The consequences of Convulsive Status Epilepticus in children

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DECLARATION

I, Marina Martinos, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed.....

ABSTRACT

Convulsive status epilepticus (CSE) is the most common neurological emergency in childhood. Approximately half of new CSE cases occur in children with no perceptible neurological priors (Chin et al., 2006). Prolonged febrile seizures (PFS), a type of CSE that occurs in neurologically normal children, have been retrospectively linked to temporal lobe epilepsy with hippocampal sclerosis. Imaging studies have revealed hippocampal abnormalities soon after PFS, yet, no neuropsychological study to date has investigated these children close to the time of insult. The present thesis investigated the effects of CSE on child development within a month of the incident and, subsequently, a year onwards. The first aim of this thesis was to investigate the effects of CSE on developmental functions using standardized assessments. The second aim was to examine children with PFS for signs of hippocampal dysfunction close to the time of incident. We hypothesized that aetiology would largely influence outcome in our CSE cohort, and, that children with PFS would reveal deficits in a delayed recognition paradigm that is thought to tap onto hippocampal processes. Eighty children were seen a mean of 38 days following CSE (34 PFS) and 50 children (24 PFS) were re-assessed a year onwards. At baseline neuropsychological impairments were evident in children following CSE associated with a PFS, as well as, children following CSE associated with other aetiologies (non-PFS), albeit, these were more pronounced in the non-PFS group. Moreover, in line with our hypothesis, the PFS group revealed deficits in a task of incidental recognition memory alluding to the presence of hippocampal dysfunction in this group. A year onwards deficits were still apparent in the two patient groups, although, the PFS group had shown some improvement on a number of measures. The implications of these findings for our understanding of CSE are discussed in this thesis.

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Chapter 1 Introduction

1.1. General Introduction

Epilepsy is the commonest neurological disorder in childhood and seizures the second most probable reason for hospital admission(Noeker, Haverkamp-Krois, & Haverkamp, 2005). Up to eight percent of the entire population will experience at least one seizure during their lifespan (Ross, 1994). Seizures are synchronized bursts of electrical activity that disrupt normal brain function. Children are more susceptible than adults to the development of seizures and this has been attributed to the hyper-excitable nature of the developing brain (Ben-Ari, 2006). The presentation of seizures during childhood has been also associated with a number of predisposing factors such as viral infections and pre-existing neurological abnormalities. Nonetheless, a large number of children present with seizures in the context of a neurologically normal development. The range of outcomes following a first ever seizure varies tremendously from child to child and is thought to be largely dependent upon the nature of the seizure itself and the presence of concomitant neurological problems (Raspall-Chaure, Chin, Neville, & Scott, 2006). In the present thesis we will investigate the nature of outcomes in a paediatric cohort following convulsive status epilepticus (CSE).

1.2. Definition of CSE

There are two types of Status Epilepticus (SE), convulsive SE (CSE) and nonconvulsive SE (non-CSE). Non-CSE is very difficult to identify from behavioural signs which makes EEG mandatory for diagnosis (Walker et al., 2005). In the present thesis we shall restrict ourselves to the study of CSE. A convulsion is a repeated contraction of the muscles that results in an uncontrolled shaking of the body. For a seizure to be classified as convulsive it needs to contain a main element of tonic and/or clonic activity.

The official definition of CSE has been a matter of ongoing debate in the past few years. In 1993, the International League Against Epilepsy (ILAE) advanced the following definition for status epilepticus: "*A seizure which shows no clinical signs of arresting after a duration encompassing the great majority of seizures of that type in most patients or recurrent seizures without resumption of baseline central nervous system function interictally*." While this was a step in the right direction that duly encompassed the prolonged nature of this seizure, the absence of a specified temporal cut-off proved somewhat confusing.

However, an unwritten consensus has emerged in the clinical setting whereby seizures as short as 5 minutes are treated as an incident of CSE as most short seizures have self-terminated by that point (Lowenstein, Bleck, & Macdonald, 1999). On the other hand, for research purposes a durational cut-off of 30 minutes has been tacitly agreed upon as animal and human studies have shown that this time limit constitutes a threshold over and above which structural changes are observed

A prolonged febrile seizure (PFS), a type of seizure with which we will concern ourselves extensively in the present thesis, has been defined as a prolonged seizure that occurs in the presence of fever and is not caused by an acute or a remote insult to the central nervous system (CNS). The age range for PFS classification has varied slightly from study to study but tends to be between the ages of 6 months and 5 years.

A simple febrile seizure is defined as an event in infancy or childhood, usually occurring between three months and five years of age, associated with fever but without evidence of intracranial infection or a defined cause (Verity, Greenwood, & Golding, 1998). If the seizure is characterized by any of the following characteristics : (a)duration over 15 minutes, (b) recurrence within 24 hours, and, (c) manifestation of focal features, then it is classified as a complex seizure (Verity, 1998)

1.3. Epidemiology of CSE

1.3.1. Epidemiology of adult CSE

There is a known bimodal distribution in the incidence of CSE with children under the age of 1 and adults over the age of 60 being the most likely candidates to have an episode (Chin, Neville, & Scott, 2004; Chin et al., 2006). Crude incidence rates of CSE in adulthood are reported to range between 6.8 to 41/100000 individuals per year. Although, adults include cases precipitated by infections with fever (a frequent trigger in children CSE), these constitute a small percentage (DeLorenzo, Pellock, Towne, & Boggs, 1995; DeLorenzo et al., 1996). Three major causes have been identified for the presentation of CSE in adults: (a) low dosage of antiepileptic medication, (b) remote symptomatic causes, and, (c) cerebrovascular accidents. Anoxia, metabolic causes, alcohol and drug withdrawal also account for a proportion of CSE episodes in adulthood. Combining cases of remote cerebrovascular accidents (classified as remote symptomatic cases) with current ones reveals that almost 50% of CSE cases in adulthood can be attributed to these (DeLorenzo et al., 1995).

Adult CSE is associated with a five-fold increase in mortality risk in patients aged less than 65 years of age and a two-fold increase in mortality risk in patients older than 65 . This is because people over the age of 65 are already at a greater risk of mortality independently of cause(Chin et al., 2004). In the group of SE without an identifiable cause, no increased mortality risk was observed, suggesting that it may the predisposing aetiology rather than CSE itself which is responsible for the increased mortality in certain groups(Logroscino et al., 2002). Seizure duration longer than 24 hours, acute symptomatic SE, and, myoclonic SE have been found to be associated with the worse mortality outcomes(Logroscino et al., 2002).

1.3.2. Epidemiology of paediatric CSE

The estimated incidence of CSE in childhood has been found to lie somewhere between 10 to 38/100.000 per year (Hesdorffer, Logroscino, Cascino, Annegers, & Hauser, 1998; Knake et al., 2001; Sadarangani et al., 2008; Vignatelli, Tonon, & D'Alessandro, 2003). Our group, which carried out the first study to determine incidence of CSE in a wholly paediatric population, (The North London Convulsive Status Epilepticus in Childhood Surveillance Study) found the incidence of CSE in the North London area to be 17to 23/100.000 per year (Chin et al., 2006). This incidence was further stratified by age with children under the age of 1 demonstrating the highest incidence (51/100.000 per year).

In the North London study more than half (56%) of first CSE episodes occurred in children with no prior neurological history, and, in turn, more than half of these children (57%) had a CSE classified as a PFS. From the remaining CSE cases, 17% were associated with an acute CNS infection or a metabolic derangement (acute symptomatic group), 16% were associated with a history of a CNS insult (remote symptomatic group), 16 % were associated with an acute CNS insult or fever in children with neurological precedents (acute on remote symptomatic group), and, 21% had idiopathic or cryptogenic epilepsy.

In population based studies, short term mortality following paediatric CSE (i.e. death within 60 days of onset) has been reported to be between 2.7 to 5.2 percent (Raspall-Chaure, Chin, Neville, Bedford, & Scott, 2007). In the North London study there was a 3% case mortality with children in the acute and the remote symptomatic groups manifesting the highest rates (Chin et al., 2006). Importantly, there were no deaths associated with PFS pointing once again to the important role played by aetiology in influencing outcome. This becomes even more evident in studies looking at

the outcome of CSE in developing countries (Molinero et al., 2009; Sadarangani et al., 2008). For example, in a study investigating CSE outcome in Kenyan children, 15 percent of children died in hospital and 21 percent of children died within 3 years of the episode (Sadarangani et al., 2008). Bacterial meningitis was found to be associated with a higher mortality risk, a condition, which is associated with a worse outcome in developing countries, though, is often found to lead to neurological sequelae in developed ones as well (Chin et al., 2006).

1.4. Pathophysiology of CSE

A detailed description of the pathophysiology of CSE is beyond the scope of this section. However, a brief introduction to what distinguishes this seizure from other ones is called for, given that the focus of this thesis is CSE. One of the things that distinguishes CSE from other seizures is the failure of seizure terminating mechanisms to take over and arrest the seizure. The observation in animal work that the longer the initial electrical stimulation of the brain the more likely the occurrence of SE combined with the fact that repeated seizures precede continuous seizure activity in humans as evidenced on their EEG suggests a role for repeated seizures in debilitating seizure terminating mechanisms (Walker, 2009). However, the mechanisms that are responsible for the ongoing nature of the seizure are believed to be different from the mechanisms responsible for the obstruction of terminating mechanisms (Walker, 2009). SE is also characterized by a time-dependent pharmacoresistance; for example, the potency of benzodiazepines may decrease 20-fold in 30 minutes (Chen, Naylor, & Wasterlain, 2007). In contrast, N-methyl-D-aspartic acid (NMDA) blockers are successful at arresting the seizure after the first 15 minutes have elapsed (Chen et al., 2007). This suggests two distinct phases during CSE; the initiation phase, and, the maintenance phase each of which can be arrested by different agents. Finally, animal studies have found evidence

for neuronal loss caused by SE, a finding which is lacking in shorter seizures (Meldrum & Brierley, 1973). Insights from the human literature on this matter will be discussed in more detail in section 1.6 of this introduction.

1.5. The effects of CSE on developmental functions

1.5.1. Animal studies

The adverse effects of SE on learning and memory during adulthood have been largely documented and are widely accepted (Rajasekaran, Zanelli, & Goodkin, 2010; Scantlebury et al., 2007). Namely, adult rats following SE manifest spatial memory impairments in tasks such as the Morris water and the radial arm mazes (Nissinen, Halonen, Koivisto, & Pitkanen, 2000; Detour, Schroeder, Desor, & Nehlig, 2005). Such cognitive deficits are often accompanied by elevated anxiety levels (Detour et al., 2005). By contrast, object recognition memory was found to be intact in rats five months post SE in one study investigating this issue (Detour et al., 2005).

In this study, rats were habituated to 3 wooden objects over a number of 3 trials with an inter-trial interval of 24 hours. On the fourth trial, a familiar object was substituted with a novel one and differences in sniffing behaviour between SE and control rats were analysed. Both groups were found to display a novelty preference as evidenced by their increased sniffing time during the novelty trial relative to the past three habituating trials. Moreover, epileptic and non-epileptic rats revealed similar interest levels in the novel object reflected by their indistinguishable total sniffing time of the novel object. Nonetheless, the novel object that was introduced on the 4th trial was a plastic one, which may have provided the rats with olfactory cues independent of recognition memory processes.

A different picture emerges in younger rats (i.e. less than 30 days old) with learning and spatial memory deficits and seizure induced damage found to be milder and milder the younger the animal is when SE occurs (Scantlebury et al., 2007; Stafstrom, Thompson, & Holmes, 1992; Stafstrom, Chronopoulos, Thurber, Thompson, & Holmes, 1993). Nonetheless, recent reports have started to strengthen the view that while the young brain may not be as susceptible to overt structural damage as the adult brain, changes in excitation and synaptic organization do occur and are accompanied by behavioural and cognitive deficits (Rutten et al., 2002a; Stafstrom, 2002; Dube et al., 2009; Sayin, Sutula, & Stafstrom, 2004). These findings persist regardless of the animal model employed to study the effects of SE, though, variations in the degree of impairments may be related to animal model specifics(Rajasekaran et al., 2010).

Recently, Dubé et al. (2009) reported moderate working and relational memory deficits as well as strategy-shifting impairments in adult rats with a history of experimental febrile seizures induced by hyperthermia while they were neonates. Abnormal T_2 relaxation times were found to be predictive of later cognitive dysfunction providing, thus, a possible biomarker for the early identification of a subgroup *at risk*. This model has been shown to be a useful one for investigating the relationship between febrile seizures and the subsequent development of TLE as some animals that undergo hyperthermia induced seizures go on to develop seizures phenomenologically identical to TLE ones (Dube et al., 2006a). Therefore, the above findings have implications for human TLE and its association with a history of febrile seizures, particularly, the prolonged kind (Baram & Shinnar, 2001).

Typically, animal studies investigating the effects of SE induce the seizure in early postnatal days and subsequently study its impact in adult rats (Stafstrom, 2002). A noted exception is a study carried out by Rutten et al. (2002) addressing the timing of cognitive dysfunction following SE. Rats underwent

lithium-pilocarpine-induced SE on postnatal day 20 (i.e. prepubescent rats) and were tested on the Morris water maze 2, 5, 10 or 30 days later. The rats were found to perform worse than the control group at all time points. More importantly, placing the animals in an enriched environment for 28 days resulted in their superior performance over non-enriched rats despite a lack of accompanying EEG and histological differences between the two groups. The finding that the provision of an enriching environment can alter functional outcome highlights the need for an early intervention following SE, but, also invites clinicians to think about intervention practices that may encompass the enrichment principle.

In sum, animal models of SE have found evidence for its adverse effects on cognition. Experimental febrile seizures suffered early on in life were also found to be associated with moderate cognitive deficits in adulthood. This is particularly important given the discrepant results in the human literature regarding the effects of febrile seizures on development. Finally, there is undoubtedly an age dependency in the degree and consistency of impairments made evident by animals following SE, though, recent data has started to challenge the previously widely held view that seizures suffered early on in life are innocuous.

1.5.2. Human adult studies

The effects of CSE, sustained during adulthood, on outcome have proven difficult to disentangle from other concomitant factors (Helmstaedter, 2007). In general, outcomes in adult patients tend to vary with the type of epilepsy, aetiology, seizure severity, seizure duration, and, age of the patient (Chin et al., 2004; Helmstaedter, 2007). For example, CSE that occurs in the context of symptomatic epilepsies has been found to have a worse outlook than CSE that occurs in the context of idiopathic epilepsies(Shorvon & Walker, 2005).

In an attempt to understand whether SE in adults with epilepsy leads to further intellectual deterioration, Adachi et al. (2005) compared the performance of epilepsy patients that experienced an episode of SE between assessments to a group of epilepsy patients that did not. No differences in performance were detected in the two groups leading the authors to postulate that SE in itself doesn't lead to intellectual decline. Moreover, seizure related variables as well as clinical characteristics were not found to be predictive of intellectual performance consolidating the absence of a seizure effect. Dodrill and Willensky (1990) came to a similar conclusion after reviewing the existing literature on status epilepticus and its impact on intellect. However, their own data on nine patients who experienced SE in a 5 year follow-up study revealed an IQ decline in those patients averaging 10-18 points. The authors concluded that "*in some individuals the effects were definitely greater, but that in many persons no effects were discernible...*"(Dodrill & Wilensky, 1990). Therefore, larger and carefully designed studies are needed to determine whether CSE in some patients but not others leads to intellectual deterioration and the factors that may contribute to this discrepancy. Moreover, many of the existing studies have utilized as their study sample patients who already have epilepsy a factor that may be clouding the presence of any direct CSE effects (Adachi et al., 2005).

Memory impairments following CSE in adults have been reported in isolated case studies (Dietl et al., 2004; Oxbury, Oxbury, Renowden, Squier, & Carpenter, 1997). Oxbury et al. (1997) described an individual who underwent left temporal lobectomy for the relief of seizures and a few years onwards experienced an episode of CSE which was repeated on 3 more occasions before the patient died. Following what must have been a particularly severe episode (3rd episode) the patient developed severe anterograde and retrograde amnesia that was accompanied by reductions in his right hippocampus compared to an MRI performed 3 months prior to the severe CSE episode.

Histopathological examinations following the patient's death revealed total neuronal loss in the CA1 region and a severe depletion in the CA4 region of the right hippocampus.

The patient had been neuropsychologically assessed on six occasions in a span of 10 years, which included assessments prior to his surgery and post the occurrence of CSE. IQ was found to be within the high average range and remained fairly stable at all time points. On the other hand, the patient's memory abilities suffered a steep decline following his 3rd episode of CSE. It is difficult to know whether memory abilities had already started declining following the 1st and 2nd episodes of CSE. Moreover, as the patient was alone when the 3rd episode occurred there is no way of determining the duration and phenomenology of this seizure, which becomes problematic for the interpretation of the above results. Therefore, while there may be some evidence that CSE may lead to memory impairments *via* hippocampal damage, carefully controlled group studies are needed to investigate this issue further.

1.5.3. Paediatric studies

Akin to animal studies, paediatric CSE is associated with an overall better outcome than adult CSE with the exception of neonatal status epilepticus which is associated with a bleak outlook (Pisani, Cerminara, Fusco, & Sisti, 2007). However, not unlike adult CSE, aetiology seems to be the main determinant of outcome in paediatric cases as well (Aicardi & Chevrie, 1970; Chin et al., 2006; Raspall-Chaure et al., 2006; Raspall-Chaure et al., 2007). One of the first studies to investigate the outlook of paediatric CSE on later development was a review of 239 cases carried out by Aicardi and Chevrie in 1970. After consideration of their patients' medical records these authors concluded that "*the prognosis of children's status epilepticus is grave, mental or neurological residua or both being present in at least 57% of our patients*".

More recent studies have painted a better picture for children experiencing CSE especially in the absence of an acute CNS insult or a prior history of neurological abnormality (Verity, Ross, & Golding, 1993; Maytal, Shinnar, Moshe, & Alvarez, 1989). More aggressive treatment and changes in the definition of CSE (e.g. duration) between older and more recent studies may be responsible for the different results. However, most studies have relied on crude outcome measures and only one study to date has utilized standardized neuropsychological tools to investigate the effects of CSE on child development (Verity et al., 1993). Therefore, there is a clear need for studies that use standardized measures to quantify outcome in CSE.

A main difference between adult and paediatric CSE is that approximately a quarter of all paediatric cases is made up from prolonged febrile seizure cases, i.e. children that were neurologically normal prior to this incident(Chin et al., 2006). Many researchers have recommended that these cases be considered separately when investigating the effects of CSE as they are associated with a different outlook (Chin et al., 2004). The effects of PFS on cognition have been usually studied *by proxy* with such patients being included within bigger studies investigating the effect of simple febrile seizures on development (Chang, Guo, Wang, Huang, & Tsai, 2001; Kolfen, Pehle, & Konig, 1998; Nelson & Ellenberg, 1978; Verity, Butler, & Golding, 1985a; Verity et al., 1998). As simple febrile seizures occur in 2% -5% of children before the age of 5, two large prospective population-based studies, one in the UK and one in the USA, have been carried out to assess the effect of such seizures on later development (Ellenberg & Nelson, 1978; Nelson & Ellenberg, 1978; Verity et al., 1998).

In the Ellenberg and Nelson (1978) study 27 patients with prolonged febrile seizures were included. The patients were tested when they reached the age of 7 on the Wechsler Intelligence Scale for Children (WISC) and their performance was compared to that of unaffected siblings. No differences were observed between the affected and the unaffected siblings in IQ. From the 431 sibling pairs

included in this study, 61 (14%) had been found to be neurologically suspect prior to the occurrence of their first febrile seizure. Characterization of neurological status in these children had been carried out prospectively and included standardized neuropsychological assessments. Children with a history of neurological abnormality performed worse than their unaffected siblings on the WISC.

Unfortunately, the authors do not disclose how many of the patients with prolonged febrile seizures were amongst the neurologically suspect patients (Ellenberg & Nelson, 1978; Nelson & Ellenberg, 1978). This would be important information as a more recent study has shown that children with prolonged febrile seizures are more likely to be neurologically abnormal from children with simple febrile seizures(Shinnar et al., 2001). Moreover, whereas the use of siblings as controls circumnavigates the issue of having to match children for maternal education and socio-economic status, genetic resemblance between the two may potentially occlude deficits in the patient group. Extensive work in the field of psychiatry has shown that unaffected siblings very often manifest similar structural and functional profiles to their affected siblings, making them, thus, unsuitable as a control comparison group when one is looking for what may be subtle differences in IQ (Woodward, Tibbo, & Purdon, 2007; Woodward et al., 2009; Wisner, Elvevag, Gold, Weinberger, & Dickinson, 2010).

In their population study, which was undertaken in the UK, Verity et al. (Verity, Butler, & Golding, 1985c; Verity et al., 1985a; Verity & Golding, 1991; Verity et al., 1993; Verity et al., 1998) prospectively enrolled 16.613 infants and followed them up till the age of 10 years to determine any history of febrile seizures and assess the children with such a history. The children were assessed twice; at the age of 5 and subsequently at the age of 10.

In the first Verity study (Verity et al., 1985c; Verity et al., 1985a), children were assessed on a small number of simple tests easily conductible at the child's home. From these tests, only 2 were further analyzed (i.e. the English Picture Vocabulary and the Copying Designs tests), as the authors reported that these afforded them with the most useful information. There were no differences in performance on these tests between the children who had experienced febrile convulsions and those who had not. This was true of both children who had experienced simple convulsions and those who had experienced complex ones. However, the authors never specified the definitions employed to classify children into one or the other category (i.e. simple or complex) nor did they clearly explain the statistics used to make their comparisons. Finally, this study reported that 42 out of the 290 patients (14.5%) had had a history of a speech problem at one point or another. From these, 11 no longer had the problem by the time the study was conducted. Moreover, children with a history of febrile convulsions were found to be more likely to have sleeping problems than children with no such history.

In their second study, reporting on the outcomes of 19 children with prolonged febrile seizures the authors reported that 12 out of the 19 children were found to be normal on neuropsychological tests(Verity et al., 1993) . From the remaining seven children, 3 had been shown to be normal at the age of 5, 1 child was found to be impaired, and, 2 children were receiving special education. The two children receiving special schooling were developmentally delayed previous to the PFS. However, as the authors concede themselves, the children were not assessed prior to their seizure and, therefore, their premorbid levels can only be speculated upon. In their larger study looking at the effects of simple febrile seizures on outcome measures no differences were observed between controls and children with a history of febrile seizures (Verity et al., 1998). However, children with simple afebrile seizures were included in their control group, which may have been a confounding factor.

Not all studies looking at prolonged febrile seizures have found them to be inconsequential to subsequent development (van, Ramlal, van Steensel-Moll, Steyerberg, & Derksen-Lubsen, 1996; Schiottz-Christensen & Bruhn, 1973; Kolfen et al., 1998). A study of 57 children with no prior history of neurological deficits found that 12 of them developed impairments following the seizure. In 5 of these patients, the impairments appeared at the time of the seizure, and, in the remaining patients a mean of 4 months following the seizure(van et al., 1996). The impairments which ranged from mild (n=9) to severe (n=3) consisted of speech defects (n=12) and motor impairments (n=5). Duration and the need for more than two drugs for seizure termination were found to be predictive of sequelae in this study.

A different study found that non-verbal intelligence was compromised in children following prolonged febrile seizures but not simple febrile seizures (Kolfen et al., 1998). Finally, a study of 14 pairs of monozygotic twins discordant for febrile convulsions revealed that the affected twins obtained a performance IQ (PIQ) score which was on average 7 points lower, and, a full scale IQ (FSIQ) which was on average 5 points lower than the unaffected twins' scores (Schiottz-Christensen & Bruhn, 1973). The unaffected twins were also shown to be significantly better from their siblings at the delayed recollection of a story (i.e. logical memory test). The ability to repeat the story in the immediate condition was indistinguishable between the two groups pointing to a recollection deficit following febrile convulsions. Unfortunately, this study does not provide details of the seizures themselves, e.g. duration. They do report, however, that age at the time of the seizure, number of febrile convulsions, and duration were not found to be related to intellectual function. This study is particularly important for its findings seem to support the notion that the seizures themselves are responsible for the IQ discrepancies in the pairs of twins.

Memory functions following prolonged febrile seizures have been scarcely studied (Chang et al., 2001; Jambaque et al., 2006; Kipp, Mecklinger, Becker, Reith, & Gortner, 2010; Schiottz-Christensen & Bruhn, 1973). This is quite surprising given the known association between prolonged febrile seizures and medial temporal sclerosis (MTS) (Scott et al., 2002; Scott, King, Gadian, Neville, & Connelly, 2003; vanLandingham, Heinz, Cavazos, & Lewis, 1998). Two group studies that have investigated the longer term effects of simple febrile seizures on memory functions have revealed no memory impairments in this group (Chang et al., 2001; Kipp et al., 2010). Strikingly, one study even revealed that the febrile seizure group outperformed their age peers in most working memory measures they were assessed on (Chang et al., 2001). Age at onset of febrile seizures before the age of 1 was the only risk factor associated with memory deficits. Finally, this study found that the febrile group was found to be more impulsive than their peers as evidenced by more jumping errors on the working memory task. While these findings may be interesting in their own right, the aforementioned study only included 8 patients with prolonged febrile seizures (out of 87). Seeing as the association advanced in the literature between MTS and seizures predominantly concerns the prolonged kind (Hesdorffer et al., 2008), focusing on this type of seizures would be more pertinent. Moreover, the choice of a working memory task to interrogate hippocampal functions is problematic as this structure is mainly known for its role in declarative memory.

A second study that investigated this issue in children with a history of febrile seizures did not reveal any memory impairments in this group but found evidence for an altered functional MTL network following febrile seizures (Kipp et al., 2010). Namely, the febrile seizure group was shown to evince an event related potential (ERP) pattern representing familiarity-based remembering during a task where controls were found to recruit recollection-based remembering processes. During the encoding phase children had to make active decisions, which seriously minimized their ability to

form strategies, and, therefore their ERP differences cannot be attributed to differences in mnemonic strategizing.

In sum, the studies to date provide conflicting evidence regarding the effect of CSE on intellectual functions. This is particularly true in the case of prolonged febrile seizures, with some studies revealing no impairments while others find evidence of cognitive and memory deficits in this group. Until this day no study has looked at the immediate aftermath of CSE on intellectual development using standardized neuropsychological tools. This poses a big gap in our understanding of CSE and its effects on human development. The current PhD thesis has been designed in part to answer this question.

1.6. The association of PFS and the development of MTS

There has been a long standing debate in the literature with regards to the association of PFS with mesial temporal sclerosis (MTS). MTS is defined as hippocampal sclerosis (HS) accompanied by more widespread abnormalities in the temporal lobe(Scott et al., 2001). HS is characterized by a stereotypical pattern whereby neuronal loss is observed in the CA3, CA1 subfields of the hippocampus and the dentate gyrus (Sutula, Hagen, & Pitkanen, 2003). TLE in adults is most commonly associated with hippocampal sclerosis (HS) (Thom, Martinian, Parnavelas, & Sisodiya, 2004) ; 65 percent of patients that undergo surgery show signs of hippocampal sclerosis (Bocti et al., 2003). Therefore, it is instrumental for both clinical and research purposes to explore the suggested link between PFS in childhood and MTS to better comprehend the pathogenesis of temporal lobe epilepsy (TLE).

There are several lines of evidence pointing to a possible link between PFS and subsequent MTS. Firstly, it has been found that 40 to 60 percent of individuals with MTS identified in epilepsy surgery

programs have had a PFS (Cendes et al., 1993). Secondly, pathological examinations of the resected temporal lobes of patients with intractable epilepsy have demonstrated a higher occurrence of PFS among those with MTS than among those with no such lesion (Falconer, 1971). Thirdly, several imaging studies have highlighted the presence of structural abnormalities in the hippocampi of patients following febrile seizures (Fernandez et al., 1998; Scott et al., 2002; Scott et al., 2003; vanLandingham et al., 1998). Whereas these findings are particularly pertinent to complex and prolonged febrile seizures (VanLandingham et al., 1998; Scott et al., 2002; Scott et al., 2006; Hesdorffer et al., 2008) evidence of hippocampal abnormalities in adults with a history of simple seizures has been recently reported (Auer et al., 2008).

