact that these reductions in hippocampal volume did not, in general take these patients outside of the range of our control cohort, as can be seen in Figure 6-15/6-16.

The children with PFS with volume loss form a heterogeneous group. There were no obvious clinical characteristics to set these children apart from the rest of the cohort at initial presentation. 3/5 had a history of previous febrile convulsions, 2 quite severe (1 had multiple PFS; the other had

recurrent FC from the age of 6 months). While 2 children have had recurrent seizures, including a further episode of CSE in one child, these have all been associated with an acute febrile illness and none of these children have developed unprovoked seizures or received a diagnosis of epilepsy.

One fact that is apparent is that MTS is not an important cause of PFS. None of our patients meet the criteria for MTS and none had hippocampal abnormalities visible on their initial MRI scan. However a sizeable proportion of children do display loss of hippocampal volume following PFS, which could be interpreted as a sign of longer-term hippocampal injury, and a potential precursor to MTS. It is difficult to draw firm conclusions about the proportion of children who will develop MTS, as it cannot be assumed that children with volume loss in the first year following PFS will necessarily progress further to clinical MTS. None of our cohort has developed unprovoked seizures to date and further follow-up is needed to determine if those children with volume loss do progress to MTS-TLE. However as progressive volume loss would appear to be a prerequisite for a normal hippocampus to transform into a sclerosed one, it seems biologically implausible that those children who are not showing signs of altered hippocampal growth will go on to develop MTS as a result of this episode of PFS if no signs of this process are detectable in the first year. This would place 19% as a plausible maximum proportion of children that are at risk of developing MTS. That this is not significantly different from the proportion of children reported by Provenzale et al(159), particularly if the two patients in their study who showed less than a 1% decline in volume are disregarded, despite our more intensive follow-up and more stringent diagnostic criteria, suggests that this is a genuine effect with longer term clinical significance.

From our limited sample, it is not possible to extract clinical predictors that could help determine which children are at risk, as our study was not powered fully to perform such subgroup analysis given the numbers enrolled. The lack of association between hippocampal growth and any of the seizure-associated variables that were tested, suggests that there may not be a simple relationship between seizure severity and hippocampal damage. In spite of previous studies pointing towards an association between febrile seizure duration and MTS-TLE(162) we did not find an association between duration of CSE and hippocampal volume loss, albeit all the children in our cohort had a seizure duration of over 30 minutes by definition. We did find an association between volume loss in children with PFS and the number of previous febrile seizures that they had sustained. This raises the possibility that “multiple hits” are required for PFS to cause lasting hippocampal damage. Some supporting evidence for this hypothesis has come from animal models of PFS, in which animals with pre-existing cortical lesions were found to be much more vulnerable to developing epilepsy after an induced PFS(233). Alternatively, certain combinations of the factors

that cause certain children to react to febrile illnesses with a PFS may also be responsible for hippocampal volume loss, with the episode of PFS perhaps being a trigger for this. Further data is needed to draw a definite conclusion, especially longer-term follow-up.

6.6.2.2 Hippocampal growth following non-PFS CSE

Volume loss is not limited to children with PFS: 5/17 (29.4%; 95%CI: 13.3-53.1%) patients with symptomatic CSE and 5/17 (29.4%; 95%CI: 13.3-53.1%) patients with other CSE also had hippocampal volume loss during the study period, a higher proportion than the children with PFS.

As well as having lower mean hippocampal volumes than the other groups and more extreme asymmetry, a sizeable proportion of children with symptomatic CSE demonstrate significant hippocampal volume loss after CSE. While only one patient in our cohort had clinical MTS, the hippocampus is known to be selectively vulnerable to a variety of neurological insults, including hypoxic injury (234;235) and brain injury associated with preterm birth (236). It can be seen from Figure 6-17/6-18 that all but one of the children showing volume loss had low initial hippocampal volumes compared to controls so it is likely that these hippocampi have sustained previous injury. Although there is relatively little known about the effects of additional neurological insults in people with pre-existing structural abnormalities, animal models have shown that pre-existing neuronal injuries or abnormalities can cause increased vulnerability to further insults(237;238). Therefore for these children to lose volume after CSE may not be unexpected. An alternative explanation is that the original injuries have caused long term disruption of hippocampal growth, resulting in small hippocampi that continue to shrink with age. The time gap between any initial brain injuries and the rate of hippocampal volume loss seen following CSE suggest that this is unlikely to have been a continuous process since initial injury, but long term damage with a more insidious time course does remain a possibility.

The 5/17 children with other CSE who showed definite volume loss all had modest bilateral losses and all received a diagnosis of epilepsy either before study entry (4/5 children) or shortly afterwards (1/5 children). Studies of adult patients with newly diagnosed epilepsy have shown that a proportion will show significant hippocampal volume loss if longitudinal MRI is performed (239;240). This has been shown to occur in both focal and generalised epilepsy, with studies by Salmenpera et al(239) and Liu et al(240) both finding that between 10-20% of adults with newly diagnosed epilepsy show decreases in hippocampal volume on longitudinal MRI. The decreases demonstrated are relatively modest and were not been shown to be related to any particular epilepsy syndrome,

seizure type or seizure frequency. Neither were they shown to lead to MTS. This effect is similar in magnitude and frequency to that displayed by our cohort. It seems reasonable to conclude that there may be a similar pathological process at work and that this is likely to be related to the underlying epilepsy, rather than the episode of CSE alone.

6.7 Hippocampal volume and cognition

6.7.1 Results

Predicted hippocampal volumes were calculated using the model generated in Section 6.3.7 and these were used to generate corrected hippocampal volumes. A similar process was used to calculate corrected ICVs, with a linear model of the form ICV = a+b*ln(age) being used to generate predicted ICV. Left and right corrected hippocampal volumes were then averaged for each scan to generate a mean corrected hippocampal volume. Linear regression was then used to investigate the relationship between hippocampal volumes, ICV, growth rates and cognitive outcome at 1 month and at 1 year.

Mean corrected hippocampal volumes at initial scan showed a strong correlation with the composite cognitive score at initial assessment (p= 0.002) and at final follow-up (p=0.006), as was corrected ICV (p=0.001/0.001; initial assessment/follow-up). Neither initial nor follow-up cognitive scores showed a significant correlation with left (p=0.465) or right (p=0.740) hippocampal growth rates. When both ICV and mean hippocampal volume were entered into a step-wise linear regression model, only corrected ICV remained in the final model for both initial and follow-up cognitive scores.

6.7.2 Discussion

Both baseline age-corrected ICV and hippocampal volume was correlated with cognitive scores at baseline and after 1 year. Modelling with step-wise linear regression showed that ICV was the stronger predictor for initial and follow-up cognitive scores. Since we have previously shown that ICV and hippocampal volume are tightly correlated, this suggests that the overall level of brain development or injury is the primary determinant of cognitive outcomes after CSE and that this masks any contribution from specific hippocampal injuries, hence little predictive power is added by including hippocampal volume into a model of cognitive scoring.

6.8 Conclusion

We have demonstrated that around 20-30% of children go on to have definite hippocampal volume loss in the year following CSE. In comparison to previous studies of hippocampal volume after CSE, we have achieved a more intensive and more complete follow-up, as well as including different aetiologies of CSE other than PFS. This has shown that volume loss after CSE is not limited to PFS, but occurs with equal, if not greater frequency in other forms of CSE. The characteristics of this volume loss appear to differ by CSE aetiology and we hypothesise that this is because of differing causes and consequences.

The most pronounced effects are in children with symptomatic CSE. These children have pre-existing brain injury, including hippocampal injury and it is likely that this plays an important part in determining the response to CSE. We hypothesise that children with pre-existing injuries are more vulnerable to the deleterious effects of CSE on the hippocampus, and this can be seen in the increased proportion of children with symptomatic CSE showing hippocampal volume loss and increasing asymmetry following CSE.

Children with CSE in the context of idiopathic or cryptogenic epilepsy appear to show bilateral hippocampal volume losses, with no increase in asymmetry. We hypothesise that this is associated with the underlying epileptic condition and is not related to the development of MTS.

Finally, children with PFS show no signs of pre-existing hippocampal injuries, and the majority show normal growth and hippocampal volumes following CSE. Around 20% will show signs of long term hippocampal injury after PFS, as evidenced by a unilateral loss of hippocampal volume over time. We hypothesise that some number of these will progress to MTS and start exhibiting spontaneous seizures. There may also be more subtle forms of hippocampal injury that occur following PFS, as evidenced by the number of children who showed a transient rise in hippocampal AI and/or reduced hippocampal growth, without actual loss of volume. Animal models of MTS show cell death and hippocampal volume loss as an early feature (53) following CSE, suggesting that although we have shown evidence of some degree of hippocampal injury, the induction of further hippocampal damage, at a time so remote from the initial episode of CSE may be less likely, though this will only become evident with longer-term follow-up of our cohort.

In the following chapters we will explore the use of diffusion tensor imaging to provide further information about brain changes following CSE, both within the hippocampus and in other brain regions.

Chapter 7: Hippocampal DTI

7.1 Introduction

As previously described, diffusion tensor imaging is a technique that provides information about the preferential diffusion of water molecules within living tissue. DTI can identify abnormalities in the micro-architecture of the brain (174;175)and is increasingly being used in the assessment of structural pathology in the brain in both clinical and experimental research (241;242). It is proposed that by being sensitive to changes in tissue microstructure, DTI can enable the detection of subtle brain abnormalities that are not observed using conventional MRI techniques.

In patients with established TLE-MTS there have been several reports of changes in MD and FA in the affected hippocampus(186-191), raising the possibility that DTI may provide an additional method of tracking hippocampal changes post-CSE and aid in detecting precursor stages to TLE-MTS. Diffusion abnormalities have been reported in the peri-ictal phase(243;244) and in the first 1-3 days following CSE using diffusion weighted imaging (DWI)(245), but no investigations using DTI have been reported.

7.2 Validation studies

7.2.1 Rationale

Although there is increasing interest in whole brain analyses of these parameters, using automated tools such as voxel-based morphometry(195) and tract-based spatial statistics(196), which will be considered in Chapter 8, selective measurement of diffusion metrics, such as MD and FA within specific brain areas using a region/volume of interest (ROI) based approach remains important.

However, even in brain structures where ROI placement should be easier than would be expected in the hippocampus, ROI placement is a non-trivial problem. The appearance of anatomical boundaries and landmarks on MD or FA maps is often unfamiliar and can be difficult to ascertain. Diffusion scans are also of a lower spatial resolution and typically have a lower signal-to-noise ratio(246) than conventional anatomical scans with greater sensitivity to susceptibility artefacts

associated with the echo planar imaging readout required for bulk motion insensitivity necessary for DTI. There is furthermore, no agreed standard for hippocampal ROI placement, and a variety of different methods have been reported throughout the literature. Some manual methods that have been used in published studies (186-191;247;248) are listed in Table 7-1:

Wieshmann et al. (1999)(188)	Hippocampus manually delineated using b_0 image
Assaf et al (2002)(186)	4x8mm ROI placed on MD map
Muller et al (2006)(247)	3X4mm ROI placed on b_0 image
Thivard et al (2005)(187)	Hippocampus manually delineated using MD map
Salmenpera et al (2006)(189)	Elliptical ROI drawn on 4 consecutive slices on b_0 image
Kimiwada et al (2006)(190)	Hippocampus manually delineated using b_0 image
Knake et al (2009)(191)	4mm diameter spherical ROI placed on b_0 image
Hong et al (2010)(248)	3.3 mm diameter spherical ROI placed on b_0 image

b_0 = Reference image without diffusion weighting

Table 7-1: Published methods of hippocampal ROI placement

As mentioned in Chapter 2, methodological differences between some of these studies have led to different conclusions as to the types and magnitude of FA changes seen in TLE-MTS, although there is broad agreement that MD tends to increase. Preliminary validation studies were therefore performed to investigate different methods of hippocampal ROI placement and optimise the method chosen for subsequent studies.

7.2.2 Methods

7.2.2.1 MRI sequences

Diffusion-weighted echo planar imaging was used to acquire 19 axial slices, 2.5mm thick, using the following parameters: TR = 96 ms, TE = 2700 ms, acquisition matrix 96x96, in-plane resolution 2.5x2.5 mm. Diffusion-weighting was performed along 20 non-collinear directions using maximum b values of 1000s/mm^2. One acquisition with no diffusion-weighting (b_0 image) was included with each set of diffusion-weighted sequences. The scan was repeated three times to improve SNR and the data merged together without averaging. MD and FA maps were then generated from this data using *TractoR* as mentioned in Chapter 3.

These maps as well as the b_0 image were used for all the studies described below. Hippocampal ROIs drawn on the 3D-FLASH images from the volumetry study as described in Chapter

6 were also used. All images were manually inspected for motion artefact and any unsuitable images were excluded from the analysis.

7.2.2.2Validation cohort

Ten sample patient scans and 6 healthy controls were chosen randomly from those children in our cohort who tolerated the DTI sequences. The patient group consisted of 4 boys and 6 girls with ages ranging from 0.93 - 15.82 years, and a mean age of 4.00 years. The group of healthy controls consisted of 4 boys and 2 girls, aged between 2.27 – 9.77 years with a mean age of 4.74 years. The controls chosen for this study were all healthy volunteers with no known neurological or developmental problems.

7.2.2.3 Manual ROI placement

FSL (http://www.fmrib.ox.ac.uk/fsl/, FMRIB, Oxford University) was the primary software package used for this analysis: *fslview* was used to visualise the MD/FA/b_0 maps as a 3-D dataset and to manually delineate the ROI; mean MD and FA values within the generated ROI were then measured using *fslstats*.

As there is no current gold standard for defining ROIs in the hippocampus, four methods of ROI placement were chosen for comparison, adapted from the manual methods that have been previously described (Table 6-1) as well as a semi-automated method that involved first drawing an ROI on a high resolution T1-weighted image and then registering this onto the MD and FA maps.

In order to create an ROI the following methods were used:

Method 1) The MD map was loaded into MRIcron and the entire hippocampus manually delineated as far as was ascertainable. Landmarks that were used to identify the hippocampus included the lateral ventricle and chroidal fissure.

Method 2) The b_0 images were loaded into MRIcron and the entire hippocampus manually delineated as far as was ascertainable using similar landmarks as Method 1.

Method 3) The slice with the largest cross-section through the anterior third of the hippocampus was identified on a coronal view and a fixed size ROI of 3x2

voxels was placed into the hippocampus on the MD map on two consecutive coronal slices.

Method 4) The hippocampus was defined using the 3-D FLASH dataset and this was then co-registered to the FA map. The technique used is described below.

Sample images of ROIs created by each method are given in Figure 7-1:

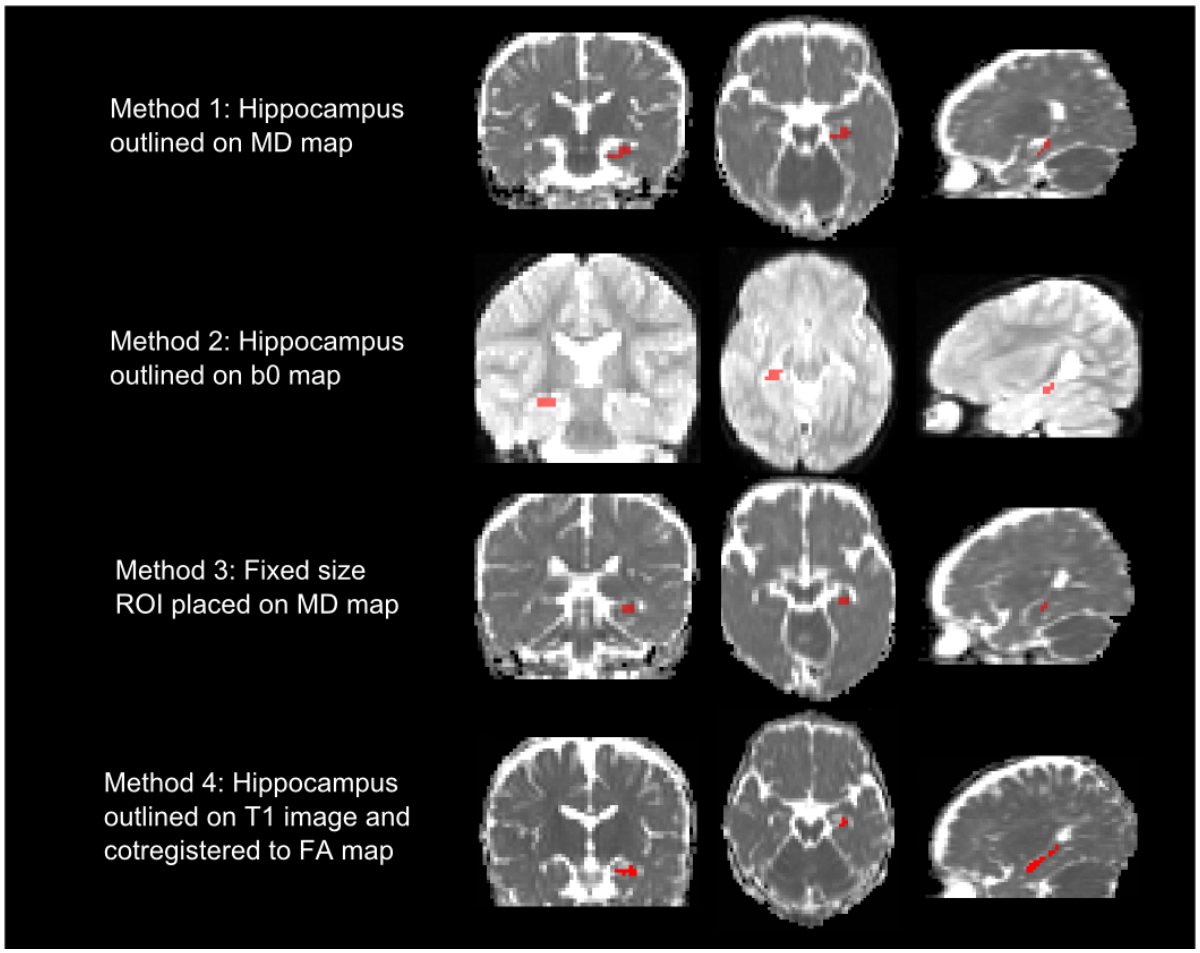


Figure 7-1: Example ROIs for each method of ROI placement

Each method was then used to generate an ROI for each hippocampus and the mean MD and FA values within the ROI recorded. This was then repeated by the same observer at least 48 hours after the first run. This generated two MD and two FA values for each method in each hippocampus.

Following this a similar comparison was performed to determine the optimum size for the ROI in Method 3. The following sized ROIs were placed into the hippocampus in MRIcron (Figure 7-2):

1) 1 voxel
2) 2x2 voxels on one coronal slice
3) 2x2 voxels on two consecutive coronal slices
4) 3x2 voxels on two consecutive coronal slices
5) 3x2 voxels on three consecutive coronal slices
6) 3x3 voxels on three consecutive coronal slices

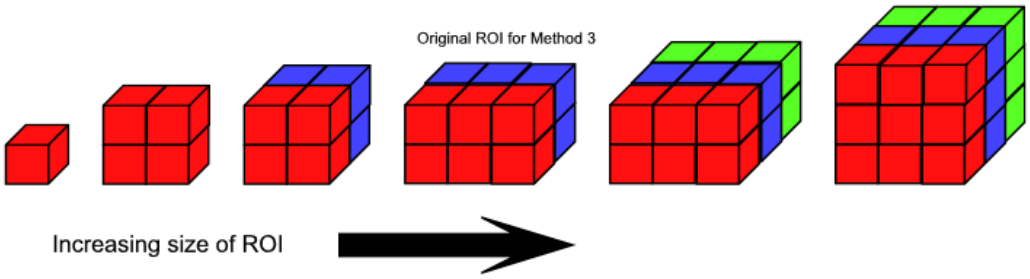


Figure 7-2: Increasing ROI sizes used for Method 3. Each cube represents one voxel.

These were similarly repeated after 48 hours and the MD and FA values compared using the coefficient of variability.

7.2.2.4 Coregistration

Method 4 used the hippocampal ROIs created on 3D-FLASH images that were used to measure hippocampal volumes described in Chapter 6. Each subject had left and right hippocampi outlined as a ROI using the 3D-FLASH sequences in MRIcron as previously described.

The FA maps were then co-registered to the 3D-FLASH images using the non-linear co-registration tools in FSL (249) (FNIRT). The resulting warp-field was inverted and used to map the ROIs from the 3D-FLASH images onto the FA maps, which are in the same image space as the MD and b_0 maps. The mean MD and FA values within each ROI were calculated in similar fashion to the ROIs created from the other methods using *fslstats*.

7.2.2.5 Qualitative assessment

Each ROI was viewed in FSLview by an experienced paediatric neuroradiologist (Dr WK Chong) who had not been involved in drawing them. They were viewed and overlaid onto MD, FA and b_0 maps. Each ROI was agreed to lie within the hippocampus as far as was ascertainable from visual inspection by the same neuroradiologist.

7.2.2.6 Statistical analysis

As described above, two MD and FA measurements were obtained for each method in each hippocampus. Since precision is not easily comparable across individuals, especially when the value being studied may vary, these two values were used to calculate the CoV separately for a method in each subject and then averaged across patients. This was performed separately for MD and FA.

The CoV from each hippocampus was then averaged across all patients to generate a mean CoV for each of the four methods. The range and standard deviation of all voxel values within each ROI was also calculated and likewise averaged across all patients for each method.

PASW 18.0 (Chicago, IL) was used to test for differences in CoV between each of the four methods of ROI placement with a Kruskal-Wallis test. Repeated Mann-Whitney U tests with Bonferroni correction for multiple comparisons were used to compare pairs of methods individually. Likewise Univariate ANOVA was used to compare the mean range and standard deviation of MD/FA values within each ROI across each of the 4 methods. The cut-off for significant values was taken as $p < 0.05$.

7.2.3 Results

7.2.3.1 Estimation of reliability

There was no significant difference between the CoV values obtained for patients and for controls for either MD or FA overall, therefore these were pooled for further analysis. There were significant differences in CoV for both MD and FA ($p < 0.001$) between methods. The results from comparisons between individual methods are given in Figures 7-3 & 7-4 and Table 7-2.