The timeline and nature of hippocampal abnormalities following febrile seizures have been described in several studies. For example, Scott et al. (2002) found that patients with PFS that were imaged within 48 hours of the episode had large hippocampal volumes suggestive of hippocampal oedema when compared to healthy controls(Scott et al., 2002). A mean of 5.5 months later, 5 out of 14 PFS patients exhibited a hippocampal asymmetry outside the 95th percentile for controls and, in 3 patients, one hippocampus was found to be significantly smaller than the 95th prediction limit for controls(Scott et al., 2003).

Another study has found evidence that hippocampal injury is associated with the nature of the prolonged seizure and not simply the occurrence of one (vanLandingham et al., 1998). Namely, in this study, patients with focal features (6 out of 15 patients), but not patients with generalized seizures (0 out of 12 patients), revealed hippocampal abnormalities on their MRI upon visual inspection within 6 days of the event. Two out of the 6 patients were suspected of chronic abnormalities due to adverse perinatal events. In the remaining 4 patients there was evidence for

larger hippocampi associated with the side of seizure origin. Finally, two out of the 4 patients that were followed up 8 to 10 months later showed advances in hippocampal atrophy.

These findings are in line with two theoretical possibilities concerning the progression from febrile seizures to TLE. The first possibility is that a pre-existent hippocampal abnormality predisposes the child to have a seizure in the first place, and, in some cases, this abnormality will progress to hippocampal sclerosis. Findings that timing of an initial precipitating injury rather than seizure characteristics *per se* were predictive of hippocampal sclerosis in a group of children with unilateral stroke and intractable seizures supports this view (Squier, Salisbury, & Sisodiya, 2003). More importantly, the presence of hippocampal abnormalities in unaffected members of families with a history of febrile convulsions and TLE provides the most important evidence to date that hippocampal abnormalities precede febrile seizures (Fernandez et al., 1998). The non-development of febrile seizures in the members with hippocampal abnormalities may be due to the absence of a trigger (e.g. a fever) during a time sensitive window. Nonetheless, febrile convulsions seem to be a prerequisite for the subsequent development of TLE in familial cases suggesting a role played by the seizure as well in the chain of events linking structural abnormalities to TLE (Maher & McLachlan, 1995).

The second theoretical possibility would be that the seizure itself leads to a structural lesion that, in rare cases, progresses to hippocampal sclerosis and TLE. Animal models have provided evidence for a progression from an initial long seizure to the development of limbic seizures in the absence of pre-existing structural lesions (Dube et al., 2006). However, the presence of a pre-existing developmental lesion (independent of lesion location) has been found to augment the epileptogenic effects of febrile convulsions in animals (Park et al., 2010). In humans, findings that an earlier age of seizure onset seems to protect against hippocampal injury support a seizure related effect though a

pre-existing hippocampal abnormality cannot be ruled out (Riney, Harding, Harkness, Scott, & Cross, 2006). However, in the same study, hippocampal sclerosis was equally seen in acquired as well as developmental pathologies something that wouldn't be expected if a pre-existing hippocampal abnormality were the case.

In sum, although progress has been made in our understanding of the relationship between febrile seizures and the pathogenesis of TLE the verdict is not in yet. A complex interplay seems to characterize this relationship consisting of genetic and/or predisposing factors coupled with environmental triggers. Moreover, who is to say that there is only one possible route that leads to the expression of febrile seizures or the expression of TLE following febrile seizures? In closing, it is important to note that whereas retrospective studies report a high rate of patients with a history of febrile seizures in TLE, prospective studies report much smaller rates and there is even a study who failed to find any evidence of MTS in 24 patients following PFS (Tarkka et al., 2003), though its small sample size precludes a premature conclusion on this issue.

1.7. The Medial Temporal Lobe and Memory

Contemporary research points strongly to the medial temporal lobes (MTL) having a highly specific role in declarative memory. However, our understanding of the roles played by the individual components of the MTL system is being continuously updated. In humans, the MTL consists of two major sets of structures. One set of structures, defined as the hippocampal formation, consists of the cornus ammonis (CA1, CA2, CA3) subfields of the hippocampus itself, the dentate gyrus (DG), the subiculum, the presubiculum, the parasubiculum and the entorhinal cortex (EC). The second set of structures consists of the perirhinal (PRC) and parahippocampal (PRH) cortices, which lie along the parahippocampal gyrus.

Much of the characterization of the MTL connectivity has been carried out in the rat and the monkey. Through this line of work we have learned that information arrives in the MTL through the PRC and the PRH cortices, which subsequently feed on the information to the EC. It is then through the EC that the information is transferred to the hippocampal formation. There is also a feedback connection from the hippocampus to the EC, which, in turn, has extensive reciprocal connections with the PRC and PRH cortices (Jeffery, 2007).

Within the hippocampal formation itself the EC innervates the DG and field CA3 through the perforant path (Insausti, Cebada-Sanchez, & Marcos, 2010). This forms the main source of afferents to the DG. DG cells project to CA3 through the mossy fibre system. CA3, in turn projects to CA1 through the Schaffer collaterals. CA1 projects to the subiculum, which, in turn projects to the presubiculum and the parasubiculum. Finally, this loop of connections terminates in the EC with inputs received from CA1, the subiculum, the presubiculum and the parasubiculum.

In what will ensue I will describe the MTL's developmental course during infancy and childhood. In this line of investigation, research conducted with rodents and primates has provided us with valuable insights into the pre- and post-natal sequence of events that leads to a fully functional MTL system. However, advances in imaging techniques have recently facilitated the investigation of these processes in humans as well. Finally, the effect of seizures on these maturational events will be discussed.

1.7.1. The Development of the Medial Temporal Lobe

Behavioural studies have pointed to a protracted maturational period of the medial temporal lobes. This is because adult-like proficiency in most MTL based memory tasks is not reached till the end stages of infancy or the early stages of childhood (Overman, Pate, Moore, & Peuster, 1996;

Overman, Ormsby, & Mishkin, 1990). On the other hand, studies of brain maturation in humans and primates have revealed that most of the hippocampal formation cells are formed during the first half of gestation (Lavenex, Banta, & Amaral, 2007).

An exception to this finding is the DG, which seems to undergo a more protracted period of development with granule cells continuing to form during the third trimester of gestation, albeit at a decreasing rate (Lavenex et al., 2007). As a consequence of their later formation, granule cells reach their maturity late in the postnatal period (Abraham et al., 2009), which, in turn, influences the development of their target cells i.e. mossy and CA3 cells. Therefore, adult like maturity in intrahippocampal circuitry appears to be delayed until the second half of the first decade (Abraham et al., 2009).

Similarly, myelination processes, particularly in the dentate gyrus, seem to continue throughout and beyond childhood following a predetermined trajectory that adheres to the developmental pattern of the hippocampal afferent and efferent pathways. Myelination processes are important for cognitive functions because they enhance conduction velocity along an axon. In other words, properly myelinated pathways lead to faster computations.

Few structural imaging studies in existence have looked at hippocampal development during childhood (Giedd et al., 1996; Gogtay et al., 2006). Moreover, due to ethical complications, the studies that do exist investigate this issue in children older than the age of 4, since they no longer require sedation for imaging purposes. Nonetheless, these studies have provided some valuable insight into the pattern of hippocampal growth. For example, Gogtay et al. (2006) have shown that the anterior and posterior hippocampal subregions follow distinct trajectories despite the lack of significant hippocampal volumetric growth within the same time frame. This group of researchers

scanned 31 children between the ages of 4 and 25 years every 2 years for 6 to 10 years obtaining 100 scans in total. They observed that during normal development the posterior region of the hippocampus seems to gain in volume whereas the anterior part seems to decrease in volume. They speculated that the volume gain observed in the posterior hippocampal region comes from the DG and CA3 regions. Whereas this proposition sits well with the findings from anatomical and cellular studies, imaging studies with a greater resolution would be needed to confirm this assumption.

Less is known about the development of the cortical areas adjacent to the hippocampus in the human brain. However, insights drawn from the primate literature point to the early maturation of these MTL structures (Alvarado & Bachevalier, 2000). For example, the EC, the main source of input to the hippocampus, has generated most of its cells within the first trimester of gestation. Moreover, projections from the EC to the subiculum and the CA1 appear to be established prenatally (Insausti, Cebada-Sánchez, & Marcos, 2010). Yet, whereas the layers of the EC are in place at birth, they are not as clearly laminated as in the adult (Grateron et al., 2003). Moreover, this structure seems to be increasing in size throughout development (Insausti, Cebada-Sánchez, & Marcos, 2010).

1.7.2. The effect of status epilepticus on the MTL circuit

The neuronal damage caused by SE in adult rats has been well documented in the literature. In the hippocampus, neuronal cell loss has been observed in the CA1, CA3 and dentate hilar regions (Scantlebury et al., 2007). Cell loss has also been reported in the amygdala, the piriform cortex and the EC (Scantlebury et al., 2007). Cell death in adults is thought to be mediated by an increased entry of calcium into the cells due to excessive activation of N-methyl-D-aspartate (NMDA) receptors and inhibition of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors

(Scantlebury, 2007). A different avenue for cell death might be the age specific increased presence of inflammatory cytokines following SE in adults (Ravizza et al., 2005). Changes in synaptic plasticity in the form of sprouting have also been documented following SE and seem to be concentrated in the dentate gyrus and CA1 (Scantlebury et al., 2007).

Akin to behavioural studies, neuronal damage caused by SE in young rats is rarer and milder than neuronal damage caused by SE in adult rats (Scantlebury et al., 2007). For example, rats following the development of spontaneous limbic seizures in the hyperthermia model were not found to have different neuronal densities from controls in the CA1 region and the hilus; areas that have been implicated in the adult models (Dube et al., 2006). There have been notable exceptions to this premise with one study describing a chronic and extensive neuronal cell loss involving hippocampal and extrahippocampal regions following SE in 14-old day rats (Sankar et al., 1998). Neuronal damage primarily involved the CA1 subfield as well as the dentate gyrus and mossy-fiber sprouting was evident in the dentate gyrus mirroring, thus, the adult findings (Sankar et al., 1998). Nonetheless, rare exceptions of extensive neuronal damage in young rats may be more indicative of a predisposition for neuronal injury in the particular strain of rats under study (Scantlebury et al., 2007) and do not seem to challenge the proposition that younger rats are more resistant to seizure induced injury .

1.7.3. Complications in the study of memory development

The study of memory functions in infancy and early childhood is still at the beginning stages. This is mainly due to major limitations in the study of memory processes during development that are not encountered when studying adults. For one, the infant brain is still immature and many of the structures and synaptic connections that are evident in the adult brain are still "under construction".

For example, the dentate gyrus which is the major source of input from extrahippocampal regions to the hippocampus does not fully develop until the end of the first year of life (Seress, 2007). These anatomical discrepancies between the adult and the immature brain render the direct comparison of the two groups problematic. Moreover, because of this early cascade of developmental events, functions that may be supported by one structure during infancy might be supported by a different one in adulthood. For example, whereas lesions in area TE in adult rhesus monkeys impair their performance on the Delayed-Non-Match-to-Sample (DNMS) task, homologous lesions suffered during infancy do not affect performance on this task (Bachevalier, 1990).

Another major obstacle to the study of memory functions during development is the paucity of expressive and receptive language in young infants. A lot of the paradigms used to study memory in adults require both the comprehension of complex verbal instructions and verbal output. One way to circumvent this issue has been to utilize memory paradigms drawn from the animal literature or those designed specifically for infants. Finally, some memory tasks require complex motor skills that are still under development in infants aged 6-12 months. However, despite these limitations, the advent of new techniques combined with a renewed interest in understanding the brain-behavior relationship during the early years has yielded some interesting results in the field of memory.

1.7.4. The development of the declarative memory systems and its neural underpinnings

The term declarative memory (or explicit memory) refers to the types of memories that can be brought to mind as an image or proposition without the need for perceptual support (Squire, 1998; Squire & Zola-Morgan, 1991). As such, declarative memory refers both to memory for events (i.e. episodic memory) and to memory for facts (i.e. semantic memory)(Squire & Zola, 1998). The recall of an event requires the mental re-enactment of this event (e.g. remembering last year's summer

holiday) and as such is distinct from the recall of a fact where such re-enactment does not take place (e.g. remembering that the capital of Greece is Athens). A similar distinction has been advanced within the field of memory research between recollection-based-recognition and familiarity-based-recognition where the definitive difference between these two processes lies in the absence (familiarity-based-recognition) or the presence (recollection-based-recognition) of encoding details within the act of recognition (Aggleton & Brown, 1999; Brown & Aggleton, 2001a; Duzel, Vargha-Khadem, Heinze, & Mishkin, 2001).

Implicit memory refers to a set of abilities that influence overt behaviour without requiring the conscious recollection of doing so (e.g., the ability to play a piano, which a pianist seems able to just 'do', without being able to verbalise how) (Buckner et al., 1995; Squire & McKee, 1993). For some time it was thought that infants relied solely on implicit memory, with explicit memory developing only later. This was partly due to the fact that many of the tasks used to assess infant memory involve indirect measures (e.g., motor performance) similar to those often used to assess adult implicit memory, and partly due to the phenomenon of infantile amnesia, wherein most adults can recall very little from the first 3-4 years of their lives(Hayne & Rovee-Collier, 1995). More recently evidence has begun to emerge suggesting that MTL based memory is present from very early in life (Collie & Hayne, 1999; deRegnier, Georgieff, & Nelson, 1997; Nelson & Collins, 1992; Pascalis & de, 1994a; Pascalis, de, Nelson, & de, 1998a). Nelson and colleagues (Nelson, 1998a) have coined the term *pre-explicit* memory to encompass all the competencies exhibited by very young infants on a number of MTL based tasks prior to the emergence of more explicit like features which we associate with declarative memory.

As the present study will use the visual paired comparison task to investigate recognition memory in our cohort, a separate section has been provided for the description of this task and its neural

recruitment. However, another commonly used task in the study of recognition memory that is believed to rely on the MTL circuit is the delayed non-match to sample task (DNMS). In this task the subject is presented with a sample object. The subject must then remove the object to reveal a reward. Following a delay, the sample object is presented along with a novel object. Opting for the novel object is rewarded. Human infants do not succeed on this version of the task until approximately the age of 15 to 21 months even with brief delays of 5-10 seconds. Interestingly, changing the demands of the task from receiving a reward for displacing the novel object to allowing the child to play with the novel object (i.e. stimulus = reward) lowers the success threshold to the age of 6 months, with infants at this age withstanding delays of 10 minutes. This has led investigators to believe that, while success on the DNMS task requires recognition memory, successful performance on this task also requires additional skills such as inhibition processes and an understanding of stimulus-reward relationships.

Further evidence for the presence of explicit-like memory processes in infants comes from studies using the deferred imitation paradigm(Bauer, Wiebe, Carver, Waters, & Nelson, 2003; Bauer, Wiebe, Waters, & Bangston, 2001). In the deferred imitation task, the examiner models a sequence of events for the child (e.g. placing a marble into a cup and then shaking the cup to make a rattle), and, following a delay, the child needs to reproduce this sequence of events without the benefit of prior practice. Research on the neural underpinnings of the deferred imitation task has pointed to the hippocampus as an important contributing structure. Namely, individuals who have sustained bilateral hippocampal damage during childhood or around the time of birth show impairments on this task (Adlam, Vargha-Khadem, Mishkin, & de-Haan M., 2005). The same holds true for individuals that have suffered damage to the MTL during adulthood (McDonough, Mandler, McKee, & Squire, 1995). Performance on this task shows a gradual improvement with age with

dramatic improvements from the age of 6 to 24 months consisting of the ability to remember longer sequences and withstand longer delays. Specifically, infants at the age of 6 months show memory for single actions after a 24 hour delay. By the age of 9 to 10 months, infants begin to show memory for the sequence of actions. Moreover, by the age of 18 months infants can perform normally, even, when the props used in the testing phase are different from the ones used in the presentation phase. This finding points to the increased flexibility of memory retrieval processes in older infants. However, similar to the DNMS task, the taxing nature of the deferred imitation task, makes it very likely that other regions such as the prefrontal cortex contribute to the development of the requisite processes for successful performance on this task.

In sum, the past 30 years in infancy research have been critical in overturning the original notion that implicit memory precedes explicit memory by showing that vestiges of the latter are evident from the very beginning and take on more and more adult-like qualities as the infants mature. Tasks such as the DNMS, which has been borrowed from the animal literature, and the deferred imitation task have offered us insights regarding the sequence of events that takes place in early memory and evidence regarding the MTL role in memory development.

1.7.5. Visual recognition memory and the visual paired comparison task

Existing data support the notion that visual recognition memory (i.e. the ability to identify and judge the prior occurrence of something that has been seen) is present from the very beginning of life (Pascalis & de, 1994b; Pascalis, de, Nelson, & de, 1998b). During infancy and early childhood recognition memory has been studied with the use of novelty paradigms. The most commonly adopted task in the study of novelty detection has been the visual paired comparison (VPC) task introduced by Fantz in 1964 (Fantz, 1964). In the VPC task, subjects are familiarized with two

identical items followed by the presentation of the familiar item coupled with a novel one. Attending to the novel stimulus for a longer duration is taken as a sign of detection of novelty and, thus, discrimination of the two visual stimuli. Applying a delay between the first presentation and the testing phase has been utilized to study memory processes.

A number of studies have shown that recognition memory is already present in newborns. For example, Pascalis and de Schonen (1994) have shown that babies 3-4 days old can detect novel stimuli following a delay of 2 minutes. As children grow older they are able to withstand longer and longer delays, with babies 3 months old withstanding delays of 24 hours on the VPC task (Pascalis et al., 1998). Moreover, as infants age, their recognition memory becomes less constrained by the perceptual similarity between the original encoding context and the testing context. In an interesting experiment, Robinson and Pascalis (2004) tested 6, 12, 18, and 24 month old babies on a variation of the VPC task. In this variation, the authors presented a different background during testing from the one presented during the encoding phase(Robinson & Pascalis, 2004). Only the 18 and the 24 month old babies were unaffected by this change. This partly reflects the early inflexibility of the nascent recognition memory system.

Longstanding investigations in the field of animal research and neuropsychology have tried to establish the brain regions that support novelty preferences. This line of research has unequivocally pointed to the medial temporal lobes (MTL) as critical structures for these processes. However, the specifics of the division of labour within the MTL are still debated. Namely, some theorists have posited the hippocampus as the critical structure for novelty detection (Nelson, 1998b; Richmond & Nelson, 2007) whereas others have argued for the pivotal role of extrahippocampal regions such as the perirhinal cortex in this function (Brown & Aggleton, 2001b; Bachevalier & Vargha-Khadem, 2005). Recently, an alternative has started to emerge which posits that both these structures can

support novelty preferences, albeit at different developmental time points (Zeamer, Heuer, & Bachevalier, 2010).

Zeamer et al. (2010) decided to investigate the development of object recognition memory in infant macaques with and without hippocampal lesions. They tested both groups at the ages of 1.5, 6 and 18 months on a standard VPC paradigm imposing delays of 10, 30, 60 and 120 seconds between familiarization and test. Both the normal and the hippocampally-lesioned animals revealed novelty preferences above chance across all ages and delays. However, at 18 months both groups evinced a delayed-dependent effect displaying significantly lower novelty preferences at the longer delays. This effect was more pronounced in the lesioned animals. Group differences were only obtained in the 18 month olds with the lesioned animals performing worse relative to controls at the 10 and the 120 second delays. Therefore, according to Zeamer et al. (2010), the above data suggest that the hippocampus is not necessary during the early stages of development for object recognition memory but gradually becomes more involved following the maturation of MTL connections. However, it remains possible that the hippocampus is needed for longer delays than 2 minutes even at younger ages.

Further evidence for the view that the hippocampus does not support recognition memory in early infancy comes from the observation that novelty preferences in the VPC task at 1 and 6 months of age in normal monkeys are the same as those in animals that have sustained hippocampal damage at 10-12 days of age (Resende et al., 2002). It has been argued, therefore, that the hippocampus is not necessary to perform a novelty detection task in early stages of development but becomes progressively involved with higher demands such as longer delays and contextual changes. In this view, earlier findings of neonatal hippocampal lesions affecting novelty preferences (Pascalis & Bachevalier, 1999) are attributed to the possibility of more extensive lesions in this sample that could

potentially have included parahippocampal regions such as the perirhinal cortex. Moreover, animals in the above study were tested in adulthood and, therefore, at a time when the hippocampus may be critical for visual recognition memory.

This view is also consistent with the human data, where hippocampal lesions in adults result in deficits in novelty detection following a delay (McKee & Squire, 1993; Pascalis, Hunkin, Holdstock, Isaac, & Mayes, 2004). Moreover, increasing the demands on the VPC by changing the background upon which the object is presented between familiarization and test leads to deficits irrespective of a delay (Pascalis, Hunkin, Bachevalier, & Mayes, 2009). This latter finding highlights the important contribution of the hippocampus to relational memory even at short lags (Eichenbaum, Yonelinas, & Ranganath, 2007; Hannula, Tranel, & Cohen, 2006).

Recently, visual recognition memory was also investigated in a group of patients with developmental amnesia (Munoz, Chadwick, Perez-Hernandez, Vargha-Khadem, & Mishkin, 2010). Developmental amnesia, a disorder associated with early circumscribed hippocampal injury, has been found to preferentially afflict episodic memory processes and spare semantic memory processes (Vargha-Khadem et al., 1997a; Vargha-Khadem, Gadian, & Mishkin, 2001). Moreover, these patients have been shown to have relatively intact recognition memory processes as evinced by their performance on the Doors and People test (Adlam, Malloy, Mishkin, & Vargha-Khadem, 2009). In contrast, their performance on a standard VPC paradigm was found to be impaired relative to normal controls when the delay between familiarization and test was greater than 30 seconds (Munoz et al., 2010). Moreover, in delays greater than 30 seconds, their novelty preferences were not significantly different from chance. Thus, age at injury, doesn't seem to predetermine performance on the VPC task during adulthood.

However, what would help settle the above issue would be the application of the VPC to infants with known hippocampal injury during the early stages of development. In our study, we have chosen to use the VPC task to explore recognition memory in infants and children with suspected hippocampal abnormalities.

1.8. Hypothesis Formulation

The present thesis was designed to investigate the shorter and longer term effects of CSE on cognitive, language, and motor development (infants only), as well as, recognition memory in a paediatric population. To that end, standardized neuropsychological assessments as well as an experimental memory paradigm were utilized to investigate these effects. Functional-structural correlates were also examined to determine the contribution of structural abnormalities to functional outcomes. However, the characterization of structural consequences following CSE is the subject matter of another PhD thesis reporting on the same paediatric cohort, and, thus, the formulation of hypotheses regarding the structural consequences of CSE will not be advanced in this thesis.

The central hypothesis of the overall project is that CSE, and in particular PFS, can cause a spectrum of hippocampal injury, and the children with the most severe hippocampal injury will develop temporal lobe epilepsy with cognitive deficits and behavioural disorders. With that bigger framework in mind and in view of the existing literature presented in this introduction we expect to find the following:

A. Etiology will largely influence the functional outcome in children following CSE. This finding has been supported by previous studies, but, this will be the first study that can quantify this effect and provide a detailed profile of the children investigated. Etiology is used here to encompass both diagnostic categories as well as brain abnormalities.

B. Children following PFS will manifest recognition memory deficits soon after the event on the VPC task. Specifically, we expect PFS children to evince deficits on the delayed aspect of the VPC task but not the immediate aspect. A prediction concerning the longer term prospect of recognition memory in these children is a bit harder to conjecture given the scarcity of MRI long term follow-up PFS studies.

2.1. Operational Definitions

We defined convulsive status epilepticus (CSE) as a tonic, clonic, or tonic-clonic seizure (continuous CSE), or two or more seizures between which consciousness was not regained (intermittent CSE), which lasted for at least 30 minutes. Two paediatric neurologists blindly reviewed each case and classified the child as a PFS or a non-PFS case. A PFS was defined as CSE in an otherwise neurologically normal child between the age of 6 months and 5 years in the presence of a fever of 38º Celsius and over. *Table 2.1* contains the classification system adopted to categorize the non-PFS patients.

2.2. Patient recruitment

For the purposes of this study, we have recruited a population cohort of children between the ages of 1 month and 16 years of age who have experienced at least one episode of CSE. In order for this cohort to be representative of the population of children with CSE we have been prospectively recruiting children from hospitals around London using a multi-tiered recruitment strategy similar to that used in the North London Convulsive Status Epilepticus Surveillance study (NLSTEPSS) (Chin et al. 2006),which achieved 86% ascertainment of cases of CSE in North London. Patients were recruited via the following methods:

1) Hospital clinicians were instructed to refer any cases of CSE via a telephone messaging service or e-mail.
2) Centralised records held by the Children's Acute Retrieval Service and by Great Ormond Street Hospital were regularly checked for referrals of patients with CSE.

3) Individual acute paediatric wards and Paediatric Intense Care Units were regularly contacted to see if they had seen any children with CSE recently.

Once consent was obtained for the children's participation, we collected baseline clinical data from the parents and arranged for the child to have an MRI scan and neuropsychological assessment at Great Ormond Street Hospital. At this appointment the child was also clinically assessed by a paediatric neurologist who asked the parents a series of questions relating to the seizure itself, their family history and the child's medical history. One standard question asked in this interview was whether the parent had noticed any behavioural differences in the child following CSE.

2.3. Participants

Two hundred and thirty one participants were referred to us for participation in our study over a recruitment period of 38 months. From these 87 took part (37.7%) and 80 patients (34.6%) were seen for a neuropsychological assessment a mean of 38 days following CSE (range 6-254 days). The remaining CSE patients were not assessed for one of the following reasons: (a) families were non-contactable, (b) patients were not suitable for sedation, and, (c) parents declined participation. A Mann Whitney test revealed no differences between the age of participants (M = 63.6, SE= 4.3) and non-participants (M = 99.4, SE= 14.7). No other clinical variables were available on non-participants for further comparison of the two groups.

2.4. Neuropsychological assessments

The children were assessed at two different time points: (a) acutely i.e. ideally, within three weeks from the episode, and, (b) as a follow-up, 8 or 12 months after the first visit. Children were randomly allocated to each follow-up group. We utilized three different neuropsychological batteries to assess the children's overall abilities which corresponded to their age range.

2.4.1. The Bayley Scales of Infant and Toddler Development

Children under the age of 42 months were assessed using the Bayley scales of infant and toddler development-3rd edition(2005). This test is divided into 5 scales. The Cognitive Scale examines how infants perceive, think, and gain an understanding of the world. The Receptive Communication Scale examines the ability to react to sound and understand language. The Expressive Communication Scale examines the ability to communicate. The Fine Motor Scale examines smaller, precise movements with the fingers, hands, and eyes. The Gross Motor Scale examines larger movements of the arms, legs, feet, or entire body. The Bayley scales provide three normative composites: one for cognitive development, one for language development and, one for motor development each with a mean of 100 and a standard deviation of 15. They also provide five scaled scores: cognitive, receptive communication, expressive communication, fine motor, and gross motor each with a mean of 10 and a standard deviation of 3.

2.4.2. The WPPSI

Children aged between 42 and 84 months were assessed using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI)-3rd UK edition(2002). The core subtests of the WPPSI consist of the information, the receptive vocabulary, the block design, the matrix reasoning, and the object

assembly. The WPPSI provides measures of verbal (VIQ), performance (PIQ) and an overall composite of VIQ and PIQ abilities (Full scale IQ: FSIQ) with a mean of 100 and standard deviation of 15.

Table 2.1 Classification system for the causes of non-febrile CSE

Acute Symptomatic: CSE in a previously neurologically normal child, within a week of an identified acute neurological insult including bacterial meningitis, viral CNS infection, metabolic derangements, drug related effects, head injury, hypoxia or anoxia, cerebrovascular disease.
Remote Symptomatic: CSE in the absence of an identified acute insult but with a history of a pre-existing CNS abnormality more than one week previously.
Acute on Remote Symptomatic: CSE that occurred within a week of an acute neurological insult or febrile illness and occurred in a child with a history of previous neurological abnormality, including epilepsy. This category included children with cerebral palsy with a febrile illness not of CNS origin, and children with obstructed ventriculoperitoneal shunts for hydrocephalus.
Idiopathic Epilepsy Related: CSE that is not symptomatic and occurred in children with a previous diagnosis of idiopathic epilepsy or when the episode of CSE is the second unprovoked that has led to a diagnosis.
Cryptogenic Epilepsy Related: CSE that is not symptomatic and occurred in a child with a previous diagnosis of cryptogenic epilepsy or when the episode of CSE is the second unprovoked seizure which has led to a diagnosis.
Unclassified: CSE that cannot be classified into any other group.

2.4.3. The WISC

Children over the age of 84 months were assessed using the Wechsler Intelligence Scale for Children-Revised (WISC)-4th UK edition(2003). Similar to the WPPSI, the WISC provides measures of verbal (VIQ), performance (PIQ), and, overall abilities (FSIQ). On top of these it also provides measures of processing speed (PSQ). The normative mean in this test is 100 and has a standard deviation of 15.

2.4.4. The CMS

Moreover, children between the age of 5 and 16 were tested on the Children's Memory Scale (CMS)(Cohen,1997). The CMS comprehensively assesses the integrity of memory functions in children and enables comparison with measures of both ability and achievement. Its 6 core subtests load onto scales tapping: (a) immediate verbal memory, (b) delayed verbal memory, (c) general memory, (d) immediate visual memory, (e) delayed visual memory. The normative mean for each scale is 100 with a standard deviation of 15.

2.5. Parental questionnaires

Parents were also given various questionnaires to provide their own insights on what the child can do.

2.5.1. Social-Emotional and Adaptive Behavior questionnaire

For children up to 42 months, parents were asked to complete the Social-Emotional and Adaptive Behavior questionnaire. The Social-Emotional questionnaire is based on the Greenspan Social Emotional Growth Chart: A Screening Questionnaire for Infants and Young Children (Greenspan, 2004) which assesses the development of functional emotional milestones in children. These

milestones include the capacity to engage with a range of emotions; to experience, express, and comprehend a variety of emotional signals; and to elaborate a range of feelings with words and symbols (e.g. pretend play). The social emotional questionnaire provides the social emotional composite score (SEC) which has a normative mean of 100 with a standard deviation of 15.

The Adaptive Behavior Scale is designed to evaluate the attainment of functional skills necessary for the increasing independence of the infant and young child. These include: (a) Communication (speech, language, listening and nonverbal communication), (b) Community use (interest in activities outside the home and recognition of different facilities), (c) Health and Safety (showing caution and keeping out of physical danger), (d) Leisure (playing, following rules and engaging in recreation at home), (e) Self Care (eating, toileting and bathing), (f) Self-Direction (self-control, following directions, and making choices), (g) Functional Pre-academics (letter recognition, counting, and drawing simple shapes), (h) Home Living (helping adults with household tasks and taking care of personal possessions), (i) Social (getting along with other people: using manners, assisting others, and recognizing emotions), and (j) Motor (locomotion and manipulation of the environment). Scores on these 10 subscales are combined together to provide the general adaptive composite (GAC) which has a mean of 100 and a standard deviation of 15.

2.5.2. Communication and Symbolic Behavior Scales

For children between the age of 6 months and 2 years of age the parents were also asked to fill out the Communication and Symbolic Behavior Scales Developmental profile (CSBS DP). This questionnaire is a norm-referenced, standardized instrument used to assess infants, toddlers and preschoolers at risk for communication delays and impairments. It provides the symbolic scaled score, the speech scaled score and the social composite score which all have a normative mean of 10

and a standard deviation of 3. Adding up the raw scores from the above areas provides a total score which when converted into a scaled score has a normative mean of 100 and a standard deviation of 15.

2.5.3. The Strengths and Difficulties questionnaire

The strengths and difficulties (SDQ) questionnaire was given to the parents of children aged between 3 and 16 years of age. It is a brief behavioural screening which assesses 5 main areas: (a) emotional symptoms, (b) conduct problems, (c) hyperactivity/inattention, (d) peer relationship problems, (e) prosocial behaviour. Adding up the first four scores (excluding the pro-social scale) provides a total difficulties score, which is considered to be within the normal range when it is below 13, in the borderline range between 14 and 16, and abnormal if the child obtains a score over 16.

2.6. The Visual Paired Comparison task

On their baseline and follow-up visits all children were also tested on the visual paired comparison task. The experimental design of this task is described in detail in Chapter 6. In brief, however, this task consisted of 3 different phases. In the first phase of the experiment, the *novelty preference phase,* we established whether the children were able to demonstrate preference for a novel face. In the second phase, the *familiarization phase*, we familiarized the child to a single face by presenting it 5 times for 10 seconds each time. Finally, in the third phase, the *recognition memory phase*, children were tested 5 minutes following the familiarization phase to determine recognition of the face they had been familiarized to during the familiarization phase.

2.7. Imaging Techniques

2.7.1. Imaging protocol in the current study

MR imaging was conducted three times within a year. The first scanning session took place within three weeks from the CSE episode, the second session was within 4 months of the episode, and the third session was planned so as to coincide with the neuropsychological assessment i.e. either at 8 or 12 months following the CSE episode .

All MR imaging of children was carried out using a Siemens Avanto 1.5 Tesla whole body system. Where possible, scans were done with the children awake in the scanner, however for younger children or those with developmental delay, sedation was deemed necessary. Sedation was either administered orally or alternatively a general anaesthetic was by the clinical sedation team. Time in the scanner for acquisition of all data was approximately 1 hour. The pulse sequences used correspond to those used for clinical scans for the routine assessment of children with epilepsy, with the addition of diffusion tensor imaging (DTI). The latter was carried out using diffusion weighted echo-planar imaging (b=1000 sec/mm^2, 20 directions; b=0 sec/ mm^2, 3 measurements).

Conventional MR imaging was viewed by two neuroradiologists who agreed by consensus on the presence and type of abnormalities. However, the development of quantitative techniques has greatly improved the sensitivity to pathology compared to qualitative visual assessment.

2.7.2. Hippocampal volumetry

Quantitative measurement of hippocampal volumes was performed using the images obtained from the 3D-FLAIR sequences. This provided 1mm isometric voxels. These images were converted from DICOM format into Analyze format using the MRIcroN (http:// www.sph.sc.edu /comd /rorden

/mricron/) DICOM converter. They were then rotated parallel to the long axis of the hippocampus and a ROI was manually drawn to encompass the entire hippocampus using MRIcroN.

This was done by two independent observers, MY and MM, blinded to the status of the child, as the images were given random numbers to obscure the child's identity. The following methodology was used to define the anatomical limits of the hippocampus (Hammers et al., 2007) (see Figure 2.1 for a detailed picture of hippocampal structures):

1) The hippocampus was visualised in all 3 orthogonal planes using MRIcroN and then this was used to guide placement of the ROI primarily on the coronal sections.
2) The anterior border of the hippocampus was defined as the most anterior coronal slice where the temporal horn (1) starts to widen and comes to sit alongside the hippocampus.
3) The posterior border was taken to be the last coronal slice on which the fornix was visible separate from the crus (23).
4) In the anterior section the amygdale (3) was used to define the superior border of the hippocampus. Where visible the alveus was identified and used to define this border (2). More posteriorly the hippocampus was bordered by the lateral ventricle (1).
5) The inferior border was taken to be variously the parahippocampal gyrus (4); uncal sulcus (6); interface of the prosubiculum and cornu ammonis; border between subiculum, and presubiculum; or sulcus hippocampalis.
6) Medially the hippocampus was taken to be bordered by the parahippocampal gyrus (4) or cerebrospinal fluid and laterally by the lateral ventricle or overlying white matter.

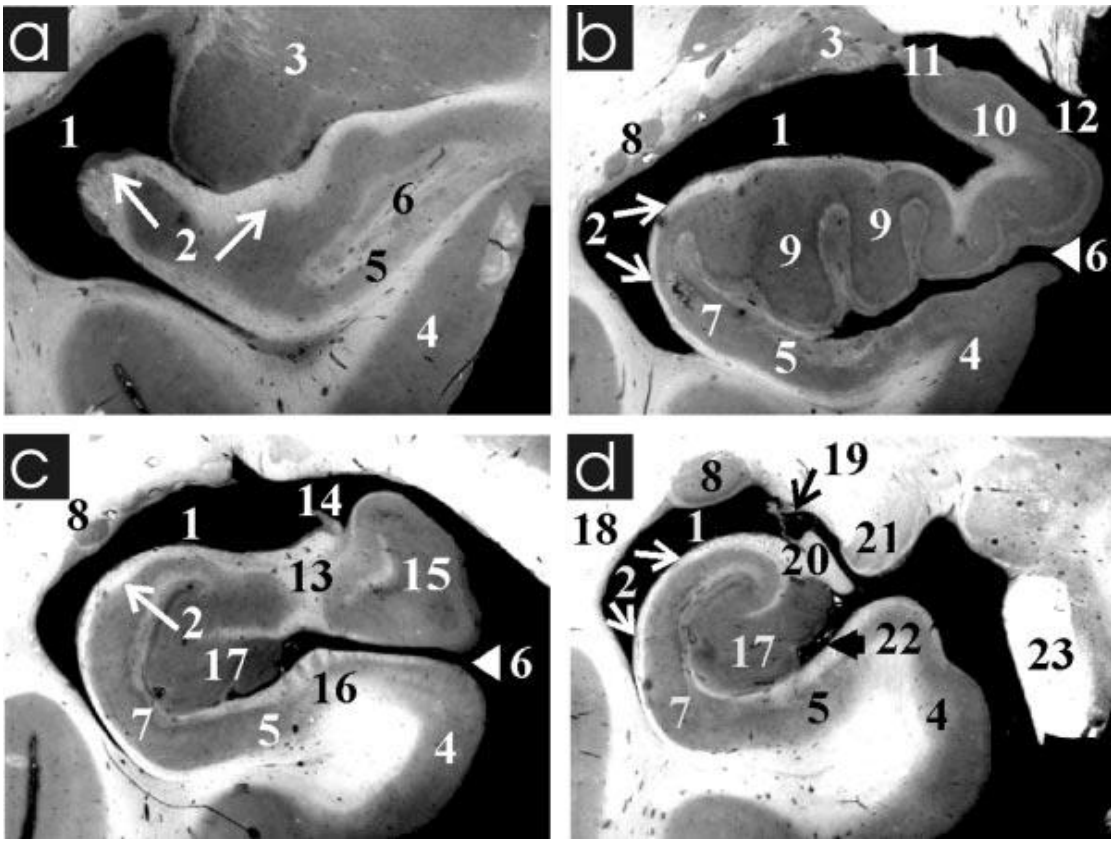


Figure 2.1. The four shape segments of hippocampus in rostro-occipital direction (a-d). Coronal paraffin sections stained with luxo fast blue and counter-stained with nuclear fast red. 1. Temporal horn of lateral ventricle; 2. Alveus; 3. Amygdaloid body; 4. Parahippocampal gyrus; 5. Subiculum; 6. Uncal sulcus; 7. Cornu ammonis; 8. Cauda nucleicaudati; 9. Digitationes hippocampi; 10. Vertical digitation; 11. Isthmus; 12. Ambient perimesencephalic cistern; 13.Basis of fimbria; 14. Taenia fimbriae; 15. Intralimbic gyrus; 16. Subiculumrpresubiculum; 17. Dentate gyrus; 18. Temporal stem; 19. Choroid plexus; 20. Fimbria hippocampi; 21.Lateral geniculate body; 22. Hippocampal sulcus; 23. Crus cerebri. Adapted from Niemann et al. (2003)

Tracing required approximately 60 minutes per dataset, i.e. both left and right hippocampi. Once a ROI was created MRIcroN was used to calculate its volume for each observer. Hippocampal volumes were taken to be the average value of both raters' measurements. We also calculated an asymmetry index (AI) which we defined as:

$$AI = \frac{|R - L|}{\frac{1}{2}(R + L)}$$

where R was the right sided hippocampal volume and L was the left sided hippocampal volume. Brain volume (including CSF in ventricles) was calculated using the automatic Brain Extraction Method (BET) available in FSL (http://www.fmrib.ox.ac.uk/fsl/). All scans were then manually inspected and adjusted as necessary by one of the researchers (MY).

2.7.3 T_2 relaxometry

Measuring the T_2 relaxation time is routinely performed as part of the clinical program for the imaging of children with epilepsy. In the current study, a clinical radiologist (T.B.) placed a ROI within each hippocampus on a coronal section of the T2 relaxation map sequence and the value within the ROI was calculated.

2.7.4 Diffusion Tensor Imaging

Each subject had left and right hippocampi outlined as a ROI using the 3D-FLASH sequences in MRIcron. A custom MATLAB (http://www.mathworks.co.uk/) script was used to create mean diffusivity (MD) maps and fractional anisotropy (FA) maps according to standard formulae (Basser, 1995; Basser, Mattiello, & LeBihan, 1994). The FA maps were then co-registered to the 3D-FLASH images using the non-linear co-registration tools in FSL (FNIRT) (Smith et al., 2004). The resulting warp-field was then inverted and used to map the hippocampal 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 using fslstats.

2.8. Statistical analysis

Statistical analysis was performed with the aid of the SPSS for Windows version 18.0.

Chapter 3 Baseline assessment of infants and children following CSE

3.1 Introduction

CSE is the most common pediatric neurological emergency (Raspall-Chaure et al., 2006). Therefore, it is surprising that the effects of pediatric CSE on mental development have not yet been systematically studied. To my knowledge, all studies that have focused on the impact of CSE on cognition and behaviour have been conducted a number of years following the seizure (Verity et al., 1998; Verity et al., 1985c) and most have not used standardized neuropsychological tests (Aicardi & Chevrie, 1970). Animal studies have shown that status epilepticus can lead to memory impairments a mere two days following status epilepticus, and, more importantly that enriching the animal's environment can modulate the degree of these impairments in the long run (Rutten et al., 2002b). These results make it imperative to assess the effects of CSE in the immediate aftermath of the seizure in a clinical population to determine whether any type of intervention is warranted.

Whereas the outlook of CSE in adults is quite grim due to the presence of co-morbidities (Helmstaedter, 2007), pediatric CSE is believed to be associated with a better outcome (Raspall-Chaure et al., 2006). However, as we discussed in detail in the introductory chapter, disentangling the effects of the seizure itself from the effects of the predisposing etiology becomes problematic. PFS may provide an opportunity to study the effects of the seizure itself on brain development because they are seizures that occur in an otherwise normally developing child. However, this approach assumes that the brain of a child that gives rise to a PFS is completely normal. Extensive animal research based on models of hyperthermia supports this assumption by showing that a previously normal brain can seize, if subjected to high temperature conditions (Dube et al., 2006b). However, only approximately 5% of children will have a febrile seizure even though almost all will experience a significant fever at the age at which febrile seizures occur. This suggests that it is more

than just a fever that is required to generate a febrile seizure and that some children have brains that are predisposed to having a seizure when exposed to a fever.

3.2 Aims

The aims of this chapter are twofold. The first aim is to investigate how children soon after CSE perform on standardized neuropsychological tests. This includes examining relationships between performance and various variables flagged by previous studies as clinically important including age. Secondly, we wanted to determine whether the performance of children is affected by a PFS, as seems to be the case in animals (Dubé et al., 2009).

3.3 Methods

3.3.1. Participants

For the purposes of this study, we recruited a population cohort of children who had experienced at least one episode of CSE. We also recruited 29 normal controls through acquaintances and by visiting areas specifically designated for mother-infant activities (e.g. playgroups, movie screenings for mothers and their babies etc.).The controls were of a similar age range to patients, with no family history of epilepsy, and, had been developing normally up to the assessment according to their primary carer. Seeing as it is unethical to sedate a young child with no clinical concerns, we were only able to scan a small proportion of the control children. Finally, we also assessed 3 of the patients' twin siblings to compare their performance with that of their affected siblings on the neuropsychological assessment. The twin siblings were not included in the larger control group.

3.3.2. Neuropsychological Assessments

A trained neuropsychologist (the current author) blind to patient diagnosis carried out the assessments. Infants were assessed using the Bayley scales of infant and toddler development-III (Bayley, 2005). The Bayley scales provide three normative composites: one for cognitive development, one for language development and, one for motor development each with a mean of 100 and a standard deviation of 15. They also provide five scaled scores: cognitive, receptive communication, expressive communication, fine motor, and gross motor scores, each with a mean of 10 and a standard deviation of 3.

Children aged between 40 and 84 months were assessed using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-3rd UK edition). Children over the age of 84 months were assessed using the Wechsler Intelligence Scale for Children Revised (WISC-4th UK edition). Both the WPPSI and the WISC provide measures of verbal (VIQ), performance (PIQ) and an overall composite of VIQ and PIQ abilities (Full scale IQ: FSIQ) with a mean of 100 and standard deviation of 15.

3.3.3. Questionnaires

For children up to the age of 42 months, parents were asked to complete the Social-Emotional and Adaptive Behavior questionnaire (Bayleys, 2005). This provides a social emotional score (SEC) and a general adaptive score (GAC) with a mean of 100 and a standard deviation of 15. For children between the age of 6 months and 2 years of age the parents were also asked to fill out the Communication and Symbolic Behavior Scales Developmental profile (CSBS DP). The CSBS DP derives three scaled scores: the symbolic composite, the speech composite and, the social composite. Similarly to the Bayley subscales the three scaled scores have a mean of 10 and a standard deviation

of 3. An overall composite is also derived from performance on all three scales (CSBS DP total score) with a mean of 100 and a standard deviation of 15.

The strengths and difficulties questionnaire (SDQ) was given to the parents of children aged between 4 and 16 years of age. The SDQ probes five main behavioural areas: (a) emotionality, (b) conduct problems, (c) hyperactivity, (d) peer problems, and (e) pro-socialization.

3.3.4 Statistical Analysis

As infants and toddlers were assessed using a different neuropsychological assessment tool from children over the age of 40 months, analyses were conducted separately for the infants-toddlers and older children. One sample t-tests were carried out to compare the performance of the PFS, the non-PFS and the control groups against the normative means provided for each index including questionnaire derived indices. For infants, a MANOVA with the cognitive, language and motor composites as dependent variables, and, group, language, and, age at the time of assessment as covariates was conducted to compare the performance of the three groups on these variables. Two ANOVAs with group as the independent variable were conducted to compare the SEC and GAC obtained by the PFS, the non-PFS and the control group. For older children, a MANOVA with VIQ, PIQ and FSIQ as the dependent variables and age as a covariate was conducted to compare the performance of the PFS, the non-PFS, and, the control group.

Subsequently, linear regression analyses were conducted separately for the two clinical groups to determine whether any clinical or demographic variables were contributing to their performance. For the PFS group, age (months), prematurity (yes or no), English as a first language (yes or no), occurrence of previous seizures (yes or no), duration of seizure (minutes), and time elapsed from seizure (days) were entered as clinical covariates. For the non-PFS group, scan abnormalities

(classified as normal or abnormal), medication at the time of the assessment (yes or no) and previous occurrence of CSE were also added as clinical covariates as these were more prominent clinical characteristics in this group. Given the small sample size of the older PFS group, linear regression analyses were conducted solely for the older non-PFS group with the above clinical variables entered as covariates. Language and prematurity were excluded from the analysis as the numbers of preterms and non-English participants in the older group were very small.

Linear regression analyses were also run separately for all PFS and all non-PFS participants irrespective of age. This was done to validate the results from the previous analyses with more participants and include patients hitherto excluded because of insufficient numbers (i.e. the older PFS group). Cognitive composites and FSIQs were used as cognitive indices and language composites plus VIQs as language indices. It should be noted, however, that as FSIQ is partly calculated based on VIQ, it is not independent of the latter. Finally, as older children were not assessed on their motor development such an analysis was not undertaken here. The same clinical variables that were entered in the previously conducted regressions were re-entered here.

Pearson's or Spearman's correlations were conducted to investigate the relationships between scores obtained in our neuropsychological assessment and scores obtained in the parental questionnaires. Given the smaller sample size of the older group (thirteen completed SDQs) we simply report descriptive statistics for this group. All results reported below were considered to be significant at the $p<0.05$ level.

3.4 Results

3.4.1. Description of all participants

Out of the 80 children seen, 34 children had experienced a CSE identified as a PFS. The remaining 46 children experienced CSE associated with other etiologies; 11 were classified as cryptogenic epilepsy related cases (CER), 9 were classified as idiopathic epilepsy related cases (IER), 13 were classified as acute on remote symptomatic cases (ARS), 7 were classified as remote symptomatic cases (RS), 3 were classified as acute symptomatic cases (AS), and, 3 cases eluded classification altogether (UNC). Thirteen out of the 80 children were born before the 36 week cut-off point for prematurity. Ten out of the 34 parents in the PFS group (29.4%), and, 13 out of the 46 parents (28.3 %) in the non-PFS group reported observing a difference in their child's behavior following CSE (see *Table 3.1* for details). Eight of the 23 parents (35%) observed increased irritability in their children.

3.4.2. Neuropsychological assessments for infant group

3.4.2.1. Comparison of the groups' performance with the normative mean

Table 3.2 provides the clinical and demographic characteristics for the infant population and *Table 3.3* provides the mean cognitive, language and motor composite scores for the 75 children (including the 21 controls) assessed using the Bayley Infant Scales (3rd edition). A one sample t-test revealed that the PFS group was performing below the normative mean of 100 on the cognitive ($t(27) = -3.044$, $p=0.005$) and the language ($t(25) = -2.961$, $p=0.007$) composites. This was true of both receptive ($t(26) = -2.101$, $p=0.046$) and expressive ($t(25) = -3.715$, $p=0.001$) language. They were also found to be performing below the mean on the motor composite but this difference did not reach statistical significance ($t(27) = -1.913$, $p=0.066$). Looking at the two motor subscales separately, fine motor skills were found to be unimpaired ($p=0.640$) whereas gross motor skills were found to be impaired ($t(27) = -4.673$, $p<0.001$). The non-PFS group was found to be performing

Table 3.1 Behavioural differences observed by parents following CSE

PFS 1	Irritability, reluctance to feed, unhappy
PFS 2	Irritability
PFS 3	Irritability, reduced concentration and interaction for 2 weeks
PFS 4	Irritability, reduced activity, lethargic
PFS 5	Lethargic, easily tired
PFS 6	Irritability, poor feeding
PFS 7	Regression, not walking as well
PFS 8	Regressed in speech and motor areas for 2 weeks
PFS 9	More aggressive, poorer concentration
PFS 10	Irritability for 2 weeks, dip in school performance
Non-PFS 1	Cranky, irritable, decreased interaction, lost her ability for head control
Non-PFS 2	Increased irritability for one week after status
Non-PFS 3	Poor development
Non-PFS 4	Left hemiparesis for 45 minutes following seizure
Non-PFS 5	No longer fixing and following
Non-PFS 6	Behavioural deterioration
Non-PFS 7	Irritable, poor concentration for 2-3 weeks in school
Non-PFS 8	Headaches and stomach sickness
Non-PFS 9	More aggressive and hyperactive
Non-PFS 10	Slurred speech and unsteady gait
Non-PFS 11	Childish
Non PFS 12	Poor memory, Poor school performance
Non PFS 13	Deterioration in Mathematics

significantly below the normative mean on all developmental composites and subscales ($p < 0.001$). The control group was found to be performing above the normative mean on the cognitive composite ($t(20) = 2.583$, $p=0.018$), and, the language composite ($t(13) = 2.604$, $p=0.022$) but was not different from the normative mean on the motor composite score ($t(13) = 1.320$, $p=0.209$)

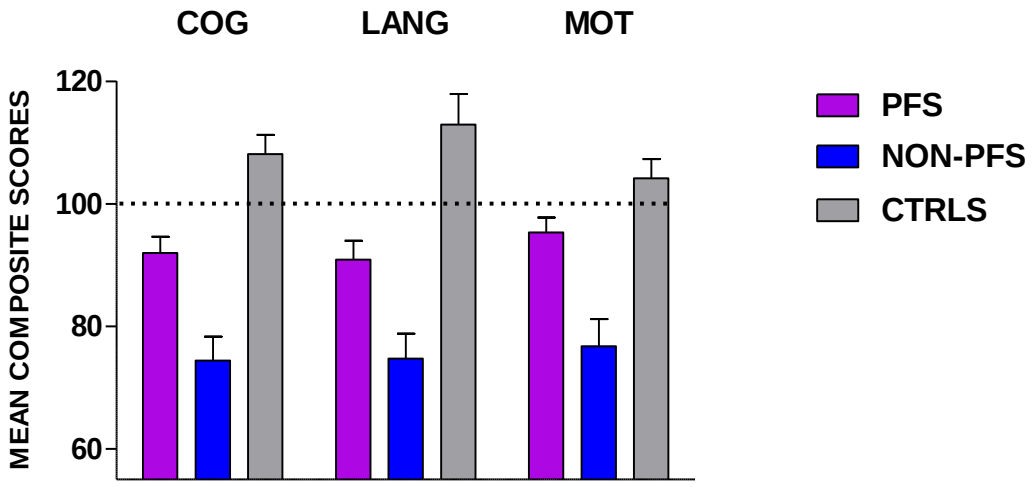
3.4.2.2. Comparison of the groups' performance with a group of healthy controls

A MANOVA with the cognitive, language, and motor composite scores as the dependent variables, group and language as the between-subjects factors, and, age as a covariate revealed a main effect of group ($F(6, 118) = 6.599$, $p < 0.001$). No other main effects were uncovered. The between subjects test revealed that age at the time of assessment had an effect on the language composite score ($F(1, 60) = 4.980$, $p=0.029$) and group had an effect on all composite scores ($p<0.001$). Bonferroni post hoc tests confirmed that the PFS group was performing significantly below controls in the cognitive ($p=0.001$) and the language ($p=0.002$) composites but not the motor composite ($p=0.433$). The non-PFS group was found to be performing below controls on all three composites ($p<0.001$). Finally, the non-PFS group was found to be performing below the PFS group, on cognition ($p=0.001$), language ($p=0.005$), and the motor composites ($p=0.001$) (see Figure 3.1).