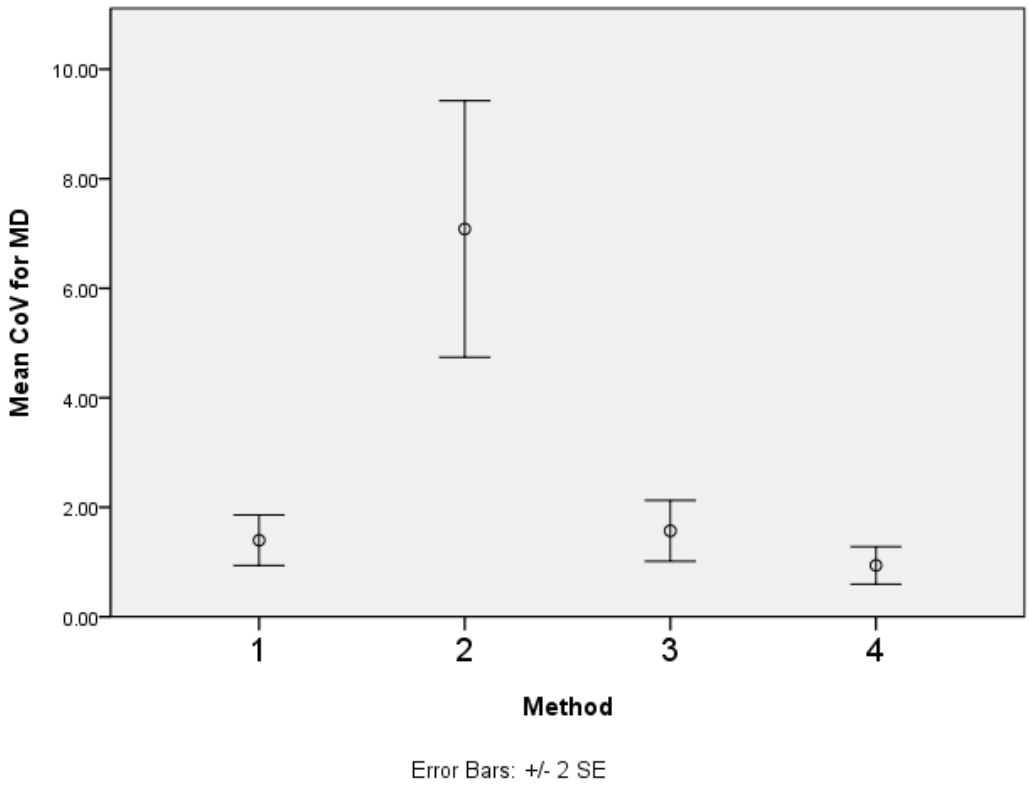


Figure 7-3; Mean coefficient of variation for MD measurements for each placement method

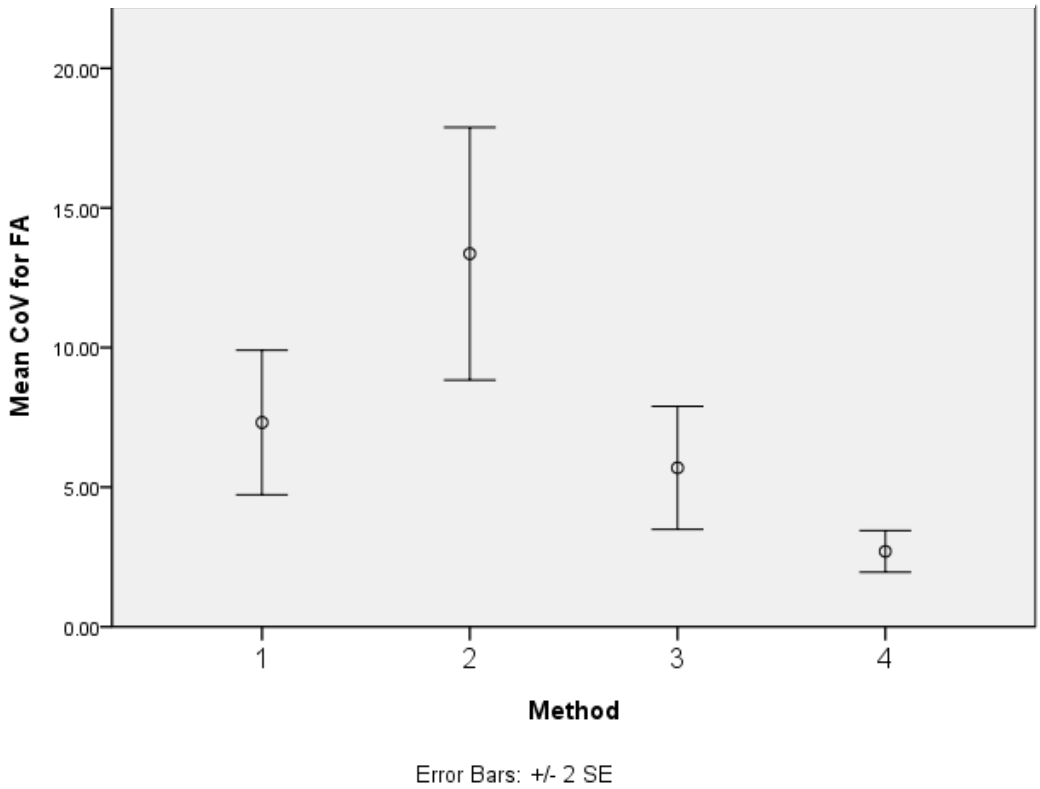


Figure 7-4: Mean coefficient of variation for FA measurements for each placement method

	Method	CoV (MD) % (s.d.)	CoV (FA) % (s.d.)
Method 1	Hippocampus outlined on MD map	1.399 (1.304)	7.318 (7.325)
Method 2	Hippocampus outlined on b0 map	7.082 (6.623)	13.362 (12.797)
Method 3	3x2x2 voxel ROI placed on MD map	1.571 (1.576)	5.692 (6.231)
Method 4	Hippocampus outlined on T1-weighted image and transformed onto FA map	0.939 (0.939)	2.701 (2.037)

Table 7-2: Comparison of different methods of ROI placement

After adjusting for multiple comparisons, Methods 3 and 4 were found to be significantly better than Method 2 for MD and FA measurements ($p < 0.001$). Method 1 was also significantly better than Method 2 for FA measurements ($p < 0.001$) but not for MD measurements. Although it did not reach statistical significance Method 4 gave lower CoV values for both MD and FA measurements than Methods 1 and 3.

7.2.3.2 Varying ROI size

The results of varying the size of the ROI in Method 3 are shown in Figure 7-5 and 7-6. There was a significant effect of ROI size on mean CoV ($p < 0.001$). The smallest CoV was obtained for a 2x2x2 voxel ROI for MD measurements and a 3x2x3 voxel ROI for FA measurements. The lowest CoV obtained by this method was still greater than that from Method 4 (dotted line in Figure 7-5/7-6).

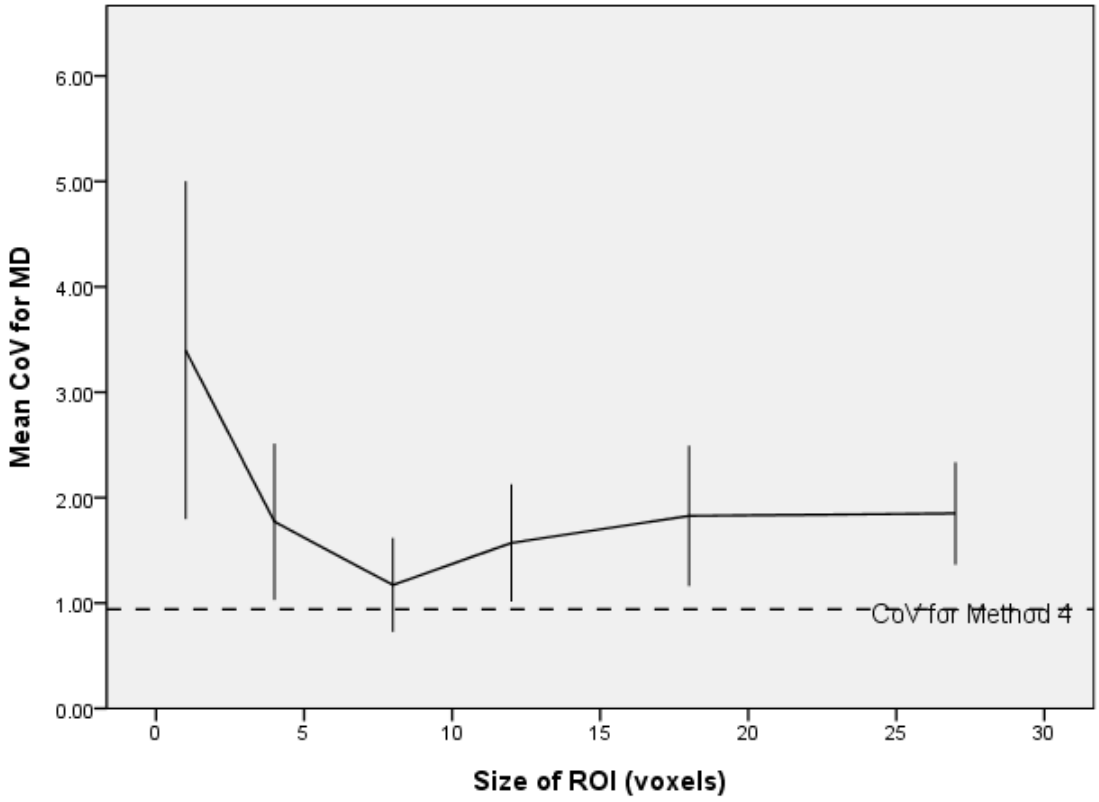


Figure 7-5: Coefficient of Variation for MD measurements by ROI size

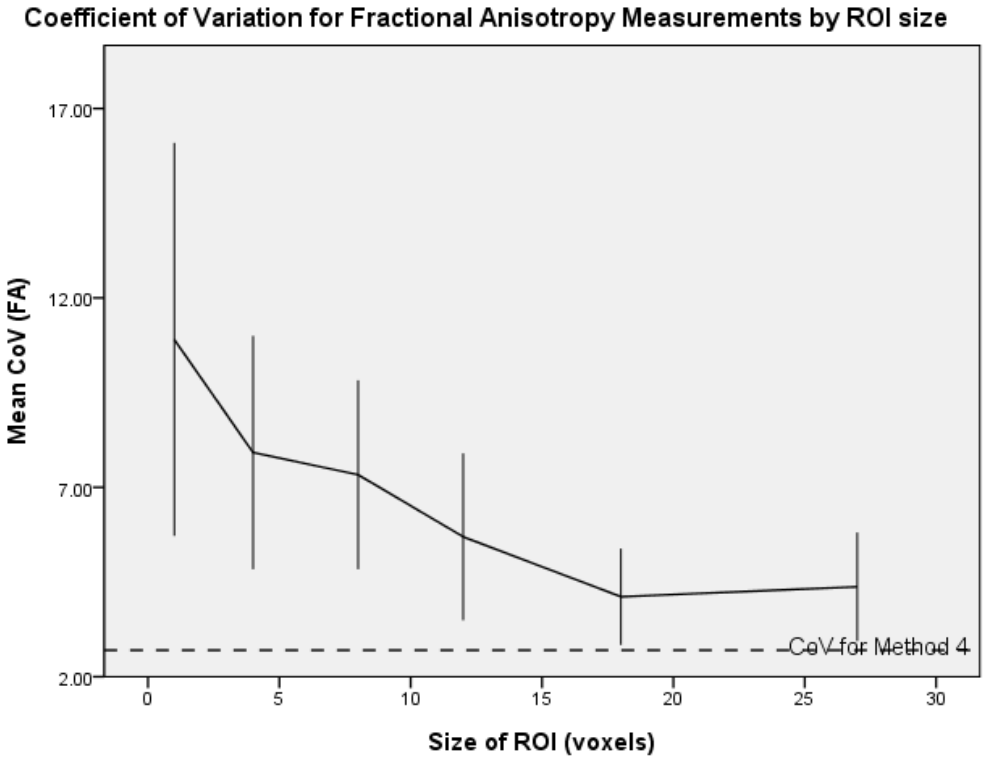


Figure 7-6: Coefficient of Variation for FA measurements by ROI size

7.2.3.3 Variance within each ROI

While the CoV values for MD or FA measurements did not significantly differentiate Methods 1, 3 and 4, there were differences in the actual MD and FA values reported. Univariate ANOVA with post-hoc analysis and Bonferroni correction for multiple comparisons was used to compare mean MD/FA values and the range of MD/FA values within each ROI across methods. Method 4 (co-registration of ROI from the T1-weighted scan) gave consistently higher values for MD ($p < 0.001$) and lower values for FA ($p < 0.001$) across all the hippocampi studied (Table 7-3). There were also differences in the standard deviation and range of MD/FA values within each ROI according to the method used to draw that ROI (Table 7-4). Method 4 produced ROIs with a significantly higher standard deviation ($p < 0.001$) and range ($p < 0.001$) of MD values within them compared to either of the other two methods. Considering FA values, Method 1 showed the greatest range and variance of voxel values within each ROI compared to either Method 3 ($p < 0.001$) or Method 4 ($p = 0.006$).

	Mean MD value (mm/s) (s.d.)	Mean FA value (s.d.)
Method 1 (ROI on MD map)	0.9161×10^{-3} (0.0428)	0.1472 (0.0343)
Method 3 (Fixed size ROI)	0.9101×10^{-3} (0.0520)	0.1391 (0.0342)
Method 4 (Co-registered ROI)	0.9860×10^{-3} (0.0496)	0.1262 (0.0124)

Table 7-3: Mean MD and FA values for Methods 1, 3 and 4

	Mean s.d. of MD	Mean MD range (mm/s)	Mean s.d. of FA	Mean FA range
Method 1 (ROI on MD map)	0.07242×10^{-3}	0.29406×10^{-3}	0.05501	0.23588
Method 3 (Fixed size ROI)	0.07941×10^{-3}	0.25971×10^{-3}	0.04052	0.13679
Method 4 (Co-registered ROI)	0.13430×10^{-3}	0.70916×10^{-3}	0.04372	0.20383

Table 7-4: The range and standard deviation of MD and FA reported from voxels within each ROI

7.2.4 Conclusion

It is apparent from our validation study that a significant increase in the reliability of hippocampal MD and FA measurements is possible by optimising the method of ROI placement. Method 2 appears to be significantly less reliable than the others and should not be recommended, despite its use in previous studies of the hippocampus (188;190). Method 2 was therefore not considered further in the more detailed ROI analysis.

Examining the values reported from individual voxels within each ROI, we found that firstly, there is a large range of MD and FA values measured within any individual ROI. Secondly, the variation in FA values between voxels is proportionally much greater than that for MD. The range of MD values from individual voxels within each ROI for Method 4 was significantly higher than that for Method 1 or 3 ($p < 0.001$). This means that the voxels within each ROI created by Method 4 contain a larger range of MD values than those created by Method 3 or Method 1. In particular, they contain more voxels with higher MD values. They also contain a larger range of FA values than ROIs created by Method 3, but these values are, on average, lower. The most likely explanation is that Method 4 suffers from partial volume effects through inclusion of edge voxels that contain a proportion of the surrounding cerebrospinal fluid (CSF) as well as hippocampus. Increased MD and decreased FA measurements would be expected if this was the case (250;251).

In contrast ROIs created by Method 1 have similar mean MD and FA values to those from Method 3 but a much higher range of FA values within each ROI. As there are some very high (over 0.4) FA values recorded using Method 1 one possible interpretation of this is extension of the ROI outside the hippocampus and into adjacent white matter tracts, with the partial volume effect from CSF acting to offset the alterations in mean values.

The final decision of which method to use must take into account many different considerations. Our results show that the most reproducible measurements can be obtained by delineating an ROI on a high resolution anatomical scan and co-registering this to the diffusion data (Method 4), although Methods 1 and 3 are not significantly less reproducible and remain viable alternatives. Both Method 1 and Method 4 attempt to cover the entire hippocampus, whereas Method 3 only samples from a portion of the hippocampal head. Therefore Method 3 will not be sensitive to changes occurring elsewhere in the hippocampus that do not affect the head. If this is considered likely then one of the other methods should be preferred. However because of its limited coverage, Method 3 is less susceptible to partial volume effects, as it avoids edge voxels, therefore if there is an *a priori* reason to believe changes may be limited to or maximal in the hippocampal head then there may be advantages to using this.

If Method 3 is used, then the size of the ROI matters; if the ROI is too small or too large then the CoV starts to increase. There appears to be a trade-off with smaller ROIs enabling more accurate placement within the hippocampus and avoiding partial volume effects. On the other hand larger ROIs are less susceptible to noise as they are able to average the signal over a larger number of voxels. For any individual set of scanning parameters and anatomical structure we suggest that there is an optimal size for an ROI. For this cohort the optimum appeared to be between 8 and 12 voxels.

Of the methods that provide whole hippocampal coverage, then Method 4 appears to be preferable to Method 1. Both will suffer from partial volume effects on edge voxels, but it seems likely that Method 1 carries a greater risk of sampling outside the hippocampus altogether due to the low resolution of the diffusion maps. By using the MD map to define an ROI, to be subsequently used to measure MD values, Method 1 also introduces another potential confounding factor. Using the high resolution T1-weighted images to define the hippocampus, as in Method 4 avoids both of these issues. It may also allow more sampling of the hippocampal body and tail, as this area is difficult to define using the MD maps alone (since at this resolution it will be only 1-2 voxels in cross-section).

Of the methods tested in this study, Methods 3 and 4 seem to be the methods of choice. Both give a CoV comparable or lower than those reported previously (247;252;253) and are therefore viable options for use in clinical studies. For our further analysis, Method 4 was preferred due to its lower variability and greater coverage of the hippocampus.

7.3 Hippocampal DTI following CSE

7.3.1 Aims

Having established a reliable method for measuring MD and FA within the human hippocampus, this present study aimed to use this technique to investigate hippocampal MD and FA following CSE. The objective was to use diffusion metrics to track hippocampal changes occurring post-CSE and identify potential biomarkers for TLE-MTS. We hypothesised that CSE will lead to increases in MD and decreases in FA in patients with CSE compared to healthy controls in patients that are at risk of developing MTS.

7.3.2 Methods

7.3.2.1 MRI sequences

The same MRI sequences were used as described in 7.2.2.1.

7.3.2.2 ROI placement

All measurements were taken using Method 4 described above in 7.2.2.4. This was applied to all patient scans who tolerated the DTI sequences and hippocampal MD/FA measurements recorded as described. Each hippocampus was measured at least once by two separate observers.

7.3.2.3 Statistical analysis

Measurements for each hippocampus were averaged to provide mean left and right hippocampal MD/FA for each MRI scan. Hippocampal MD and FA were plotted against age for control subjects and tested for possible dependencies with Spearman's Rank correlation and linear regression. Cross-sectional analysis was then performed between aetiological groups and controls at each time-point using univariate ANOVA with appropriate corrections for covariates. Bonferroni post-hoc tests were used to compare each aetiological group with controls individually.

Spearman's Rank correlation was used to test the age dependency of MD in each aetiological group after removal of obvious outliers. Correction for multiple comparisons was not performed in order to maintain the sensitivity of a negative result.

Linear regression was performed to estimate the rate of change of left and right hippocampal MD and FA in each child over time in a similar manner as described in Chapter 6 for estimating hippocampal growth rates. A standard error was estimated from each linear regression and values greater than 1.96 S.E. above zero were reported as significant increases in MD. Similarly for FA, values greater than 1.96 S.E above or below zero are reported as significant increases or decreases in FA over time respectively.

Univariate ANOVA, t-tests and χ-squared tests were used as appropriate to test for differences in the effects of clinical factors on the rates of change of MD/FA. MD/FA measurements and rates of change were also compared with cognitive scores using linear regression with age correction as appropriate.

7.3.3 Subjects

25 control subjects and 56 patients tolerated DTI at their initial scan and were entered into this analysis. A detailed breakdown of patient numbers for each aetiological group is given in Table 7-5.

	Controls	PFS	Symptomatic CSE	Other CSE
Initial MRI	25	30	19	17
6 month follow-up	-	18	12	13
1 year follow-up	-	19	15	9

Table 7-5: Number of patients tolerating DTI by group

7.3.4 Normal changes in MD and FA

7.3.4.1 Results

Hippocampal MD on both sides in healthy controls showed a clear age dependency, with a sharp decrease in MD in the first few months of life followed by a steady slower decline thereafter (Figure 7-7). One control subject was excluded from further analysis as an outlier (left and right hippocampi circled in red on Figure 7-7). Linear regression showed a strong linear relationship between MD and ln(age). The regression coefficients and p-values are given in Table 7-6.

	Regression coefficient ($10^{-3}mm^3/s/ln(years)$)	95% confidence interval	p-value
Left hippocampus	(1.027) -0.032	-0.052 – -0.012	0.003
Right hippocampus	(1.031) -0.042	-0.060 – -0.024	<0.001

Table 7-6: Regression coefficients for MD vs. ln(age) in control subjects

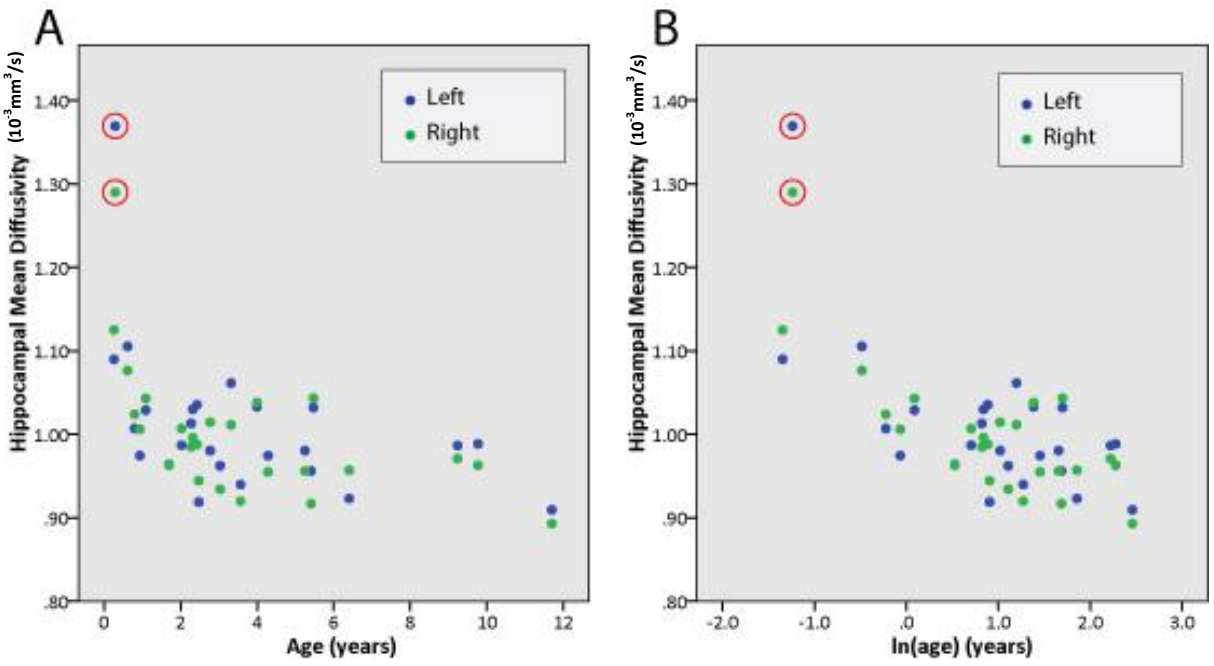


Figure 7-7: Graphs showing hippocampal mean diffusivity against age (A) and log-transformed age (B) in control subjects (Red circles show outliers excluded from the regression analyses)

In contrast, our control cohort did not appear to show a relationship between age and hippocampal FA on either side (Figure 7-8). There was no significant correlation between FA and age or ln(age) with linear regression.

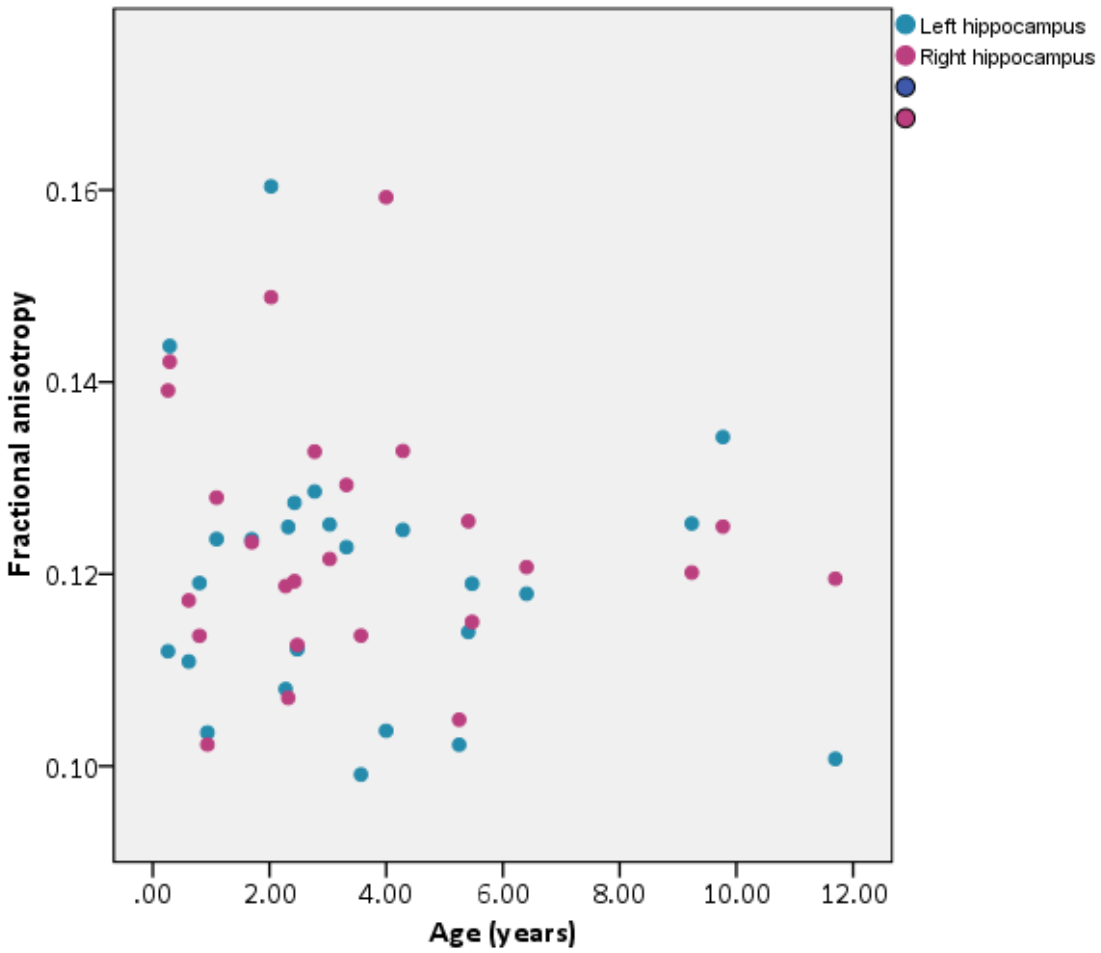


Figure 7-8: Hippocampal fractional anisotropy against age in control subjects

7.3.4.2 Discussion

Previous studies of MD and FA in normal populations have shown that they continue to develop as children grow older, with reductions in MD and increases in FA being seen with increasing age(254), although few studies have been able to look closely at children between 1 and 3 years of age. Our study is unique in including such a broad age range of children, from 1 month up to 16 years and therefore exact comparison with previous studies of normal development, which typically contain a larger number of children in a more tightly constrained age range, is difficult. The timing and magnitude of MD/FA changes alters between brain regions and is thought to reflect the differential rates of maturation of different brain structures(255). In addition there have been no

longitudinal studies performed that specifically look at the normal development of MD/FA within the hippocampus.

The control hippocampal FA values reported in this study are lower than those reported in previous studies on adults (186;187;189), which have typically reported mean control FA values of 0.15-0.23. In the one study of children with TLE (190), hippocampal FA values of 0.17 – 0.25 were reported in healthy controls, with a mean of 0.21. By contrast FA in our control sample was between 0.10 - 0.16, with a mean of 0.12. Children are known to have lower FA values across a number of brain areas than adults; FA increases with age as their brains mature (255-257). The very young nature of our control group, with a mean age of 4.14 years, meant that lower absolute FA values would be expected compared to either adult studies or the previous report of hippocampal MD/FA in children by Kimiwada et al.(190), which included control children with a mean age of 11.0 years. Furthermore, the signal-to-noise ratio (SNR)of the particular MRI sequences used to calculate FA have a large effect on the absolute values obtained, with increased SNR leading to lower FA(258). As the majority of previous studies used relatively fewer repetitions and diffusion directions, while 20 non-co-linear diffusion directions, were used in this study, each repeated 3 times and averaged, the improved SNR obtained by this DTI sequence provides a further factor causing lower absolute FA values.

It is apparent that, despite efforts to optimise the methodology used to measure hippocampal MD/FA, this remains difficult. Unmeasurable errors, such as partial volume effects mean that there is a greater degree of uncertainty over the measurements, and it is difficult to be certain if this is the reason we were unable to detect a significant relationship between age and hippocampal FA in control children or if there is truly no age effect in our cohort.

7.3.5 Cross-sectional mean MD and FA after CSE

7.3.5.1 Results – mean MD

There was a significant effect of group on MD at 1 month post-CSE for both left ($p < 0.001$) and right ($p = 0.007$) hippocampi after correcting for age. There was a significant interaction between aetiology and age ($p=0.007$), Post-hoc analysis with correction for multiple comparisons showed that children with symptomatic CSE had significantly higher hippocampal MD than controls on both left ($p = 0.001$, mean difference $0.13x10^{-3}mm^2/s$) and right ($p<0.001$, mean difference

0.11x10^{-3}mm^2/s) sides. However, neither of the other groups had significantly different MD values in the hippocampus from controls.

At 6 months post-CSE children with symptomatic CSE continued to have significantly higher MD than controls (p=0.022, mean difference 0.04 x10^{-3}mm^2/s , left; p=0.002, mean difference 0.09x10^{-3}mm^2/s, right). At 1 year post-CSE this difference was still present, but did not reach significance on the left (p=0.317, mean difference 0.04x10^{-3} mm^2/s) though it remained significant on the right (p=0.015, mean difference 0.07x10^{-3}mm^2/s) hippocampal MD.

Summaries of hippocampal MD at each time-point are given in Table 7-7. p-values represent difference from control subjects.

Group	Mean adjusted left hippocampal MD (10^{-3} mm^2/s)	p-value	Mean adjusted right hippocampal MD (10^{-3}mm^2/s)	p-value
1 month post-CSE			(MD is evaluated at ln(age)=0.92)	
Controls	1.001 (0.959 - 1.043)		0.993 (0.960 - 1.027)	
PFS	1.005 (0.967 - 1.043)	NS	1.000 (0.970 - 1.031)	NS
Symptomatic CSE	1.131 (1.083 - 1.178)	**0.001***	1.106 (1.069 - 1.144)	**< 0.001***
Other CSE	1.047 (0.995 - 1.099)	NS	1.026 (0.985 - 1.068)	NS
6 months post-CSE			(MD is evaluated at ln(age)=1.05)	
Controls	0.993 (0.978 - 1.009)		0.987 (0.961 - 1.013)	
PFS	1.004 (0.986 - 1.022)	NS	1.013 (0.982 - 1.044)	NS
Symptomatic CSE	1.034 (1.012 - 1.056)	**0.022***	1.074 (1.036 - 1.111)	**0.002***
Other CSE	1.020 (0.999 - 1.042)	0.284	1.002 (0.965 - 1.039)	NS
1 year post-CSE			(MD is evaluated at ln(age)=1.17)	
Controls	0.988 (0.962 - 1.014)		0.982 (0.952 - 1.012)	
PFS	0.977 (0.948 - 1.006)	NS	0.982 (0.949 - 1.015)	NS
Symptomatic CSE	1.030 (0.997 - 1.062)	0.317	1.058 (1.020 - 1.095)	**0.015***
Other CSE	0.996 (0.953 - 1.040)	NS	0.999 (0.950 - 1.049)	NS

Figures in brackets represent 95% confidence intervals
p-values quoted represent differences from control means with Bonferroni correction for multiple comparisons
***statistically significant at p < 0.05 NS: Not statistically significant**

Table 7-7: Age-adjusted hippocampal mean diffusivity compared with control values

7.3.5.2 Results – mean FA

There were no significant differences detected in hippocampal FA between each aetiological group at 1 month or 6 months post-CSE. At 1 year post-CSE there was a significant effect of aetiology on FA in the left hippocampus (p = 0.001) but not right (p = 0.289). Post-hoc analysis with correction for multiple comparisons showed that children with symptomatic CSE had significantly reduced left hippocampal FA compared to children with PFS (p = 0.008) and a trend towards lower FA than control children (p = 0.051). No significant effect was seen in the right hippocampus.

7.3.5.3 Discussion

Children with symptomatic CSE were found to have higher hippocampal MD than controls. While this did not reach statistical significance after correction for multiple comparisons for the left hippocampus at 12 months follow-up (Table 7-7), left hippocampal MD was significantly higher in children with symptomatic CSE than controls at one month and also at 6 months post-CSE. This is in keeping with the reductions in hippocampal volume demonstrated in Chapter 6 and is similarly likely to represent the effect of the previous neuronal injury or abnormality. It is difficult to attribute this to a direct effect of CSE given the high proportion of this group who had widespread pre-existing structural abnormalities.

Children with PFS or other CSE did not differ systematically from controls in hippocampal MD, again similar to the previous analysis of hippocampal volumes.

No significant differences were found in hippocampal FA, which may be a result of the natural variation in hippocampal FA at this age masking any individual alterations.

While, as previously discussed, there have been a number of studies showing increases in hippocampal MD and possible reductions in FA in established MTS in both adults(186-189;191) and children(190), it is unknown whether this is an early or late change in the course of the disease. Only one of the children in our cohort had clinical evidence of MTS and this child had hippocampal MD on the affected side much greater than any of the other children in this study (Figure 7-11). No children developed MTS over the course of this study, therefore it not surprising that we did not find significant alterations in hippocampal MD or FA except in the group of children with pre-existing neuronal damage/abnormality (symptomatic CSE). This would point towards the increase in MD and decrease in FA being a relatively late change in the evolution of seizure-associated neuronal injury.

7.3.6 Developmental changes in MD

7.3.6.1 Results

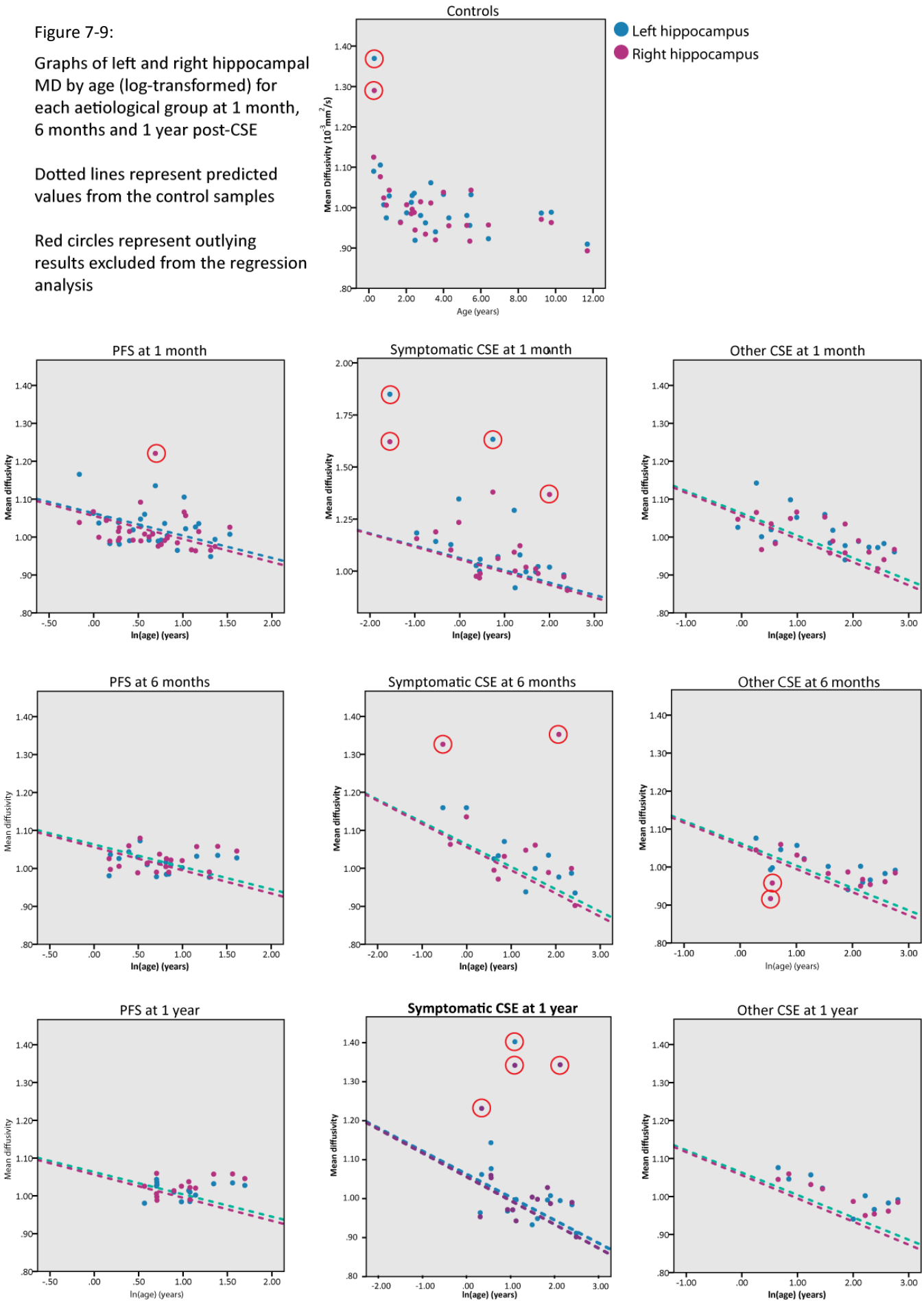
Graphs of left and right hippocampal MD against ln(age) for each aetiological group at each time point are shown in Figure 7-9. The dotted lines represent the predicted values from control measurements.

Figure 7-9:

Graphs of left and right hippocampal MD by age (log-transformed) for each aetiological group at 1 month, 6 months and 1 year post-CSE

Dotted lines represent predicted values from the control samples

Red circles represent outlying results excluded from the regression analysis



After removal of outliers (circled in red on Figure 7-9), Spearman's Rank Correlation was performed on each patient group at each time point in order to test the relationship between age and MD within each group. Bootstrapping using 1000 random samples with replacement was used to generate 95% confidence intervals for ρ. The results are given in Table 7-8 with uncorrected p-values.

	Left		Right	
	ρ (95% CI)	**p-value**	**ρ (95% CI)**	**p-value**
Controls	-0.429 (-0.739 - -0.003)	**0.037***	-0.568 (-0.822 - -0.162)	**0.004***
1 month post-CSE				
PFS	-0.323 (-0.631 - 0.103)	0.081	-0.354 (-0.681 - 0.104)	0.055
Symptomatic CSE	-0.730 (-0.914 - -0.378)	**0.001***	-0.522 (-0.895 - -0.007)	**0.032***
Other CSE	-0.750 (-0.894 - -0.472)	**0.001***	-0.598 (-0.814 - -0.163)	**0.011***
6 months post-CSE				
PFS	-0.172 (-0.711 - 0.422)	0.494	0.160 (-0.306 - 0.636)	0.526
Symptomatic CSE	-0.813 (-0.983 - -0.355)	**0.001***	-0.564 (-0.915 - 0.218)	0.090
Other CSE	-0.637 (-0.885 - -0.138)	**0.019***	-0.773 (-0.981 - -0.139)	**0.005***
1 year post-CSE				
PFS	-0.418 (-0.748 - 0.039)	0.075	-0.407 (-0.685 - --0.028)	0.084
Symptomatic CSE	-0.349 (-0.782 - 0.310)	0.221	-0.203 (-0.861 - 0.605)	0.527
Other CSE	-0.700 (-1.000 - 0.077)	**0.036***	-0.933 (-1.000 - -0.568)	**<0.001***

*** p < 0.05 (uncorrected values)**

Table 7-8: Correlations between MD and age in children following CSE

Unlike the control group, children with PFS did not show a significant correlation between age and MD at any time point; children with symptomatic CSE also did not show a significant

correlation between age and hippocampal MD at 1 year post-CSE, although they did before this time point; children with other CSE showed significant correlations at all points.

7.3.6.2 Discussion

Although hippocampal MD was strongly correlated with age in control subjects, and this remained true for children with other CSE, this was not the case for children with PFS, who did not show a significant relationship between age and MD at any time point.

Similar findings were noted in a previous study of children with PFS by Scott et al(232), which investigated a cohort of 14 children 4-8 months after PFS. They found that the children with PFS did not show an age dependency of hippocampal MD, whereas MD in control subjects was strongly correlated to $\log_{10}$(age). We have now shown that this remains a consistent finding up to 1 year post-CSE, and that it appears to only occur in children with PFS, as opposed to those with other types of CSE: children with epilepsy-related and other non-symptomatic CSE displayed a strong age dependency of hippocampal MD at all stages and, while children with symptomatic CSE did not show such a strong correlation at their final follow-up scan, the large number of outlying measurements that were excluded from the correlation analysis suggest that in many cases the hippocampus was involved in the underlying pathology as has previously been discussed.

The rate of change of MD is greatest during the first 2 years of life, and decreases thereafter, therefore the strongest correlations should be found in younger children. This makes the lack of age dependency in the children with PFS, who were the youngest of all the groups, more striking.

For this to be a seizure-related effect, an age-dependant effect of PFS on hippocampal MD would have to be hypothesised, such that younger children show a decrease in hippocampal MD following PFS, whereas older children show an increase. However, given that children with other forms of CSE are not so affected, this seems biologically implausible. One alternative hypothesis would be that hippocampal MD develops differently in children with PFS: i.e. there is a developmental abnormality in children with PFS, likely caused by genetic factors, such as have been shown to play an important role in susceptibility to febrile seizures(259). This would imply that brain maturation is different in some children, rendering them susceptible to having prolonged seizures with certain febrile illnesses. This difference in developmental trajectory is then expressed as a lower hippocampal MD at a younger age and a subsequent failure of hippocampal MD to then reduce at the child gets older.

This effect appears to be specific to children with PFS, as children with epilepsy-related and other, non-symptomatic forms of CSE continued to show a strong age dependency at all time-points. We would therefore suggest that this represents evidence to support the hypothesis that there is a transient developmental abnormality in children with PFS rather than an effect of CSE.

7.3.7 Changes in MD and FA over time

7.3.7.1 Changes in MD over time

Serial plots of MD in individual children are shown in Figure 7-10, 7-11 and 7-12. Children who showed significant decreases in hippocampal volume (Chapter 6) are highlighted in red/light blue.

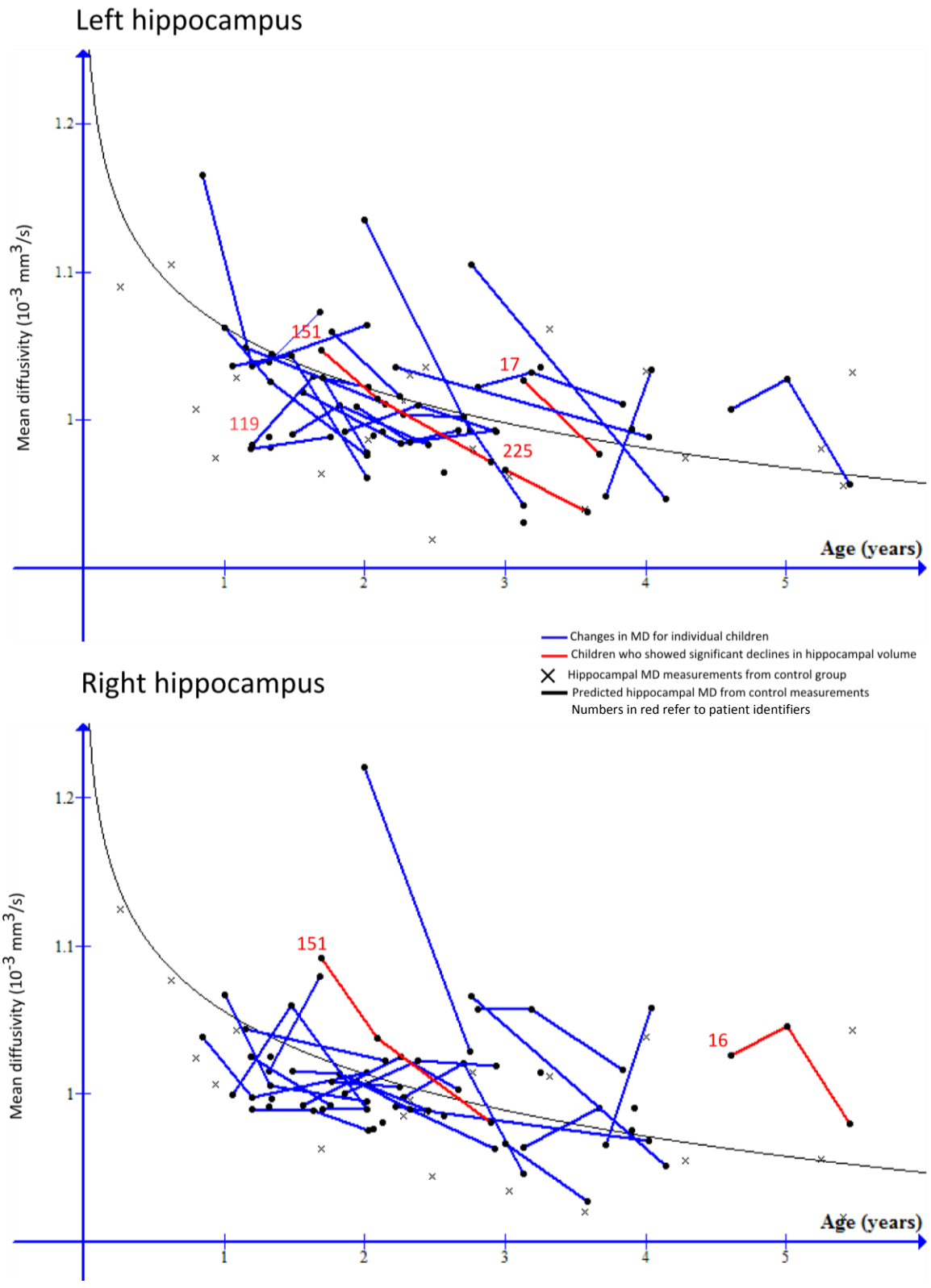


Figure 7-10: Hippocampal MD in children with PFS

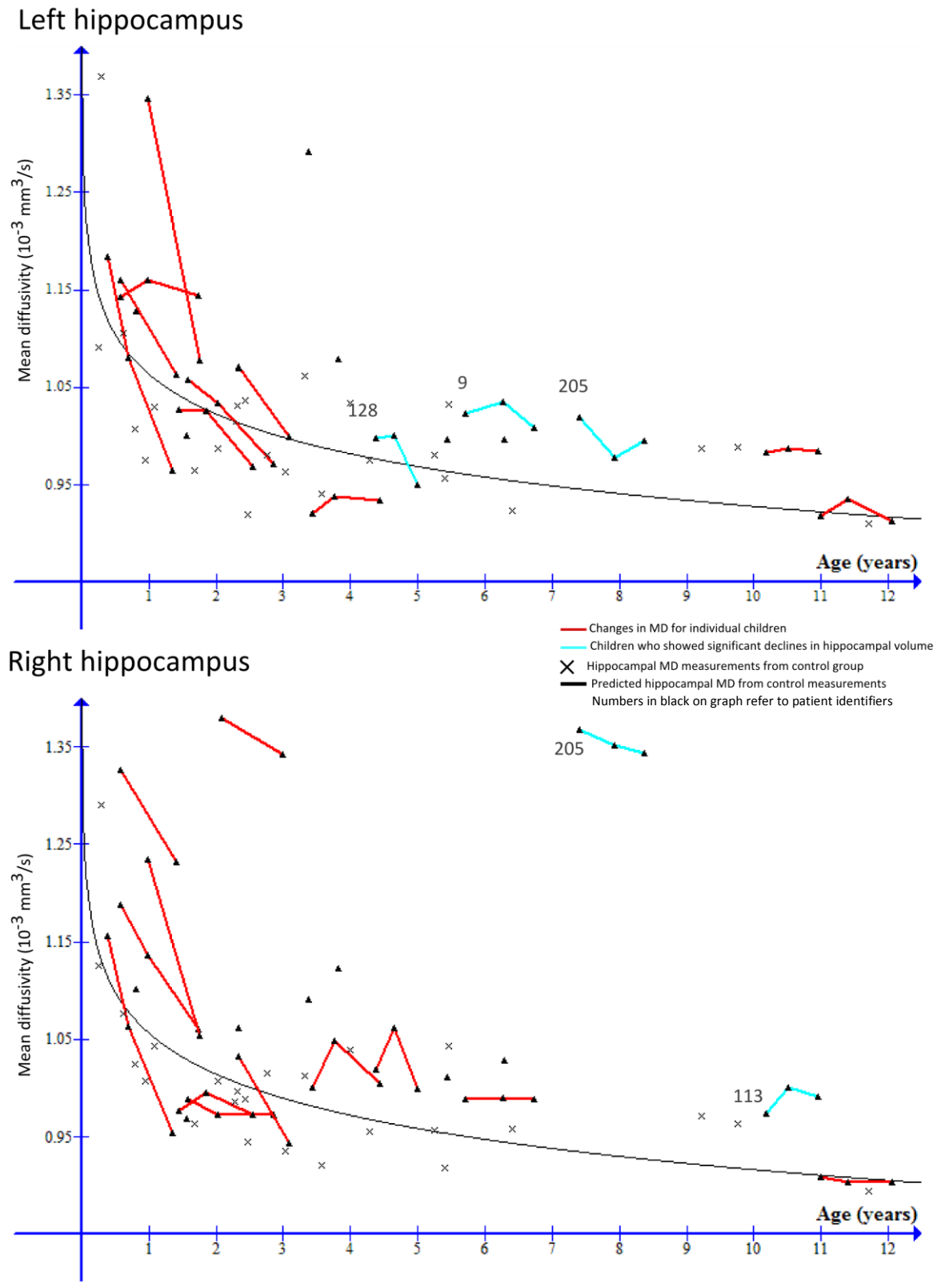


Figure 7-11: Hippocampal MD in children with symptomatic CSE

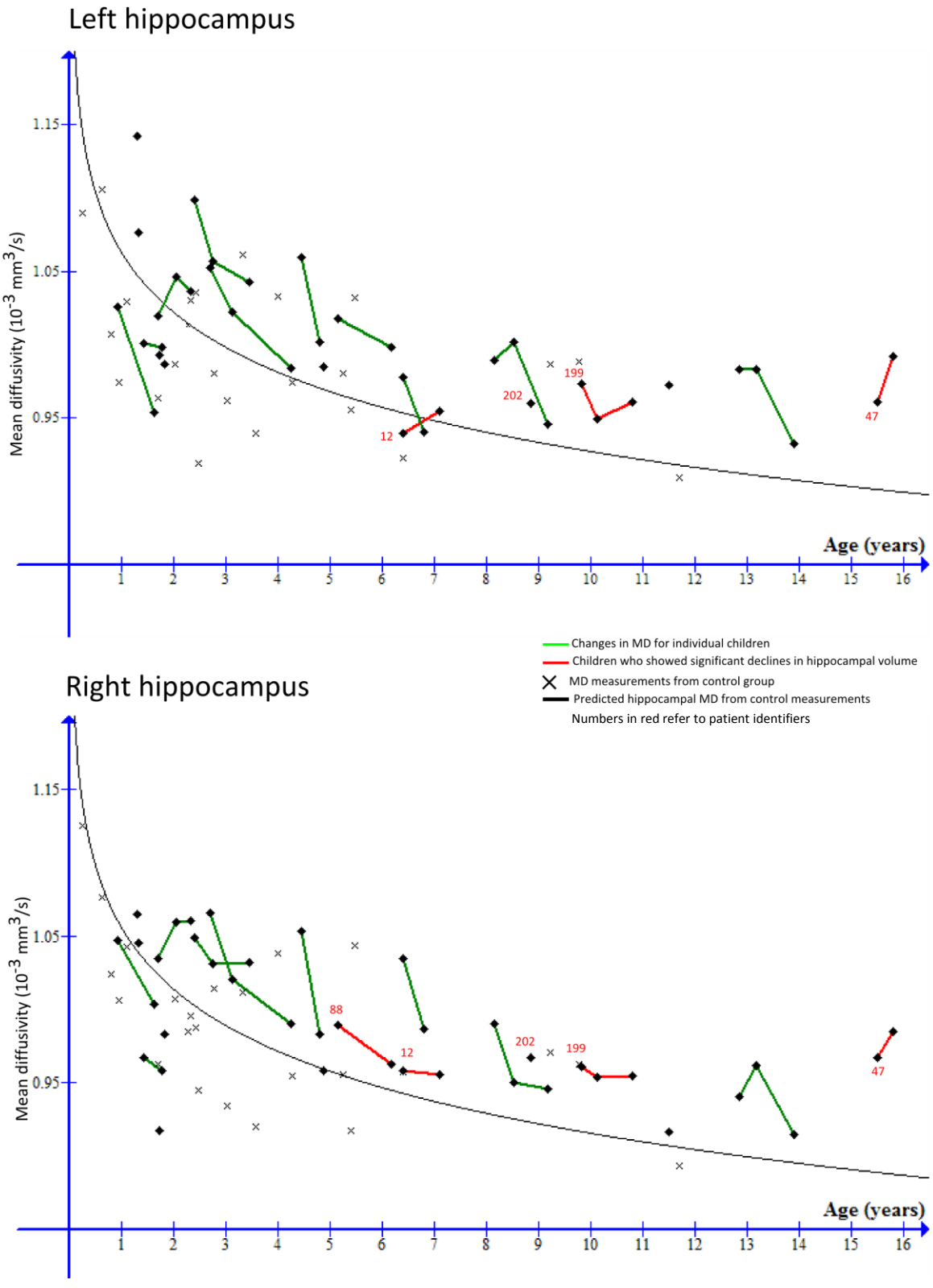


Figure 7-12: Hippocampal MD in children with other CSE

The change in hippocampal MD over time was estimated in each child using similar methodology as that described in Chapter 6 to estimate hippocampal growth rates. There was no significant effect of aetiological group on the rate of change of hippocampal MD after adjusting for age, although there was a trend for children with PFS to show smaller rates of reduction in MD than either of the other groups (Univariate ANOVA, p=0.063). Of the 50 children who had serial DTI enabling longitudinal analysis, 6 showed a significant increase (defined as >1.96 S.E. above 0) in hippocampal MD over the course of their follow-up. 4 of these were children with prolonged febrile seizures and 2 were children with other CSE. Their details are summarised in Table 7-9, of note all but one child had subsequent recurrent seizures (whether febrile or otherwise) during the follow-up period, although the numbers were too small for formal statistical analysis.