Table 3.2 Clinical and demographic variables for the infant groups

	PFS	Non-PFS	Controls
N	28	26	21
Male	10 (35.7%)	17(65.4%)	14(66.7%)
Age at test (months)	18.26 (1.29)	15.51(1.93)	22.49(2.29)
English as a 1st language	11 (39.3%)	20 (76.9%)	20(95.24%)
Preterms	4(14.3%)	6 (23.1%)	1(4.7%)
Scan abnormalities	3/27 (11.1%)	15/23(65.2%)	0/4 (0%)
AED	1 (3.6%)	19(73.1%)	N/A
Days from CSE	45.54 (8.42)	30.62 (3.53)	N/A
Previous seizures	9 (32.14%)	20 (76.9%)	N/A
Previous CSE	1 (3.6%)	11 (42.3%)	N/A
Continuous	16 (57.1%)	15(57.9%)	N/A
Duration (minutes)	83.32(6.94)	66.81(9.56)	N/A
Behaviour change post CSE	9(32.14%)	5 (19.23%)	N/A

Figure 3. 1 Performance of the three groups on the Bayley Scales of Infant Development



3.4.2.3. The contribution of clinical and demographic variables to the groups' performance

Linear regression analyses carried out to investigate the contribution of clinical variables to PFS performance revealed that age at the time of assessment was a significant predictor of language performance (p=0.002). No other variables were found to be significant predictors of performance. In the non-PFS group, scan abnormalities were found to be a significant predictor of cognitive composite scores (p=0.010), language composite scores (p=0.031) and motor composite scores (p=0.021). Duration was also found to be a significant predictor of motor composite scores in this group (p=0.019). Pearson's correlations confirmed the positive correlation between age and language composite scores in the PFS group (r=0.571, p=0.002). Mann Whitney tests confirmed that non-PFS children with abnormal scans (n=15) performed worse than children with normal scans (n=8) on cognition (p=0.009), language (p=0.009), and motor (p=0.038) scales. Linear regression analyses conducted on control participants with age as the only independent variable revealed that age was a significant predictor of language (p=0.029).

Table 3.3 Mean cognitive, language, and motor composite scores for the three groups

	PFS	Non-PFS	Controls
Cognitive composite (SE)	91.96(2.64) N=28	74.42(3.89) N=26	108.1 (3.13) N=21
Language composite (SE)	90.89(3.08) N=26	74.77 (4.06) N=26	112.93(4.96) N=14
Motor composite (SE)	95.32(2.45) N=28	76.73 (4.45) N=26	104.14 (3.14) N=14

3.4.2.4. The performance of unaffected twin siblings

Table 3.4 describes the performance of three patients (2 PFS) and their twins. From this table it is evident that the healthy twins of PFS patients outperformed their siblings on the neuropsychological assessment. Specifically, one of the healthy twins performed better on all three areas of assessment, i.e. cognition, language and motor development. The second twin performed better in the cognitive and the motor scales and performed similarly with the PFS twin on the language scale. The difference in performance ranged from 3 to 25 points and was most pronounced for both twins in the cognitive scale. On the other hand, the healthy non-PFS twin performed worse than its affected sibling on the language and the motor assessment. However, it should be noted that 3 months later, the healthy non-PFS twin had a CSE and became enrolled as a patient in our study. Moreover, the child had had shorter seizures in the past (i.e. prior to our assessment). This child has now been formerly diagnosed as having epilepsy.

Table 3.4 Cognitive, language, and motor composites for 3 patients and their healthy twins

	PFS 1	Twin 1	PFS 2	Twin 2	Non-PFS 1	Twin1
Cognitive Composite	95	105	70	95	90	90
Language Composite	109	112	83	83	89	86
Motor Composite	110	115	67	73	103	94

3.4.2.5. Parental questionnaires

Table 3.5 provides information regarding the means and standard deviations obtained in the parental questionnaires. One sample t-tests revealed that the SEC wasn't different from the normative mean in all three groups (*PFS group:* t (23) = 0.191, p=0.850, *non-PFS group:* t (14) = -0.528, p=0.606, *control group:* t (14) = -0.843, p=0.414). The GAC, on the other hand, was found to be lower than the norm in the two patient groups (*PFS group:* t (23) = -2.159, p=0.042; *non-PFS group*: t (12) = -2.419, p=0.032), but not the control group (t (12) =0.236, p =0.817). No differences between the 3 groups were uncovered by conducting two ANOVAs with GAC and SEC as the dependent variables and group as the fixed factor. Pearson's correlations didn't reveal any significant correlations between the SEC and any of the composite scores, whereas, the GAC was found to be positively correlated with all of them (*cognitive:* r = 0.313, p=0.027; *language:* r = 0.391, p=0.010; *motor:* r=0.299, p=0.048).

Only one control provided data for the CSBS questionnaire. His results are described in *Table 3.5* and have been included only in the correlational analysis. One sample t-tests revealed no significant differences between the scores obtained by both patient groups and the norms provided by the CSBS questionnaire. Only the non-PFS group made evident a trend for a lower speech composite

scaled score (t (7) = -2.043, p= 0.080). A Mann Whitney test revealed no differences in any of the CSBS indices between the two groups. Pearson's correlations determined that the cognitive composite scores were positively correlated with all the CSBS derived measures (*total scaled score:* r=0.607, p=0.002; *social composite:* r= 0.427, p=0.042; *speech composite:* r=.671, p<0.001; *symbolic composite*: r=0.627, p<0.001). The same was true of the language composite which was positively correlated with all of the above measures (*total scaled score:* r=0.648, p=0.001; *social composite:* r= 0.594, p=0.004; *speech composite:* r=.664, p=0.001; *symbolic composite*: r=0.671, p=0.001).

Table 3.5 Means and standard deviations obtained by the two groups on the Bayley and the CSBS DP

	PFS	Non-PFS	Controls
Social Emotional composite (SEC)	100.83(4.37) N=24	96.67(6.32) N=15	94.4(6.65) N=15
General adaptive score (GAC)	89.04 (5.08) N=24	86.23 (5.69) N=13	100.85 (3.58) N=13
CSBS DP Total score	93.43(6.27) N=14	88 (8.58) N=8	106 N=1
CSBS DP Social composite	9.01 (1.18) N=14	8.25 (1.72) N=8	8 N=1
CSBS DP Speech composite	9.79 (1.32) N=14	7.13 (1.41) N=8	13 N=1
CSBS DP Symbolic composite	8.64 (1.20) N=14	7.13 (1.75) N=8	12 N=1

3.4.3. Neuropsychological assessments for older group

3.4.3.1. Comparison of the groups' performance with the normative mean

Table 3.6 provides the clinical and demographic characteristics for the older children, and, *Table 3.7* provides the mean VIQ, mean PIQ and mean FSIQ for the 34 children (including 8 normal controls) assessed on the WIPPSI or the WISC.

Table 3.6 Clinical and demographic variables for the older groups

	PFS	Non-PFS	Controls
N	6	20	8
Male	1 (16.7%)	13(65%)	2(25%)
Age at test (months)	40.17 (3.75)	93.8 (8.88)	49.13 (4.76)
English as a 1st language	6 (100%)	3 (15%)	8 (100%)
Preterms	0(0%)	6 (23.1%)	0(0%)
Scan abnormalities	2/6 (33.3%)	8/19(42.1%)	0/4 (0%)
AED	1 (16.7%)	8(40%)	N/A
Days from CSE	44.83 (11.74)	34.05 (7)	N/A
Previous seizures	3 (50%)	16 (80%)	N/A
Previous CSE	0 (0 %)	6 (30%)	N/A
Continuous	3 (50%)	10(50%)	N/A
Duration (minutes)	45(7.30)	73.45(12.15)	N/A
Behaviour change post CSE	1(16.7%)	8(40%)	N/A

A one sample t-test revealed one significant difference in the performance of the PFS group from the normative mean of 100. Namely, the PFS group was found to have a significantly higher FSIQ (t (5) =2.815, p=0.037) from the mean. The non-PFS group was found to be performing significantly below the normative mean across the board, obtaining a significantly lower VIQ (t (18) = -2.830, p= 0.011), PIQ (t (19) = -2.596, p=0.018) and FSIQ (t (17) = -2.280, p=0.036). The control group obtained a higher than average VIQ (t (7) = 3.256, p=0.014), PIQ (t (7) = 5.041, p=0.001), and FSIQ (t (7) =5.326, p=0.001).

Table 3.7 Mean VIQ, PIQ, and FSIQ for the three groups

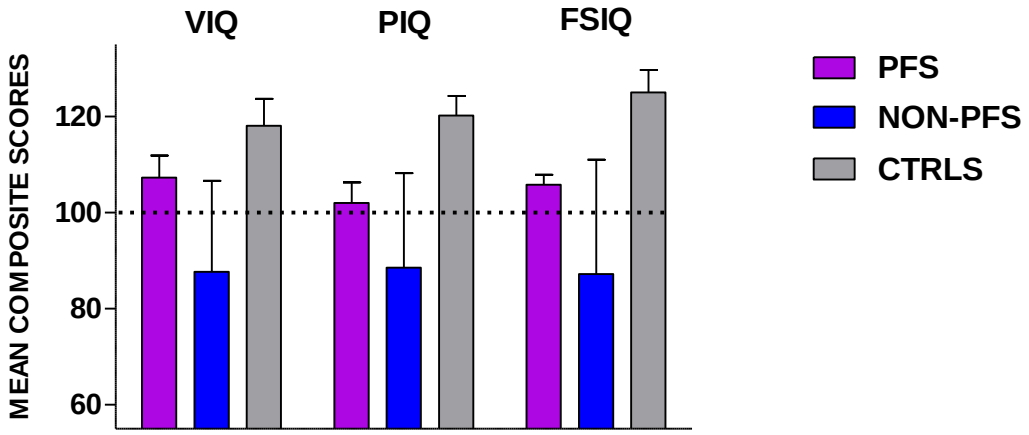
	PFS	Non-PFS	Controls
VIQ (SE)	107.33(4.54) N=6	87.68(4.35) N=19	118.13 (5.57) N=8
PIQ (SE)	102(4.3) N=6	88.60 (4.39) N=20	120.25(4.02) N=8
FSIQ(SE)	105.83(2.07) N=6	87.22 (5.61) N=18	125 (4.69) N=8

3.4.3.2. Comparison of the groups' performance with a group of healthy controls

A MANOVA with VIQ, PIQ and FSIQ as the dependent variables and age as a covariate revealed a trend for a main effect of group (F (6, 54) = 2.165, p=0.061) (see Figure 3.2). The between subjects-effects test revealed that group affected VIQ (F (2, 28) = 4.233, p=0.025), PIQ (F (2, 28) = 5.819, p=0.008), as well as FSIQ (F (2, 32) = 5.595, p=0.009). Bonferroni post hoc comparisons revealed that the PFS and the control group were comparable on all three measures. The same was true for

the PFS and the non-PFS group. On the other hand, the non-PFS group was found to have lower VIQs (p=0.021), PIQs (p=0.007), and FSIQs (p=0.008) from controls.

Figure 3.2 VIQ, PIQ and FSIQ for the three groups



3.4.3.3. The contribution of clinical and demographic variables to the groups' performance

The linear regression analyses which were conducted to assess the contribution of clinical variables to the performance of the non-PFS group on the WIPPSI or the WISC revealed no significant predictors of performance.

3.4.3.4. Parental Questionnaires for the older group

Table 3.8 provides information regarding the means and standard deviations obtained in the SDQs. Two out of the two completed questionnaires for the PFS group were found to be within the normal range when considering their overall score. However, 1 of the 2 PFS children was found to have abnormal scores on the emotional and the peer problems scales, and, the other one obtained borderline scores on the peer problems scale. In the non-PFS group, 6 children were found to be within the normal range, 3 children were found to be within the abnormal range, and, 2 children

were found to be within the borderline range according to their total SDQ scores. Finally, in the control group, 3 children were categorized as normal and one obtained scores that placed them within the borderline range.

Table 3.8 Percentage of normal scores achieved on the SDQ for each group

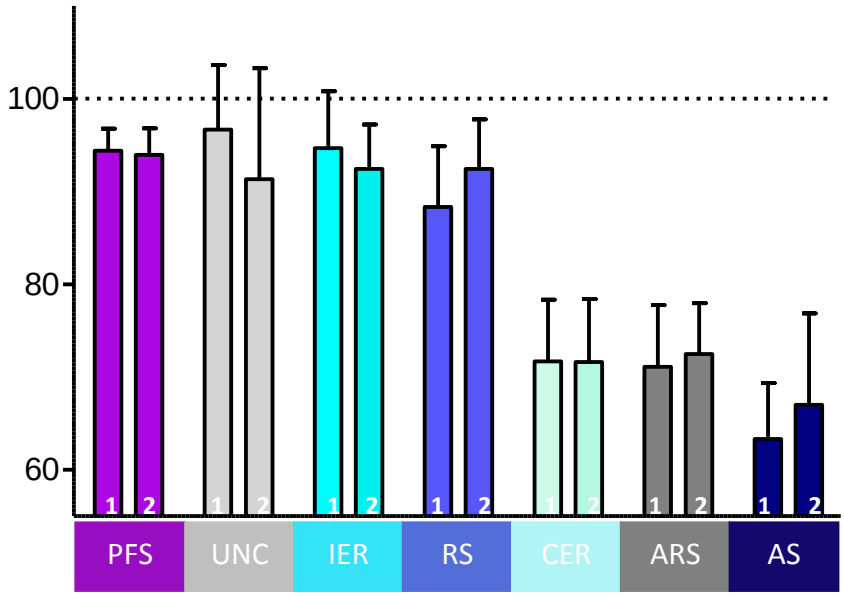
	PFS	Non-PFS	Controls
SDQ Total	2 (100%) N=2	6 (50%) N=12	3 (75%) N=4
SDQ Emotion	1 (50%) N=2	5 (41.7%) N=12	4 (100%) N=4
SDQ Conduct	2(100%) N=2	7(58.3%) N=12	3 (75%) N=4
SDQ Hyper	2 (100%) N=2	9 (75%) N=12	3 (75%) N=4
SDQ Peer	0 (0%) N=2	5(41.7%) N=12	3 (75%) N=4
SDQ Social	2 (100%) N=2	10 (83.3%) N=12	4 (100%) N=4

3.4.4. All participants

Since the small sample sizes of certain etiological groups prohibited the use of formal statistics, Figure 3.3, has been provided instead to give a sense of the groups' hierarchical performance on cognitive and language measures. Cognitive composites have been combined with FSIQs and language composites have been combined with VIQs to provide a measure of performance in these areas. What becomes immediately obvious from this graph is that children with acute symptomatic

causes (AS) (n=3) and children with acute on remote symptomatic causes (ARS) (n=13) are obtaining means which place them approximately 2 SDs from the normative mean. This also seems to be true of children with a cryptogenic epilepsy related diagnosis (CER) (n=10). The remaining 4 groups (i.e., PFS, unclassified cases (UNC), idiopathic epilepsy related cases (IER), and remote symptomatic cases (RS)) seem to be performing on a par with each other with slight differences in their means and distributions.

Figure 3.3 Means for cognitive (1) and language (2) performance of all the patient subgroups



Linear regression analyses conducted on all PFS participants revealed that age (p=0.006) and prematurity (p=0.025) were significant predictors of cognition (F (2, 31) = 7.256, p=0.003) and age (p=0.002) was a significant predictor of language (F (1, 30) = 11.744, p=0.002). In the non-PFS group, scan abnormalities (p=0.005) and medication (p=0.006) were both found to affect cognition (F (2, 37) = 7.438, p=0.002). Scan abnormalities (p=0.006), medication (p=0.003) and language (p=0.019) were shown to affect performance on the language measures (F (3, 37) = 6.428, p=0.001).

Linear regression analyses conducted on control participants with age as the only independent variable revealed that age was a significant predictor of cognition ($p=0.002$) and language ($p=0.010$).

3.5 Discussion

The current study has shown that approximately a month following CSE, all infants reveal impairments on the Bayley scales. Namely, even infants that have experienced a PFS, a long seizure in an otherwise neurologically normal child, perform below normative standards and normal controls on cognitive, language and gross motor measures. Nonetheless, infants following CSE associated with a PFS are still performing significantly better than infants with a history of neurological insult or an existing diagnosis of epilepsy.

From a preliminary look at the data it becomes obvious that the children with an acute symptomatic etiology are the ones who perform worse on the neuropsychological tests. This finding is not surprising as neurological outcomes in this group have been shown to be adverse (Chin et al., 2006; Molinero et al., 2009; Sadarangani et al., 2008), and, confirms views that suggest the exclusion of such groups from studies looking at the effects of convulsive status epilepticus *per se* on development (Chin, Neville, & Scott, 2005). The same applies to children with an acute on remote symptomatic classification who were also found to be impaired on the neuropsychological tests. Finally, children with a cryptogenic epilepsy diagnosis appeared to perform lower than their non-PFS peers, though no formal statistics were applied to investigate this issue because of small sample sizes.

When we investigated the contribution of clinical variables to the non-PFS group, performance as a whole, scan abnormalities and antiepileptic medication came out as significant predictors of performance. Specifically, for the infant group, scan abnormalities were revealed to be predictors of

performance for cognition, language and motor development. Duration of CSE was also found to independently have an impact on motor development. When all participants were combined together, medication also came out as a significant predictor of cognitive and language performance as did English as a first language. The above findings are not counterintuitive and add to the existing literature regarding the effects of a pre-existing abnormality and medication on developmental processes.

No previous study has assessed the development of motor skills following CSE using a standardized assessment. Therefore, this is the first study to document the impact of duration on motor abilities. Nonetheless, early neurological studies investigating CSE outcome have reported motor deficits following long seizures which in many cases were severe, e.g. hemiplegia (Aicardi & Chevrie, 1970). Nowadays, such severe instances of motor impairments are rare to come across as seizures are treated more aggressively and CSE is arrested before it can prove damaging to motor abilities. This is not the first time that duration has been flagged as a potential predictor of future development but this has been done so more in the context of mortality and morbidity investigations (Drislane, Blum, Lopez, Gautam, & Schomer, 2009). However, the specificity of the effect, i.e. that duration seems to only affect motor skills in our cohort, is hard to interpret, and, could be related to the fact that tonic-clonic seizures engage motor areas to manifest themselves, though, this remains a mere speculation and requires further investigation.

Whereas the means obtained by the PFS group place them within the normal range of performance, it does so at the lower end. Moreover, the comparison of the PFS group to the control group confirms the findings obtained by comparing the patients' performance against the normative mean. These results do not come in direct conflict with previous studies. This is because all previous population studies have tested children a few years following PFS (Nelson & Ellenberg, 1978;

Pascalis et al., 2009; Verity et al., 1985c; Verity et al., 1993; Verity et al., 1998). For example, Verity et al. (1985, 1998) studied children at the age of 5 and then again at the age of 10 to determine the long term consequences of febrile convulsions and concluded that these seizures are benign with regards to subsequent development. However, in their first study, these authors did report that 14.5% of children with a history of febrile convulsions had a history of a speech problem (Verity, 1985). The problem had been resolved in 11 out of the 42 children by the time of the assessment. Therefore, it is possible that the PFS cohort under investigation in this study may outgrow their "deficits" at some point during the development.

Whereas a conclusion regarding the child's premorbid developmental trajectory is not readily available to us, the better performance of the two PFS twin controls points to a decline in the patients' performance following the seizure. In their study, Schiotzz-Christensen and Bruhn (1973) found that the unaffected monozygotic twin performed on average 7 points higher than the affected twin on the performance scale IQ. The current study was not designed to answer this question, however, the superiority of the unaffected dizygotic twins seems to suggest that the seizure might have affected the patient's performance. Alternatively, the PFS in the affected twin was predated by an existing brain abnormality that is dually responsible for the seizure and the lower performance on the neuropsychological assessment when compared to the healthy twin. Our one year follow-up study may help us decide between these two possibilities. Namely, if children are found to perform according to normative standards at follow-up, one may safely conclude that their previous underperformance was a transient effect of the seizure. However, if they are still underperforming by that time, none of the two alternatives can be ruled out.

A third of parents observed a behavioural difference in their child following the seizure. This supports the idea that the PFS has at the very least a transient effect on the child's behaviour. This is

not the first study to record behavioural complications following febrile seizures. In a German cohort of 80 children with a history of febrile convulsions, 22% of those evaluated at ages 6 to 9 years exhibited behavioural disturbances, primarily in the form of hyperactivity, compared with 6% of the control group (Kolfen et al., 1998). Moreover, in the British National Cohort study (Verity, Butler, & Golding, 1985b) when children with a history of febrile convulsions were tested at the age of 5, they were found to suffer from more sleep problems than their normal peers. These sleeping problems seemed to no longer be present at the age of 10 years (Verity et al., 1998). Both these studies uncovered behavioural complications in those with a history of febrile seizures a few years following the event. Nonetheless, both studies seem to suggest that febrile convulsions may affect behavioural outcomes.

The strong positive correlations obtained between our own neuropsychological assessments and the questionnaires filled out by the patients' parents provide a further validation for our conclusions. Positive correlations were obtained for all main areas we assessed, i.e. cognition, language and motor development. Whereas standardized neuropsychological assessments probe each area meticulously and in a rigorous manner, the examiner gets to assess the child only once. Therefore, it is possible that the child might be assessed on an unrepresentative day. This becomes particularly true in the case of infants, who are more likely to get distressed than older children. Thus, the agreement between parent and examiner becomes important in these age groups and makes us more confident about the conclusions drawn from the neurodevelopmental assessments.

Conclusions regarding the performance of older children following CSE cannot be drawn as readily as with the infant group. For one, the small sample size, particularly, when it comes to the PFS children, makes it difficult to determine whether performance is affected by the seizure. The smaller sample size of the PFS group is a direct result of the nature of these seizures, i.e. they have their

peak incidence around 18 months (Chin et al., 2006). Moreover, PFS are by definition seizures that occur between the age of 6 months and 5 years.

Nonetheless, some preliminary conclusions can still be drawn from the available data. Firstly, the non-PFS group is still performing significantly worse from the normal controls on all areas. They are also performing below normative means in the same areas, i.e. verbal ability, non-verbal ability, overall abilities. On the other hand, the PFS children seem to have a better outcome than their infant counterparts. Firstly, the performance of the PFS group was not found to be significantly different from the normative mean, the sole exception being the FSIQ where their performance was found to be better than the average. Comparison with the normal controls revealed no differences in their FSIQ, PIQs and VIQs. Therefore, whereas the infants reveal a significant difference in cognitive and verbal abilities when compared to both a group of normal controls and the standardization sample, older children following a PFS perform on a par with both these groups.

This discrepancy in results between the infant and the older children alludes to an age dependency relationship whereby younger children are more vulnerable to the effects of a long seizure and older ones might be more resistant. This suggestion is further supported by the correlation obtained between age at the time of the assessment, and, therefore, by proxy age at the time of the incident, and cognitive and language indices. This age effect has been suggested many times in the literature. For example, Chang et al. (2001) found that children that had a febrile seizure before the age of 1 made evident learning and memory deficits at a later age. However, the fact that our control group also revealed an age correlation with language, argues against a seizure specific effect, and raises the possibility of an administration bias.

Two findings argue against this possibility. Firstly, a similar correlation is not obtained in the non-PFS group, even if one only includes the non-PFS children who score over 74 on the language scale (i.e. the minimum score obtained by children in the PFS group). This is reflected in their distribution of language scores when they are plotted against age (see Figures 3.4, 3.5). Namely, it can be seen from the scatterplots that whereas both patient groups had a large subsection of their population score below 100, in the case of the PFS group only, was an age dependency evident in this subgroup. Moreover, the control scatterplot (see Figure 3.6) reveals an underrepresentation of children aged between 10 and 30 months old in our sample, a finding which makes the association of age and language in this group difficult to interpret. The differential impact of age at the time of assessment on language abilities between the two patient groups may reflect the tendency for etiology to override all other factors in the non-PFS group.

There is another possibility which may be responsible for the discrepancy observed in our two age groups, i.e. the use of different assessment tools. However, the Bayley infant scales of development (3rd edition) seem to be highly correlated with scores obtained on the WIPPSI-III. The correlation between the language composite score and the VIQ has been reported to be very high (r = .83) (Bayley Manual). More importantly, however, a correlation between age and language, with older children achieving higher scores than younger ones, was also obtained when the Bayley's results were investigated separately. Therefore, it is quite likely that despite the different tools employed in this study, the differences observed in the younger and the older PFS group reflects a pathophysiological difference. This possibility remains even after establishing the age dependency in the control population given the observation that whereas PFS patients are different from the normative means and controls as infants they do not seem to be different when compared against the same benchmark as older children.

Figure. 3.4 The Relationship between Age and Language in the PFS group

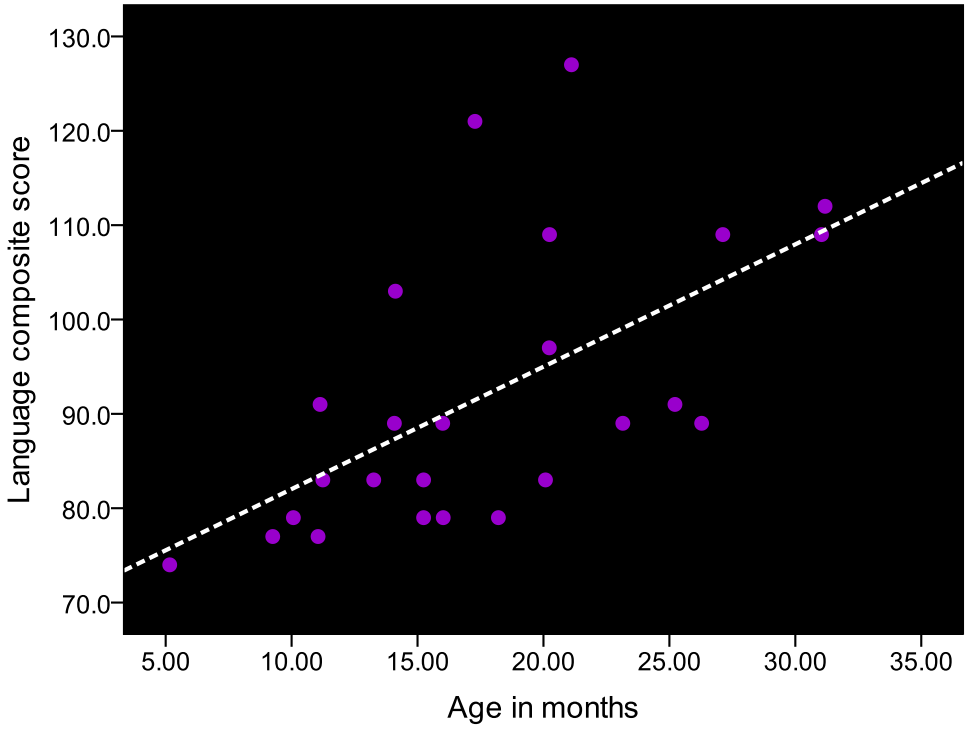


Figure. 3.5 The Relationship between Age and Language in the NON- PFS group

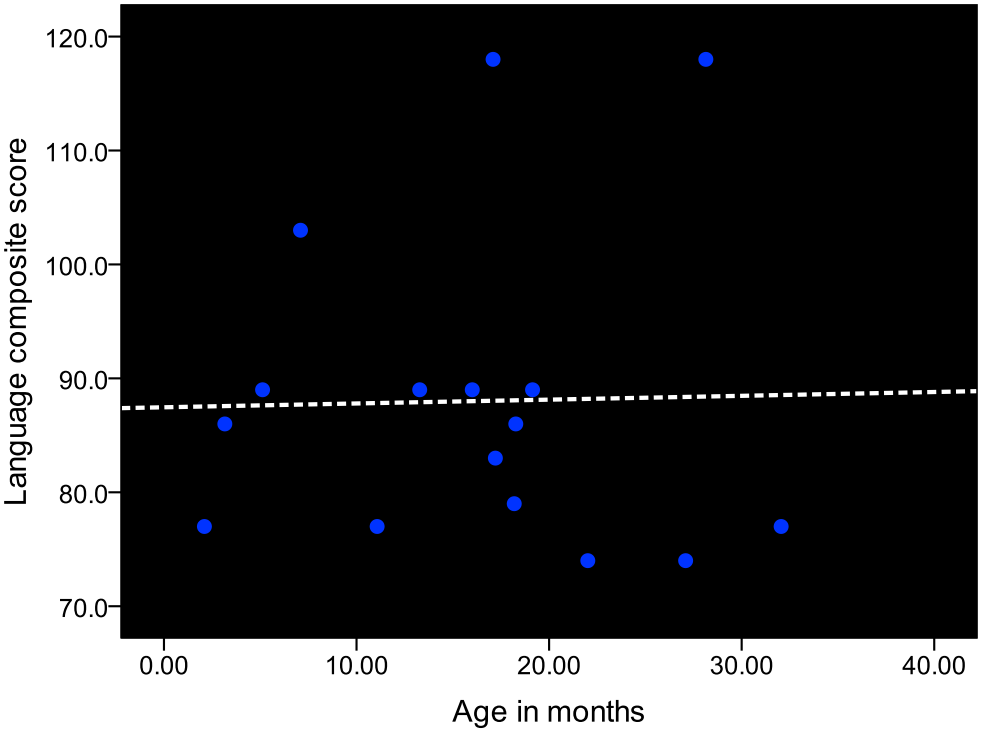
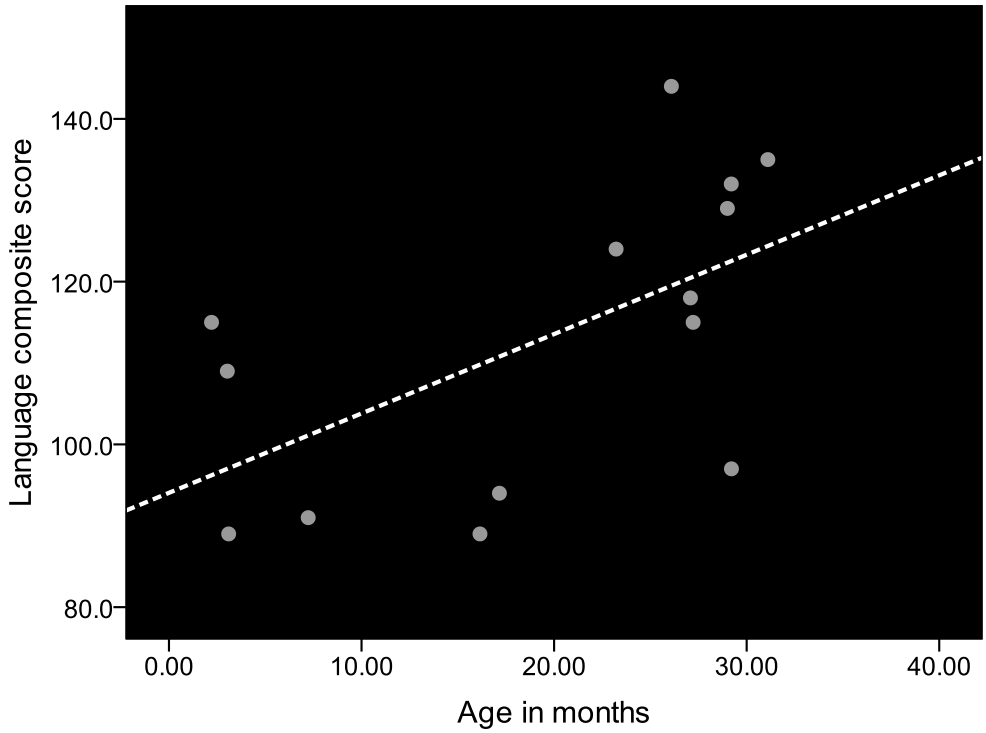


Figure. 3.6 The Relationship between Age and Language in the control group



3.6 Conclusions

At the beginning of this chapter, we set out to answer two questions. The first one was related to whether CSE leads to functional impairments close to the time of the incident. And the second question was related to whether the seizure can affect children with no previous record of epilepsy or other neurological abnormalities. In infants, both patient groups are found to underperform when compared to their age peers and the normative standards set by the neuropsychological tests administered. Whether this is a direct effect of the incident itself is a bit harder to conclude. The fact that duration of CSE was discovered to have an effect on motor development in the non-PFS group seems to suggest that there may be a direct effect of the seizure. In the PFS infant group, on the other hand, the possibility still remains that children are performing at their premorbid levels. When it comes to the older children, the sample size is too small to reach a conclusive verdict. However, it

seems quite likely that the performance of the PFS group is affected to a lesser degree, if at all, than the performance of their infant counterparts. The non-PFS group continues to manifest impairments throughout their childhood span attesting to the possibility that it is their ongoing or pre-existent neurological problems that are responsible for this rather than their seizure.