Patient ID	Age at CSE (yrs)	Duration CSE (mins)	Previous seizure history	Focal	Continuous	Clinical information	Medication	Seizure recurrence	Hippocampal changes
2	3.72	32	4 previous SFS	N	Y	PFS	N	2 further SFS	Large bilateral increases in MD
7	1.32	40	None	N	N	PFS	N	None	Bilateral increases in MD
17	3.13	45	4 previous SFS, 1 previous PFS	N	Y	PFS	N	2 further PFS	Increase in right MD and decrease in left hippocampal volume
56	1.20	40	None	Y	N	PFS	N	1 further SFS	Small increase in left MD
47	15.5	100	Daily seizures	N	N	Cryptogenic epilepsy	VPA, TPM	Daily myoclonic jerks	Bilateral increases in MD and decreases in hippocampal volume
121	1.70	45	2 previous afebrile seizures	Y	N	Idiopathic epilepsy	VPA	Continuing seizures every 1-2 months	Small increase in right MD

SFS: Short febrile seizure (<30 mins duration)
PFS: Prolonged febrile seizure
VPA: Sodium Valproate
TPM: Topiramate

Table 7-9: Clinical details of patients showing increases in hippocampal mean diffusivity following CSE

7.3.7.2 Changes in FA over time

Serial plots of FA in individual children are shown in Figure 7-13, 7-14 and 7-15. Children who showed significant decreases in hippocampal volume (Chapter 6) are highlighted in red/light blue. As there was no significant difference between left and right hippocampal FA rates of change over time (paired t-test, p =0.760), rates of change were averaged for comparison. There was no significant effect of aetiology on the rate of hippocampal FA change (Univariate ANOVA, p = 0.309), but those children with recurrent seizures did show greater reductions in FA than those who did not have subsequent seizures (2-sample t-test; -0.002/year vs. -0.031/year, p = 0.044).

28/50 children with longitudinal FA measurements showed decreases in hippocampal FA greater than 1.96 standard errors over the study period and 13/50 showed increases greater than 1.96 standard errors. There were no children who showed a significant decrease on one side and an increase in the contralateral hippocampus. Again, there was no significant difference between aetiological groups in the proportion of children showing either a decrease (Fisher's exact test, p = 0.270) or increase (Fisher's exact test, p=0.919) in hippocampal FA, however a significantly greater proportion of those showing decreases in FA had recurrent seizures (Chi-squared, p=0.044). Numbers are summarised in Table 7-10.

		Number	Decreases in hippocampal FA	Increases in hippocampal FA
Aetiological group	PFS	23	10 (43.5%)	6 (26.1%)
	Symptomatic CSE	14	10 (71.4%)	3 (21.4%)
	Other CSE	13	8 (61.5%)	4 (30.8%)
Recurrent seizures		26	18 (69.2%)	5 (19.2%)
No further seizures		24	10 (41.7%)	8 (33.3%)

Table 7-10: Children showing decreases and increases in hippocampal FA following CSE

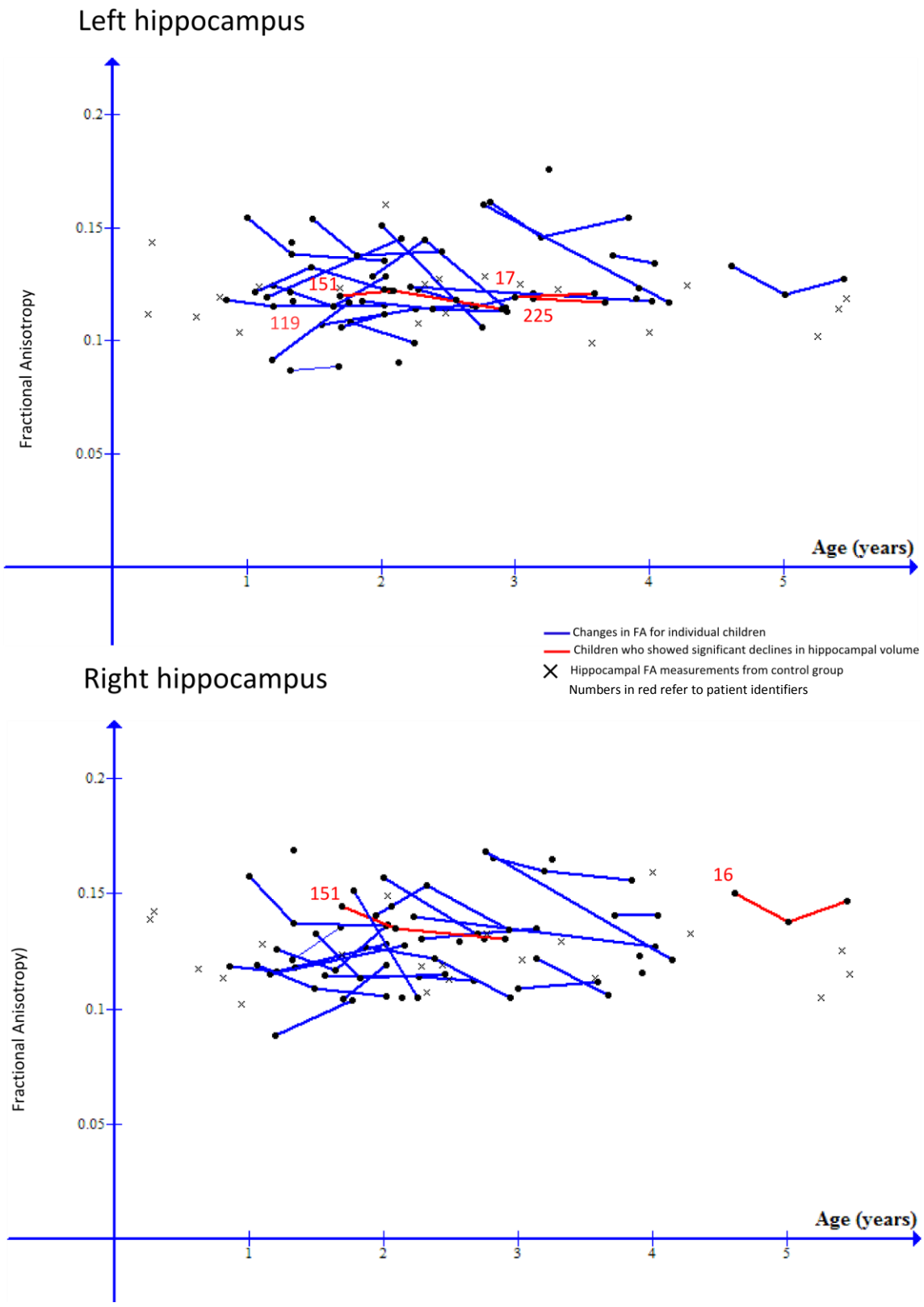


Figure 7-13: Hippocampal Fractional anisotropy in children with PFS

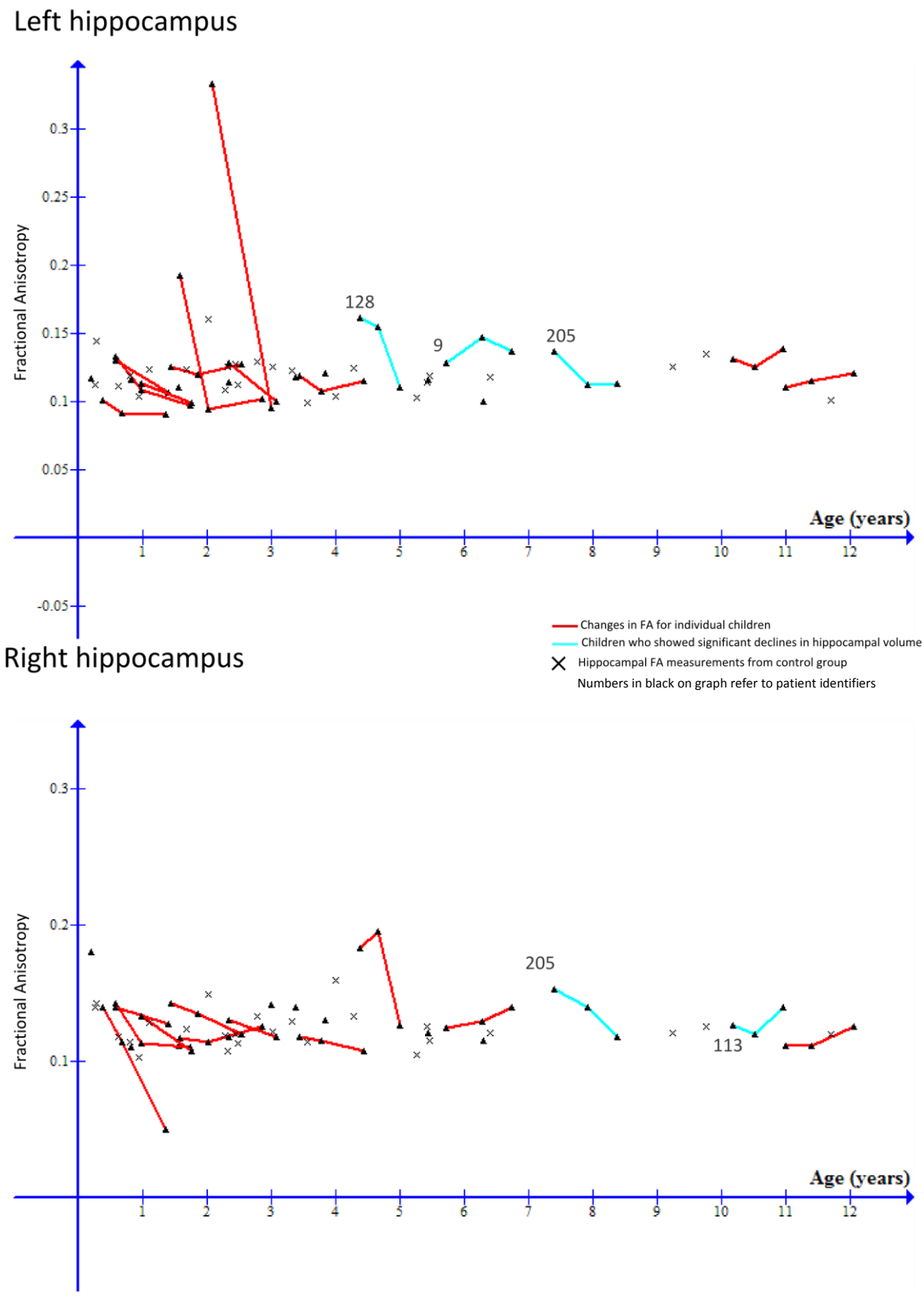


Figure 7-14: Hippocampal FA in children with symptomatic CSE

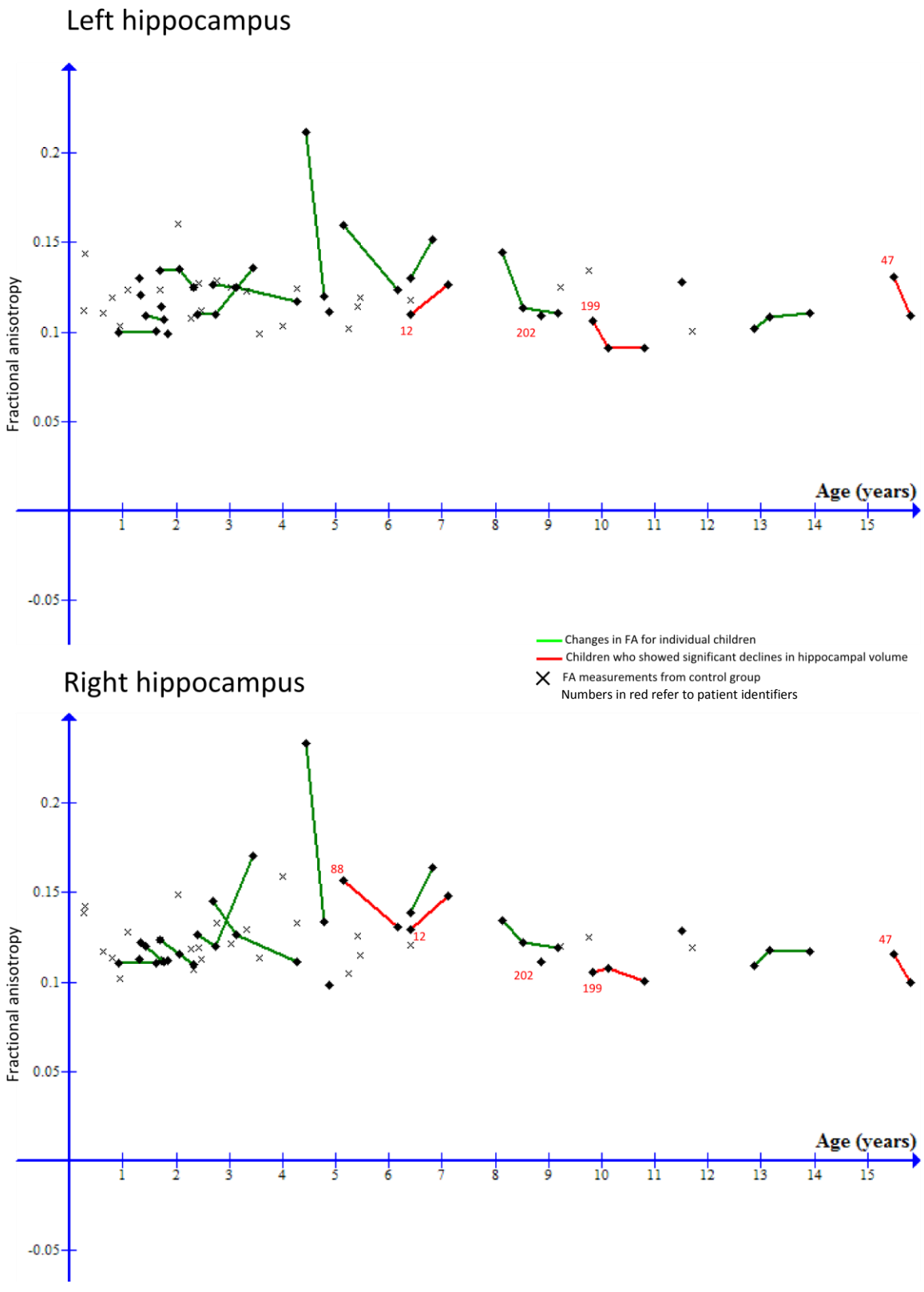


Figure 7-15: Hippocampal FA in children with other CSE

7.3.7.3 Discussion

Looking more closely at changes in individual children, we know that hippocampal MD decreases with age in normal children. The same was true for the majority of our patient cohort, who also showed decreasing MD with age. Only 6 children showed a significant increase (>1.96 S.E. above 0) in hippocampal MD over the course of the study. This was bilateral in 3 and unilateral in 3 and occurred predominantly in children with PFS (4/6) or cryptogenic or idiopathic epilepsy (2/6) with further seizures following their episode of CSE. Only 1 child had had no subsequent seizures during the follow-up period and they showed the smallest increase in MD. There also appeared to be an effect of seizure recurrence on changes in FA, with those children with recurrent seizures showing a significantly greater decline in FA over the study period. This suggests that a small proportion of children will show increases in MD and decreases in FA following CSE, changes suggestive of an evolving hippocampal injury that may be a precursor to TLE-MTS, and that subsequent seizure activity may play an important part in this evolution.

Figure 7-16 summarises the relationship between changes in MD, FA and hippocampal volume. Interestingly the children showing abnormal MD/FA trajectories were not necessarily the same children who showed loss of hippocampal volume. This raises the possibility that the pathological processes occurring following CSE can be separated; with neuronal reorganisation, causing changes in MD/FA not necessarily dependant on the neuronal cell loss that is thought to underlie volume loss. Since the hippocampus in established MTS has been shown to display both neuronal cell loss(112) and signs of structural reorganisation such as gliosis, mossy fibre sprouting and neurogenesis(260) it may be that both processes need to be activated in order for MTS to occur.

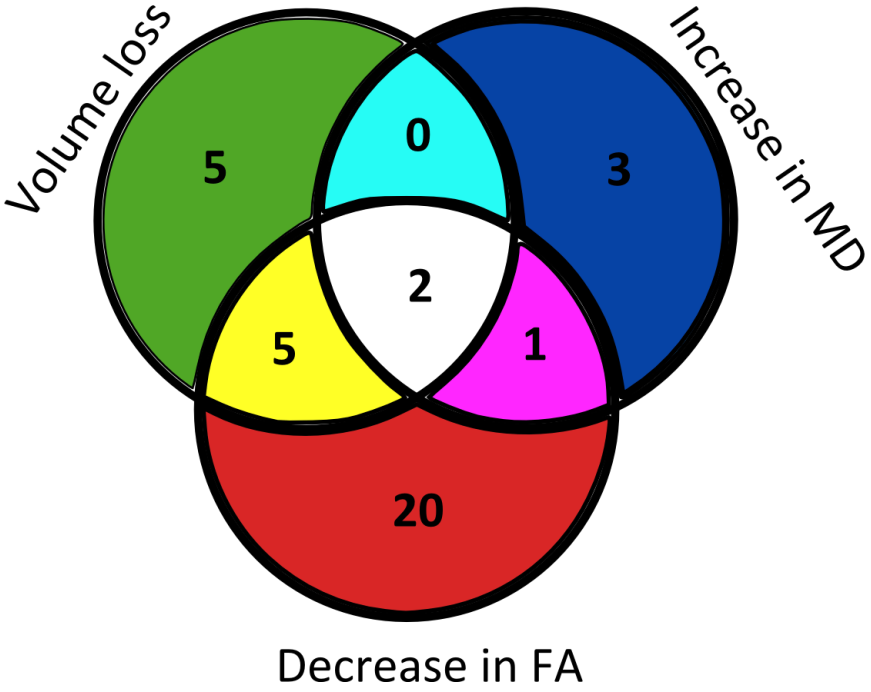


Figure 7-16: Venn diagram summarising the numbers of children with changes in hippocampal MD, FA and/or volume

7.3.5 Cognitive outcome

7.3.5.1 MD/FA and cognition

Mean hippocampal MD and FA at each time point, were tested against cognitive scores at 1 month and at 1 year. No significant correlations were found. There were likewise no significant correlations between the rate of change in MD and either cognitive score.

There was however a significant association between both initial and final cognitive scores and the rate of change in FA, with greater increases in FA being associated with improved cognitive scores at both 1 month ($p = 0.005$) and 1 year ($p = 0.041$). The association with cognitive scores at 1 month remained even after adjusted ICV was added into the model ($p = 0.008$) but disappeared by 1 year ($p = 0.221$).

7.3.5.2 Discussion

Given that we have previously shown that the most important determinants of cognitive outcome appear to be overall ICV and the presence/absence of structural abnormalities, the finding that greater rates of increase in hippocampal FA were associated with improved cognitive scores needs to be interpreted carefully. FA is thought to reflect the microstructural organisation of the brain and has previously been shown to increase as the brain develops (255;261). Increases in FA are therefore thought to represent maturation of brain structures and increased organisation.

One possibility may be that greater increases in hippocampal FA are related to or indicative of improved neurodevelopment and therefore lead to improved cognitive outcomes. However, as the relationship between the rates of increase of hippocampal FA over the study period was stronger for the initial cognitive scores rather than final ones, there are some problems with this interpretation. An alternative explanation is that it is the better cognitive ability at study entry that provides an improved substrate for subsequent neurodevelopment and therefore leads to larger increases in hippocampal FA over the study period. Since initial and final cognitive scores are correlated, there would then be a residual association between the rate of increase in FA and cognitive outcome 1 year post-CSE. Some evidence for this may come from the fact that if age-adjusted ICV is added to the model as well then while there remains a significant association between the rate of FA change and the initial cognitive score, this is no longer significant at 1 year post-CSE.

7.4 Conclusion

In this chapter we have shown that despite developing a reproducible and reliable method of measuring hippocampal MD and FA, measurement remains problematic. We were able to demonstrate a proportion of children displaying abnormal trajectories in DTI metrics following CSE, with some children showing increases in MD and others decreases in FA, suggestive of those changes seen in MTS. Since these are not the same children that were demonstrated in Chapter 6 to show loss of hippocampal volume post-CSE, apart from 2 children who showed volume loss, increases in MD and decreases in FA (Figure 7-16), we speculate that there may be separate processes at work, all of which are required for the development of clinical TLE:MTS.

We have also shown that children with PFS do not show the normal relationship between age and hippocampal MD in contrast to healthy controls and children with other CSE. One plausible explanation for this is that there is a developmental abnormality in children with PFS leading to different trajectories in the development of neuronal organisation and that this is one of the factors that renders them vulnerable to having PFS at this age.

The focus of our study so far has been on the hippocampus, because of its central role in MTS. However it is well recognised that MTS is rarely limited to the hippocampus. In the subsequent chapter we will expand our focus and investigate broader changes in the rest of the brain following CSE.

Chapter 8: Tract-based Spatial Statistics

8.1 Introduction

In the previous two chapters we have focussed on hippocampal changes following CSE, looking both at hippocampal volume measurements and DTI metrics. We have shown that there are progressive losses of hippocampal volume in a proportion of children post-CSE and that this occurs in CSE of any aetiology.

There is a body of evidence that MTS is not limited to the hippocampus but often affects other, extra-hippocampal structures (262;263). Animal studies have suggested that several other cortical and subcortical regions are affected by CSE, in addition to the hippocampal changes seen (54;264). In addition CSE in humans has been associated with widespread changes in cortical grey matter(265) and subcortical white matter tracts(266;267). It is likely therefore that any initial damage sustained in an episode of childhood CSE is not restricted to the hippocampus but rather that pathological changes elsewhere in the brain may play an important part in the pathogenesis of TLE-MTS. The aim of this chapter is to consider structural brain changes outside of the hippocampus.

As using an ROI based approach for whole brain analysis is time consuming and difficult to standardise for multiple brain regions, an automated, whole-brain analysis technique, tract-based spatial statistics (TBSS) was used to investigate MD and FA changes following CSE across the entire white matter skeleton. The results from the TBSS investigations were then cross-validated using manual ROI-based measurements of MD/FA in selected white matter tracts.