Chapter 4 Follow-up assessment of infants and children following CSE

4.1 Introduction

There is no dispute that the presence of chronic epilepsy has adverse effects on intellectual abilities (Elger, Helmstaedter, & Kurthen, 2004; Jokeit & Ebner, 2002). What is more controversial is whether epileptic activity in itself is responsible for the observed deterioration of intellectual functioning or whether factors such as aetiology and medication are the main culprits. To that end, there has been a rise in the number of studies examining cognition in children with new onset epilepsy (Hermann et al., 2006; Fastenau et al., 2009). What is slowly emerging from these studies is the possibility that subtle neuropsychological impairments may antedate the presentation of seizures (Hermann et al., 2006; Fasteneau et al., 2009). In one study, 27% of children that had only experienced a single seizure were manifesting neuropsychological deficits compared to their unaffected siblings (Fastenau et al., 2009).

In light of these findings, it becomes imperative to systematically characterize the effects of CSE on a child's future neurodevelopment for two reasons. Firstly, CSE is a self-sustaining long seizure resistant to common avenues of cessation, which has been shown to cause structural damage in animals (Meldrum & Brierley, 1973), and, may be associated with structural abnormalities in humans (Scott et al., 2003; vanLandingham et al., 1998). Secondly, a recent paediatric epidemiological study has shown that more than half of the reported CSE cases occur in previously neurologically normal children (Chin et al., 2006).

To date, no study has systematically investigated the neuropsychological effects of CSE in a paediatric population during the acute stage. In the current study, we have prospectively investigated the effects of paediatric CSE by assessing children close to the time of the incident and a year later.

Results from our baseline assessment have pointed to the prevalence of neuropsychological impairments in children with prior neurological abnormalities. These seem to be mainly dictated by the presence of structural abnormalities in these children. However, children with PFS were also found to underperform when compared to a group of healthy controls and the normative indices provided by the neuropsychological tests utilized. Importantly, this was less so for older children than for younger ones as was highlighted by a positive correlation between age and performance indices. Given the suggested relationship between PFS and TLE studying the longitudinal development of this select group and identifying potential markers of dysfunction is clinically very important.

The aim of the current chapter is to investigate the neuropsychological development of children following CSE a year after their incident. This will allow us to disentangle the immediate effects of CSE from the longer lasting ones. In the Scott et al. (2003) study, where a group of children following PFS were tracked longitudinally, a significant reduction in hippocampal volumes and T_2 relaxation times occurred between the first investigations (i.e. within 5 days of PFS) and the second ones (i.e. 4-8 months following PFS). These reductions, however, had given way to increases in hippocampal volume asymmetry compared to the initial data. Moreover, three PFS patients were found to have one hippocampal volume outside the 95^{th} prediction limit set by the control data. In view of the established role of the hippocampus in learning and memory and the recent contention of its involvement in language processes (Schumann et al., 2007; DeLong & Heinz, 1997) we were keen to delineate the performance of these children a year following their injury.

We predicted that children with previous neurological abnormalities would continue to manifest impairments in standardized neuropsychological tests. A prediction regarding the neurodevelopment

of the PFS group was more difficult to formulate given the lack of systematic studies in this area and the presence of conflicting evidence in the literature.

4.2. Methods

4.2.1. Participants

Families that participated in the baseline phase of our study were re-invited 4 months later for an MRI scan, and, a year later for a developmental assessment plus an MRI scan. Four month developmental assessments were not conducted so as to minimize test-retest practice effects. At the follow-up appointment, one pediatric neurologist took full medical history to determine the child's medical progression since CSE. Namely, the parents were interrogated regarding the recurrence of seizures and any changes in the child's medication regime. For the purposes of the current analysis, the classification of patients into diagnostic subgroups as determined at baseline was utilized. Four month and one year follow-up MRI T1 scans were classified by a radiologist and three paediatric neurologists into normal or abnormal.

4.2.2. Neuropsychological assessments

A detailed description of the neuropsychological assessments and the composite scores derived from these has been provided in Chapter 3 and shall be omitted here. However, in brief, infants that were assessed on the Bayley Scales of Infant Development (3rd edition) at baseline were re-assessed on this instrument at follow-up, except in the rare cases where the child had outgrown the scale by the time of their follow-up, in which case the WIPPSI was used (n=2). Similarly, children who had outgrown the WIPPSI at their follow-up assessment were assessed on the WISC (n=1). However, the WIPPSI and the WISC have many subtests in common and both provide VIQ, FSIQ, PIQ and

PSQ indices. Therefore, a direct comparison of WIPPSI and WISC results does not pose a methodological issue.

4.2.3. Questionnaires

A detailed description of the parental questionnaires used in our study and the composite scores derived from these has been provided in Chapter 3 and shall be omitted here.

4.2.4. Statistical analysis

As infants and toddlers were assessed using different neuropsychological tests from older children, analyses were conducted separately for these two age groups. Chi square, independent t-tests and when necessary Mann Whitney tests were utilized to compare clinical and demographic variables between the groups. One sample t-tests were carried out to compare the performance of the two patient groups against the normative means provided for each index including questionnaire derived indices. MANOVAs comparing the performance of the two patient groups against that of a group of controls were conducted. However, it should be noted that our control group was only assessed once and, therefore, we utilized their results in a cross-sectional manner. In the infant group, we also carried out 3 repeated measures ANOVAs to investigate the development of the two groups from time point 1 to time point 2 in the three measured areas (i.e. cognition, language and motor development). As the older PFS sample was too small, their progression through time is not analysed using statistics but simply described. Paired sample t-tests were used to investigate any changes from baseline to follow-up in the non-PFS group.

In a final analysis, all follow-up participants were investigated together irrespective of age. The reasons were two-fold. Firstly, we wanted to validate the findings from the earlier repeated measures

analysis by also including the individuals who were excluded from such an analysis previously because of small sample sizes. Secondly, we wanted to investigate the contribution of demographic and clinical variables to the performance of the two patient groups separately as some variables weren't applicable to both groups (e.g. medication). To that end we used linear regression to assess the contribution of variables to cognition (combined cognitive composite and FSIQ) and language (combined language composite and VIQ).

4.3 Results

4.3.1. Neuropsychological assessments for infant group

Thirty-four out of the 54 children in the original cohort were seen for a follow-up assessment. As this is an ongoing study, recruitment is still under way and 5 children from the infant group are still awaiting follow-up. From the remaining children, 1 died, 1 family moved outside the UK, 1 family was unreachable, 2 children provided unusable data, and 8 families declined to participate in the follow-up stages of this study. Finally, two children had outgrown the Bayley scales at follow-up and were assessed using the WIPPSI. Their results will be discussed in the older children group. Therefore, we were able to conduct follow-up Bayley assessments on 72.3% (34/47) of the original cohort.

4.3.1.1. Description of the non-participating infant patient sample

Clinical and demographic variables for the participants and non-participants along with the means and standard errors on the three assessment scales from baseline can be found in *Table 4.1*. From the 13 non-participants, 5 children had been classified as PFS, 4 children had been classified as acute on remote symptomatic (ARS), 3 had been classified as cryptogenic epilepsy related cases (CER), and, 1

had been classified as an idiopathic epilepsy case (IER). Chi-square tests revealed no differences in the gender, scan results, medication, and seizure history between the two groups. However, the two groups were significantly different in prematurity ($p=0.055$) and language ($p=0.044$) with the non-participants having a larger proportion of infants born prematurely and more infants with English as their first language. A Mann-Whitney test revealed no differences in the baseline cognitive ($p=0.152$) language ($p=0.458$) and motor scores ($p=0.467$) between follow-up participants and non-participants.

A Mann Whitney test revealed no differences between the PFS participants ($n=19$) and the PFS non-participants ($n=5$) in the three composite scores. This was also true of the non-PFS group, with both participating ($n=15$) and non-participating ($n=8$) parties achieving similar scores. A Mann Whitney test comparing the two non-participating patient groups revealed a significant difference in the motor scale ($p=0.045$) and a trend for a difference in the cognitive scale ($p=0.065$), but did not make evident such a trend in the language scale where the two groups were not found to be different from each other ($p=0.284$).

In sum, the non-participating sample was found to be similar to the participating sample in most demographic and clinical characteristics. More importantly, the non-participating sample was shown to perform similarly to the follow-up sample on the developmental assessments, thus, providing assurance that the results described here forth are representative of the whole sample.

Table 4.1 Comparison of participants and non-participants

	FOLLOW-UP	NO FOLLOW-UP
N	34	13
Male	19 (55.9%)	6 (46.2%)
Age at baseline (months)	15.3 (1.25)	18.18 (10.7)
English as a first language	15 (44.1%)	10 (76.9%)
Preterms	4 (11.8%)	5 (38.5%)
Scan abnormalities	12 (35.3%)	4/10 (40%)
AED	11(32.4%)	7 (53.8%)
Previous seizures	16 (47.1%)	8 (61.5%)
Previous CSE	5 (14.7%)	4 (30.8%)
Continuous CSE	19 (55.9%)	7 (53.8%)
Duration (minutes)	76.85 (7.94)	69.38 (12.13)
Cognitive composite (SE)	85.15 (3.17)	76.92 (5.02)
Language composite (SE)	82.81 (2.81)	76.39 (6.6)
Motor composite (SE)	87.38 (3.18)	80.23 (7.37)

4.3.1.2. Description of the follow-up patient sample

Table 4.2 provides clinical and demographic data for the follow-up sample. Nineteen out of the 34 children had been classified as PFS at baseline. From the remaining 15, 6 patients had been classified as acute on remote symptomatic cases, 3 patients had been classified as cryptogenic epilepsy related

cases, 3 patients had been classified as idiopathic epilepsy related cases, 2 patients had been classified as acute symptomatic cases, and, 1 patient had been classified as a remote symptomatic case.

Out of the 32 children who were scanned at baseline, 25 children were scanned for a second time between the baseline and the follow-up appointment, and 25 children were rescanned at the 1 year follow up assessment. Even though identical in numbers, the two follow-up scanning groups did not consist of the exact same individuals. From the 25 individuals scanned at the one-year follow up assessment 7 were discovered to have an abnormality on their scan. This proportion is commensurate to the baseline proportion where 10 out of the 32 children were found to have abnormal scans.

Time from assessment ($t(32) = 0.143$, $p=0.888$), time from seizure ($t(32) =0.949$, $p=0.350$), presence of scan abnormalities on their follow-up scans ($\chi^2(1) = 2.968$, $p=.177$), prematurity ($\chi^2(1) = 0.064$, $p=1$), and occurrence of CSE between the 1st and the 2nd assessment ($\chi^2(1) = 0.679$, $p=0.571$) were not found to differ between the PFS and the non-PFS group. There was a trend for the PFS group to be older at the time of assessment ($t(32) =1.771$, $p=0.086$) and for the non-PFS group to contain a larger proportion of males than the PFS group ($\chi^2(1) = 3.316$, $p=0.091$). The PFS and the non-PFS group were found to be different in the occurrence of seizures between the two assessments ($\chi^2(1) = 8.591$, $p=0.005$), their medication history ($\chi^2(1) = 17.007$, $p<0.001$) and language ($\chi^2(1) =5.384$, $p=0.024$). Namely, the non-PFS group included more patients that experienced seizures between the two assessments, and, more individuals that were currently on antiepileptic medications (only one child from the PFS group was on antiepileptic medication). On the other hand, the PFS group included fewer individuals with English as a first language.

Table 4.2 Clinical and demographic variables for the two infant patient groups

	PFS	NON-PFS
N	19	15
Male	8 (42.1%)	11 (73.3%)
Age at follow-up (months)	28.84 (1.46)	24.73 (1.82)
Time from seizure (months)	13.16 (0.44)	12.47 (0.60)
Time from 1st assessment	11.58 (0.50)	11.47 (0.62)
English as a first language	4 (21.1%)	9 (60%)
Preterms	2 (10.5%)	2 (13.3%)
Scan abnormalities 1st scan	2/17 (11.8%)	8/15 (53.3%)
Scan abnormalities 3rd scan	2/14 (14.3%)	5/11 (45.5%)
AED	1 (5.3%)	11 (73.3%)
Seizures between assessments	7 (36.8%)	13 (86.7%)
CSE between assessments	1 (5.3%)	2 (13.3%)

4.3.1.3. Neuropsychological results for the infant group

4.3.1.3.1. Comparison of the groups' performance with the normative mean

Table 4.3 provides the mean cognitive, language, and motor composite scores for the 34 children assessed using the Bayley Scales of Infant Development (3rd edition). Details on the neuropsychological results of healthy controls were provided in Chapter 3 and shall be omitted here. However, one sample t-tests on the baseline scores of the patient groups shall be conducted as this is a subset of the original cohort.

One sample t-tests revealed that the PFS group was performing below the normative mean of 100 on the cognitive scale at the baseline assessment (t (18) =-2.805, p=0.012) as well as the follow-up assessment (t (17) =-3.462, p=0.003). However, the PFS group improved on language measures from the baseline (t (16) =-3.224, p=0.005) to the follow-up assessment (t (16) =-1.201, p=0.247) as no difference between their scaled scores and the normative mean was detected at their latter assessment. This was also the case with the patients' performance on the motor scales, albeit to a lesser degree, with the PFS group obtaining a significantly different mean from the norm at the baseline (t (18) =-2.140, p=0.046) but not at the follow-up assessment (t (17) =-1.784, p=0.092) where they revealed a non-significant trend for a difference with the norm.

The non-PFS group was found to be different from the normative mean on all scales at both time points. Specifically, at baseline the non-PFS patients were found to perform significantly below the normative mean on the cognitive (t (14) =-4.080, p=0.001), the language (t (14) =-6.189, p<0.001), and the motor (t (15) =-3.617, p=0.003) measures. Similarly, at follow up, the non-PFS patients were found to be impaired relative to the mean on the cognitive (t (14) = -5.807, p<0.001), the language (t (14) = -5.797, p<0.001), and the motor (t (13) =-3.313, p=0.006) measures.

4.3.1.3.2. Comparison of the groups' performance with a group of healthy controls

Two MANOVAs with the three composite scores as the dependent variables, group and language as the between-subjects factors, and, age at the time of assessment as a covariate were conducted. The MANOVA on the baseline data was carried out so as to validate the results obtained in Chapter 3 as the current sample was made up of fewer individuals.

Both MANOVAs revealed an effect of group at both time points (*baseline:* F (6, 78) =3.687, p=0.003; *follow-up:* F (6, 74) =4.628, p<0.001). Bonferroni post hoc tests revealed that the control group was performing better than the PFS group on the cognitive scale (*baseline*: p=0.010, *follow-up*: p=0.004) and the language scale (*baseline:* p<0.001, *follow-up:* p=0.006) at both time points. Motor performance was found to be different from controls at baseline (p=0.038) with the control group outperforming the PFS group. At their follow-up assessment the two groups were found to be in line (p=0.199).

Table 4.3 Mean cognitive, language, and motor composite scores for the two patient groups

	PFS	NON-PFS
Cognitive Composite 1 (SE)	91.05 (3.19) N=19	77.67 (5.47) N=15
Cognitive Composite 2 (SE)	91.94 (2.33) N=18	74 (4.48) N=15
Language Composite 1 (SE)	89.29 (3.32) N=17	75.47 (3.96) N=15
Language Composite 2 (SE)	94.29 (4.75) N=17	71.67 (4.89) N=15
Motor Composite 1 (SE)	93.21 (3.17) N=19	80 (5.53) N=15
Motor Composite 2 (SE)	95.94 (2.27) N=18	79.07 (6.32) N=14

The non-PFS group was found to be different from the control group on all scales at both assessments (p<0.001). The two patient groups were found to be different from each other on

language measures at both time points (*baseline:* p=0.027, *follow-up:* p=0.011) with the PFS group obtaining higher scores on these measures. In addition, the two patient groups were shown to perform similarly on cognitive measures at the baseline assessment (p=0.182), but were found to be different at their follow-up assessment (p=0.049) with the PFS group outperforming the non-PFS group. On their motor assessment, the two patient groups were shown to obtain similar scores at baseline (p=0.246) and revealed a trend for a different performance on their follow-up assessment (p=0.085).

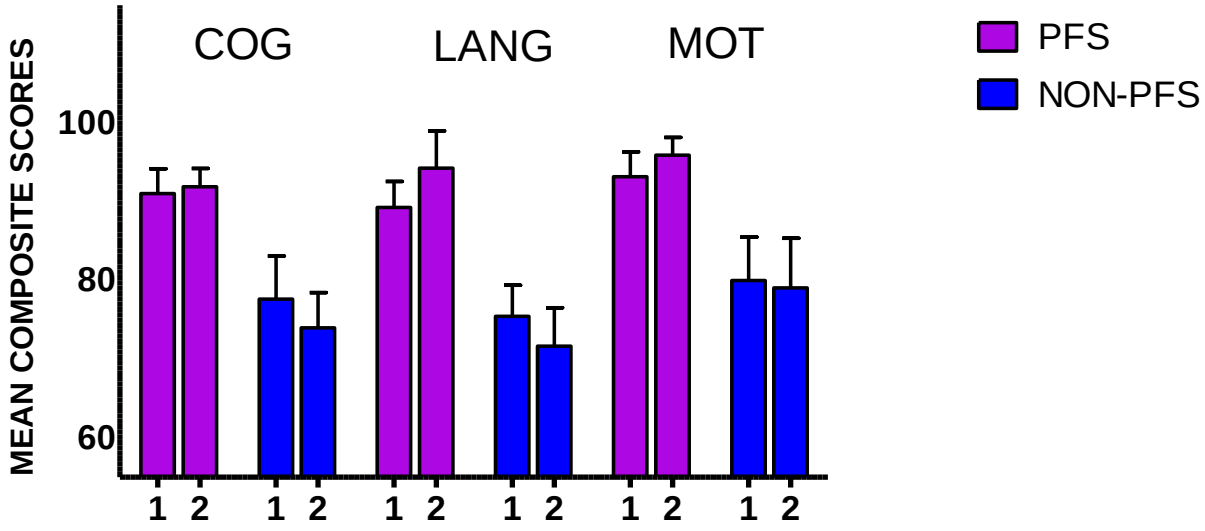
On top of a group effect, the MANOVA conducted on the follow-up scores also revealed a main effect of age at the time of assessment on performance (F (3, 36) =3.976, p=0.015). Moreover, age at the time of assessment was found to have a specific effect on language performance at both time points (*baseline:* F (1) =6.812, p=0.013; *follow-up:* F (1) =11.149, p=0.002) as revealed by the between subjects test. Pearson's tests confirmed the correlation between age of assessment and language performance for the baseline (r=0.472, p=0.001) and the follow-up assessment (r=0.440, p=0.012). Control data was excluded in the second correlation analysis as they were seen once. Conducting correlations for the two groups separately revealed that this relationship was true for the PFS group (*baseline:* r=0.577, p=0.015; *follow-up:* r=0.589, p=0.013) but was not evident at any time point in the non-PFS group (*baseline:* r=-0.047, p=0.868; *follow-up:* r=0.101, p=0.721)

4.3.1.3.3. Comparison of the two patient group's trajectories over time

Figure 4.1 depicts the means and standard errors for the two patient groups for both assessments. A repeated measures ANOVA with cognitive performance on the baseline and the follow-up assessment as a repeated measure and group, seizures and language as between subject factors revealed no main effect of follow-up (F(1,25)=0.019, p=0.890) suggesting that the patient groups

didn't significantly change from their baseline to their follow-up assessment. None of the interaction factors came out as significant. The between subjects test revealed no differences between the two patient groups (F (1) =1.603, p=0.217). There was a trend for a difference between patients who had experienced seizures in between assessments to those who hadn't with the latter group obtaining a higher mean (F (1) =3.877, p=0.060).

Figure 4.1 Change in performance in the two patient groups from baseline to follow-up



A repeated measures ANOVA with language performance on the baseline and the follow-up assessment as the repeated measure, and group, seizures, and language as a between subject factors revealed a main effect of follow-up(F(1,23)=5.078, p=0.034). There was also a significant interaction between language performance, group and seizures (F (1, 23) =4.559, p=0.044). Breaking down the means along these three interaction terms it was revealed that whereas PFS patients did better at their follow-up assessment regardless of whether they had a seizure in between or not, the non-PFS group improved only if they didn't experience a seizure in between assessments. The between

subjects test revealed a significant difference between the two patient groups in their language performance (F (1) =7.883, p=0.010).

A repeated measures ANOVA with motor performance on the baseline and the follow-up assessment as a repeated measure and group and seizures as between subject factors revealed no main effect of follow-up (F(1,28)=7.64, p=0.369). No interaction terms were significant and the two patient groups were not found to differ from each other in their motor performance (F (1) =1.357, p=0.254).

4.3.1.3.4. Questionnaires

Table 4.4 contains the means and standard errors for the SEC and the GAC scores obtained by the two patient groups at the baseline and follow-up assessments. One sample t-tests revealed that the PFS group's SEC score was not different from the normative mean at the baseline assessment (t (14) = 0.975, p=0.346) but was found to be significantly above the norm at the follow-up assessment (t (14) = 2.145, p=0.050). Their GAC score was found to edge towards a difference at their baseline assessment (t (12) = -2.031 p=0.065) and was significantly lower from the norm at their follow-up assessment (t (16) = -2.752, p=0.014).

There was no difference between the SEC score and the norm for the non-PFS group at the baseline assessment (t (10) =-1.547, p=0.153) but this was no longer the case at their follow-up assessment where they obtained a significantly lower SEC mean from the norm (t (9) =-2.236, p=0.052). Their mean GAC score was found to be lower than the norm at both time points (*baseline:* t (8) =-2.896, p=0.020; *follow-up:* t (9) =-4.144, p=0.003).

Table 4.4 Mean SEC and GAC scores for the two patient groups

	PFS	NON-PFS
Social Emotional Composite 1 (SE)	106 (6.16) N=15	89.55 (6.76) N=11
Social Emotional Composite 2 (SE)	113.67 (6.37) N=15	87.5 (5.59) N=10
General Adaptive Composite 1 (SE)	91.92 (3.98) N=13	81.78 (6.29) N=9
General Adaptive Composite 2 (SE)	90.82 (3.98) N=17	64.6 (5.59) N=10

Pearson's correlations for the whole group (i.e. both patient groups) revealed a trend for a positive correlation between the cognitive composite score and the SEC score at the baseline assessment ($r=0.338$, $p=0.092$) and a significant correlation between the two at the follow-up assessment ($r=0.477$, $p=0.016$). No other correlations between the remaining composite scores and the SEC were revealed. Cognitive composite scores were found to be positively correlated with the GAC scores at the baseline assessment ($r=0.512$, $p=0.015$) and the follow-up assessment ($r=0.688$, $p<0.001$). A positive correlation was also evident between the language composite scores and the GAC scores at both time points (*baseline:* $r=0.560$, $p=0.010$, *follow-up:* $r=0.612$, $p=0.001$). Finally, positive correlations were revealed between motor composite scores and GAC scores at both time points (*baseline:* $r=0.609$, $p=0.003$; *follow-up:* $r=0.516$, $p=0.007$).

4.3.2. Neuropsychological assessments for older group

Fourteen children out of the 26 originally assessed on the WIPPSI or the WISC at baseline were seen for a follow-up assessment. Moreover, two children that had outgrown the Bayley scales at their follow-up assessment were assessed using the WIPPSI and their results are analyzed here. From the remaining 12 children, 9 are still due for a follow-up and 3 families have declined participation in the follow-up stages of the study. Therefore, we have been able to conduct assessments on 82.4% (14/17) of the original cohort. Given the small number of non-participants in this older group a description of the non-participating patient sample shall not be included in this section.