8.1.1 Tract-based Spatial Statistics

Tract-based Spatial Statistics (TBSS) (196) is an operator independent, automated technique for performing whole-brain analysis of diffusion data. It relies on the identification of a common white matter skeleton, containing orientation independent information about the major white matter tracts from subjects' FA maps.

This has several advantages over other automated techniques for the comparison of DTI-based metrics, such as voxel based morphometry (VBM) or traditional manual techniques involving ROI placement. Unlike manual ROI placement, it allows analysis of the entire white matter skeleton and

avoids having to make a priori assumptions about the likely location of white matter differences. Since it is not operator dependent, it also avoids subjective errors of ROI placement. In contrast to VBM-style approaches, TBSS does not require perfect alignment between images as location information is summarised by projection onto the common white matter skeleton, hence it may be less prone to inaccuracies caused by mis-registration(196). Altering the size of the smoothing kernel used in VBM can drastically alter the results of the analysis, especially when processing DTI data(268), as TBSS does not require smoothing of the image, this removes a further element of subjectivity.

Previous use of TBSS in patients with TLE has shown decreases in FA in several white matter tracts including the anterior corpus callosum, parahippocampal gyrus and temporal gyri(269) and so there are reasons to believe that white matter abnormalities may be present following PFS.

8.1.2 Region of interest analysis

Although TBSS will identify areas along the white matter skeleton where there is a significant deviation in FA or MD from a control population, it does not provide information as to the magnitude of such differences, nor is it able to take into account of longitudinal information. It is useful therefore to combine TBSS analysis with an independent approach to quantitative measurements of DTI metrics. To this end a region of interest was placed in the genu of the corpus callosum on each scan in order to quantitate the MD/FA changes in this region. The genu was chosen out of all the tracts where differences were shown on the initial TBSS analysis because its large size and anatomy allowed consistent placement of an ROI. The mean MD/FA were also calculated across the entire white matter skeleton This allows cross-validation of the results from the TBSS analysis, as well as providing a quantitative insight into how these metrics are changing across time points.

8.2 Methods

As these automated techniques rely on accurate transformation of MRI images from disparate subjects into a common image space (co-registration) in order to allow like for like comparisons, this analysis was restricted to comparison of children with PFS with healthy controls in the same age range. The reasons for this were three-fold. Firstly the heterogeneous nature of the other two patient groups and specifically the high incidence of structural brain abnormalities in

children with non-PFS CSE (as discussed in Chapter 5) meant that accurate co-registration was not possible using standard tools. Secondly the time taken to perform the analysis rises exponentially with the number of subjects therefore this was restricted in order to complete this within a reasonable time period. Finally, limiting the number of different comparisons performed reduces the probability of a type-I error

8.2.1 TBSS

TBSS was performed using standard scripts contained within the FSL software package (249). Using the FA maps generated during the previous study, all subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT(270). As the age range of our subjects precluded using a standard target image, TBSS was used to perform a search for the most representative image in our cohort and each subject was then aligned to that image. Finally that image is affine-transformed into 1x1x1mm MNI52 space and the same transformation applied to all of the other subjects. This was done separately for each time-point. Visual inspection of each subject was then used to check the validity of the registration and any subjects where the registration had not worked were removed from the analysis.

Next, the mean FA composite image was created by averaging all the warped FA images and thinned to create a mean FA skeleton which represents the centres of all tracts common to the group using a threshold of FA > 0.2. Each subject's aligned FA data was then projected onto the mean FA skeleton. A similar transformation was applied to each subjects' MD data and both datasets were analysed using voxelwise cross-subject statistics.

The *randomise* tool from FSL was used to perform voxel-wise permutation-based nonparametric two-sample permutation tests(271) with 5000 permutations on the aligned white matter skeleton to look for significant differences in MD or FA between children with PFS and healthy controls. Threshold-free cluster enhancement(272) was used to detect significant clusters of voxels and correct for multiple comparisons. Gender, age and ICV were entered as covariates and coded as nuisance variables. The results were stored as statistical maps with $p < 0.05$ taken as the level of significance. Results were visualised as colour-coded masks thresholded at $p < 0.05$ and superimposed on the mean FA map and skeleton. Localisation of differences was performed with the aid of a reference white matter atlas.

Each of these stages was repeated for each time point, thus 3 separate TBSS analyses were made comparing patients at each time point with controls. As the age of the patients was changing at each time point, control subjects were chosen from our cohort to approximately match the age range of the patients at each time point, so each comparison was made to a different, albeit overlapping set of control subjects.

8.2.2 Region of interest analysis

The genu of the corpus callosum was chosen for the region of interest analysis. Since defining the entirety of a specific white matter tract using manual methods is not possible from a structural MRI scan(273), a small, fixed-size ROI (similar to Method 3 from Section 7.2.2.3) was placed within the centre of the genu using the co-registered T1-weighted image as this provided the best contrast for white matter visualisation without confounding FA measurements.

A 3x2x2 voxel ROI was placed within the genu of the corpus callosum. This was defined as the most anterior coronal slice where the two halves of the corpus callosum meet in the midline. A 3x2 voxel ROI was drawn on this slice and the slice immediately posterior to it (Figure 8-1).

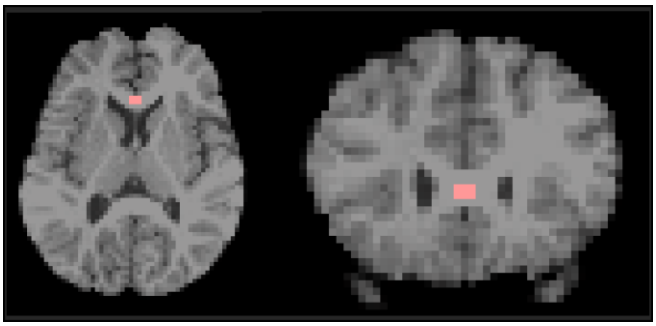


Figure 8-1: Example ROI placed in the genu of the corpus calllosum

The mean white matter skeleton generated during the TBSS analysis was also saved and used as an ROI to measure the mean MD and FA over the entire skeleton in each subject.

fslview was used for image visualisation and ROI placement. *fslstats* was then used to calculate the mean MD and FA within each ROI.

Univariate ANOVA was used to compare MD/FA within each ROI between control and patient groups at each time point. Age, ICV and gender were entered as co-variates to mimic the TBSS analysis. Adjusted MD/FA values were determined after correction for age/ICV. Spearman's

rank correlation with Bonferroni correction for multiple comparisons was used to explore the age dependencies of both MD and FA within each ROI.

8.3 Results

29/33 children with PFS had imaging suitable for use in this analysis. The remaining 4 children did not tolerate the DTI sequence and so were excluded. 18 children from the control group tolerated DTI and were in a suitable age range. The numbers of children with PFS at each time point and their ages are summarised in Table 8-1.

	Number	Mean age (years)	Median age (years)	Age range (years)
Controls	18	2.76	2.45	0.62 – 5.47
Patients 1 month post-PFS	29	2.06	1.86	0.85 – 4.61
Patients 6 months post-PFS	17	2.40	2.25	1.19 – 5.01
Patients 1 year post-PFS	19	2.99	2.90	1.76 – 5.45

Table 8-1: Age ranges and numbers of children at each time point

For the TBSS analysis the control group was split into three overlapping cohorts to approximately match the age range of the patients at each time point. The numbers of children and ages for each control cohort are given in Table 8-2.

	Number	Mean age (years)	Age range (years)
Controls for 1 month group	15	2.24	0.62 – 4.28
Controls for 6 months group	12	2.95	1.69 – 5.25
Controls for 1 year group	14	3.31	1.69 – 5.47

Table 8-2: Age ranges for control group at each time point

8.3.1 TBSS

8.3.1.1Fractional Anisotropy

The results of the TBSS analysis comparing FA between patient and control groups are shown in Figure 8-2 and 8-3. There were widespread reductions in FA at 1 month post-CSE in multiple white matter tracts. These reductions were largely still present at 6 months post-CSE. However by 1 year post-CSE there were no longer any significant differences between patients and controls. A summary

of the tracts found to be involved at each time point is given in Table 8-3. There were no areas found with significantly higher FA in patients than controls at any time point.

In order to ascertain if these reductions were an artefact due to the selection of the control groups, the analysis was repeated comparing patients at each time point against the entire control group. Reductions were still apparent at 1 and 6 months post-CSE and the regions affected did not appear to differ to any great extent.

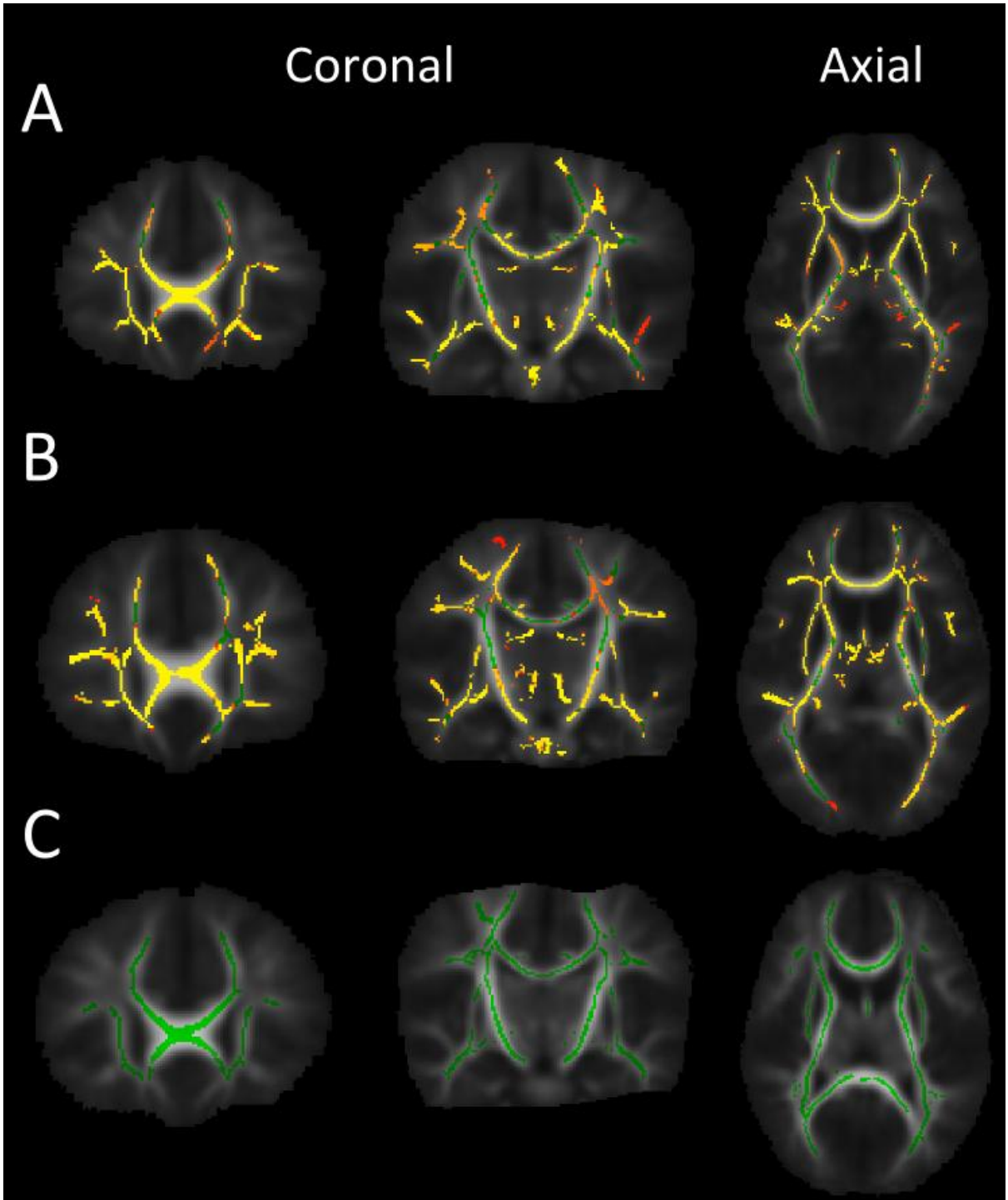


Figure 8-2: TBSS analysis at A) 1 month B) 6 months and C) 1 year post PFS in coronal and axial section Yellow-Red areas show significant reductions in FA in patients compared to controls ($p < 0.05$)

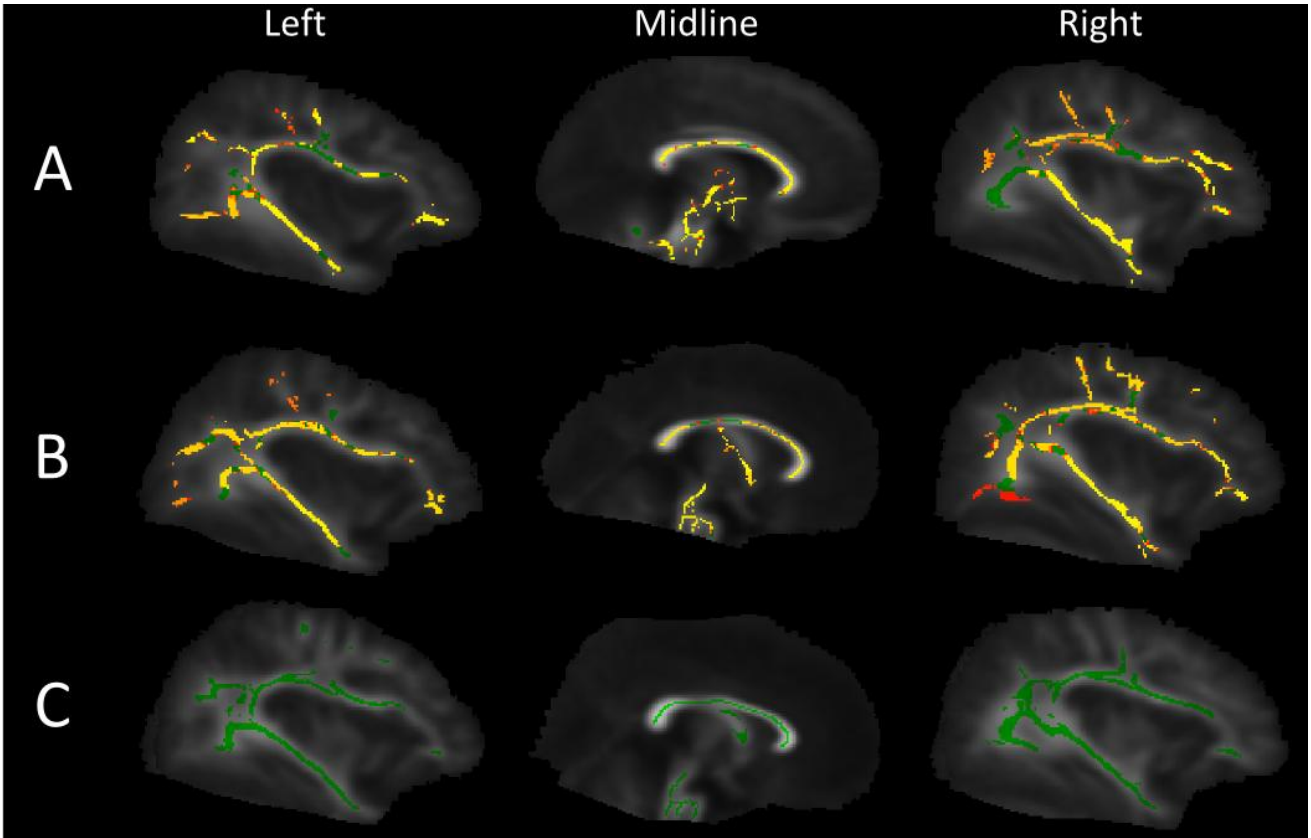


Figure 8-3: TBSS analysis at A) 1 month, B) 6 months and C) 1 year post PFS in sagittal sections
Yellow-Red areas show areas of significant reductions in FA in patients compared to controls ($p < 0.05$)

Major white matter tracts showing decreases in FA
Anterior thalamic radiation (left + right)
Inferior fronto-orbital fasciculus (left + right)
Anterior corona radiata (left + right)
Genu of the corpus callosum
Body of the corpus callosum
Splenium of the corpus callosum
Internal capsule
External capsule
Inferior fronto-occipital fasciculus (left + right)
Uncinate fasciculus (left + right)

Table 8-3: White matter tracts showing significant decreases in FA at 1 and 6 months post-PFS

8.3.1.2 Mean Diffusivity

There were no significant differences in MD along the white matter skeleton found between patients and controls at any time point.

8.3.2 Regions of Interest

Mean values for FA within the genu of the corpus callosum and over the entire FA skeleton for each of the groups are given in Table 8-4. FA within the genu was significantly lower in patients than controls 1 month post-PFS (p=0.006). It then increased at 6 months post-PFS, but was still significantly below control values (p=0.042) before appearing to normalise by 1 year post-PFS. FA values over the entire TBSS skeleton were also significantly below control values at 1 month (p = 0.012) and 6 months (p = 0.021) post-PFS, but had normalised by 1 year post-PFS.

	Mean FA adjusted for age, ICV and gender (95%CI)			
	Genu	**p-value**	**Mean skeleton**	**p-value**
Controls	0.648 (0.649 – 0.718)		0.367 (0.356 - 0.378)	
Patients 1 month post –PFS	0.607 (0.577 – 0.637)	**0.006***	0.346 (0.338 - 0.355)	0.012*
Patients 6 months post –PFS	0.618 (0.579 – 0.656)	**0.042***	0.345 (0.334 - 0.356)	0.021*
Patients 1 year post –PFS	0.676 (0.638 – 0.715)	1	0.360 (0.349 - 0.370)	1

p-values represent significance levels for differences from control values and are adjusted for multiple comparisons

*** p < 0.05 after Bonferroni correction for multiple comparisons**

Table 8-4: Mean adjusted FA within ROIs and comparison with control values

Mean values for MD within each ROI for each of the groups are given in Table 8-5 below.

	Mean MD adjusted for age, ICV and gender within each ROI (95% CI)			
	Genu	**p-value**	**Mean skeleton**	**p-value**
Controls	0.989 (0.930 – 1.048)		0.897 (0.881 - 0.913)	
Patients 1 month post –PFS	1.017 (0.966 – 1.068)	1	0.905 (0.892 - 0.918)	1
Patients 6 months post –PFS	1.000 (0.935 – 1.065)	1	0.911 (0.894 - 0.927)	**0.726**
Patients 1 year post – PFS	0.954 (0.890 – 1.019)	1	0.892 (0.876 - 0.908)	1

p-values represent significance levels for differences from control values and are adjusted for multiple comparisons

Table 8-5: Mean adjusted MD within ROIs and comparison with control values

8.4 Discussion

8.4.1 MD and FA changes following PFS

Widespread reductions in FA were detected in multiple white matter tracts following PFS using TBSS analysis. These changes were apparent at 1 month post-PFS, and remained until at least 6 months post-PFS. However, by 1 year they appeared to have resolved. Manual region of interest analysis also showed similar reductions in FA in the genu, with gradual normalisation over time, as did analysis over the entire white matter skeleton. No differences in MD were detected using TBSS and, this was also the case using the ROI analyses.

8.4.2 Methodological comparisons

Comparison of different methods of measuring white matter FA and MD is important, as neither TBSS nor an ROI-based approach can truly be held as a "gold standard". There was good concordance between the results of the TBSS and the ROI analysis. Manual ROI placement is subject to problems with reproducibility as discussed in Chapter 7.2, which is a potential source of Type II error, and this has been commented on by other research groups(274;275); TBSS, although it avoids any subjective bias from ROI selection and placement relies on automated co-registration techniques can be difficult to assess and validate. That concordant results have been achieved using these different techniques allows greater confidence to be placed on these findings.

8.4.3 Interpretation

Apart from isolated case reports (158;266;276), there have been no studies looking at diffusion metrics following CSE to date, and none have used whole brain analysis techniques such as TBSS. Those cases that have been reported in the literature have shown acute changes in cortical grey and sub-cortical white matter on DWI, with a reduced apparent diffusion coefficient (ADC). These acute MRI changes have generally been observed to resolve on follow-up imaging by 1 month, although the majority of the cases reported have had residual neurological problems with associated chronic MRI changes. These reports therefore represent the most severe extreme outcomes of CSE and cannot be taken as being representative of the general population, nor can they prove causality. They do show, however, that permanent neurological alterations can occur following CSE, and changes in DWI and ADC form part of the acute response to CSE. No reports of reductions in FA

following CSE have been reported, however FA is rarely measured in a clinical context and the one study that did calculate FA(158) only reported FA values from the fornix, which remained constant.

A number of more systematic DTI studies have taken place in patients with established TLE, which are summarised in Table 8-6. These studies have used a mixture of manual ROI measurements, VBM and TBSS to compare various diffusion metrics in patients with TLE (both with and without MTS) and controls. There have been consistent reports of reduced FA in portions of the corpus callosum, external capsule, ipsilateral temporal lobe and frontal white matter tracts.

Study	Method	Patient group	Summary of findings
Arfanakis et al 2002(263)	Manual ROI	15 patients with TLE	Reduced FA in external capsule and posterior corpus callosum
Thivard et al 2005(187)	SPM-DTI Manual ROI	35 patients with TLE:MTS	Reduced FA in corpus callosum and ipsilateral temporal stem. Increased MD in ipsilateral temporal lobe
Gross et al 2006(277)	Manual ROI	11 patients with TLE:MTS	Reduced FA in genu of corpus callosum and external capsule. Increased MD in genu, splenium and external capsule
Focke et al 2008(278)	TBSS/VBM	33 patients with TLE:MTS	Reduced FA in ipsilateral temporal lobe, external capsule, temporal lobe white matter and frontal white matter tracts
Riley et al 2010(279)	TBSS	12 patients with TLE	Reduced FA in ipsilateral anterior and mesial temporal lobe, cerebellum and contralateral frontoparietal lobe
Afzali et al 2011(269)	TBSS	19 patients with TLE (seizure-free)	Reduced FA in ipsilateral temporal lobe, anterior corpus callosum, external capsule and frontal gyri

Table 8-6: DTI studies of patients with TLE

None of our cohort of 29 children with PFS has a diagnosis of epilepsy, nor do they show any visible clinical changes on MRI or gross neurological abnormalities, as previously discussed in Chapter 5. It is then perhaps not surprising that our findings differ from previous reports of patients with established TLE or case reports of CSE. While reductions in FA were found in all of the areas highlighted in previous studies of TLE, children with PFS showed more dramatic FA reductions across many more portions of the white matter skeleton compared to the limited regional changes that have been reported in TLE. Unlike the case reports of CSE, they did not display focal abnormalities on DWI or increases in ADC/MD, but as the mean time of our first scan was 1 month post-CSE, any acute changes would have been expected to have resolved.

The predominant changes reported post-CSE have been increases in hippocampal volume(46), T2 hyper-intensities(159) and increases in ADC(266), all of which have been linked with transient and self-resolving oedema(46;232). If the reductions in FA are a direct effect of the PFS and represent seizure-related neuronal damage, then as FA remains reduced past the time when the acute oedematous changes are expected to resolve, it would appear to represent a longer term disruption of white matter, with a slower time-course to resolution. Reductions in FA may represent other aspects of this initial injury that cause more long lasting impairments of white matter integrity, such as a degree of myelin breakdown or axonal degeneration (174;280) and therefore recovery of FA may lag behind the other measures. That it does recover may be a reflection of the resilience of the developing brain or the relatively mild nature of any PFS associated injury, and suggest that neuronal repair and reorganisation following PFS is by and large a successful process, although as demonstrated in Chapter 6, failure to detect differences at a group level does not necessarily exclude the possibility that there may be a proportion of individuals in which changes do not resolve.

However given the time course of FA reductions noted on ROI analysis, there may be alternative explanations that are also biologically compelling: there are reasons to believe that the reductions in FA may not represent seizure-associated neuronal injury, but rather may be a transient developmental stage in children with a predisposition to PFS.

Febrile seizures are most common between the ages of 6 months-5 years, with a peak incidence at 18 months(281). They are also known to have a large genetic component to their aetiology(282). Furthermore epidemiological data shows that if children who had one PFS have a further febrile convulsion, it is likely to also be prolonged(283), reflecting a possible underlying predisposition to prolonged seizures. The implications of this are that children who suffer a PFS are highly likely to have pre-existing abnormalities that predispose them to have a prolonged seizure in response to a febrile illness and that these abnormalities are likely to have a genetic basis. It is possible that this increased susceptibility is related to aberrant white matter development, and it is this that is causing the global reductions in white matter FA. As children grow and develop, their susceptibility to febrile seizures decreases and it may be expected that any associated structural abnormality will also normalise - i.e. that the abnormality predisposing these children to PFS represents an aberrant developmental stage that normalises with age. If this is the case, then as the children in our cohort were a year older by the end of the study and all were over 18 months in chronological age, then the normalisation of FA values taking place during this time should represent an end to their risk of further febrile seizures.