4.3.2.1. Description of the follow-up patient sample

Table 4.5 provides clinical and demographic data for the follow-up sample. Five out of the 16 children had been classified as PFS at baseline. From the remaining 11, 3 patients had been classified as acute on remote symptomatic cases, 2 patients had been classified as cryptogenic epilepsy related cases, 3 patients had been classified as idiopathic epilepsy related cases, 2 patients had been classified as remote symptomatic cases, and 1 patient had eluded classification altogether.

Out of the 14 children who were scanned at baseline, 10 children were scanned for a second time between the baseline and the follow-up appointment, and 13 children were rescanned at the 1 year follow up assessment. From the 13 individuals scanned at the one-year follow up assessment 5 were found to have an abnormality on their scan. This proportion is commensurate to the baseline proportion where 5 out of the 14 children were shown to have abnormal scans.

The two patient groups were found to be different in their age at the time of assessment (t (14) = -2.559, p=0.023) with the non-PFS group being significantly older than the PFS group. Time elapsed from the baseline assessment (t (14) = 3.675, p=0.002), and CSE (t (13) =4.086, p=0.001) was also found to differ among the groups with the non-PFS group having been assessed earlier than the PFS group. Moreover, the PFS group at follow-up contained no males whereas 8 out of the 11 non-PFS patients were male, making the two groups different in their gender distribution (χ^2 (1) = 7.273, p=0.026). Finally, a trend was revealed for a difference in medication (χ^2 (1) =4.364, p=0.093) with 6 out of the 11 non-PFS patients being currently on AEDs and none of the PFS patients. No other clinical or demographic variables were found to differ between the two groups.

Table 4.5 Clinical and demographic variables for the two older patient groups

	PFS	NON-PFS
N	5	11
Male	0 (0%)	8 (72.7%)
Age at follow-up (months)	52.6 (4.82)	104.45 (13.24)
Time from seizure (months)	17 (1.64)	11.5 (0.52)
Time from 1st assessment	15.8 (1.59)	10.8 (0.58)
English as a first language	5 (100%)	10 (90.9%)
Preterms	1 (20%)	2 (18.2%)
Scan abnormalities 1st scan	1/4 (25%)	6/10 (60%)
Scan abnormalities 3rd scan	1/4 (25%)	5/9 (55.5%)
AED	0 (0%)	6 (54.5%)
Seizures between assessments	0 (0%)	3/10 (30%)
CSE between assessments	0 (0%)	0 (0%)

4.3.2.2. Neuropsychological results for the older patient group

4.3.2.2.1. Comparison of the groups' performance with the normative mean

Table 4.6 provides the mean VIQ, PIQ, PSQ and FSIQ for the two patient groups. A one sample t-test on the PFS baseline composite scores was not conducted as the sample group was too small (n=3). This was the case, as two of the PFS patients that have been included in the follow-up analysis were assessed on the Bayley Scales for their baseline assessment. However, the 3 PFS patients that were assessed using the WIPPSI at baseline seemed to perform on a par with the normative mean at baseline with FSIQs ranging from 105 to 109.

One sample t-tests on the composite scores obtained at the follow-up assessment revealed that the PFS group had a higher FSIQ from the norm ($t(4) = 8.101$, $p=0.001$), and showed a similar trend for their VIQ ($t(4) = 2.609$, $p=0.059$) and their PIQ ($t(4) = 2.414$, $p=0.073$) scores. PSQ wasn't found to differ from the mean ($t(3) = -1.269$, $p=0.294$).

For the non-PFS group, one sample t-tests revealed a trend for a difference from the norm in FSIQ ($t(10) = -1.870$, $p=0.094$) at baseline and a statistically significant difference at follow-up ($t(10) = -2.286$, $p=0.045$). The same was true of their VIQ scores with a trend for a difference from the norm at baseline ($t(10) = -2.092$, $p=0.063$) and a significant difference at follow-up ($t(10) = -2.556$, $p=0.028$). The opposite pattern was observed in their PIQ scores with a significant difference from the mean at baseline ($t(10) = -2.413$, $p=0.037$) and a trend for a lower mean from the norm at follow-up ($t(10) = -2.099$, $p=0.062$). PSQ was also found to edge towards a difference at baseline ($t(6) = -2.042$, $p=0.087$) and be significantly different from the norm at follow-up ($t(10) = -5.064$, $p<0.001$).

Table 4.6 Mean VIQ, PIQ, PSQ and FSIQ scores for the two patient groups

	PFS	NON-PFS
VIQ 1 (SE)	110.33 (7.67)	85.73 (6.82)
	N=3	N=11
VIQ 2 (SE)	107 (2.68)	84.27 (6.13)
	N=5	N=11
PIQ 1 (SE)	100.67 (9.21)	85.36 (6.07)
	N=3	N=11
PIQ 2 (SE)	109 (3.73)	85.82 (6.76)
	N=5	N=11
PSQ 1 (SE)	-	77.71 (10.92)
	N=0	N=7
PSQ 2 (SE)	96.75 (2.56)	77.09 (2.56)
	N=4	N=11
FSIQ 1 (SE)	107 (1.16)	83 (9.09)
	N=3	N=10
FSIQ 2 (SE)	114.4 (1.78)	83.27 (7.32)
	N=5	N=11

4.3.2.2.2. Comparison of the groups' performance with a group of healthy controls

Given that the PFS sample size at baseline was too small, a comparison between the three groups was not possible. A MANOVA with the baseline FSIQ, VIQ, and PIQ as the dependent variables and group (i.e. non-PFS and controls) as the between subjects factor revealed a main effect of group ($F(3, 14) = 5.290$, $p=0.012$). Specifically, the two groups were found to have different VIQs ($F(1,$

16) =11.132, p=0.004), PIQs (F (1, 16) = 16.912, p=0.001), and FSIQs (F (1, 16) = 14.465, p=0.002).

Table 4.7 Description of VIQ, PIQ and FSIQ scores obtained by the PFS follow-up patients

	PFS1	PFS2	PFS3
VIQ1	95	118	118
VIQ2	110	104	113
PIQ1	119	90	93
PIQ2	108	101	123
FSIQ1	109	105	107
FSIQ2	111	111	120

A MANOVA with the follow-up FSIQ, VIQ and PIQ as the dependent variables and group (all groups) as the between subject factor revealed a main effect of group (F (6, 40) = 2.814, p=0.022). The three groups were found to perform differently on VIQ (F (2, 21) =9.768, p=0.001), PIQ (F (2, 21) =9.800, p=0.001), and FSIQ (F (2, 21) = 12.848, p<0.001) measures. Bonferroni post hoc tests revealed a difference between the non-PFS group and the control group in VIQ (p=0.001), PIQ (p=0.001) and FSIQ (p<0.001). The two patient groups were found to have a different FSIQ (p=0.016) and a trend was revealed for a difference in their VIQ (p=0.064) and their PIQ (p=0.062). No differences were revealed between the control group and the PFS group.

4.3.2.2.3. The groups' trajectories over time

Given the small sample size of the PFS group, a repeated measures analysis with group as a fixed factor was not possible. However, *Table 4.7* has been provided which contains the VIQs, PIQs, and FSIQs obtained by the 3 PFS patients on their two visits. In brief, 2 out of the 3 patients obtained lower VIQs at their follow-up assessment whereas the third PFS patient obtained an improved VIQ. The contrary was observed for PIQ measures where the patients whose VIQs had declined saw an improvement and the patient that had seen an improvement in their VIQ previously saw a decline in their PIQ. FSIQs improved in all PFS patients somewhere between 2 and 13 points.

The non-PFS group trajectory in time was investigated using a paired samples t-test. This test revealed no differences in VIQ ($t(10) = 0.552$, $p = 0.593$), PIQ ($t(10) = -0.111$, $p = 0.914$) and FSIQ ($t(9) = -0.140$, $p = 0.892$) performance from baseline to follow-up in this group.

4.3.2.2.4. Parental Questionnaires

Table 4.8 contains the percentages of normal scores achieved by the 2 groups on the SDQ. One PFS patient provided a complete SDQ questionnaire at the baseline assessment and this was found to lie within the normal range. At the follow-up assessment, 3 out of the 5 PFS patients provided completed SDQ questionnaires. One patient was found to have a score placing her within the normal range, one patient was found to be within the borderline range and one patient was found to be within the abnormal range. Within the non-PFS group 3 out of the 6 patients were found to be within the abnormal range at baseline. The remaining three were determined to lie within the normal range. At their follow-up assessment 3 out of the 7 non-PFS patients were revealed to lie within the abnormal range with the remaining being characterized as normal. Four non-PFS patients provided completed SDQ questionnaires at baseline as well as follow-up. The characterization of non-PFS

patients who provided questionnaires at both time points remained unchanged from baseline to follow-up.

Table 4.8 Percentage of normal scores achieved on the SDQ for each group

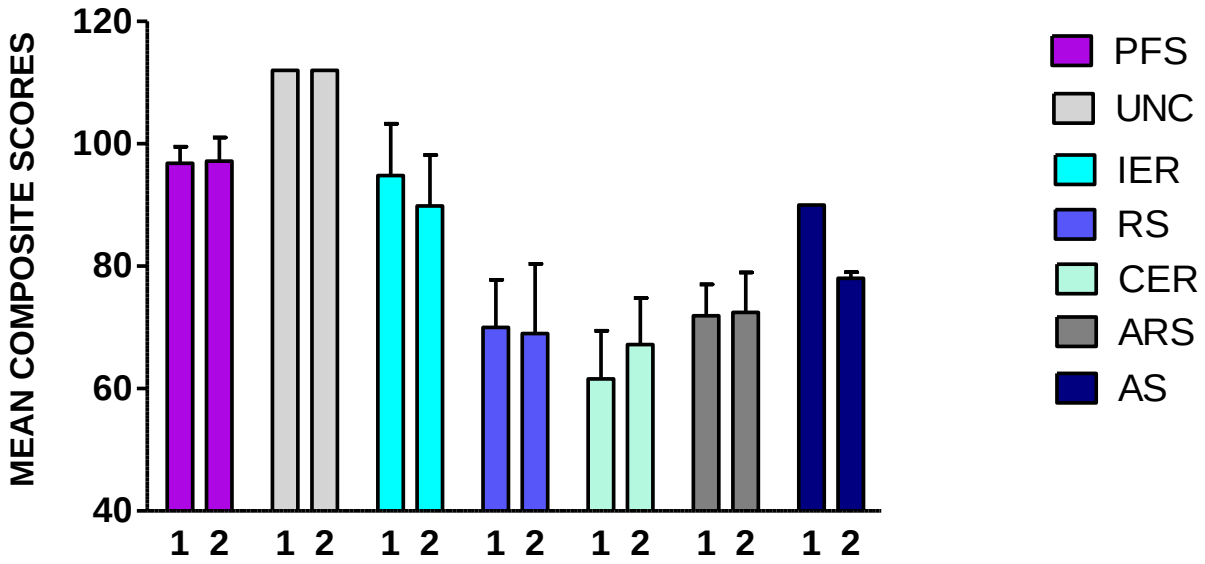
	PFS	NON-PFS
SDQ Total 1	100% (N=1)	50% (N=6)
SDQ Total 2	33.3% (N=3)	50% (N=8)
SDQ Emotion 1	100% (N=1)	66.7% (N=6)
SDQ Emotion 2	66.7% (N=3)	62.5% (N=8)
SDQ Conduct 1	100% (N=1)	66.7% (N=6)
SDQ Conduct 2	0 % (N=3)	50% (N=8)
SDQ Hyper 1	100% (N=1)	66.7% (N=6)
SDQ Hyper 2	33.3% (N=3)	75% (N=8)
SDQ Peer 1	0% (N=1)	50% (N=6)
SDQ Peer 2	100% (N=3)	62.5% (N=8)
SDQ Social 1	100% (N=1)	66.7% (N=6)
SDQ Social 2	100% (N=3)	87.5 % (N=8)

4.3.3. All participants

In total, 50 participants were seen for a follow-up assessment a mean of 13 months following CSE and a mean of 11.8 months following their baseline assessment. This count includes the 2 patients that were assessed on the Bayley Scales at baseline and the WIPPSI at follow-up, as the results were not altered by excluding them. Twenty-four patients had been classified as PFS at baseline. Within the non-PFS group, 2 had been classified as acute symptomatic cases, 3 as remote symptomatic cases, 6 as idiopathic epilepsy related cases, 5 as cryptogenic epilepsy related cases, and 1 eluded classification altogether.

Whereas the small sample size of classification sub-groups disallows the use of formal statistics, Figure 4.2 has been created to provide a sense of performance in each subgroup in cognition and language. One thing that is quite striking is the betterment of the acute symptomatic group (AS) (2 out of the 3 from the original cohort have been assessed so far) from time point 1 to time point 2. When we looked at the 2 individuals separately, 1 was found to have improved in all 3 measures (i.e. cognition, language and motor measures) and the other was found to have improved in cognition but not the other 2 scales where they were found to be worse. In the remaining groups there have been some downward/upward shifts in means (e.g. cryptogenic epilepsy related cases (CER), and, unclassified cases(UNC)), however, still half or more of the original cohort remains to be seen in these subgroups. The idiopathic group (IER), which is the only other group where a sufficient proportion of the original cohort (6/9) were followed up seems to be performing in line with their baseline assessment with small increases witnessed in their language means.

Figure 4.2 Change in performance from baseline to follow-up in the CSE subgroups



Age at the time of assessment (t (28.32) =-2.479, p=0.019), time from seizure (t (47) =2.670, p=0.010), gender (χ^2 (1) =7.936, p=0.010), first scan (χ^2 (1) =5.903, p=0.026), language (χ^2 (1) = -6.411), occurrence of seizures in between assessments(χ^2 (1) = 5.965, p=0.022), and medication (χ^2 (1) = 20.300, p<0.001) were found to be significantly different between the two groups. Namely, the non-PFS group was older and included more males and more English speakers than the PFS group. Moreover, the non-PFS group included more patients on medication, more patients experiencing seizures between assessments and more patients with abnormalities on their scans than the PFS group.

4.3.3.1. Comparison of the two patient group's trajectories over time

A repeated measures analysis with cognitive assessment at the baseline and the follow-up assessment as the repeated measure and group, gender, scan, seizures and language as fixed factors, and, time from seizure and age as covariates revealed no main effect of follow-up (F(1,23)=0.016, p=0.552).

None of the interaction terms were found to be significant. The test of between subjects effects revealed a difference between people who had experienced seizures between assessments to those who hadn't ($F(1) = 4.769$, $p=0.039$) and a trend for a difference between PFS and non-PFS patients ($F(1) = 3.569$, $p=0.072$). Planned pairwise comparisons with a Bonferroni correction revealed a significant difference between the two patient groups ($p=0.040$).

An identical repeated measures design with the language assessment at the baseline and the follow-up as the repeated measure revealed no main effect of follow-up ($F(1, 21) = 0.005$, $p=0.742$). The test of between subjects effects revealed a difference between PFS and non-PFS patients ($F(1) = 4.743$, $p=0.041$).

4.3.3.2. Investigation of variables affecting the two groups at follow-up

To determine the contribution of specific demographic and clinical variables to the performance of the entire non-PFS group at the follow up assessment we conducted a stepwise linear regression with the follow-up cognitive composite/FSIQ and language composite/VIQ scores as the dependent variables and scan abnormalities (1 year follow-up), occurrence of seizures between assessments, occurrence of CSE between assessments, medication, English as a first language, and, age as covariates. Medication came out from both analyses (i.e. cognition and language) as a strong predictor of performance ($p<0.001$). To examine whether different predictors would emerge if we looked at the infant group alone we ran linear stepwise regressions on the follow-up cognitive and language scores. Occurrence of CSE between assessments wasn't entered in this analysis as there were only 2 participants who experienced one. Medication ($p=0.005$) was found to affect cognition, and, scan abnormalities ($p=0.005$) and the occurrence of seizures ($p<0.001$) between assessments were found to be predictors of language scores. Occurrence of seizures between assessments and scan abnormalities impacted performance in an expected fashion, i.e. patients with abnormal scans

performed worse than patients with normal ones and patients who experienced seizures in between assessments performed worse than patients who didn't.

The stepwise linear regression examining the contribution of variables to PFS performance with the follow-up cognitive and language indices as dependent variables and language, age, occurrence of seizures, and, prematurity as independent variables revealed that age was a strong predictor of cognitive scores ($p=0.001$) and English as a first language a predictor of follow-up language performance ($p=0.010$). To investigate whether different predictors were highlighted in the infant group we ran a stepwise linear regression for the PFS group solely on their Bayley scores. Both predictors were no longer significant.

4.4 Discussion

In the current study we assessed 50 children a mean of 13 months following CSE using standardized neuropsychological assessments. These children are a subset of the 80 children seen a mean of 38 days from CSE. Therefore, this is the first study to longitudinally track the progression of a paediatric population following CSE. As this is an ongoing study, a number of participants are still due for a follow-up visit. However, a preliminary analysis has determined that this is a representative sample of the original cohort.

A year onwards the non-PFS group are still underperforming relative to normative standards and healthy controls. This finding applies to both age groups, i.e. infants and older children. The non-PFS group is also found to perform worse than the PFS group on most indices with the exceptions of the motor composite, VIQ and FSIQ where they are found to edge towards a difference. If anything, the performance of the non-PFS group seems to have declined from the baseline to the follow-up assessment with trends towards significance being translated into significant differences. However, the paired sample t-test revealed no statistical differences from time point 1 to time point 2 on the Bayley assessment.

The above results are further corroborated by the parental questionnaires with the non-PFS children achieving lower GAC scores relative to the norm at both time points and a lower SEC score at follow-up. Positive correlations between the GAC score and the three Bayley composite scores attest to the validity of our findings. The SEC score was only found to correlate with the cognitive composite score at follow-up. However, the SEC score has been designed to measure the social and emotional growth of a child and as such assesses things like emotional engagement to the mother, ability to be comforted, use of gestures etc. The GAC, on the other hand, contains 10 subscales

which probe into the abilities of a child as evidenced in their daily lives. Therefore, things like pre-academic abilities, motor abilities, and adherence to health and safety precautions are being interrogated by this measure. It, therefore, seems commonsensical that the GAC correlates strongly to the performance of the children on our standardized assessments.

Regression analyses conducted to investigate the effect of clinical variables on performance revealed that medication was the only clinically significant determinant of performance when all non-PFS participants were lumped together. When the infant data was analysed separately, medication was again found to affect cognitive performance. Scan abnormalities, and, recurrence of seizures, but not medication, were discovered to affect language development in the infant group. The effect of seizures on language development was further highlighted by the interaction between language, group, and, seizures obtained in our infant repeated measures analysis, which confirmed the group selectivity of this effect, i.e. PFS children were unaffected by the occurrence of seizures between assessments. An effect of seizure was also obtained in our repeated measures analysis of cognitive measures with a significantly lower performance of the group who had experienced seizures in between assessments. No group specificity was revealed for the effect of seizures on cognition suggesting that whether a PFS or a non-PFS patient, occurrence of seizures between assessments adversely affects one's performance in cognitive assessments.

Finally, the clinical variables obtained in our follow-up analysis agree with our baseline results where scan abnormalities and medication were both highlighted as clinical variables that have an effect on performance. This finding attests to the long term impact of these variables and suggests that it is these variables rather than CSE itself that account for the majority of impairments in this group.

Whereas the small sample size of the non-PFS groups makes it difficult to reach firm conclusions regarding their trajectory through time, some preliminary remarks can still be formulated. Firstly, our acute symptomatic group, which consisted of 2 patients with a presentation of meningitis at baseline, has improved significantly from time point 1 to time point 2. This was particularly true of their cognitive scores, which had improved in both patients markedly. Whereas one cannot discount the theoretical possibility that the seizure affected their performance at baseline, given the profile of the remaining non-PFS subgroups, it is much more likely that their acute neurological insult was the responsible agent. The idiopathic group which is the only other group where a sufficient proportion of the original cohort was followed up (6/9) seems to be performing in line with their baseline assessment.

When one turns to the PFS group the story becomes more complicated. Firstly, there is a clear discrepancy between the performance of the younger and the older group. Namely, whereas the younger group still show impairments in cognition at follow-up, the older group are obtaining higher FSIQs than the norm and are not different from normal controls. They are also showing trends for a higher mean than the norm in their VIQs and their PIQs. These results mirror the results obtained in the baseline chapter where the older PFS group was statistically indistinguishable from the normal controls.

It is the contention of this author that the differences observed between the older and younger groups are not related to the administration of different neuropsychological assessments. Both groups were also measured against a group of normal controls and whereas the infant group performed worse than their normal peer group in most measures, the older PFS group performed in line with the controls. Another possibility is that the older PFS group assessed in our study is not representative of the older PFS population as a whole. This cannot be discounted by the present

data because the sample studied is very small. However, given the age-specific incidence of CSE, much fewer children have one after the age of 3 and, therefore the smaller sample in our study does reflect an existing population difference. It is, however, plausible that the occurrence of CSE at a younger age may differently affect later development than the occurrence of CSE at an older age. The fact that the older PFS group appears to be completely normal seems to argue against the view that a pre-existing abnormality in children with PFS is the dual cause of CSE and the presence of subtle deficits.

However, the infant PFS group does demonstrate some improvements from their baseline to their follow-up assessment, i.e. in their language and their motor abilities. Namely, whereas a difference from the norm is discovered at baseline in both composites, no such difference is obtained at their follow-up assessment. When we turn to their comparison with a group of normal controls, this finding is confirmed for their motor abilities but not their language abilities. The improvement observed could be theoretically attributed to a test-retest practice effect but this is highly unlikely for two reasons. Firstly, when the children are assessed on the Bayley scales a year onwards, they are tested on different items from the ones used in their baseline assessment as the starting point of administration has shifted upwards. Secondly, if that were the case, we would expect to witness similar improvements in their cognitive scores, which hasn't been the case.

Another possibility that might account for the differences from baseline to follow-up in the language domain is the age dependency uncovered in our baseline and our follow-up chapter. In other words, it is possible that the PFS infants are achieving better scores because they are older at their follow-up assessment. Therefore, one cannot argue definitively that language faculties were transiently affected by CSE at baseline and, as the transient effects of CSE "retreated", language faculties improved. Nonetheless, PFS children were still found to lag behind normal controls in language. This finding

cannot be attributed to the different distribution of native English speakers in the two groups as this factor did not emerge in any of the infant group analyses.

Motor abilities, on the other hand, which haven't been shown to be dependent on age may have been transiently affected by CSE at baseline and as time progressed gotten better. This mostly applies to the gross motor abilities as fine motor abilities were the only ones found to be unimpaired at baseline. The fact that a relationship between duration of CSE and motor development was discovered in the non-PFS group in our baseline assessment further attests to the possibility that CSE may temporarily affect motor abilities.

As suggested above, parental questionnaires for the infant group were found to strongly correlate with our neuropsychological findings. The fact that GAC scores for the PFS group at follow-up were found to be significantly below the mean, corroborate our findings regarding cognition and language in this group. This is particularly important given the methodological issues raised above. Moreover, despite the lack of functional impairments in the older PFS group there is some evidence for behavioural issues as attested by the SDQ where 2 out of the 3 children were found to lie outside the normal range. However, the small sample size in the older PFS group prohibits further discussion of this issue.

In closing, it is important to note, that whereas the PFS infants are found to underperform, their scores do not place them more than a standard deviation away from the mean, i.e. they are clinically normal as defined by the tests administered. This may be the reason why studies in the literature seem to be in disagreement regarding the outcome of prolonged febrile seizures. For example, in the Schiotzz Christensen study children following PFS were found to lag behind their monozygotic twins by 7 PIQ points. This difference in points still places the affected twins within the normal

range of performance with a PIQ of 103. Therefore, one needs to be cautious when interpreting the literature regarding the definition of normality.

4.5 Conclusions

In sum, our yearly follow-up study has determined that the non-PFS group is still manifesting functional impairments. Children who have abnormal scans and are taking medication seem to be the most impaired in this group. These findings seem to apply to both age groups. Moreover, the infant PFS group, but not the older group, is still manifesting subtle deficits relative to the norm and a group of healthy controls a year onwards. This is particularly true of their cognitive development. Therefore, at least a year following PFS, two possibilities remain. The first one is that PFS affected the children's development in the longer term rather than in a transient fashion. Alternatively, these children may have been pre-morbidly performing at this level and will continue to do so. However, their motor abilities and language abilities do seem to have progressed somewhat from the baseline to the follow-up assessment pointing to the possibility that some functions may have been transiently affected by CSE at baseline.

Chapter 5 Investigating the structural-functional relationships in children following CSE

5.1 Introduction

The aim of the previous two chapters was to investigate in detail the neuropsychological performance of a group of children following CSE, close to the time of incident and a year later. The purpose of the current chapter is to investigate the relationship between neuropsychological performance and structural MR indices in this paediatric cohort at baseline and follow-up. In addition, we wanted to investigate whether any MR measures at baseline were predictive of follow-up performance since this would enable the early identification of biomarkers of future neurodevelopment. This is particularly important given the association between PFS and TLE.

A detailed examination of structural-functional correlates in human CSE has never been carried out. However, previous imaging studies have been critical in establishing the presence and progression of structural abnormalities following CSE, and more particularly, PFS. For example, Scott et al. (2003) have shown that a mean of 5.5 months following a PFS, children show hippocampal volume and T_2 relaxation time reductions when compared to values recorded within 5 days of the incident. Moreover, a significant increase in hippocampal volume asymmetry is present in the paediatric patients at 5.5 months when compared to the initial data. Scott et al. (2006) also investigated apparent diffusion coefficient (ADC) hippocampal measurements in this paediatric cohort and discovered a reduction in ADC from the baseline to the follow-up investigations. Moreover, in direct contrast to controls, the patients in this study did not show an age dependency in their hippocampal ADC measurements (Scott, King, Gadian, Neville, & Connelly, 2006).

In view of the above findings, we were primarily interested in interrogating the relationship of hippocampal "integrity" and later development in our paediatric CSE cohort. Research on developmental amnesia has pointed to the functional importance of this structure for memory development (Vargha-Khadem et al., 1997b). However, recent studies have begun to reveal the importance of the hippocampus for the development of other higher cognitive processes aside from memory. For example, studies looking at the effect of prematurity on later development have highlighted the hippocampal contribution to cognitive outcomes (Thompson et al., 2008; Lodygensky et al., 2005; Abernethy, Palaniappan, & Cooke, 2002), and, even, motor outcomes (Thompson et al., 2008) during the first few years of life. Moreover, a relationship between hippocampal size and verbal IQ was recently reported in healthy developing boys (Schumann et al., 2007). Therefore, whereas, the hippocampus has been traditionally viewed in the literature as a memory subserving structure, it is becoming increasingly clear that during development this structure contributes to a number of developmental functions.

While structural-functional relationships have not been probed following CSE in humans, Dubé and colleagues (2009) investigated this issue in rats following experimental febrile seizures. These authors induced febrile seizures (FS) in previously healthy rat pups and studied the impact of these seizures on memory and executive functions in adulthood. They found that rat pups with a history of febrile seizures exhibited working and reference memory deficits as well as strategy shifting deficits when compared to normally developing rats. More importantly, these authors demonstrated that rats with significantly elevated hippocampal T_2 values as indicated on their 1-month post FS scan performed worse than rats with no significant elevation in their hippocampal T_2 values. The elevated T_2 values were associated with ipsilateral hippocampal and ventricular volume losses. It is of note, however, that these authors did not obtain correlations with performance for all their rats with a history of

febrile seizures, pointing to a spectrum of injury following a febrile seizure. Therefore, given the reported relationship between T_2 relaxation times and degree of impairments in the above study, we wanted to investigate whether a similar relationship is obtained in a human paediatric cohort following CSE.

In the current study, structural-functional relationships following CSE have also been explored using DTI as it has been shown to be a promising avenue in studying the developing brain (Cascio, Gerig, & Piven, 2007). Specifically, relationships between fractional anisotropy (FA), mean diffusivity (MD) and neuropsychological measures were considered. FA measures the directionality of diffusion within a voxel and MD is a scalar measure of its total diffusion within a voxel. These measures are commonly used clinically to localize white matter lesions that do not show up on other forms of clinical MRI. The specific DTI measures were selected as they have been shown to be very effective in detecting subtle structural deficits in paediatric populations and relating these to cognitive impairments (Wozniak et al., 2007).