8.4.4 Conclusions

In this chapter, we have shown that there are widespread reductions in FA detected following PFS and that these are still present at 6 months post-PFS, but normalise by 1 year post-PFS. We have advanced two possible explanations for these observations: namely that they may represent longer term seizure-induced injury, with subsequent healing and normalisation; alternatively that they represent an aberrant developmental phase that is related to these children's susceptibility to PFS and that subsequently normalises as their risk of febrile seizures decreases. Both of these hypotheses would provide a plausible explanation for the data presented in this chapter, however further data will be required to determine which should be preferred.

CHAPTER 9 - DISCUSSION

The aim of this thesis has been to explore the structural and functional consequences of childhood CSE in the first year post-CSE. In this concluding section I will attempt to draw together the separate strands of evidence that have been presented in the separate preceding chapters and set out the conclusions that we have drawn as to our original hypotheses. I will also discuss the potential limitations of our study and suggest improvements that could be made to address these in the future. Finally I will advance some suggestions for future research to build on the findings of this study.

9.1 CSE and structural brain abnormalities

One of the initial findings to come out of this study was an estimate of the incidence of structural brain abnormalities in children with CSE. 31.2% of the children in this study were found to have an abnormality on MRI following their episode of CSE, although it seems clear that the majority of these predate the seizure and are likely to represent causes rather than outcomes of CSE. This is a similar proportion to that reported in previous studies of neuroimaging after CSE(202), although this is the first reported cohort to have complete MRI coverage. It was also possible to demonstrate that abnormalities picked up on CT in the acute situation differ from those detected by MRI.

Of note no children with PFS were found to have clinically significant abnormalities on their initial MRI scan and none had evidence of pre-existing MTS. This implies that detectable, pre-existing hippocampal damage is not a significant cause of PFS and that children with PFS have grossly normal hippocampi at the time of CSE.

9.2 Hippocampal injury following CSE

One of the primary aims of this thesis was to investigate hippocampal injury following CSE and specifically any link between PFS and the development of MTS. To this end detailed measurements of hippocampal volume were made in children over the initial year following an episode of CSE. A pre-requisite for the development of MTS is a loss of hippocampal volume and we found that 20-30% of children show evidence of hippocampal volume loss in the year following CSE. This is the first

study to demonstrate hippocampal volume loss in an unselected cohort of children with CSE and is an important first step to quantifying the risk of developing TLE-MTS following CSE.

The proportion of children showing volume loss was not significantly different between aetiological groups, showing that hippocampal injury following CSE is not limited to PFS, but can occur with all types of prolonged seizures. There are qualitative differences in the pattern of injury between children with CSE of different aetiologies, with unilateral damage being more common in PFS and bilateral damage in other CSE. We hypothesise that the consequences of this injury may differ as TLE-MTS is usually a lateralised condition.

Volume loss is not the only marker of hippocampal damage, and another change that has been reported in TLE-MTS is an increase in hippocampal MD. Evidence of increases in hippocampal MD was found in a smaller proportion of children, not all of whom showed significant volume loss. The majority of these children had PFS, although overall numbers were too small for formal statistical analysis. Increases in hippocampal MD would be consistent with developing MTS and it is possible that the disassociation with hippocampal volume loss indicates separate pathological processes at work. If both are necessary for the development of TLE-MTS then the increased incidence of the latter in PFS may explain the association between TLE-MTS and PFS but not other forms of CSE.

DTI changes were associated with an increased incidence of recurrent seizures (both febrile and afebrile) over the follow-up period, and there was a borderline association between volume loss and the number of previous febrile seizures, so it may be that further episodes of seizure activity play an important part in the evolution to MTS.

9.3 Developmental abnormalities in children with PFS

It is recognised that febrile seizures show an extremely strong familial inheritance, with studies estimating the heritability at around 65-75%(284;285). Likewise animal models and genetic linkage studies have demonstrated the contribution of individual genes to febrile seizure susceptibility(259). As seizures, including febrile seizures are a neurological phenomenon, it seems reasonable to assume that the genetic contribution to febrile seizure susceptibility is modulated via an effect on brain structure and/or development. There is therefore reason to believe that, although children who are classified as having a PFS were believed to be neurologically and developmentally normal prior to their episode of CSE, there may in fact be subtle differences in brain structure and/or neuronal connectivity. We have previously reported on functional differences in the form of

impaired cognitive function and impaired performance on specific tests of hippocampal memory in children with PFS compared to healthy controls and to population norms, both at 1 month post-PFS and persisting at 1 year(173).

Since the occurrence of febrile seizures and PFS is age limited, the underlying abnormalities may likewise be transient and normalise as the child grows older. Two findings from our study lead us to believe that this may be the case:

Firstly we found widespread reductions in FA compared to healthy controls across the white matter skeleton in children following PFS. These reductions persisted at 6 months post-PFS but were no longer apparent at 1 year. While this could represent a long-lasting, but ultimately transient seizure effect, we believe that it is more plausible that this represents abnormal white matter development in children with PFS that normalises as the susceptibility to febrile seizures wanes.

Secondly in most children hippocampal MD decreases with increasing age, however this age dependency is not apparent in children with PFS, unlike healthy controls or those with other forms of CSE. This finding would be consistent with children with PFS having a different developmental trajectory. While only limited conclusions can be drawn from a negative result, this finding would be consistent with a developmental abnormality in children with PFS rather than a seizure-related insult.

9.4 Cognitive changes post-CSE

We have previously reported impaired cognitive function in children with CSE, both at 1 month post-CSE and persisting at 1 year(173). Children with PFS had better outcomes than children with symptomatic or other CSE, but were still under-performing compared to both healthy controls and population norms.

Since children with an abnormal MRI had significantly reduced cognitive outcome, a large part of the cognitive impairment in children with symptomatic CSE is likely to be attributable to structural brain abnormalities, which largely pre-date the episode of CSE. The importance of this was further demonstrated by volumetric analysis, which showed that while both hippocampal volume and overall intracranial volume were predictive of cognitive outcome, intracranial volume was the stronger predictor, suggesting that is the overall condition of the brain that is the primary determinant rather than any specific injury, whether CSE-induced or not.

9.5 Limitations

There are a number of weaknesses in our current study that set limits on the strength of the conclusions that can be drawn.

The most important limitation of this study was the lack of longitudinal assessment of our control group. Ideally serial cognitive assessment and imaging would have been done on the control group as well as our patient cohort to enable a direct comparison with normal growth and development. However, given the difficulty of performing MRI in this age group, it was not feasible to repeat these investigations in our control group. Repeating the general anaesthesia for those children who were having MRI for clinical reasons would not have been appropriate. Repeating an MRI scan on those controls that had their MRI during natural sleep after one year would have required additional sedation. It may have been possible to repeat imaging on the older children who were able to tolerate an awake scan, but this would not have covered the age range most of interest to us in this study and was not judged worthwhile to attempt on only a small proportion of our control group.

The lack of longitudinal data on a control population limits the comparisons that are possible with patient groups. For longitudinal measurements, such as hippocampal growth rates, we have been restricted to indirect comparisons with an estimated model of normal growth. While this is a valid technique, and has resulted in the detection of significant differences, direct comparison with a control group may offer greater statistical power and robustness.

Cross-sectional comparisons have also involved the serial comparison of a changing patient population against a static control group. While the children contained in our control cohort have been adjusted to match the age range of patients at each time point, we recognise that this may be prone to bias and therefore it is difficult to be completely certain that the differences that have been found on sequential cross-sectional analyses are not due to a developmental process taking place in patients, but which does not show up in our static control sample. Since the majority of our cross-sectional analyses found no difference between patients and controls, the practical impact of this may be limited for this study.

A further limitation was the large proportion of children who were notified to us with CSE but did not take part in the study, whether for clinical reasons or because of parental reluctance. While every effort was made to recruit as representative a sample of children as possible, inevitably

we were not able to collect full details from those children who were either not willing or not able to attend GOSH to take part in the study and therefore it is not possible to completely exclude other unknown areas of bias in our cohort. From the details available to us the clinical characteristics of participants did not differ significantly from non-participants except in the aetiology of CSE, as previously discussed in Chapter 3. This does limit the applicability of our findings to children with the most severe acute or previous neuronal injuries, who were underrepresented in our cohort, but in these children the severity of the injury would be expected to overwhelm any additional significance of CSE itself.

Although the duration of CSE did not appear to have a significant effect on any of the parameters measured in this study, it remains a possibility that seizure duration is important but that a ceiling effect is seen at 30 minutes. Assessment of this would require the recruitment of a cohort of children with brief seizures for comparison, alongside the existing group of healthy controls.

9.6 Future directions

This current study has provided important information about the consequences of childhood CSE; nevertheless, some questions remain unanswered. Given the initial abnormalities identified during this study, extended follow-up of this cohort of children over the following 10-15 years will be necessary to answer the ultimate question of which, if any, go on to develop clinical TLE-MTS. We hope to repeat the clinical, neuroimaging and cognitive assessments in these children after a suitable time period of at least 10 years. This will help to resolve which of our speculations as to the nature of hippocampal injury post-CSE are correct and which are not. In the meantime further relevant information will be produced from an extended 8 year follow-up of a previous cohort of children with CSE that is being collected by other members of our research group.

Further analysis of the data available from this cohort of children also has the potential to provide more valuable answers and clarification of some of the hypotheses generated from this thesis. The use of techniques such as tractography to further probe the white matter changes identified following PFS in Chapter 8 would allow further delineation of the magnitude and time-course of these changes.

In addition, if the speculation about underlying developmental abnormalities in children with PFS is correct, it is possible that not all children with these abnormalities will encounter the

required environmental triggers to have a PFS. Similar abnormalities may be present in children at risk of developing PFS, such as siblings or those with a strong family history of febrile seizures. Investigation of these children may be of benefit in exploring whether similar developmental abnormalities are present independent of the initiating factor of CSE and identify the role of the seizure itself in the pathology previously identified. Investigation of genetic factors and associating these with structural or functional brain changes may also help to elucidate the underlying pathology behind PFS.

9.7 Conclusion

In conclusion, through the analysis of this unique cohort of children, a number of important findings have been discovered:

- 31.2% of children with CSE have structural abnormalities on their MRI scan
    - The majority of these are pre-existing abnormalities and clinically significant abnormalities are rare in children with PFS
- Children with symptomatic CSE have reduced hippocampal volumes and increased hippocampal MD compared with controls and children with other forms of CSE
- Long term hippocampal damage, as represented by hippocampal volume loss, occurs in 20-30% of children following CSE and is not restricted to children with PFS
- Decreases in FA are found across multiple white matter tracts in the months following PFS and appear to resolve by 1 year
- There may be developmental abnormalities in the evolution of hippocampal MD in children with PFS

Over the course of this thesis we have shown that hippocampal injury following CSE is not just associated with PFS and that brain abnormalities following PFS are not just limited to the hippocampus. Rather both concepts need to be widened to include the risk of hippocampal injury in non-PFS CSE and the presence of other developmental abnormalities found in children with PFS.

Changes in metrics such as hippocampal growth, mean diffusivity and fractional anisotropy following CSE may also herald pathological changes within the hippocampus and are likely to play a part in the evolution of MTS. However, while we have demonstrated that such changes occur in almost a third of children, the ultimate significance of these changes will only be apparent with

further follow-up of our cohort. It does appear that the development of MTS may be a multi-step process and that recurrent seizures or CSE may play a role.

Subtle cognitive impairments and developmental abnormalities found in children with PFS suggest that these children are not entirely normal compared to healthy controls, and that this likely pre-dates the episode of CSE. Whilst the development of TLE-MTS remains a concern, attention also needs to be paid to the underlying problem that renders these children susceptible to PFS and the potential ramifications of this.

Of our original hypotheses, we have shown that:

1. Children with symptomatic CSE do indeed have worse structural and clinical outcomes than children with other forms of CSE
2. Hippocampal injury following CSE however, does not appear to be specifically associated with PFS, but occurs across all forms of CSE
3. While cognitive performance was associated with hippocampal volume, overall ICV appeared to be a stronger predictor and the degree of volume loss did not appear to be predictive of cognitive outcomes.

Further investigation of the link between CSE, MTS and eventual clinical as well as cognitive outcomes will hopefully answer many of these questions. This study has provided important evidence that long term hippocampal damage does occur following CSE and that children with PFS display significant abnormalities that are likely developmental in origin. Extended follow-up from this and other similar cohorts should aid in drawing definitive conclusions about the likelihood of developing TLE-MTS following childhood CSE as well as the significance of the other abnormalities presented as part of this thesis.

APPENDIX A: LIST OF PARTICIPATING HOSPITALS

- Great Ormond Street Hospital, Great Ormond Street, London WC1N 3JH
- Barnet Hospital, Wellhouse Lane, Barnet, Herts EN5 3DJ
- Chase Farm Hospital, The Ridgeway, Enfield, Middlesex EN2 8JL
- Chelsea & Westminster Hospital, 369 Fulham Road London SW10 9NH
- Central Middlesex Hospital, Acton Lane, Park Royal, London NW10 7NS
- Evelina Children's Hospital, Westminster Bridge Road, London, SE1 7EH
- Hammersmith Hospital, Du Cane Road , London W12 0HS
- Hillingdon Hospital, Pield Heath Road, Uxbridge, UB8 3NN
- Homerton University Hospital, Homerton Row, London E9 6SR
- King George Hospital, Barley Lane, Ilford IG3 8XE
- Newham University Hospital, Glen Road, Plaistow, London E13 8SL
- North Middlesex University Hospital, Sterling Way, London N18 1QX
- Northwick Park Hospital, Watford Road, Harrow, Middlesex HA1 3UJ
- Queen's Hospital, Rom Valley Way, Romford, Essex RM7 0AG
- Royal Free Hospital, Pond Street, London NW3 2QG
- Royal London Hospital, Whitechapel Road, Whitechapel, London E1 1BB
- St George's Hospital, Blackshaw Road, Tooting, London SW17 0QT
- St Mary's Hospital, Praed Street, Paddington London W2 1NY
- University College Hospital , 235 Euston Road , London NW1 2BU
- Watford General Hospital, Vicarage Road, Watford, Herts WD18 0HB
- Whipp's Cross Universiyt Hospital, Whipps Cross Road, London E11 1NR
- The Whittington Hospital, Magdala Avenue, London, N19 5NF

APPENDIX B: MRI PROTOCOLS

Scan sequence for children under 2 years of age

Duration (mins)	Sequence	No. slices	Echo time (ms)	Repeat time (ms)	Thickness	Gap	Matrix	Field of view	Flip	Inversion time
05:34	**3dFLASH**	1 slab (176 slices)	4.94	11	1mm	20% (0.2mm)	224x256	256x224	15	0
06:04	**T2 MAPS Relaxometry**	1	16 echoes from 22ms to 352ms	2400	5	-	150x256	210x157.5	180	-
03:19	**T2 DESTIR AXIAL**	22	14	6170	4	40% (1.6mm)	216x320	200x200	150	130
03:19	**T2 DESTIR CORONAL**	22	14	6170	4	40% (1.6mm)	216x320	200x200	150	130
06:27	**TIR Coronal**	20	68	7000	5	10% (0.5mm)	192x256	200x200	150	350
12:96	**3x20 DTI**	45	89	6300	2.5 mm	none	96x96	240x240	N/A	
01:04	**DWI**	19	96	2700	5mm	30% (1.5 mm)	128x128	230x230	-	-

Scan sequence for children over 2 years of age

Duration (mins)	Sequence	No. slices	Echo time (ms)	Repeat time (ms)	Thickness	Gap	Matrix	Field of view	Flip	Inversion time
05:34	**3dFLASH**	1 slab (176 slices)	4.94	11	1mm	20% (0.2mm)	224x256	256x224	15	0
06:04	**T2 MAPS Relaxometry**	1	16 echoes from 22ms to 352m	2400	5mm	-	150x256	210x157.5	180	-
03:13	**Axial TSE T2**	25	101	4920	4mm	40% of 4mm = 1.6mm	245x384	220x175.3	150	-
03:13	**Coronal TSE T2**	25	101	4920	4mm	40% of 4mm = 1.6mm	245x384	220x175.3	150	-
08:08	**3DFLAIR**	176 Slices	353	6000	1mm	none	256x256	256	150	2200
12:96	**3x20 DTI**	45	89	6300	2.5 mm	none	96x96	240	N/A	-
01:04	**DWI**	19	96	2700	5mm	30% (1.5 mm)	128x128	230	-	-

APPENDIX C: SEDATION PROTOCOL

Patient Preparation

All patients must:

- Have hospital notes
- Be weighed
- Have local anaesthetic cream applied
- Be fasted: 4 hours for food/milk, 2 hours for clear fluids / breast milk
- Have baseline observations recorded inc. SpO_2
- A metal check must be completed
- A full patient assessment should be conducted to establish suitability for sedation
- Sleep deprivation should be encouraged

Ward patients must be accompanied by a parent / guardian AND a ward nurse

General Protocol

- Small infants under 5 kgs = feed and wrap
- Co-operative children of any age: may be persuaded to lie still without sedation, please seek the assistance of play therapist
- Children with severe developmental delay, behavioural difficulties, OR over the age of 8 may need a GA
- All other patients should be considered for sedation

Sedation Medications

Under 5 kgs -Nil, feed and wrap

5-15 kgs	-Chloral Hydrate 100mg/kg (max 1 g)
12-20 kgs	-Triclofos 100mg/kg (max 2 g) + Alimemezine 2mg/kg (max 60 mg)
20 kgs +	-Triclofos 100mg/kg (max 2g) + Alimemizine 2 mg/kg (max 60 mg)

Intravenous 'Top-Up'

To be considered if oral sedation is not effective after 45-60 minutes

IV Diazemuls 1mg/kg (max dose 20mg)

[Given slowly, in increments]

Sedation Reversal

Flumazenil 10-20 mcg/kg

APPENDIX D: PUBLICATIONS ARISING FROM THIS THESIS

Published research papers:

1. **Management of convulsive status epilepticus in children.** Yoong M, Chin RF, Scott RC. *Arch Dis Child Educ Pract Ed. 2009 Feb;94(1):1-9.*
2. **The role of magnetic resonance imaging in the follow-up of children with convulsive status epilepticus.** Michael Yoong, Rodica Mardari, Marina Martinos, Christopher Clark, Kling Chong, Brian Neville, Richard Chin, Rod Scott. *Developmental Medicine & Child Neurology - Early online publication Jan 2012* http://dx.doi.org/10.1111.j.1469-8749.2011.04202.x.

Abstracts:

1. **"Hippocampal growth following childhood convulsive status epilepticus"** M Yoong, MM Martins, CA Clark, RFM Chin, RC Scott *DMCN 54:S1 58 2012*
2. **"The Developmental Profiles of Children Following CSE"** Martinos M, Yoong M, Scott RC, de Haan M *Epilepsia 50:288 2009*
3. **"Measuring diffusion tensor parameters in the human hippocampus: region of interest placement"** Yoong M, Martinos M, Clark CA, Chin RF, Scott RC. In *Proceedings of the ISMRM 17th Scientific Meeting & Exhibition, Honolulu, USA*
4. **"Hippocampal volume is reduced in children with other forms of convulsive status epilepticus compared to children with prolonged febrile seizures."** Yoong M, Martinos M, Clark CA, Chin RF, Scott RC. Abstracts from the 9th European Congress on *Epilepsy Epilepsia June 2010 Volume 51 Supplement s4: 138*

Reference List

(1) Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League Against Epilepsy. Epilepsia 1993 Jul;34(4):592-6.

(2) Pinto DJ, Patrick SL, Huang WC, Connors BW. Initiation, propagation, and termination of epileptiform activity in rodent neocortex in vitro involve distinct mechanisms. J Neurosci 2005 Sep 7;25(36):8131-40.

(3) Lopes da SF, Blanes W, Kalitzin SN, Parra J, Suffczynski P, Velis DN. Epilepsies as dynamical diseases of brain systems: basic models of the transition between normal and epileptic activity. Epilepsia 2003;44 Suppl 12:72-83.

(4) Schevon CA, Ng SK, Cappell J, Goodman RR, McKhann G, Jr., Waziri A, et al. Microphysiology of epileptiform activity in human neocortex. J Clin Neurophysiol 2008 Dec;25(6):321-30.

(5) Worrell GA, Parish L, Cranstoun SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. Brain 2004 Jul;127(Pt 7):1496-506.

(6) Chagnac-Amitai Y, Connors BW. Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. J Neurophysiol 1989 Nov;62(5):1149-62.

(7) Tyvaert L, LeVan P, Dubeau F, Gotman J. Noninvasive dynamic imaging of seizures in epileptic patients. Hum Brain Mapp 2009 Dec;30(12):3993-4011.

(8) Tyvaert L, Chassagnon S, Sadikot A, LeVan P, Dubeau F, Gotman J. Thalamic nuclei activity in idiopathic generalized epilepsy: an EEG-fMRI study. Neurology 2009 Dec 8;73(23):2018-22.

(9) Swartzwelder HS, Anderson WW, Wilson WA. Mechanism of electrographic seizure generation in the hippocampal slice in Mg2+-free medium: the role of GABAa inhibition. Epilepsy Res 1988 Jul;2(4):239-45.

(10) WANG RI, SONNENSCHEIN RR. PH of cerebral cortex during induced convulsions. J Neurophysiol 1955 Mar;18(2):130-7.

(11) Lux HD, Heinemann U, Dietzel I. Ionic changes and alterations in the size of the extracellular space during epileptic activity. Adv Neurol 1986;44:619-39.

(12) Ziemann AE, Schnizler MK, Albert GW, Severson MA, Howard MA, III, Welsh MJ, et al. Seizure termination by acidosis depends on ASIC1a. Nat Neurosci 2008 Jul;11(7):816-22.

(13) Loscher W, Kohling R. Functional, metabolic, and synaptic changes after seizures as potential targets for antiepileptic therapy. Epilepsy Behav 2010 Oct;19(2):105-13.

(14) Schuele SU, Bermeo AC, Alexopoulos AV, Burgess RC. Anoxia-ischemia: a mechanism of seizure termination in ictal asystole. Epilepsia 2010 Jan;51(1):170-3.

(15) Young D, Dragunow M. Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. Neuroscience 1994 Jan;58(2):245-61.

(16) Tsuchida TN, Barkovich AJ, Bollen AW, Hart AP, Ferriero DM. Childhood status epilepticus and excitotoxic neuronal injury. Pediatr Neurol 2007 Apr;36(4):253-7.

(17) Nixon J, Bateman D, Moss T. An MRI and neuropathological study of a case of fatal status epilepticus. Seizure 2001 Dec;10(8):588-91.

(18) Sisodiya SM, Thom M. Widespread upregulation of drug-resistance proteins in fatal human status epilepticus. Epilepsia 2003 Feb;44(2):261-4.

(19) ter Maaten JC, Strack van Schijndel RJ, Heimans JJ, Schreuder WO. Ten patients with refractory status epilepticus in an intensive care department. Neth J Med 1998 Dec;53(6):260-5.

(20) Neligan A, Shorvon SD. The history of status epilepticus and its treatment. Epilepsia 2009 Mar;50 Suppl 3:56-68.

(21) Clark L, Prout T. Status epilepticus: a clinical and pathological study in epilepsy. Am J Insanity 1903 Oct;60(2):291-306.

(22) Gastaut H, Roger J, Lob H. Les etats de mal epileptique: compte rendu de la reunion europene d'information lectroencphalographique.: Masson, Paris; 1962.

(23) Fujikawa DG. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. Brain Res 1996 Jun 24;725(1):11-22.

(24) Lowenstein DH, Bleck T, Macdonald RL. It's time to revise the definition of status epilepticus. Epilepsia 1999 Jan;40(1):120-2.

(25) Shinnar S, Berg AT, Moshe SL, Shinnar R. How long do new-onset seizures in children last? Ann Neurol 2001 May;49(5):659-64.

(26) Hesdorffer DC, Benn EK, Bagiella E, Nordli D, Pellock J, Hinton V, et al. Distribution of febrile seizure duration and associations with development. Ann Neurol 2011 Jan 11.

(27) Shinnar S. Who is at risk for prolonged seizures? J Child Neurol 2007 May;22(5 Suppl):14S-20S.

(28) Chin RF, Neville BG, Peckham C, Bedford H, Wade A, Scott RC. Incidence, cause, and short-term outcome of convulsive status epilepticus in childhood: prospective population-based study. Lancet 2006 Jul 15;368(9531):222-9.

(29) DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, et al. A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. Neurology 1996 Apr;46(4):1029-35.

(30) Hesdorffer DC, Logroscino G, Cascino G, Annegers JF, Hauser WA. Incidence of status epilepticus in Rochester, Minnesota, 1965-1984. Neurology 1998 Mar;50(3):735-41.

(31) Coeytaux A, Jallon P, Galobardes B, Morabia A. Incidence of status epilepticus in French-speaking Switzerland: (EPISTAR). Neurology 2000 Sep 12;55(5):693-7.

(32) Raspall-Chaure M, Chin RF, Neville BG, Bedford H, Scott RC. The epidemiology of convulsive status epilepticus in children: a critical review. Epilepsia 2007 Sep;48(9):1652-63.

(33) Chin RF, Neville BG, Scott RC. A systematic review of the epidemiology of status epilepticus. Eur J Neurol 2004 Dec;11(12):800-10.

(34) Raspall-Chaure M, Chin RF, Neville BG, Scott RC. Outcome of paediatric convulsive status epilepticus: a systematic review. Lancet Neurol 2006 Sep;5(9):769-79.

(35) Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of GABAA receptors. J Neurosci 2005 Jun 8;25(23):5511-20.

(36) Goodkin HP, Sun C, Yeh JL, Mangan PS, Kapur J. GABA(A) receptor internalization during seizures. Epilepsia 2007;48 Suppl 5:109-13.

(37) Feng HJ, Mathews GC, Kao C, Macdonald RL. Alterations of GABA A-receptor function and allosteric modulation during development of status epilepticus. J Neurophysiol 2008 Mar;99(3):1285-93.

(38) Meldrum B. Physiological changes during prolonged seizures and epileptic brain damage. Neuropadiatrie 1978 Aug;9(3):203-12.

(39) Blennow G, Brierley JB, Meldrum BS, Siesjo BK. Epileptic brain damage: the role of systemic factors that modify cerebral energy metabolism. Brain 1978 Dec;101(4):687-700.

(40) Cavus I, Kasoff WS, Cassaday MP, Jacob R, Gueorguieva R, Sherwin RS, et al. Extracellular metabolites in the cortex and hippocampus of epileptic patients. Ann Neurol 2005 Feb;57(2):226-35.

(41) Kanamori K, Ross BD. Chronic electrographic seizure reduces glutamine and elevates glutamate in the extracellular fluid of rat brain. Brain Res 2011 Jan 31;1371:180-91.

(42) Korngut L, Young GB, Lee DH, Hayman-Abello BA, Mirsattari SM. Irreversible brain injury following status epilepticus. Epilepsy Behav 2007 Sep;11(2):235-40.

(43) Men S, Lee DH, Barron JR, Munoz DG. Selective neuronal necrosis associated with status epilepticus: MR findings. AJNR Am J Neuroradiol 2000 Nov;21(10):1837-40.

(44) Pohlmann-Eden B, Gass A, Peters CN, Wennberg R, Blumcke I. Evolution of MRI changes and development of bilateral hippocampal sclerosis during long lasting generalised status epilepticus. J Neurol Neurosurg Psychiatry 2004 Jun;75(6):898-900.

(45) VanLandingham KE, Heinz ER, Cavazos JE, Lewis DV. Magnetic resonance imaging evidence of hippocampal injury after prolonged focal febrile convulsions. Ann Neurol 1998 Apr;43(4):413-26.

(46) Scott RC, King MD, Gadian DG, Neville BG, Connelly A. Hippocampal abnormalities after prolonged febrile convulsion: a longitudinal MRI study. Brain 2003 Nov;126(Pt 11):2551-7.

(47) Scott RC, Gadian DG, King MD, Chong WK, Cox TC, Neville BG, et al. Magnetic resonance imaging findings within 5 days of status epilepticus in childhood. Brain 2002 Sep;125(Pt 9):1951-9.

(48) Meldrum BS, Brierley JB. Prolonged epileptic seizures in primates. Ischemic cell change and its relation to ictal physiological events. Arch Neurol 1973 Jan;28(1):10-7.

(49) Covolan L, Mello LE. Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. Epilepsy Res 2000 Apr;39(2):133-52.

(50) Engelhorn T, Weise J, Hammen T, Bluemcke I, Hufnagel A, Doerfler A. Early diffusion-weighted MRI predicts regional neuronal damage in generalized status epilepticus in rats treated with diazepam. Neurosci Lett 2007 May 7;417(3):275-80.

(51) Menini C, Meldrum BS, Riche D, Silva-Comte C, Stutzmann JM. Sustained limbic seizures induced by intraamygdaloid kainic acid in the baboon: Symptomatology and neuropathological consequences. Ann Neurol 1980 Nov;8(5):501-9.

(52) Schwob JE, Fuller T, Price JL, Olney JW. Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid: a histological study. Neuroscience 1980;5(6):991-1014.

(53) Holopainen IE. Seizures in the developing brain: cellular and molecular mechanisms of neuronal damage, neurogenesis and cellular reorganization. Neurochem Int 2008 May;52(6):935-47.

(54) Choy M, Wells JA, Thomas DL, Gadian DG, Scott RC, Lythgoe MF. Cerebral blood flow changes during pilocarpine-induced status epilepticus activity in the rat hippocampus. Exp Neurol 2010 Sep;225(1):196-201.

(55) Fabene PF, Merigo F, Galie M, Benati D, Bernardi P, Farace P, et al. Pilocarpine-induced status epilepticus in rats involves ischemic and excitotoxic mechanisms. PLoS ONE 2007;2(10):e1105.

(56) Klitgaard H, Matagne A, Vanneste-Goemaere J, Margineanu DG. Pilocarpine-induced epileptogenesis in the rat: impact of initial duration of status epilepticus on electrophysiological and neuropathological alterations. Epilepsy Res 2002 Sep;51(1-2):93-107.

(57) Convulsive status epilepicus in infants, children and adolescents, Starship Children's Hospital, (2004).

(58) Babl FE, Sheriff N, Borland M, Acworth J, Neutze J, Krieser D, et al. Emergency management of paediatric status epilepticus in Australia and New Zealand: Practice patterns in the context of clinical practice guidelines. J Paediatr Child Health 2009 Jul 20.

(59) The Advanced Life Support Group. Advanced Paediatric Life Support: The Practical Approach. 4th ed. Blackwell publishing group; 2004.

(60) Riviello JJ, Jr., Holmes GL. The treatment of status epilepticus. Semin Pediatr Neurol 2004 Jun;11(2):129-38.

(61) Sugai K. Treatment of convulsive status epilepticus in infants and young children in Japan. Acta Neurol Scand Suppl 2007;186:62-70.

(62) Yoong M, Chin RF, Scott RC. Management of convulsive status epilepticus in children. Arch Dis Child Educ Pract Ed 2009 Feb;94(1):1-9.

(63) Goodkin HP, Kapur J. Responsiveness of Status Epilepticus to Treatment with Diazepan Decreases Rapidly as Seizure Duration Increases. Epilepsy Curr 2003 Jan;3(1):11-2.

(64) Chin RF, Neville BG, Peckham C, Wade A, Bedford H, Scott RC. Treatment of community-onset, childhood convulsive status epilepticus: a prospective, population-based study. Lancet Neurol 2008 Jul 2.

(65) Appleton R, Martland T, Phillips B. Drug management for acute tonic-clonic convulsions including convulsive status epilepticus in children. Cochrane Database Syst Rev 2002;(4):CD001905.

(66) Abend NS, Gutierrez-Colina AM, Dlugos DJ. Medical treatment of pediatric status epilepticus. Semin Pediatr Neurol 2010 Sep;17(3):169-75.

(67) Lowenstein DH. The management of refractory status epilepticus: an update. Epilepsia 2006;47 Suppl 1:35-40.

(68) Mehta V, Singhi P, Singhi S. Intravenous sodium valproate versus diazepam infusion for the control of refractory status epilepticus in children: a randomized controlled trial. J Child Neurol 2007 Oct;22(10):1191-7.

(69) Patel NC, Landan IR, Levin J, Szaflarski J, Wilner AN. The use of levetiracetam in refractory status epilepticus. Seizure 2006 Apr;15(3):137-41.

(70) van Gestel JP, Blusse van Oud-Alblas HJ, Malingre M, Ververs FF, Braun KP, van NO. Propofol and thiopental for refractory status epilepticus in children. Neurology 2005 Aug 23;65(4):591-2.

(71) Claassen J, Hirsch LJ, Emerson RG, Mayer SA. Treatment of refractory status epilepticus with pentobarbital, propofol, or midazolam: a systematic review. Epilepsia 2002 Feb;43(2):146-53.

(72) Lambrechtsen FA, Buchhalter JR. Aborted and refractory status epilepticus in children: A comparative analysis. Epilepsia 2007 Dec 13.

(73) Riviello JJ, Jr., Ashwal S, Hirtz D, Glauser T, Ballaban-Gil K, Kelley K, et al. Practice parameter: diagnostic assessment of the child with status epilepticus (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology 2006 Nov 14;67(9):1542-50.

(74) Neligan A, Shorvon SD. Frequency and prognosis of convulsive status epilepticus of different causes: a systematic review. Arch Neurol 2010 Aug;67(8):931-40.

(75) Aicardi J, Chevrie JJ. Convulsive status epilepticus in infants and children. A study of 239 cases. Epilepsia 1970 Jun;11(2):187-97.

(76) Maytal J, Shinnar S, Moshe SL, Alvarez LA. Low morbidity and mortality of status epilepticus in children. Pediatrics 1989 Mar;83(3):323-31.

(77) Logroscino G, Hesdorffer DC, Cascino GD, Annegers JF, Bagiella E, Hauser WA. Long-term mortality after a first episode of status epilepticus. Neurology 2002 Feb 26;58(4):537-41.

(78) Shinnar S, Pellock JM, Berg AT, O'Dell C, Driscoll SM, Maytal J, et al. Short-term outcomes of children with febrile status epilepticus. Epilepsia 2001 Jan;42(1):47-53.

(79) Pujar SS, Neville BG, Scott RC, Chin RF. Death within 8 years after childhood convulsive status epilepticus: a population-based study. Brain 2011 Oct;134(Pt 10):2819-27.

(80) Logroscino G, Hesdorffer DC, Cascino G, Annegers JF, Hauser WA. Short-term mortality after a first episode of status epilepticus. Epilepsia 1997 Dec;38(12):1344-9.

(81) DeLorenzo RJ, Garnett LK, Towne AR, Waterhouse EJ, Boggs JG, Morton L, et al. Comparison of status epilepticus with prolonged seizure episodes lasting from 10 to 29 minutes. Epilepsia 1999 Feb;40(2):164-9.

(82) Eriksson KJ, Koivikko MJ. Status epilepticus in children: aetiology, treatment, and outcome. Dev Med Child Neurol 1997 Oct;39(10):652-8.

(83) Chevrie JJ, Aicardi J. Convulsive disorders in the first year of life: etiologic factors. Epilepsia 1977 Dec;18(4):489-98.

(84) Shinnar S, Berg AT, Moshe SL, O'Dell C, Alemany M, Newstein D, et al. The risk of seizure recurrence after a first unprovoked afebrile seizure in childhood: an extended follow-up. Pediatrics 1996 Aug;98(2 Pt 1):216-25.

(85) Shinnar S, Berg AT, Moshe SL, Petix M, Maytal J, Kang H, et al. Risk of seizure recurrence following a first unprovoked seizure in childhood: a prospective study. Pediatrics 1990 Jun;85(6):1076-85.

(86) Barnard C, Wirrell E. Does status epilepticus in children cause developmental deterioration and exacerbation of epilepsy? J Child Neurol 1999 Dec;14(12):787-94.

(87) Verity CM, Ross EM, Golding J. Outcome of childhood status epilepticus and lengthy febrile convulsions: findings of national cohort study. BMJ 1993 Jul 24;307(6898):225-8.

(88) Stroink H, Geerts AT, van Donselaar CA, Peters AC, Brouwer OF, Peeters EA, et al. Status epilepticus in children with epilepsy: Dutch study of epilepsy in childhood. Epilepsia 2007 Sep;48(9):1708-15.

(89) Nelson KB, Ellenberg JH. Predictors of epilepsy in children who have experienced febrile seizures. N Engl J Med 1976 Nov 4;295(19):1029-33.

(90) Verity CM, Golding J. Risk of epilepsy after febrile convulsions: a national cohort study. BMJ 1991 Nov 30;303(6814):1373-6.

(91) Nelson KB, Ellenberg JH. Prognosis in children with febrile seizures. Pediatrics 1978 May;61(5):720-7.

(92) Sapir D, Leitner Y, Harel S, Kramer U. Unprovoked seizures after complex febrile convulsions. Brain Dev 2000 Dec;22(8):484-6.

(93) Offringa M, Bossuyt PM, Lubsen J, Ellenberg JH, Nelson KB, Knudsen FU, et al. Risk factors for seizure recurrence in children with febrile seizures: a pooled analysis of individual patient data from five studies. J Pediatr 1994 Apr;124(4):574-84.

(94) Dodrill CB, Wilensky AJ. Intellectual impairment as an outcome of status epilepticus. Neurology 1990 May;40(5 Suppl 2):23-7.

(95) Neligan A, Shorvon SD. Prognostic factors, morbidity and mortality in tonic-clonic status epilepticus: a review. Epilepsy Res 2011 Jan;93(1):1-10.

(96) Ellenberg JH, Nelson KB. Febrile seizures and later intellectual performance. Arch Neurol 1978 Jan;35(1):17-21.

(97) Kolfen W, Pehle K, Konig S. Is the long-term outcome of children following febrile convulsions favorable? Dev Med Child Neurol 1998 Oct;40(10):667-71.

(98) Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 1957 Feb;20(1):11-21.

(99) Lah S, Miller L. Effects of temporal lobe lesions on retrograde memory: a critical review. Neuropsychol Rev 2008 Mar;18(1):24-52.

(100) Lacruz ME, Valentin A, Seoane JJ, Morris RG, Selway RP, Alarcon G. Single pulse electrical stimulation of the hippocampus is sufficient to impair human episodic memory. Neuroscience 2010 Jul 17.

(101) Burgess N. Spatial cognition and the brain. Ann N Y Acad Sci 2008 Mar;1124:77-97.

(102) Ji J, Maren S. Hippocampal involvement in contextual modulation of fear extinction. Hippocampus 2007;17(9):749-58.

(103) Bremner JD. The relationship between cognitive and brain changes in posttraumatic stress disorder. Ann N Y Acad Sci 2006 Jul;1071:80-6.

(104) McKinnon MC, Yucel K, Nazarov A, MacQueen GM. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J Psychiatry Neurosci 2009 Jan;34(1):41-54.

(105) Macqueen G, Frodl T. The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? Mol Psychiatry 2010 Jul 27.

(106) Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 2010 Jan 14;65(1):7-19.

(107) Kobayashi E, Li LM, Lopes-Cendes I, Cendes F. Magnetic resonance imaging evidence of hippocampal sclerosis in asymptomatic, first-degree relatives of patients with familial mesial temporal lobe epilepsy. Arch Neurol 2002 Dec;59(12):1891-4.

(108) Benbadis SR, Wallace J, Reed MF. MRI evidence of mesial temporal sclerosis in subjects without seizures. Seizure 2002 Jul;11(5):340-3.

(109) Moore KR, Swallow CE, Tsuruda JS. Incidental detection of hippocampal sclerosis on MR images: is it significant? AJNR Am J Neuroradiol 1999 Oct;20(9):1609-12.

(110) Ng YT, McGregor AL, Duane DC, Jahnke HK, Bird CR, Wheless JW. Childhood mesial temporal sclerosis. J Child Neurol 2006 Jun;21(6):512-7.

(111) Sommer W. Erkrankung des Ammonshornes als aetiologisches Moment der Epilepsie. Archiv für Psychiatrie und Nervenkrankheiten , 361-375. 1880.
Ref Type: Abstract

(112) Thom M. Hippocampal Sclerosis: Progress Since Sommer. Brain Pathol 2008 Aug 29.

(113) Cascino GD, Jack CR, Jr., Parisi JE, Marsh WR, Kelly PJ, Sharbrough FW, et al. MRI in the presurgical evaluation of patients with frontal lobe epilepsy and children with temporal lobe epilepsy: pathologic correlation and prognostic importance. Epilepsy Res 1992 Mar;11(1):51-9.

(114) Jack CR, Jr., Sharbrough FW, Cascino GD, Hirschorn KA, O'Brien PC, Marsh WR. Magnetic resonance image-based hippocampal volumetry: correlation with outcome after temporal lobectomy. Ann Neurol 1992 Feb;31(2):138-46.

(115) Deblaere K, Achten E. Structural magnetic resonance imaging in epilepsy. Eur Radiol 2008 Jan;18(1):119-29.

(116) Reutens DC, Stevens JM, Kingsley D, Kendall B, Moseley I, Cook MJ, et al. Reliability of visual inspection for detection of volumetric hippocampal asymmetry. Neuroradiology 1996 Apr;38(3):221-5.

(117) Cascino GD. Clinical correlations with hippocampal atrophy. Magn Reson Imaging 1995;13(8):1133-6.

(118) FALCONER MA. Clinical manifestations of temporal lobe epilepsy and their recognition in relation to surgical treatment. Br Med J 1954 Oct 23;2(4894):939-44.

(119) Semah F, Picot MC, Adam C, Broglin D, Arzimanoglou A, Bazin B, et al. Is the underlying cause of epilepsy a major prognostic factor for recurrence? Neurology 1998 Nov;51(5):1256-62.

(120) Guimaraes CA, Li LM, Rzezak P, Fuentes D, Franzon RC, Augusta MM, et al. Temporal lobe epilepsy in childhood: comprehensive

neuropsychological assessment. J Child Neurol 2007 Jul;22(7):836-40.

(121) Berg AT. The natural history of mesial temporal lobe epilepsy. Curr Opin Neurol 2008 Apr;21(2):173-8.

(122) Sperling MR, O'Connor MJ, Saykin AJ, Plummer C. Temporal lobectomy for refractory epilepsy. JAMA 1996 Aug 14;276(6):470-5.

(123) Fogarasi A, Jokeit H, Faveret E, Janszky J, Tuxhorn I. The effect of age on seizure semiology in childhood temporal lobe epilepsy. Epilepsia 2002 Jun;43(6):638-43.

(124) Berg AT, Engel J, Jr. Hippocampal atrophy and the prognosis of epilepsy: some answers, more questions. Neurology 2006 Jul 11;67(1):12-3.

(125) Alarcon G, Valentin A. Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis. In: Panayiotopoulos CP, editor. Atlas of Epilepsies. 1 ed. SpringerLink; 2010. p. 1171-5.

(126) Grattan-Smith JD, Harvey AS, Desmond PM, Chow CW. Hippocampal sclerosis in children with intractable temporal lobe epilepsy: detection with MR imaging. AJR Am J Roentgenol 1993 Nov;161(5):1045-8.

(127) Bocti C, Robitaille Y, Diadori P, Lortie A, Mercier C, Bouthillier A, et al. The pathological basis of temporal lobe epilepsy in childhood. Neurology 2003 Jan 28;60(2):191-5.

(128) Sztriha L, Gururaj AK, Bener A, Nork M. Temporal lobe epilepsy in children: etiology in a cohort with new-onset seizures. Epilepsia 2002 Jan;43(1):75-80.

(129) Valentin A, Alarcon G. Mesial Temporal Epilepsy due to Etiologies Other than Hippocampal Sclerosis. Atlas of the Epilepsies. 1 ed. Springer; 2010. p. 1177-82.

(130) Labate A, Gambardella A, Andermann E, Aguglia U, Cendes F, Berkovic SF, et al. Benign mesial temporal lobe epilepsy. Nat Rev Neurol 2011 Apr;7(4):237-40.

(131) Santana MT, Jackowski AP, da Silva HH, Caboclo LO, Centeno RS, Bressan RA, et al. Auras and clinical features in temporal lobe

epilepsy: A new approach on the basis of voxel-based morphometry. Epilepsy Res 2010 Mar 22.

(132) Currie S, Heathfield KW, Henson RA, Scott DF. Clinical course and prognosis of temporal lobe epilepsy. A survey of 666 patients. Brain 1971;94(1):173-90.

(133) Harvey AS, Berkovic SF, Wrennall JA, Hopkins IJ. Temporal lobe epilepsy in childhood: clinical, EEG, and neuroimaging findings and syndrome classification in a cohort with new-onset seizures. Neurology 1997 Oct;49(4):960-8.

(134) Cersosimo R, Flesler S, Bartuluchi M, Soprano AM, Pomata H, Caraballo R. Mesial temporal lobe epilepsy with hippocampal sclerosis: study of 42 children. Seizure 2011 Mar;20(2):131-7.

(135) Janszky J, Janszky I, Ebner A. Age at onset in mesial temporal lobe epilepsy with a history of febrile seizures. Neurology 2004 Oct 12;63(7):1296-8.

(136) Dlugos DJ, Sammel MD, Strom BL, Farrar JT. Response to first drug trial predicts outcome in childhood temporal lobe epilepsy. Neurology 2001 Dec 26;57(12):2259-64.

(137) Spooner CG, Berkovic SF, Mitchell LA, Wrennall JA, Harvey AS. New-onset temporal lobe epilepsy in children: lesion on MRI predicts poor seizure outcome. Neurology 2006 Dec 26;67(12):2147-53.

(138) Berg AT, Langfitt J, Shinnar S, Vickrey BG, Sperling MR, Walczak T, et al. How long does it take for partial epilepsy to become intractable? Neurology 2003 Jan 28;60(2):186-90.

(139) Engel J, Jr. Mesial temporal lobe epilepsy: what have we learned? Neuroscientist 2001 Aug;7(4):340-52.

(140) McIntosh AM, Kalnins RM, Mitchell LA, Fabinyi GC, Briellmann RS, Berkovic SF. Temporal lobectomy: long-term seizure outcome, late recurrence and risks for seizure recurrence. Brain 2004 Sep;127(Pt 9):2018-30.

(141) Micallef S, Spooner CG, Harvey AS, Wrennall JA, Wilson SJ. Psychological outcome profiles in childhood-onset temporal lobe epilepsy. Epilepsia 2010 Oct;51(10):2066-73.

(142) Helmstaedter C, Kockelmann E. Cognitive outcomes in patients with chronic temporal lobe epilepsy. Epilepsia 2006;47 Suppl 2:96-8.

(143) Yasuda CL, Tedeschi H, Oliveira EL, Ribas GC, Costa AL, Cardoso TA, et al. Comparison of short-term outcome between surgical and clinical treatment in temporal lobe epilepsy: a prospective study. Seizure 2006 Jan;15(1):35-40.

(144) Liu Z, Mikati M, Holmes GL. Mesial temporal sclerosis: pathogenesis and significance. Pediatr Neurol 1995 Jan;12(1):5-16.

(145) Labate A, Gambardella A, Aguglia U, Condino F, Ventura P, Lanza P, et al. Temporal lobe abnormalities on brain MRI in healthy volunteers: a prospective case-control study. Neurology 2010 Feb 16;74(7):553-7.

(146) FALCONER MA, SERAFETINIDES EA, CORSELLIS JA. ETIOLOGY AND PATHOGENESIS OF TEMPORAL LOBE EPILEPSY. Arch Neurol 1964 Mar;10:233-48.

(147) Harvey AS, Grattan-Smith JD, Desmond PM, Chow CW, Berkovic SF. Febrile seizures and hippocampal sclerosis: frequent and related findings in intractable temporal lobe epilepsy of childhood. Pediatr Neurol 1995 Apr;12(3):201-6.

(148) French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, et al. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol 1993 Dec;34(6):774-80.

(149) Neurodiagnostic evaluation of the child with a simple febrile seizure. Pediatrics 2011 Feb;127(2):389-94.

(150) Choy M, Cheung KK, Thomas DL, Gadian DG, Lythgoe MF, Scott RC. Quantitative MRI predicts status epilepticus-induced hippocampal injury in the lithium-pilocarpine rat model. Epilepsy Res 2009 Dec 29.

(151) Toth Z, Yan XX, Haftoglou S, Ribak CE, Baram TZ. Seizure-induced neuronal injury: vulnerability to febrile seizures in an immature rat model. J Neurosci 1998 Jun 1;18(11):4285-94.

(152) Dube C, Yu H, Nalcioglu O, Baram TZ. Serial MRI after experimental febrile seizures: altered T2 signal without neuronal death. Ann Neurol 2004 Nov;56(5):709-14.

(153) Dube C, Richichi C, Bender RA, Chung G, Litt B, Baram TZ. Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. Brain 2006 Apr;129(Pt 4):911-22.

(154) Dube C, Chen K, Eghbal-Ahmadi M, Brunson K, Soltesz I, Baram TZ. Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. Ann Neurol 2000 Mar;47(3):336-44.

(155) Dube CM, Zhou JL, Hamamura M, Zhao Q, Ring A, Abrahams J, et al. Cognitive dysfunction after experimental febrile seizures. Exp Neurol 2009 Jan;215(1):167-77.

(156) Sokol DK, Demyer WE, Edwards-Brown M, Sanders S, Garg B. From swelling to sclerosis: acute change in mesial hippocampus after prolonged febrile seizure. Seizure 2003 Jun;12(4):237-40.

(157) Tanabe T, Hara K, Shimakawa S, Fukui M, Tamai H. Hippocampal damage after prolonged febrile seizure: one case in a consecutive prospective series. Epilepsia 2011 Apr;52(4):837-40.

(158) Gong G, Shi F, Concha L, Beaulieu C, Gross DW. Insights into the sequence of structural consequences of convulsive status epilepticus: A longitudinal MRI study. Epilepsia 2008 May 20.

(159) Provenzale JM, Barboriak DP, VanLandingham K, MacFall J, Delong D, Lewis DV. Hippocampal MRI signal hyperintensity after febrile status epilepticus is predictive of subsequent mesial temporal sclerosis. AJR Am J Roentgenol 2008 Apr;190(4):976-83.