FA decreases in the hippocampus have been described in rats following hypoxia-ischemia and have been shown to represent white matter volume losses emanating from the alveus (Stone et al., 2008). Moreover, FA has been shown to be an effective detector of a wide spectrum of structural abnormalities (Wieshmann et al., 1999a; Wieshmann et al., 1999b). More importantly, decreased FA and increased MD has been reported in the temporal lobes of TLE patients ipsilateral to their epileptogenic focus (Thivard et al., 2005). Interestingly, patients with a history of febrile seizures were found to have higher FA values than those with no such history. The authors interpreted this finding as evidence of an initial precipitating injury affecting the whole brain or alternatively a genetic predisposition which led to both the occurrence of febrile seizures and TLE.

Unfortunately, the above study did not investigate the correlation of these water diffusion abnormalities and neuropsychological performance. However, other studies that have undertaken this type of analysis have revealed that decreases in FA are associated with neuropsychological impairments (Wozniak et al., 2007). Moreover, even during normal development, performance on the WISC has been shown to positively correlate with FA and negatively correlate with MD values in the frontal and the occipito-parietal areas (Schmithorst, Wilke, Dardzinski, & Holland, 2005). Therefore, in the current study we would expect that possible reductions in FA soon after CSE may be associated with neuropsychological impairments in our paediatric cohort.

Finally, whereas the previous chapters were instrumental in demonstrating the presence of deficits in children following either PFS or CSE associated with other aetiologies, they were less helpful in establishing whether the seizure itself had a transient effect on performance. Two exceptions were the finding that seizure duration was an independent predictor of motor performance in the non-PFS group and the observation that language and motor performance seemed to recover from the baseline to the follow-up assessment in the PFS group, both results that point to an effect of the seizure itself. Investigating structure-function relationships might help us shed more light into whether the seizure in itself is having a direct effect on performance. For example, finding that T_2 relaxation times correlate with performance at the baseline but not the follow-up assessment would be consistent with a transient effect.

We were expecting to witness correlations between hippocampal MR measures and performance measures in the PFS group despite the absence of overt structural abnormalities upon visual inspection in this group. As already discussed, previous studies have pointed to the presence of hippocampal abnormalities in a subset of children following PFS and given the existing association between PFS and TLE, this is a group whose progress through time is of great interest to

researchers and clinicians alike. However, given the overrepresentation of structural abnormalities in the non-PFS group, we were also expecting to observe correlations between their performance on neuropsychological tests and brain volume, which is a reliable measure of gross abnormalities.

5.2. Methods

5.2.1. Participants

Children soon after a CSE were studied using a full set of neuropsychological and MRI investigations. A year following their baseline assessment the same set of investigations was carried out to track the children's progress through time following a CSE. Normal controls were assessed only once using the above investigations. However, due to ethical constraints few controls were able to provide a full set of investigations. Therefore, in the analysis presented below, normal controls have been included for the baseline MR comparisons but have been excluded from all other analyses.

5.2.2. MR Imaging

The imaging parameters and procedure followed for our imaging protocol have been described in the General Methods section and shall not be repeated here. In brief, two independent researchers (MM and MY) who were blind to patient diagnosis circumscribed the hippocampus for each participant at least once. The anatomical landmarks used to define the hippocampal boundaries have been described in the General Methods section and shall be omitted here. The average of all available hippocampal measurements was computed to arrive at the present hippocampal volumes. Namely, if there were 3 separate values for a participant's left hippocampus, then all 3 were averaged to arrive at the current value. Subsequently, the left, the right, and, the mean hippocampal volumes were divided by the total brain volume (BV) to provide left, right and mean hippocampal ratios

(HPCR). It was felt that this measure would bypass a possible confounding relationship between age and hippocampal size seeing as we were dealing with a pathological population. The value derived was then multiplied by 10000 for simplicity of use within SPSS. An asymmetry index (AI) was also calculated according to the formula described in the General Methods section. A plus value signified a right-to left hippocampal asymmetry, and, a negative value, a left-to-right hippocampal asymmetry. Akin to the hippocampal volumetrics the average of all available DTI hippocampal measurements was taken to arrive at the current fractional anisotropy (FA) and mean diffusivity (MD) values. Finally, FA and MD values were multiplied by 10000000 for ease of manipulation by SPPS.

5.2.3. Analyses

In the present chapter, the relationships between cognition, language indices and MR measures were investigated in all participants where these were available. Cognition was measured by the cognitive composite provided by the Bayley Infant Scales of Development (3rd edition) in the infant population and the FSIQ provided by the WISC or the WPPSI in the older population. Similarly, language was measured by the language composite provided by the Bayley Infant Scales of Development (3rd edition) in the infant population and the VIQ provided by the WISC or the WPPSI in the older population. For the infant population, relationships between motor composite scores and MR measures were also explored.

As there are well documented age dependencies in the MR measures under investigation in this study, we also looked at the relationships between age and MR measures in our cohort to understand whether the same principles were being adhered to following CSE. Namely, a positive correlation between BV and age as well as a similar correlation between age and hippocampal volume in the first few years of life has been amply documented. The relationship between DTI measurements and age have not yet been extensively studied in infants and young children yet,

however, given the ongoing myelination processes in the first few years of life such associations are definitely present.

5.2.4. Statistical Analysis

A detailed analysis of the MR differences between the 3 groups is not included in this chapter as these data will be presented in Dr Michael Yoong's PhD thesis. However, for the purposes of establishing whether the three groups differed on the MR variables used in this chapter, a MANOVA with hippocampal volumes and AI as dependent variables and age and BV as covariates was carried out. A separate MANOVA with T_2 relaxation times, MD, FA and mean HPCRs as dependent variables and age as a covariate was conducted. Bonferroni post hoc corrections were carried out for multiple comparisons. One way-ANOVAs and chi-square tests were used to compare the three groups on demographic and clinical variables. For the follow-up data, independent sample t-tests were used instead of ANOVAs as the control group was excluded from this analysis.

Linear regressions were conducted to assess the contribution of MR measures to neuropsychological performance controlling for age. We used p values of .05 and .10 to enter or remove, respectively. In these analyses we considered the effect of BV, HPCR, hippocampal T_2 relaxation times, and hippocampal MD and FA values on performance. When needed, logarithmic or square root transformations were applied to ensure the normal distribution of the data. For all regression analyses, diagnostics included the investigation of normality and residual plots. Pearson's or Spearman Rho's correlations were carried out to investigate relationships of *a priori* interest between hippocampal and outcome measures. Repeated measures ANOVAs were conducted to assess changes in MR measures from baseline to follow up.

5.3 Results

5.3.1. Baseline Results

5.3.1.1. Description of the patient sample tested at baseline

Eighty two children provided a full set of hippocampal and neuropsychological measurements. These consisted of 32 children classified as PFS, 42 children classified as non-PFS, and, 8 healthy controls. A subset of these children also provided FA, MD and T_2 values. *Table 5.1* contains the means and standard errors for all MR derived indices as well as the demographic and neuropsychological means for the three groups. The 3 groups were found to be different in age ($F(2, 79) = 5.248$, $p=0.007$), gender ($\chi^2(2) = 9.757$, $p=0.008$) (with a bigger proportion of males in the non-PFS group), cognitive scores ($F(2, 77) = 22.154$, $p<0.001$), language scores ($F(2, 76) = 15.711$, $p<0.001$), and scan abnormalities ($\chi^2(2) = 16.968$, $p<0.001$). Bonferroni post hoc corrections revealed that the two patient groups were different in age with the non-PFS group being significantly older ($p=0.005$). Cognition and language composite scores were different amongst all groups with the non-PFS group performing worse than all groups and the PFS group performing worse than controls.

The MANOVA carried out to investigate any differences between hippocampal volumes and asymmetry indices in the three groups revealed a main effect of BV ($F(2, 73) = 41.795$, $p<0.001$). Pairwise comparisons did not reveal any statistical differences between the three groups. The MANOVA conducted to investigate differences in the remaining MR measures revealed a main effect of age ($F(4, 40) = 6.464$, $p<0.001$) and a main effect of group ($F(8, 82) = 2.268$, $p=0.030$). Pairwise comparisons revealed that the non-PFS group had significantly higher T_2 relaxation times than the PFS ($p=0.005$) and the control ($p=0.039$) groups. No other significant differences between the three groups were uncovered.

Table 5.1 Comparison of PFS and non-PFS patients at baseline

	PFS	NON-PFS	Controls
N	32	42	8
Male	10 (31.3%)	27 (64.3%)	2 (25%)
Age at baseline (months)	22.31 (2.02)	49.81 (7.41)	38 (9.13)
English as a first language	16 (50%)	35 (83.3%)	8 (100%)
Scan abnormalities	5 (15.6%)	23 (54.8%)	0 (0%)
AED	2 (6.3%)	23 (54.8%)	Not Applicable
Cognitive composite (SE)	94.69 (2.51)	80.9 (3.5)	127.38 (2.56)
Language composite (SE)	94.37 (3.01)	80.81 (3.1)	118.88 (6.41)
Motor composite (SE)	95.54 (2.62)	77.48 (4.49)	106 (9.08)
Mean Hippocampal Volume (mm^3)	1943.06 (47.9)	1846.58 (98.12)	1840.31 (174.29)
Mean Hippocampal Ratio	16.45 (0.24)	15.60 (0.42)	14.82 (0.8)
BV (mm^3)	1183547(26454.5)	1182090 (44885.95)	1218570 (76742.36)
Asymmetry Index (AI)	0.04 (0.01)	0.04 (0.02)	-0.03 (0.04)
Mean T_2 values	123.47 (0.89)	125.9 (1.66)	117.16 (2.08)
Mean Mean Diffusivity (MD)	10185.40 (69.2)	10486.24 (243.94)	9756.88 (251.45)
Mean Fractional Anisotropy (FA)	1287745 (36863.65)	1303888 (56109.08)	1221770 (53647.0)

Table 5.2 Comparison of PFS and non-PFS patients at follow-up

	PFS	NON-PFS
N	17	18
Male	5 (29.4%)	12 (66.7%)
Age at follow-up (months)	33.2 (3.31)	63.1 (12.39)
English as a first language	6 (35.3%)	13(72.2%)
Scan abnormalities	3 (17.6%)	10 (55.6%)
AED	0(0%)	13 (72.2%)
Seizures	5 (29.4%)	11 (61.1%)
Cognitive composite (SE)	95.94 (3.16)	73.89 (5.14)
Language composite (SE)	95.94 (5.14)	73.67 (5.07)
Motor composite (SE)	92.31 (3.08)	70.78 (8.60)
Mean Hippocampal Volume (mm^3)	2158.32 (71.02)	1977.41 (127.91)
Mean Hippocampal Ratio	16.4 (0.37)	15.93 (0.84)
BV (mm^3)	1319179 (42874.08)	1243736 (57749.93)
Asymmetry Index (AI)	0.06 (0.02)	0.04 (0.05)
Mean T_2 values	120.6 (1.16)	122.81 (1.69)
Mean Mean Diffusivity (MD)	7439.64 (1883.12)	7739.17 (1038.76)
Mean Fractional Anisotropy (FA)	1234306 (28635.15)	1226133 (44879.43)

5.3.1.2. Investigation of the relationship between age and MR measures

Age was positively correlated with BV and mean hippocampal volumes in all groups. Mean hippocampal ratio was found to positively correlate with age in the non-PFS group (r=0.351, p=0.023) and the controls (r=0.777, p=0.023), but not the PFS group. Hippocampal AI was found to correlate with age only in the control group (r=0.713, p=0.047). The direction of this correlation suggests that older controls had a bigger right to left asymmetry than younger ones.

Only 4 controls provided T2, FA and MD values, so they were excluded from this analysis. Mean T_2 values were revealed to be inversely correlated with age in the PFS (r=-0.681, p<0.001) and the non-PFS (r=-0.720, p<0.001) groups. This was also the case with mean MD values which were found to be inversely correlated with age in both the PFS (r=-0.402, p=0.038) and the non-PFS (r=-0.494, p=0.008) groups. Mean FA was not found to correlate with age in either of the two patient groups.

5.3.1.3. The relation of MR measures to baseline performance

In the PFS group, mean FA was revealed to significantly contribute to performance on the motor assessments (F (1, 17) =6.077, p=0.025) (see Figure 5.1) and age was found to significantly contribute to language performance (F (1, 18) =5.776, p=0.027). In the non-PFS group, BV was found to significantly contribute to motor composite scores (F (1, 8) =6.635, p=0.033). No significant contributions of MR measures to cognitive performance were revealed at baseline.

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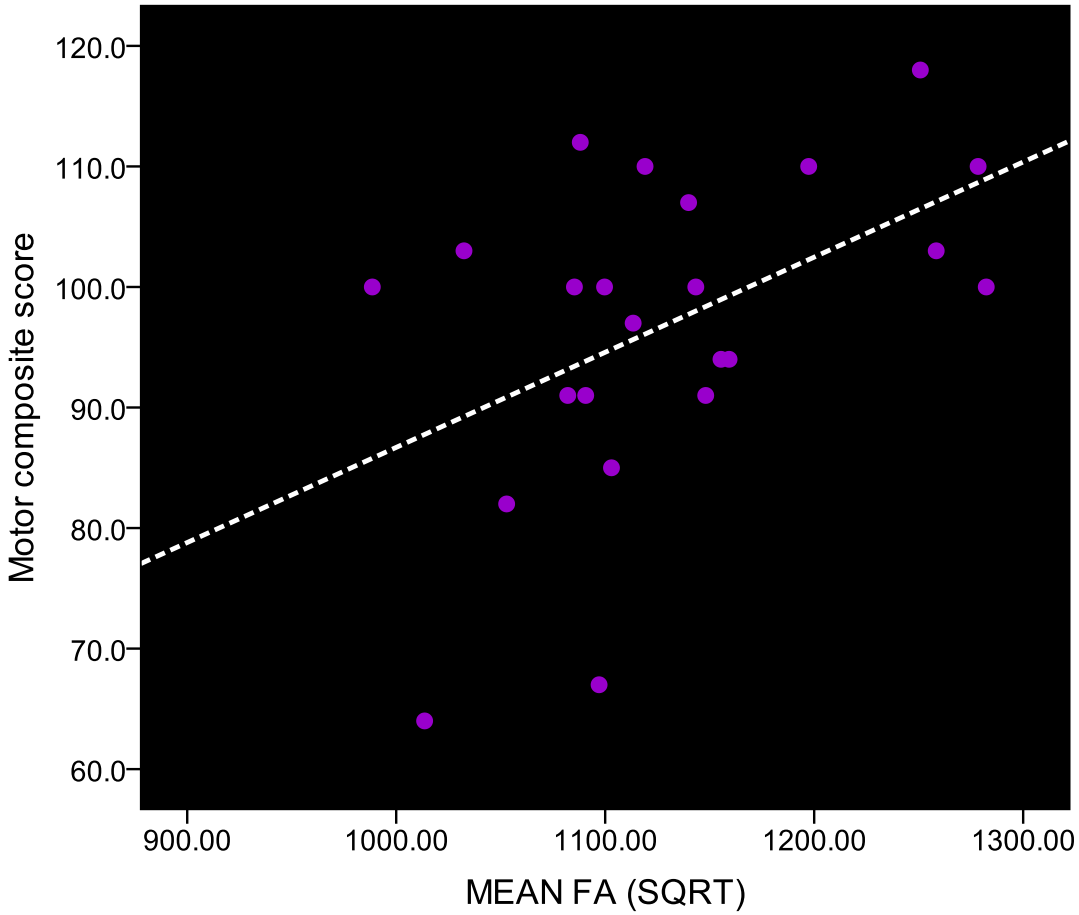
5.3.1.4. Specific contribution of the hippocampus to performance soon after CSE

As we were interested in specifically investigating the relationship between hippocampal volumes and T_2 values with neuropsychological performance on our standardized tests we ran separate correlations for these. Age corrections and non-parametric tests were performed when necessary.

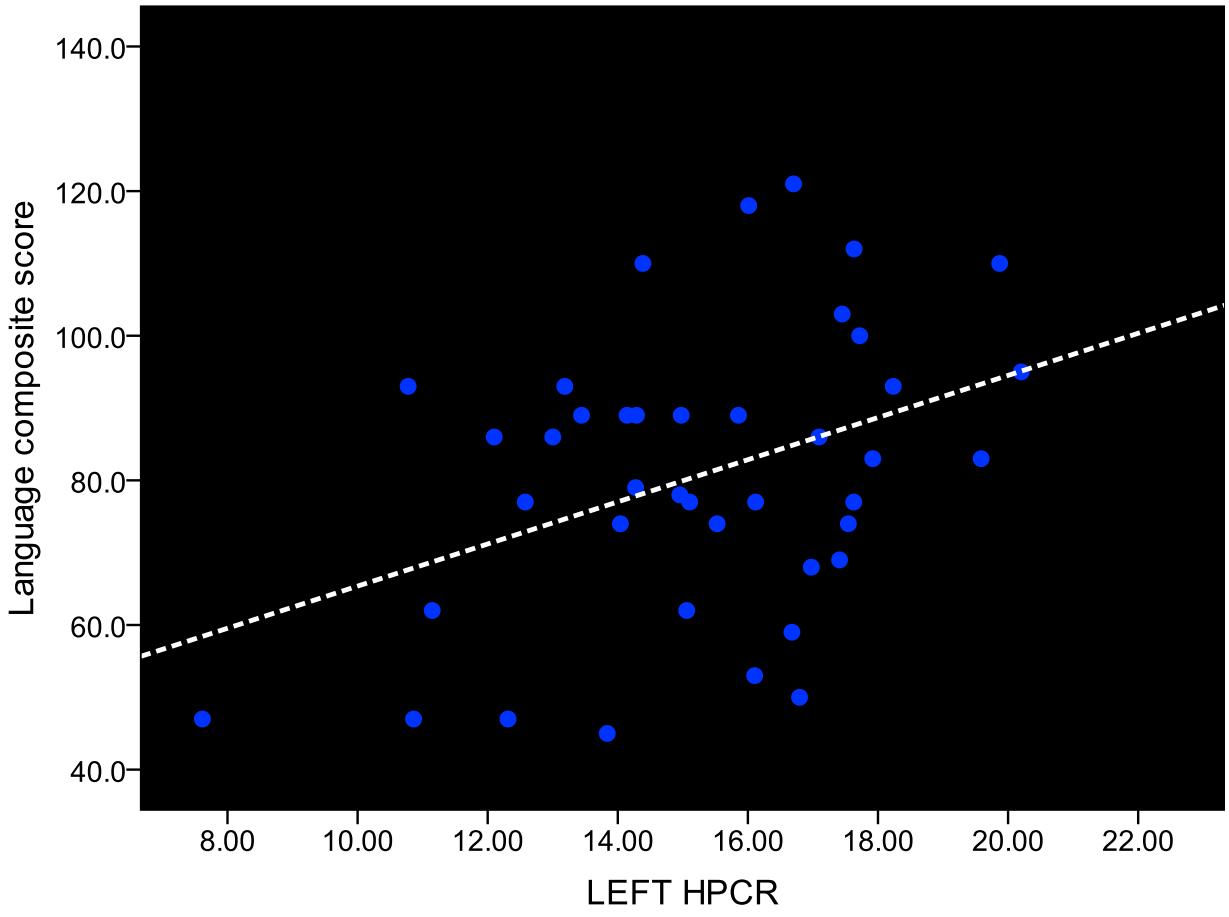
Figure. 5.1 The Relationship between mean FA and motor skills in the PFS group



In the PFS group, we observed no correlations between hippocampal and performance measures. In the non-PFS group, on the other hand, a significant correlation between mean HPCR and language composite scores (r=0.307, p=0.054) was revealed (Figure 5.2). This correlation stemmed from the left HPCR (p=0.048) and not the right HPCR (p=0.111) attesting for the widely acknowledged role

of the left hemisphere in language functions. No correlations between mean T_2 values and performance measures were obtained at baseline.

Figure. 5.2 The Relationship between left HPCR and Language in the non-PFS group



5.3.2. Follow-up results

5.3.2.1. Description of the patient sample tested at follow-up

Thirty-five children provided a full set of hippocampal and neuropsychological measurements at follow-up. These consisted of 17 children classified as PFS, and, 18 children classified as non-PFS. A subset of these children also provided FA, MD and T_2 values. *Table 5.2* contains the means and standard errors for all MR derived indices as well as the demographic and neuropsychological means

for the two patient groups. The two groups were found to be different in age (t (33) =-2.275, p=0.030), gender (χ^2 (1) = 4.858, p=0.044), cognitive scores (t (32) = 3.656, p<0.001), language scores (t (32) = 3.078, p<0.004), motor composite scores (t (20) =2.356, p=0.040), and, antiepileptic medication (χ^2 (1) = 19.533, p<0.001). Namely, the non-PFS group in the follow-up cohort was older, contained more males, and, was more impaired in the neuropsychological assessments than the PFS group. There was a trend for a difference in the number of patients experiencing seizures between the two patient groups with the non-PFS children being more likely to have experienced seizures in between assessments (χ^2 (1) = 3.540, p=0.092). A MANOVA with group as a fixed factor and age as a covariate revealed that BV was smaller in the non-PFS group when compared to the PFS group (p=0.027). A trend was also revealed for higher T2 values in the non-PFS group (p=0.065). In contrast, the mean HPCR, the mean AI, the mean MD, and the mean FA values were not found to differ in the two patient groups.

5.3.2.2. Relationship between age and MR measures in the two patient groups

Age and mean hippocampal volumes revealed a positive correlation in the PFS group (r=0.535, p=0.033) and a trend for a similar correlation in the non-PFS group (r=0.471, p=0.057). The mean hippocampal ratio wasn't revealed to be correlated with age in any of the two patient groups. Akin to the baseline results, mean T_2 values were found to be inversely related to age in the PFS (r=-0.638, p=0.008) and the non-PFS groups (r=-0.630, p=0.005). Similarly, BVs were revealed to be positively correlated with age in the PFS (r=0.685, p=0.014) and the non-PFS (r=0.743, p=0.001) groups. Mean MD and mean FA values were not correlated with age in the PFS group. However, mean FA values revealed a trend for a positive correlation with age in the non-PFS group (r=0.505, p=0.078).

5.3.2.3. Changes in MR measures from baseline to follow-up

As specified earlier a detailed analysis of the longitudinal MR measures is beyond the scope of this chapter. These data have been explored in detail and shall be presented in Dr. Michael Yoong's thesis where a larger cohort of patients has been investigated. However, for the purpose of evaluating whether MR measures in the current sample have changed through time, simple repeated measures ANOVAs were conducted with group as a between subjects factor. These revealed that mean hippocampal volumes increased with time ($F(1, 30) = 14.744$, $p=0.001$) as did BVs ($F(1, 26) = 16.078$, $p<0.001$). T_2 values were found to significantly decrease with time in both patient groups ($F(1, 23) = 17.069$, $p<0.001$). Mean HPCR, MD, and, FA values were not found to significantly change from the baseline to the follow-up assessment. No interactions between group and the dependent variables were obtained pointing to the similar behaviour of both groups through time. Because the control group was assessed only once, an analysis of their data longitudinally was not possible.

5.3.2.4. The relation of MR measures to performance at follow-up

In the PFS group, BV was found to positively correlate with language performance ($F(1, 7) = 19.397$, $p=0.003$). It is important to note that BV acted as an independent predictor from age as the latter variable had been included in the model and was not a significant predictor. In the non-PFS group, BV and mean FA were significant predictors of cognitive performance ($F(2, 10) = 18.047$, $p=0.001$) and language performance ($F(3, 8) = 27.645$, $p<0.001$). Mean T_2 was also a significant predictor of language performance ($p=0.042$). Finally, motor performance in this group was related to BV ($F(1, 5) = 8.483$, $p=0.033$).

Table 5.3 Summation of all significant correlations obtained in this chapter

	COG2	LANG1	LANG2	MOT1	MOT2
AGE		**PFS**			
BV_1		**NON-PFS**		**NON-PFS**	**NON-PFS**
BV_2	**NON-PFS**		**PFS** **NON-PFS**		**NON-PFS**
HPCR_1		**NON-PFS**			**NON-PFS**
HPCR_2	**PFS**				
T_2_2	**NON-PFS**		**NON-PFS**		
FA_1		**NON-PFS**		**PFS**	
FA_2	**NON-PFS**		**NON-PFS**		

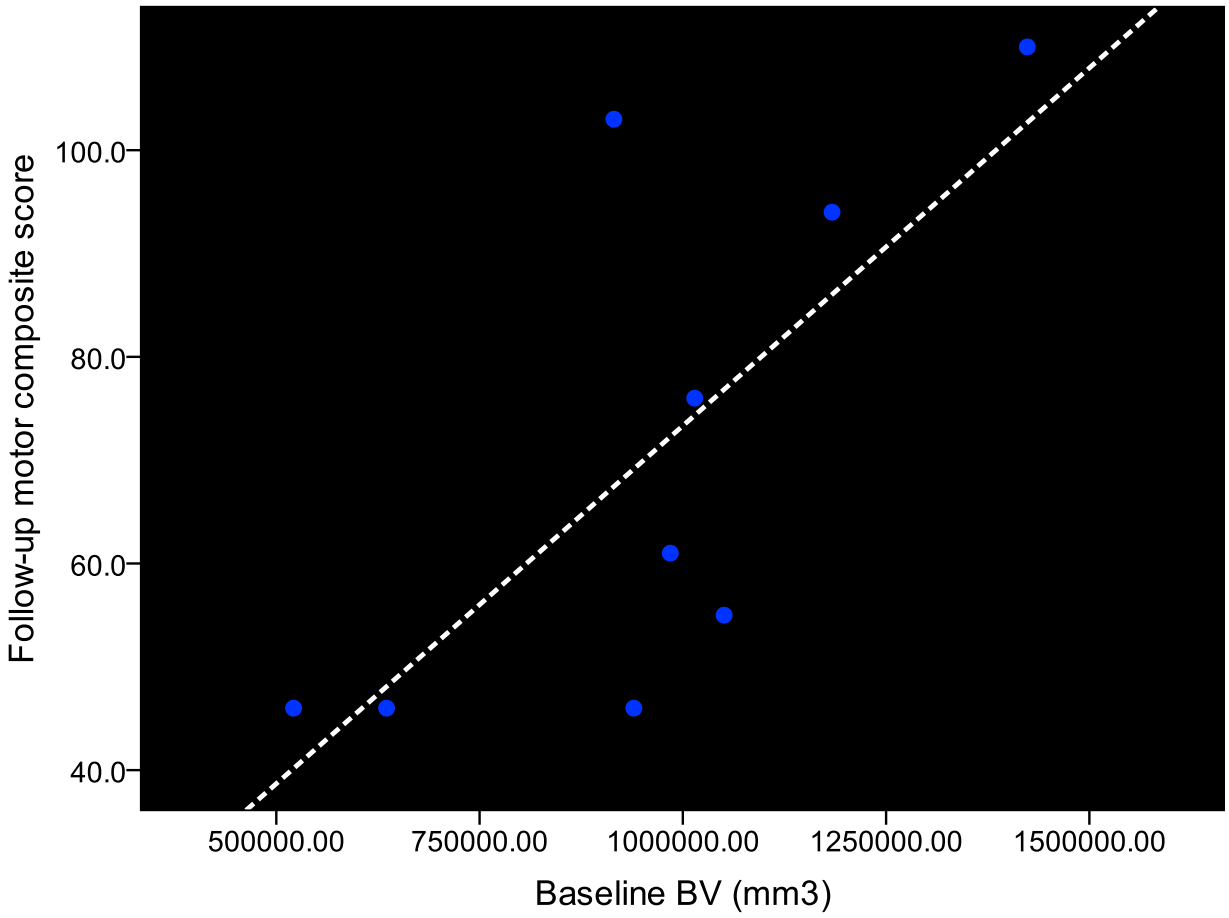
5.3.2.5. Specific contribution of the hippocampus to performance a year following CSE

At follow-up, mean HPCR was positively correlated with cognitive performance in the PFS group (r=0.587, p=0.052). No other correlations between mean HPCR and performance measures were obtained. Mean T_2 values showed a trend for an inverse correlation with cognition (r=-0.471, p=0.056) and were correlated with language (r=-0.514, p=0.035) in the non-PFS group.