(160) Tarkka R, Paakko E, Pyhtinen J, Uhari M, Rantala H. Febrile seizures and mesial temporal sclerosis: No association in a long-term follow-up study. Neurology 2003 Jan 28;60(2):215-8.

(161) Rocca WA, Sharbrough FW, Hauser WA, Annegers JF, Schoenberg BS. Risk factors for complex partial seizures: a population-based case-control study. Ann Neurol 1987 Jan;21(1):22-31.

(162) Maher J, McLachlan RS. Febrile convulsions. Is seizure duration the most important predictor of temporal lobe epilepsy? Brain 1995 Dec;118 (Pt 6):1521-8.

(163) Camfield P, Camfield C, Gordon K, Dooley J. What types of epilepsy are preceded by febrile seizures? A population-based study of children. Dev Med Child Neurol 1994 Oct;36(10):887-92.

(164) Lee K, Diaz M, Melchior JC. Temporal lobe epilepsy -- not a consequence of childhood febrile convulsions in Denmark. Acta Neurol Scand 1981 Apr;63(4):231-6.

(165) Rocca WA, Sharbrough FW, Hauser WA, Annegers JF, Schoenberg BS. Risk factors for generalized tonic-clonic seizures: a population-based case-control study in Rochester, Minnesota. Neurology 1987 Aug;37(8):1315-22.

(166) Rocca WA, Sharbrough FW, Hauser WA, Annegers JF, Schoenberg BS. Risk factors for absence seizures: a population-based case-control study in Rochester, Minnesota. Neurology 1987 Aug;37(8):1309-14.

(167) Annegers JF, Blakley SA, Hauser WA, Kurland LT. Recurrence of febrile convulsions in a population-based cohort. Epilepsy Res 1990 Apr;5(3):209-16.

(168) Millichap JJ, Koh S, Laux LC, Nordli DR, Jr. Child Neurology: Dravet syndrome: when to suspect the diagnosis. Neurology 2009 Sep 29;73(13):e59-e62.

(169) Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. Brain 1997 Mar;120 (Pt 3):479-90.

(170) Singh R, Scheffer IE, Crossland K, Berkovic SF. Generalized epilepsy with febrile seizures plus: a common childhood-onset genetic epilepsy syndrome. Ann Neurol 1999 Jan;45(1):75-81.

(171) Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O. Severe myoclonic epilepsy in infancy: Dravet syndrome. Adv Neurol 2005;95:71-102.

(172) Offringa M, Hazebroek-Kampschreur AA, Derksen-Lubsen G. Prevalence of febrile seizures in Dutch schoolchildren. Paediatr Perinat Epidemiol 1991 Apr;5(2):181-8.

(173) Martinos M. The consequences of Convulsive Status Epilepticus in children Institute of Child Health, University College London; 2010.

(174) Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002 Nov;15(7-8):435-55.

(175) Neil J, Miller J, Mukherjee P, Huppi PS. Diffusion tensor imaging of normal and injured developing human brain - a technical review. NMR Biomed 2002 Nov;15(7-8):543-52.

(176) Mori S. Introduction to diffusion tensor imaging. 1 ed. Elsevier; 2007.

(177) Moseley ME, Cohen Y, Kucharczyk J, Mintorovitch J, Asgari HS, Wendland MF, et al. Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system. Radiology 1990 Aug;176(2):439-45.

(178) Basser PJ, Mattiello J, LeBihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson B 1994 Mar;103(3):247-54.

(179) Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. Neurotherapeutics 2007 Jul;4(3):316-29.

(180) Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed 1995 Nov;8(7-8):333-44.

(181) Hammers A, Heckemann R, Koepp MJ, Duncan JS, Hajnal JV, Rueckert D, et al. Automatic detection and quantification of hippocampal atrophy on MRI in temporal lobe epilepsy: a proof-of-principle study. Neuroimage 2007 May 15;36(1):38-47.

(182) Hammers A, Allom R, Koepp MJ, Free SL, Myers R, Lemieux L, et al. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. Hum Brain Mapp 2003 Aug;19(4):224-47.

(183) Geuze E, Vermetten E, Bremner JD. MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed. Mol Psychiatry 2005 Feb;10(2):147-59.

(184) Yucel K, Macqueen G. Hippocampal volumetry: Is a consensus definition of the posterior boundaries possible? Hippocampus 2006;16(8):682-3.

(185) Jack CR, Jr., Bentley MD, Twomey CK, Zinsmeister AR. MR imaging-based volume measurements of the hippocampal formation and anterior temporal lobe: validation studies. Radiology 1990 Jul;176(1):205-9.

(186) Assaf BA, Mohamed FB, bou-Khaled KJ, Williams JM, Yazeji MS, Haselgrove J, et al. Diffusion tensor imaging of the hippocampal formation in temporal lobe epilepsy. AJNR Am J Neuroradiol 2003 Oct;24(9):1857-62.

(187) Thivard L, Lehericy S, Krainik A, Adam C, Dormont D, Chiras J, et al. Diffusion tensor imaging in medial temporal lobe epilepsy with hippocampal sclerosis. Neuroimage 2005 Nov 15;28(3):682-90.

(188) Wieshmann UC, Clark CA, Symms MR, Barker GJ, Birnie KD, Shorvon SD. Water diffusion in the human hippocampus in epilepsy. Magn Reson Imaging 1999 Jan;17(1):29-36.

(189) Salmenpera TM, Simister RJ, Bartlett P, Symms MR, Boulby PA, Free SL, et al. High-resolution diffusion tensor imaging of the hippocampus in temporal lobe epilepsy. Epilepsy Res 2006 Oct;71(2-3):102-6.

(190) Kimiwada T, Juhasz C, Makki M, Muzik O, Chugani DC, Asano E, et al. Hippocampal and thalamic diffusion abnormalities in children with temporal lobe epilepsy. Epilepsia 2006 Jan;47(1):167-75.

(191) Knake S, Salat DH, Halgren E, Halko MA, Greve DN, Grant PE. Changes in white matter microstructure in patients with TLE and hippocampal sclerosis. Epileptic Disord 2009 Sep;11(3):244-50.

(192) Engelhorn T, Hufnagel A, Weise J, Baehr M, Doerfler A. Monitoring of acute generalized status epilepticus using multilocal diffusion MR imaging: early prediction of regional neuronal damage. AJNR Am J Neuroradiol 2007 Feb;28(2):321-7.

(193) Wieshmann UC, Symms MR, Shorvon SD. Diffusion changes in status epilepticus. Lancet 1997 Aug 16;350(9076):493-4.

(194) Farina L, Bergqvist C, Zimmerman RA, Haselgrove J, Hunter JV, Bilaniuk LT. Acute diffusion abnormalities in the hippocampus of children with new-onset seizures: the development of mesial temporal sclerosis. Neuroradiology 2004 Apr;46(4):251-7.

(195) Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000 Jun;11(6 Pt 1):805-21.

(196) Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 2006 Jul 15;31(4):1487-505.

(197) Eltze CM. Epilepsy in infancy study: A population based study on epilepsies with onsent in the first two years of life UCL Institute of Child Health; 2010.

(198) Appleton R, Macleod S, Martland T. Drug management for acute tonic-clonic convulsions including convulsive status epilepticus in children. Cochrane Database Syst Rev 2008;3(3).

(199) Barsi P, Kenez J, Solymosi D, Kulin A, Halasz P, Rasonyi G, et al. Hippocampal malrotation with normal corpus callosum: a new entity? Neuroradiology 2000 May;42(5):339-45.

(200) Bajic D, Kumlien E, Mattsson P, Lundberg S, Wang C, Raininko R. Incomplete hippocampal inversion-is there a relation to epilepsy? Eur Radiol 2009 Oct;19(10):2544-50.

(201) Gamss RP, Slasky SE, Bello JA, Miller TS, Shinnar S. Prevalence of hippocampal malrotation in a population without seizures. AJNR Am J Neuroradiol 2009 Sep;30(8):1571-3.

(202) Singh RK, Stephens S, Berl MM, Chang T, Brown K, Vezina LG, et al. Prospective study of new-onset seizures presenting as status epilepticus in childhood. Neurology 2010 Feb 23;74(8):636-42.

(203) Rigby AS. Statistical recommendations for papers submitted to Developmental Medicine & Child Neurology. Dev Med Child Neurol 2010 Mar;52(3):299-304.

(204) Berg AT, Testa FM, Levy SR, Shinnar S. Neuroimaging in children with newly diagnosed epilepsy: A community-based study. Pediatrics 2000 Sep;106(3):527-32.

(205) Maytal J, Krauss JM, Novak G, Nagelberg J, Patel M. The role of brain computed tomography in evaluating children with new onset of seizures in the emergency department. Epilepsia 2000 Aug;41(8):950-4.

(206) Doescher JS, deGrauw TJ, Musick BS, Dunn DW, Kalnin AJ, Egelhoff JC, et al. Magnetic resonance imaging (MRI) and electroencephalographic (EEG) findings in a cohort of normal children with newly diagnosed seizures. J Child Neurol 2006 Jun;21(6):491-5.

(207) Morris Z, Whiteley WN, Longstreth WT, Jr., Weber F, Lee YC, Tsushima Y, et al. Incidental findings on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ 2009;339:b3016.

(208) Guideline development group: National Institute for Health and Clinical Excellence. Sedation in children and young people. National Institue for Clinical Excellence, editor. Sedation for diagnostic and therapeutic procedures in children and young people . 2010. National Clinical Guideline Centre.
Ref Type: Online Source

(209) Cendes F. Febrile seizures and mesial temporal sclerosis. Curr Opin Neurol 2004 Apr;17(2):161-4.

(210) Raininko R, Bajic D. "Hippocampal malrotation": no real malrotation and not rare. AJNR Am J Neuroradiol 2010 Apr;31(4):E39.

(211) Scottish Intercollegiate Guidelines Network. The diagnosis and management of epilepsy in young people. [81]. 2005.
Ref Type: Serial (Book,Monograph)

(212) Hirtz D, Ashwal S, Berg A, Bettis D, Camfield C, Camfield P, et al. Practice parameter: evaluating a first nonfebrile seizure in children: report of the quality standards subcommittee of the American Academy of Neurology, The Child Neurology Society, and The American Epilepsy Society. Neurology 2000 Sep 12;55(5):616-23.

(213) Brenner D, Elliston C, Hall E, Berdon W. Estimated risks of radiation-induced fatal cancer from pediatric CT. AJR Am J Roentgenol 2001 Feb;176(2):289-96.

(214) Davies HE, Wathen CG, Gleeson FV. The risks of radiation exposure related to diagnostic imaging and how to minimise them. BMJ 2011 Mar 12;342(7797):589-93.

(215) Bronen RA, Cheung G, Charles JT, Kim JH, Spencer DD, Spencer SS, et al. Imaging findings in hippocampal sclerosis: correlation with pathology. AJNR Am J Neuroradiol 1991 Sep;12(5):933-40.

(216) Barnes J, Foster J, Boyes RG, Pepple T, Moore EK, Schott JM, et al. A comparison of methods for the automated calculation of volumes and atrophy rates in the hippocampus. Neuroimage 2008 May 1;40(4):1655-71.

(217) Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 2002 Jan 31;33(3):341-55.

(218) Jack CR, Jr., Barkhof F, Bernstein MA, Cantillon M, Cole PE, Decarli C, et al. Steps to standardization and validation of hippocampal volumetry as a biomarker in clinical trials and diagnostic criterion for Alzheimer's disease. Alzheimers Dement 2011 Jul;7(4):474-85.

(219) Gousias IS, Rueckert D, Heckemann RA, Dyet LE, Boardman JP, Edwards AD, et al. Automatic segmentation of brain MRIs of 2-year-olds into 83 regions of interest. Neuroimage 2008 Apr 1;40(2):672-84.

(220) Shi F, Yap PT, Wu G, Jia H, Gilmore JH, Lin W, et al. Infant brain atlases from neonates to 1- and 2-year-olds. PLoS ONE 2011;6(4):e18746.

(221) Pantel J, O'Leary DS, Cretsinger K, Bockholt HJ, Keefe H, Magnotta VA, et al. A new method for the in vivo volumetric measurement of the human hippocampus with high neuroanatomical accuracy. Hippocampus 2000;10(6):752-8.

(222) Cook MJ, Fish DR, Shorvon SD, Straughan K, Stevens JM. Hippocampal volumetric and morphometric studies in frontal and temporal lobe epilepsy. Brain 1992 Aug;115 (Pt 4):1001-15.

(223) Jack CR, Jr., Theodore WH, Cook M, McCarthy G. MRI-based hippocampal volumetrics: data acquisition, normal ranges, and optimal protocol. Magn Reson Imaging 1995;13(8):1057-64.

(224) Utsunomiya H, Takano K, Okazaki M, Mitsudome A. Development of the temporal lobe in infants and children: analysis by MR-based volumetry. AJNR Am J Neuroradiol 1999 Apr;20(4):717-23.

(225) Szabo CA, Wyllie E, Siavalas EL, Najm I, Ruggieri P, Kotagal P, et al. Hippocampal volumetry in children 6 years or younger: assessment of children with and without complex febrile seizures. Epilepsy Res 1999 Jan;33(1):1-9.

(226) Obenaus A, Yong-Hing CJ, Tong KA, Sarty GE. A reliable method for measurement and normalization of pediatric hippocampal volumes. Pediatr Res 2001 Jul;50(1):124-32.

(227) Gogtay N, Nugent TF, III, Herman DH, Ordonez A, Greenstein D, Hayashi KM, et al. Dynamic mapping of normal human hippocampal development. Hippocampus 2006;16(8):664-72.

(228) Knickmeyer RC, Gouttard S, Kang C, Evans D, Wilber K, Smith JK, et al. A structural MRI study of human brain development from birth to 2 years. J Neurosci 2008 Nov 19;28(47):12176-82.

(229) Pfluger T, Weil S, Weis S, Vollmar C, Heiss D, Egger J, et al. Normative volumetric data of the developing hippocampus in children based on magnetic resonance imaging. Epilepsia 1999 Apr;40(4):414-23.

(230) Jaccard P. La distribution de la flore dans la zone alpine. Rev Gen Sci Pures Appl 1907;18:961-7.

(231) Dice L.R. Measure of the amount of ecological association between species. Ecology 1945;26:297-302.

(232) Scott RC, King MD, Gadian DG, Neville BG, Connelly A. Prolonged febrile seizures are associated with hippocampal

vasogenic edema and developmental changes. Epilepsia 2006 Sep;47(9):1493-8.

(233) Ahmad S, Marsh ED. Febrile status epilepticus: current state of clinical and basic research. Semin Pediatr Neurol 2010 Sep;17(3):150-4.

(234) Maneru C, Serra-Grabulosa JM, Junque C, Salgado-Pineda P, Bargallo N, Olondo M, et al. Residual hippocampal atrophy in asphyxiated term neonates. J Neuroimaging 2003 Jan;13(1):68-74.

(235) Caine D, Watson JD. Neuropsychological and neuropathological sequelae of cerebral anoxia: a critical review. J Int Neuropsychol Soc 2000 Jan;6(1):86-99.

(236) Thompson DK, Wood SJ, Doyle LW, Warfield SK, Lodygensky GA, Anderson PJ, et al. Neonate hippocampal volumes: prematurity, perinatal predictors, and 2-year outcome. Ann Neurol 2008 May;63(5):642-51.

(237) Lucas MM, Lenck-Santini PP, Holmes GL, Scott RC. Impaired cognition in rats with cortical dysplasia: additional impact of early-life seizures. Brain 2011 Jun;134(Pt 6):1684-93.

(238) Jensen FE, Holmes GL, Lombroso CT, Blume HK, Firkusny IR. Age-dependent changes in long-term seizure susceptibility and behavior after hypoxia in rats. Epilepsia 1992 Nov;33(6):971-80.

(239) Salmenpera T, Kononen M, Roberts N, Vanninen R, Pitkanen A, Kalviainen R. Hippocampal damage in newly diagnosed focal epilepsy: a prospective MRI study. Neurology 2005 Jan 11;64(1):62-8.

(240) Liu RS, Lemieux L, Bell GS, Sisodiya SM, Bartlett PA, Shorvon SD, et al. Cerebral damage in epilepsy: a population-based longitudinal quantitative MRI study. Epilepsia 2005 Sep;46(9):1482-94.

(241) White T, Nelson M, Lim KO. Diffusion tensor imaging in psychiatric disorders. Top Magn Reson Imaging 2008 Apr;19(2):97-109.

(242) Rollins NK. Clinical applications of diffusion tensor imaging and tractography in children. Pediatr Radiol 2007 Aug;37(8):769-80.

(243) Goyal MK, Sinha S, Ravishankar S, Shivshankar JJ. Peri-ictal signal changes in seven patients with status epilepticus: interesting MRI observations. Neuroradiology 2009 Mar;51(3):151-61.

(244) Chatzikonstantinou A, Gass A, Forster A, Hennerici MG, Szabo K. Features of acute DWI abnormalities related to status epilepticus. Epilepsy Res 2011 Jul 27.

(245) Natsume J, Bernasconi N, Miyauchi M, Naiki M, Yokotsuka T, Sofue A, et al. Hippocampal volumes and diffusion-weighted image findings in children with prolonged febrile seizures. Acta Neurol Scand 2007 Apr;115(4 Suppl):25-8.

(246) Heim S, Hahn K, Samann PG, Fahrmeir L, Auer DP. Assessing DTI data quality using bootstrap analysis. Magn Reson Med 2004 Sep;52(3):582-9.

(247) Muller MJ, Mazanek M, Weibrich C, Dellani PR, Stoeter P, Fellgiebel A. Distribution characteristics, reproducibility, and precision of region of interest-based hippocampal diffusion tensor imaging measures. AJNR Am J Neuroradiol 2006 Feb;27(2):440-6.

(248) Hong YJ, Yoon B, Shim YS, Cho AH, Lim SC, Ahn KJ, et al. Differences in microstructural alterations of the hippocampus in Alzheimer disease and idiopathic normal pressure hydrocephalus: a diffusion tensor imaging study. AJNR Am J Neuroradiol 2010 Nov;31(10):1867-72.

(249) Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 2004;23 Suppl 1:S208-S219.

(250) Falconer JC, Narayana PA. Cerebrospinal fluid-suppressed high-resolution diffusion imaging of human brain. Magn Reson Med 1997 Jan;37(1):119-23.

(251) Alexander AL, Hasan KM, Lazar M, Tsuruda JS, Parker DL. Analysis of partial volume effects in diffusion-tensor MRI. Magn Reson Med 2001 May;45(5):770-80.

(252) Ciccarelli O, Parker GJ, Toosy AT, Wheeler-Kingshott CA, Barker GJ, Boulby PA, et al. From diffusion tractography to quantitative

white matter tract measures: a reproducibility study. Neuroimage 2003 Feb;18(2):348-59.

(253) Pfefferbaum A, Adalsteinsson E, Sullivan EV. Replicability of diffusion tensor imaging measurements of fractional anisotropy and trace in brain. J Magn Reson Imaging 2003 Oct;18(4):427-33.

(254) Cascio CJ, Gerig G, Piven J. Diffusion tensor imaging: Application to the study of the developing brain. J Am Acad Child Adolesc Psychiatry 2007 Feb;46(2):213-23.

(255) Snook L, Paulson LA, Roy D, Phillips L, Beaulieu C. Diffusion tensor imaging of neurodevelopment in children and young adults. Neuroimage 2005 Jul 15;26(4):1164-73.

(256) Lebel C, Walker L, Leemans A, Phillips L, Beaulieu C. Microstructural maturation of the human brain from childhood to adulthood. Neuroimage 2008 Apr 15;40(3):1044-55.

(257) Provenzale JM, Isaacson J, Chen S, Stinnett S, Liu C. Correlation of apparent diffusion coefficient and fractional anisotropy values in the developing infant brain. AJR Am J Roentgenol 2010 Dec;195(6):W456-W462.

(258) Pierpaoli C, Basser PJ. Toward a quantitative assessment of diffusion anisotropy. Magn Reson Med 1996 Dec;36(6):893-906.

(259) Hessel EV, van Gassen KL, Wolterink-Donselaar IG, Stienen PJ, Fernandes C, Brakkee JH, et al. Phenotyping mouse chromosome substitution strains reveal multiple QTLs for febrile seizure susceptibility. Genes Brain Behav 2009 Mar;8(2):248-55.

(260) Rakhade SN, Jensen FE. Epileptogenesis in the immature brain: emerging mechanisms. Nat Rev Neurol 2009 Jul;5(7):380-91.

(261) Lebel C, Beaulieu C. Longitudinal development of human brain wiring continues from childhood into adulthood. J Neurosci 2011 Jul 27;31(30):10937-47.

(262) Bernasconi N, Bernasconi A, Caramanos Z, Antel SB, Andermann F, Arnold DL. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. Brain 2003 Feb;126(Pt 2):462-9.

(263) Arfanakis K, Hermann BP, Rogers BP, Carew JD, Seidenberg M, Meyerand ME. Diffusion tensor MRI in temporal lobe epilepsy. Magn Reson Imaging 2002 Sep;20(7):511-9.

(264) Sloviter RS. The neurobiology of temporal lobe epilepsy: too much information, not enough knowledge. C R Biol 2005 Feb;328(2):143-53.

(265) Milligan TA, Zamani A, Bromfield E. Frequency and patterns of MRI abnormalities due to status epilepticus. Seizure 2008 Aug 22.

(266) Okamoto R, Fujii S, Inoue T, Lei K, Kondo A, Hirata T, et al. Biphasic clinical course and early white matter abnormalities may be indicators of neurological sequelae after status epilepticus in children. Neuropediatrics 2006 Feb;37(1):32-41.

(267) Kim JA, Chung JI, Yoon PH, Kim DI, Chung TS, Kim EJ, et al. Transient MR signal changes in patients with generalized tonicoclonic seizure or status epilepticus: periictal diffusion-weighted imaging. AJNR Am J Neuroradiol 2001 Jun;22(6):1149-60.

(268) Jones DK, Symms MR, Cercignani M, Howard RJ. The effect of filter size on VBM analyses of DT-MRI data. Neuroimage 2005 Jun;26(2):546-54.

(269) Afzali M, Soltanian-Zadeh H, Elisevich KV. Tract based spatial statistical analysis and voxel based morphometry of diffusion indices in temporal lobe epilepsy. Comput Biol Med 2011 May 24.

(270) Klein A, Andersson J, Ardekani BA, Ashburner J, Avants B, Chiang MC, et al. Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. Neuroimage 2009 Jan 13.

(271) Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 2002 Jan;15(1):1-25.

(272) Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 2009 Jan 1;44(1):83-98.

(273) Melhem ER, Mori S, Mukundan G, Kraut MA, Pomper MG, van Zijl PC. Diffusion tensor MR imaging of the brain and white matter tractography. AJR Am J Roentgenol 2002 Jan;178(1):3-16.

(274) Snook L, Plewes C, Beaulieu C. Voxel based versus region of interest analysis in diffusion tensor imaging of neurodevelopment. Neuroimage 2007 Jan 1;34(1):243-52.

(275) Nguyen D, Vargas MI, Khaw N, Seeck M, Delavelle J, Lovblad KO, et al. Diffusion tensor imaging analysis with tract-based spatial statistics of the white matter abnormalities after epilepsy surgery. Epilepsy Res 2011 Mar 3.

(276) Kassem-Moussa H, Provenzale JM, Petrella JR, Lewis DV. Early diffusion-weighted MR imaging abnormalities in sustained seizure activity. AJR Am J Roentgenol 2000 May;174(5):1304-6.

(277) Gross DW, Concha L, Beaulieu C. Extratemporal white matter abnormalities in mesial temporal lobe epilepsy demonstrated with diffusion tensor imaging. Epilepsia 2006 Aug;47(8):1360-3.

(278) Focke NK, Yogarajah M, Bonelli SB, Bartlett PA, Symms MR, Duncan JS. Voxel-based diffusion tensor imaging in patients with mesial temporal lobe epilepsy and hippocampal sclerosis. Neuroimage 2007 Dec 27.

(279) Riley JD, Franklin DL, Choi V, Kim RC, Binder DK, Cramer SC, et al. Altered white matter integrity in temporal lobe epilepsy: association with cognitive and clinical profiles. Epilepsia 2010 Apr;51(4):536-45.

(280) Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. Neuroimage 2003 Nov;20(3):1714-22.

(281) Nordli DR, Jr. Febrile seizures. In: Panayiotopoulos CP, editor. The Atlas of Epilepsies.London: SpringerLink; 2010.

(282) Nakayama J. Progress in searching for the febrile seizure susceptibility genes. Brain Dev 2009 May;31(5):359-65.

(283) Berg AT, Shinnar S. Complex febrile seizures. Epilepsia 1996 Feb;37(2):126-33.

(284) Tsuboi T. Genetic analysis of febrile convulsions: twin and family studies. Hum Genet 1987 Jan;75(1):7-14.

(285) Kjeldsen MJ, Kyvik KO, Friis ML, Christensen K. Genetic and environmental factors in febrile seizures: a Danish population-based twin study. Epilepsy Res 2002 Sep;51(1-2):167-77.