5.3.3. Exploration of MR predictors of later outcome

Baseline BV and HPCR were predictive of motor development at follow up in the non-PFS group (see Figure 5.3).

Figure. 5.3 The Relationship between BV and motor abilities in the non-PFS group



5.4 Discussion

In the present chapter we have interrogated structure function relationships in a paediatric cohort soon after CSE and a year onwards. Hippocampal integrity in this cohort has been shown to be predictive of performance in all developmental domains as evinced by several MR measures. The same was true of BV which was shown to be positively correlated with performance after correcting for age. This relationship was particularly evident in the non-PFS group. Mean FA at baseline was found to be predictive of motor performance in the PFS group at the baseline but not the follow-up assessment. This finding is interesting given earlier suggestions that motor performance may have been transiently influenced by the seizure.

A preliminary analysis revealed no differences between the three groups in any of the MR measures at baseline with the sole exception of mean T_2 values, which were found to be higher in the non-PFS group when compared to the PFS and the control groups. The absence of differences between the mean hippocampal volumes of the three groups is not surprising given previous reports in the literature in patients imaged 4 to 8 months following a PFS who revealed no volumetric differences to normal controls (Scott et al., 2003). The current patients were imaged approximately 1 month post CSE and its possible that any oedema, which may have been responsible for enlarged PFS hippocampi in patients imaged within 5 days of status epilepticus, had subsided.

At their one year follow-up, the non-PFS group revealed smaller BVs than the PFS groups and a trend for elevated T_2 values. A previous study investigating hippocampal T_2 relaxation times in children imaged within 5 days of a PFS discovered that these were significantly elevated relative to controls when imaging was conducted within 2 days of the initial event (Scott et al., 2002). However, when PFS patients were imaged 3 to 5 days after the event they didn't reveal elevated T_2 values relative to controls. The afebrile CSE patients investigated in the same study, however, revealed

elevated T_2 values relative to normal controls irrespective of when they were imaged. This finding led the authors to postulate that the elevated T2 values observed in the PFS group were a result of oedema whereas the elevated T2 values in the afebrile CSE group were a reflection of pre-existing brain abnormalities.

The current findings extend these results by demonstrating that approximately a month following CSE the PFS patients still show normal T_2 values. The non-PFS group, on the other hand, have elevated T_2 relaxation times compared to both groups. This finding confirms the suspicion that elevated T_2 values may be a result of pre-existing abnormalities in this group. It is also possible that ongoing epileptic activity may be responsible for the observed elevated T_2 values. However, previous studies have established no association between seizure related variables and T_2 relaxation times making this an unlikely alternative (Grunewald, Jackson, Connelly, & Duncan, 1994). The finding that the non-PFS group is still manifesting a trend for elevated T_2 values relative to the PFS group at their one year follow-up attests to the effect of pre-existing abnormalities on their T_2 relaxation times rather than an effect of the seizure itself as does the finding that the non-PFS group have smaller BVs than PFS patients .

Both groups in our study revealed age dependencies in BVs and hippocampal volumes. This observation confirms previous findings in the literature in both normally developing children (Groeschel, Vollmer, King, & Connelly, 2010; Giedd et al., 1996) and pediatric cohorts following CSE (e.g. Scott et al., 2002, 2003, 2006). Moreover, the repeated measures analysis in the current paediatric cohort revealed growth in hippocampal volumes and BVs in both patient groups. These findings have also been confirmed in a bigger sample of the current CSE patients including controls and will be presented in the American Epilepsy Society meeting in December 2010 (Yoong et al., unpublished). Therefore, CSE does not seem to arrest the development of these structures in our

paediatric cohort. Mean T2 values were also shown to be age dependent and decrease from baseline to follow-up. Previous studies with fewer patients following CSE have revealed an age dependency in T2 relaxation times and a decrease of these values through time at least in PFS patients (Scott et al., 2002, 2003).

Thirteen out of the seventeen significant uncovered associations between MR measures and developing functions in this chapter were observed in the non-PFS group. This makes sense given the bigger variability in performance expressed within this group and the range of etiologies associated with the occurrence of CSE. In this group, BV was revealed to be an independent predictor of performance for all tested domains, though not at all time points. BV as measured during our first assessment was found to be predictive of motor abilities a year onwards alluding to its potential use as a biomarker for later neurodevelopment in this group.

The mean HPCR was also revealed to be positively correlated with language abilities at the baseline assessment in the non-PFS group. This correlation was strongest for the left hippocampal ratio, a finding that fits well with the known predilection of language faculties for the left hemisphere. The role of the hippocampus in language development is slowly starting to emerge in the literature (De long & Heinz, 1997, Schumann et al., 2007). Recent studies have established positive correlations between hippocampal size and VIQ in healthy developing males between the ages of 8 and 18 years of age (Schumann et al., 2007). Moreover, a clinical observational study of four patients with afebrile CSE and confirmed circumscribed bilateral hippocampal sclerosis described the lack of development of language abilities in two of their patients and the loss of such faculties in the other two (De Long & Heinz, 1997). These authors reported adequate motor and sensory functions in their patients and considered the language effect to be an isolated one, ascribed to the presence of bilateral hippocampal sclerosis in these patients. However, the patients in this study neither were assessed

using standardized measures nor were correlational analyses conducted between MR measures and outcome. In contrast, in the current study we have shown that non-PFS patients with bigger left hippocampal ratios perform better than patients with smaller left hippocampal ratios on standardized language assessments.

Mean T_2 values were also found to be inversely correlated with language faculties in our non-PFS cohort at follow-up. The same finding was observed for cognitive follow-up scores which were shown to be inversely correlated to T_2 relaxation times. Therefore, akin to the Dubé study, elevated T_2 values were associated with impaired performance in the non-PFS group. However, contrary to the Dubé study (2009), baseline T_2 values were not predictive of follow-up performance making them less useful as potential biomarkers of future development. What is slightly surprising, however, is that baseline T_2 values were not correlated with any baseline neuropsychological measures. Moreover, children following PFS, i.e. the phenomenon being modeled in the Dubé study (2009), didn't manifest any correlations between T_2 values and neuropsychological performance. Given previous findings it is plausible that such correlations may have been uncovered if we had imaged the children within 48 hours of CSE. However, in the Dubé study (2009), rats were imaged 1 month following the seizure which makes the above explanation less likely. The other possibility is that the PFS children may make evident correlations between baseline T_2 relaxation times and cognitive indices if tested as adults, as was the case in the Dubé study (2009). Alternatively, hippocampal T_2 values in the PFS group may be more attuned to selective memory deficits and this hypothesis will be addressed in Chapter 6 where we have tested PFS children on a recognition memory paradigm.

Mean FA values were found to predict motor abilities in the PFS group close to the time of incident. This is of particular interest given the findings in the previous chapter which suggested a transient effect of CSE on motor performance. FA is a measure of the directionality of water diffusion and is

thought to be an indicator of loss of structural organization, expansion of the extracellular space or increased permeability of membranes (Wieshmann et al., 1999a; Wieshmann, 2003). Stone et al. (2008) have shown that following a hypoxic-ischemic episode in rats, the initial FA decreases observed in the fimbria are superseded by FA increases after a number of days pointing to a recovery process. This is potentially what we are seeing here, with a subgroup of PFS children showing decreases in FA which are correlated with impaired motor performance at baseline and a year onwards discovering no such association because FA has recovered. An alternative explanation would be that a subset of PFS children had pre-existing FA decreases which are responsible for seizure and neuropsychological performance alike. However, that would not explain why at follow-up no association between the motor performance and mean FA was observed. A bigger control group which would allow the systematic exploration of FA values in our patient groups would help shed more light on this matter.

At their one year assessment the mean hippocampal ratio was found to be predictive of cognition and BV was found to be predictive of language performance in the PFS group. The latter result is of special note given findings in previous chapters regarding the interrelationship between age and language. Therefore, it becomes plausible that some of the correlations obtained in previous chapters between age and language, and in particular, the correlation obtained in our follow-up chapter may have been driven by differences in BV. This finding highlights the methodological urgency of investigating structure function relationships to avoid the misattribution of observed effects.

The relationship obtained between mean HPCR and cognition at follow-up is not surprising given previous reports of such correlations in premature infants assessed on earlier editions of the Bayley Scales of Infant Development (Thompson et al., 2008; Lodygensky et al., 2008). The lack of a similar

relationship at baseline is, however, puzzling. Two alternatives may be considered here. One is the possibility that a year onwards some PFS children have not shown commensurate hippocampal growth relative to their BV growth and this is reflected in their cognition scores. The other alternative would be that the follow-up sample is made-up of individuals who make this relationship evident whereas at baseline this relationship was obscured by the lack of it in the other individuals that made up the group.

One outstanding question is whether the correlations obtained in this study are a result of isolated hippocampal dysfunction or also a reflection of extra-hippocampal abnormalities. Animal studies have shown that following status epilepticus areas outside the hippocampus such as the piriform and the parietal cortices as well as the thalamus are also affected(Choy et al., 2010). Moreover, recent models of hypoxia-ischemia have shown how neurodegeneration follows a systems coherent pathway with injuries sourced at the hippocampus spreading outwards from this structure in a time delayed fashion (Stone et al., 2008). Evidence that more areas may be involved in the neuropsychological impairments witnessed in the non-PFS group comes from the finding that BV strongly influences performance in this group. However, to further address these issues whole brain voxel-based techniques need to be applied and have been scheduled following the termination of follow-up assessments.

6.1 Introduction

The aim of the present chapter is to investigate visual recognition memory processes in children following CSE. To my knowledge, this is the first study to look at recognition memory processes in this paediatric population shortly after their incident. Moreover, this is the first study to use the visual paired comparison (VPC) task, i.e. a task specifically designed to query recognition memory development across the lifespan (Rose, Feldman, & Jankowski, 2001; Rose & Feldman, 1997a; Rose, Feldman, & Wallace, 1992; Rose & Wallace, 1985). The investigation of recognition memory processes in children following CSE is particularly important given the association between PFS and TLE. Numerous studies have established the presence and material specificity of recognition memory deficits in children with TLE (Mabbott & Smith, 2003; Nolan et al., 2004), which makes the early exploration of these processes in a population believed to be "at risk" a pressing issue.

Memory processes in children following febrile seizures have been scarcely studied. This is quite surprising given the importance of the hippocampal formation in the association between febrile seizures and TLE. One of the few exceptions is a recent study looking at working memory processes in 87 children following febrile seizures (Chang et al., 2001). These authors quite remarkably discovered that working memory processes were superior in the FS group relative to their normally developing peers. Nonetheless, an onset of seizures prior to the age of 1 was found to be a significant risk factor for memory problems later on, including problems in delayed recognition. While these may be interesting results in their own right, working memory processes *per se* do not traditionally engage the hippocampus, and therefore, do not constitute the best avenue for exploring the functionality of this structure. What's more, only 10% of individuals in this cohort had

experienced a PFS which are the types of seizures that have been linked with TLE (Baram & Shinnar, 2001).

A more recent study specifically investigating episodic memory processes and recognition memory following febrile seizures discovered a different ERP pattern in the FS group compared to a control group in the absence of any behavioural differences (Kipp et al., 2010). Namely, the FS group was found to evince an ERP pattern suggestive of ongoing familiarity-based processes whereas the control group was found to evince an ERP pattern suggestive of recollection-based processes during the performance of the same recognition memory task. The absence of any behavioural based differences between the groups led the authors to suggest that the FS group is compensating for a deficit in traditional recall mechanisms by resorting to familiarity based mechanisms. The same study did not detect any differences in volumetric hippocampal measurements between the two groups suggesting that simple febrile seizures do not result in gross structural deficiencies. Correlations between left and right hippocampal volumes and performance on the behavioural recognition memory task were observed in this study when hippocampal volumes were collapsed together for both groups.

No prior studies of CSE have investigated memory using the VPC task, even though it has been used extensively to study the normal development of recognition memory in infants and animals and has been shown to rely on the hippocampus. This task capitalizes on the natural proclivity of infants and certain animals to orient themselves to novel stimuli in their environment. Introducing a delay between the familiarization and the test phases transforms the paradigm into a recognition memory one. Infants as young as 3 days old have been shown to orient to the novel stimulus following a 2 minute retention period (Pascalis & de Schonen, 1994). As infants grow older they are able to withstand longer delays, require shorter familiarization periods and begin to form abstract

representations of the stimuli presented (Rose, Feldman & Jankowski, 2004; Robinson & Pascalis, 2004). Risk factors such as prematurity, specifically, when the latter is associated with hypoxic events, compromise performance on this task, a finding, which has been eloquently demonstrated time and time again in a series of studies conducted by Rose and colleagues (Rose et al., 2001; Rose, Feldman, McCarton, & Wolfson, 1988; Rose & Wallace, 1985; Rose, 1983).

Most importantly, for the purposes of this study, the VPC task has been shown to be very sensitive to the presence of hippocampal damage especially when a delay is introduced between familiarization and test. For example, Y.R., a patient with confined damage to the hippocampus, reveals no impairments in the immediate VPC paradigm but does so when a delay as short as 5 seconds is introduced between familiarization and test (Pascalis et al., 2004). More recently, the VPC task was also used to study recognition memory in a group study of developmental amnesiacs ((Munoz et al., 2010)). These patients have usually suffered a hypoxic episode early on in life resulting in circumscribed hippocampal damage which compromises their declarative memory development (Vargha-Khadem et al., 1997). Namely, developmental amnesic patients grow into severe episodic memory problems in the context of a preserved semantic memory system. Moreover, these patients have been shown to have relatively intact recognition memory processes as evinced by their performance on the Doors and People test (Adlam et al., 2009). Despite the above finding the developmental amnesic patients did reveal impairments relative to normal controls on the VPC task in a delay-dependent manner (Munoz et al., 2010).

Apart from its unique role in the study of memory development the VPC task has also been shown to be of predictive value in terms of later cognitive, language and memory development (Fagan, III, 1990; Rose & Feldman, 1997b; McCall & Carriger, 1993). Namely, many studies have shown that novelty preference (at immediate test) proportions are predictive of later FSIQ, VIQ and

standardized memory measures. One study has even discovered that novelty preferences exhibited at the age of 7 months are able to predict FSIQ and academic achievement at 21 years of age regardless of levels of parental education (Fagan, Hollan & Wheeler, 2007). Moreover, the predictive validity of novelty preference has been also shown to apply to populations at risk such as preterm children (Rose & Feldman, 1995).

In the present study we used the VPC to study recognition memory processes in children following CSE shortly after the event and a year onwards. The children were assessed twice to determine the presence or absence of seizure related effects. A subset of these children (the PFS group) is suspected of hippocampal abnormalities which may or may not be structurally identifiable. Therefore, we hypothesized that children following PFS will perform worse than normal controls in the recognition memory measure but not the immediate novelty preference measure which relies less, if at all, on the presence of functionally intact hippocampi. We also hypothesized that recognition memory performance in this group will correlate with hippocampal volumetric measurements. We expected the non-PFS group to perform worse than controls across the board reflecting our general findings regarding this group.

6.2 Methods

6.2.1. Participants

Children following an episode of CSE and normally developing children within a similar age range were recruited to participate in this study. Patients were assessed twice on the VPC task, i.e. close to the time of incident and approximately a year onwards. On the same visit, all participants were also assessed on standardized neuropsychological tests and underwent detailed MRI investigations. Control children were only seen once, and, as it is unethical to sedate young children for MRI

investigations in the absence of clinical concerns, only a subset of the controls tested on the VPC were able to undergo scanning.

6.2.2. Stimuli

Two sets of stimuli depicting female faces were developed for the VPC task. Half of the children were tested on set A first, and, then set B, on their follow up visit, and, vice versa for the remaining half. This was done to avoid any trace of recollection for the faces (especially in older children) in the follow up visit. The actual size of the images was 19 centimetres in width and 22 centimetres in height. No articles of clothing appeared in the images and the images were obtained under standard lighting conditions. The images were not further matched on low visual perceptual characteristics as this was not the primary question being asked in this study.

6.2.3. Experimental procedure

Stimuli were presented on a flat screen located within a dark cubicle to minimize peripheral visual distractions. Children viewed the stimuli from a chair placed approximately 60 cm from the display screen. From this position the child's visual angle was calculated to be 23.5 degrees. However, given that we were dealing with infants it was not always possible to maintain a 60 cm distance throughout the experiment. Depending on their age the children either sat on their caregiver's lap or on their own. A digital camera positioned on top of the screen recorded their eye movements throughout the experiment. The output from the camera was visible to the experimenter online on their control screen. Parents were instructed to ensure that children were looking centrally at the initiation of each trial but to allow them to gaze freely following that. Following the novelty preference and the familiarization trials described in detail below, a 5 minute break ensued during which children were

allowed to play freely with a toy of their choice prior to the delayed memory test. The whole experiment lasted approximately 8 minutes.

6.2.3.1. Novelty preference task

In the first stage of the VPC task subjects were tested to determine whether they are able to show a novelty preference in the context of minimal memory demands. The novelty preference task consisted of twelve 10-second trials during which familiarisation trials alternated with test trials. A prior study using the same procedure showed that normal 1- and 3-month-olds are able to show a novelty preference in this time frame (de Haan et al., 2001), thus we expected to be able to detect such preferences if present even in the youngest infants tested. Trial length was set to 10 seconds per presentation as this trial length is commonly used in VPC studies. At the beginning of each trial a cartoon character was shown centrally on the screen to attract the child's attention. Trials were initiated once it was determined that the child was looking centrally. Six trials in the novelty preference task consisted of the presentation of an identical face placed left and right of the fixation point. The remaining six displayed the original face presented alongside a trial-unique novel face. Position of the novel and the familiar face were counterbalanced across trials. The presentation of the familiarisation and test trials was alternated, i.e. the identical pair was followed by the novel/familiar pair followed by the identical pair and so on. This permitted us to determine how long each participant needs to inspect a stimulus before they showed a novelty preference.

6.2.3.2. Recognition Memory task

In the second stage of the VPC task participants were familiarized to a single face with the aim of determining whether a single face can be remembered over a 5 minute delay. For learning, the child was exposed to five 10 second trials. On each trial, the to-be-learned face was presented to the left

and right of fixation. Following a 5 minute delay, the subjects were shown the to-be-remembered face with a novel face for two 10-second trials counterbalancing for the left/right position of the novel/familiar face. The identity of the familiar and novel face was counterbalanced across participants i.e., Face A was the familiar face and Face B the novel face for half of the participants, and the opposite was true for the other half.

6.2.4. Coding of the VPC

Two independent researchers coded the participants' performance on the VPC task. Namely, half of the VPC recordings were coded by an investigator blind to patient diagnosis (M.D.) and the other half by the current author (M.M). It must be noted, nonetheless, that while coding both experimenters were blind with regards to the positioning of the novel face (i.e. left or right of fixation point) in the novelty preference and recognition memory trials. The subjects' were judged to be looking left or right of the fixation point according to the corneal reflection of the images in their eyes. Trials where the child became increasingly fussy or gaze direction was obstructed by external factors such as the reflection of light on eyeglasses were excluded from further analysis.

6.2.5. Dependent variables derived from the VPC task

For each novelty preference trial, the proportion of novelty preference was computed by dividing looking time to the novel face by the total looking time for the trial (i.e. attendance to both the novel and the familiar face). Averaging proportion of novelty preference across both test trials in the recognition memory paradigm provided an overall novelty preference proportion (novpref-test). We also used the novelty preference proportion on the first memory trial (novpref-first) as this is the first time the child is faced with the novel face following the delay and may be a more sensitive measure of recognition memory than that obtained from combining the test trials. For the

familiarization trials in the no-delay paradigm, the proportion of looking to the to-be-familiarized face was calculated by dividing the time spent looking at the faces by the total trial time (i.e. 10 seconds). Total familiarization time in the familiarization paradigm and total looking time in the recognition memory paradigm were also calculated and used as dependent variables in our group analysis.

6.2.6. Dependent variables derived from the neuropsychological and the MRI assessments

Given suggestions in the literature regarding the association between FSIQ, language measures, and novelty preference also we investigated these relationships in our current study. Cognition and language were measured with the Bayley Scales of Infant Development (3rd edition) in children aged less than 42 months and the WPPSI or WISC in children aged more than 42 months. Eight children from the non-PFS group were also tested on the Children's Memory Scale. Correlations between general memory, visual memory (immediate and delayed), attention and performance on the VPC task were explored. Moreover, we also looked at the relationship between performance on this task and the hippocampally based MRI measures described in Chapter 5. This analysis included hippocampal FA and MD measurements. Given the predictive value of BV, which was highlighted in Chapter 5, the relationship between BV and performance was also investigated.

6.2.7. Statistical analysis

One sample t-tests were used to determine attendance to novelty against the chance level of 0.50. Consistently being found to achieve scores above this benchmark signifies attendance to novelty whereas the opposite signifies attendance to the familiar face. No difference to chance level signals that there is no preference for either the novel or the familiar face on the part of the participant. MANOVAs were used to compare the performance of the three groups on all the dependent

variables derived in the VPC task. Given that our main hypothesis concerns the performance of the PFS group against that of controls, separate MANOVAS were run to look at the isolated performance of these two groups. Pearson's or Spearman's tests were used to investigate correlations between performance, neuropsychological and MR measures. For the baseline data we also looked at whether time from seizure had any effect on VPC performance.

6.3 Results

6.3.1. Baseline Results

6.3.1.1. Description of the patient sample tested at baseline

Eighty five children were tested on the VPC task of which 25 comprised the PFS group, 34 comprised the non-PFS group, and, 26 children comprised the normal control group (see *Table 6.1*). The patients were seen a mean of 43 days (range: 7-254 days) following CSE. The three groups were found to be different in age with the non-PFS group being significantly older than the PFS ($p<0.001$) and the control ($p=0.012$) groups. The three groups were also found to be different in gender with the PFS group containing a higher female to male ratio than the other two groups ($p=0.050$). Mirroring our findings from the developmental assessments the controls achieved higher cognitive scores than the two patient groups. No differences between the three groups were obtained in any of the MR measurements assessed in this study (see *Table 6.2*).

6.3.1.2. Are the groups able to show novelty preference without a delay?

Table 6.1 contains the means and standard errors for the total familiarization and the total novelty preference proportions exhibited by each group during the novelty preference task. One sample t-tests revealed that all groups showed a mean novelty preference when virtually no delay was imposed between familiarization and test ($p<0.001$).

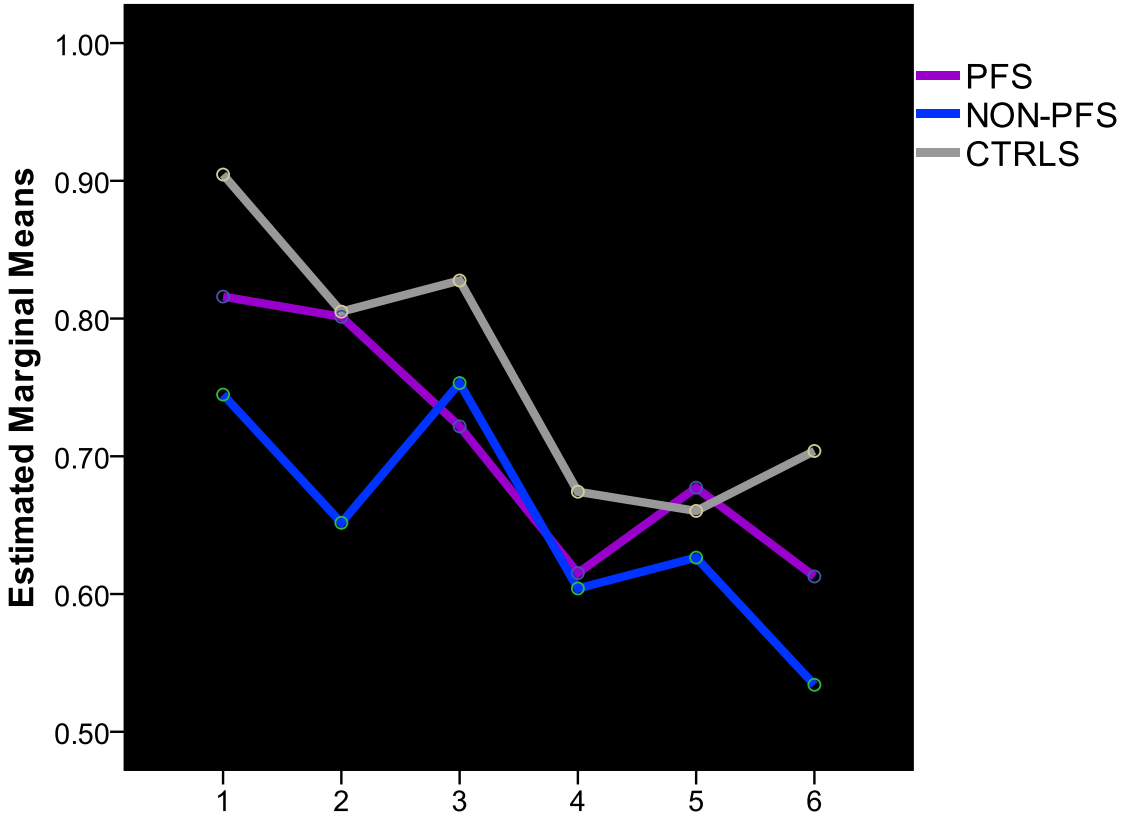
Table 6.1 The performance of the three groups on the VPC task at baseline

	PFS	Non-PFS	Controls
N	25	34	26
Male	8 (32%)	22 (64.71%)	14(66.7%)
Age at test (months)	23.4 (2.46)	56.74 (8.03)	32.15(3.18)
Days from CSE	48.48 (9.89)	39.12 (3.75)	Not applicable
Mean familiarization proportion (immediate)	0.70 (0.03)	0.66 (0.03)	0.76 (0.03)
Mean novelty proportion (immediate)	0.61 (0.02)	0.62 (0.02)	0.59 (0.02)
Mean familiarization looking time (delayed)	28.18 (1.78)	30.31 (1.87)	31.74 (1.69)
Mean looking time during test (sec)	11.54 (1.08)	12.59 (0.74)	12.75 (0.79)
Mean novelty preference proportion (test)	0.48 (0.02)	0.51 (0.03)	0.56 (0.02)
Mean novelty preference proportion on 1st trial (test)	0.44 (0.04)	0.51 (0.03)	0.59 (0.04)
Cognition	96 (2.95)	82.48 (4.23)	109.42 (3.49)
Language	95. 22 (3.19)	82.93 (4.03)	115.33 (18.47)

A repeated measures analysis with the proportion of time attending to the familiar face during the 6 familiarization trials and group as a between subjects factor revealed a main effect of familiarization ($F(5,74) = 6.589$, $p < 0.001$) establishing that all children were spending less and less time looking at the familiar face during the familiarization trials (see Figure 6.1). No interactions were revealed between age, group and familiarization patterns suggesting that all children reduced the time spent

looking at the face displayed from trial to trial to the same extent regardless of age and group. The between subjects' test revealed that age and group had a significant effect on overall familiarization time. Pairwise comparisons revealed that the non-PFS group had a lower mean from controls in the overall familiarization proportion across the 6 trials.

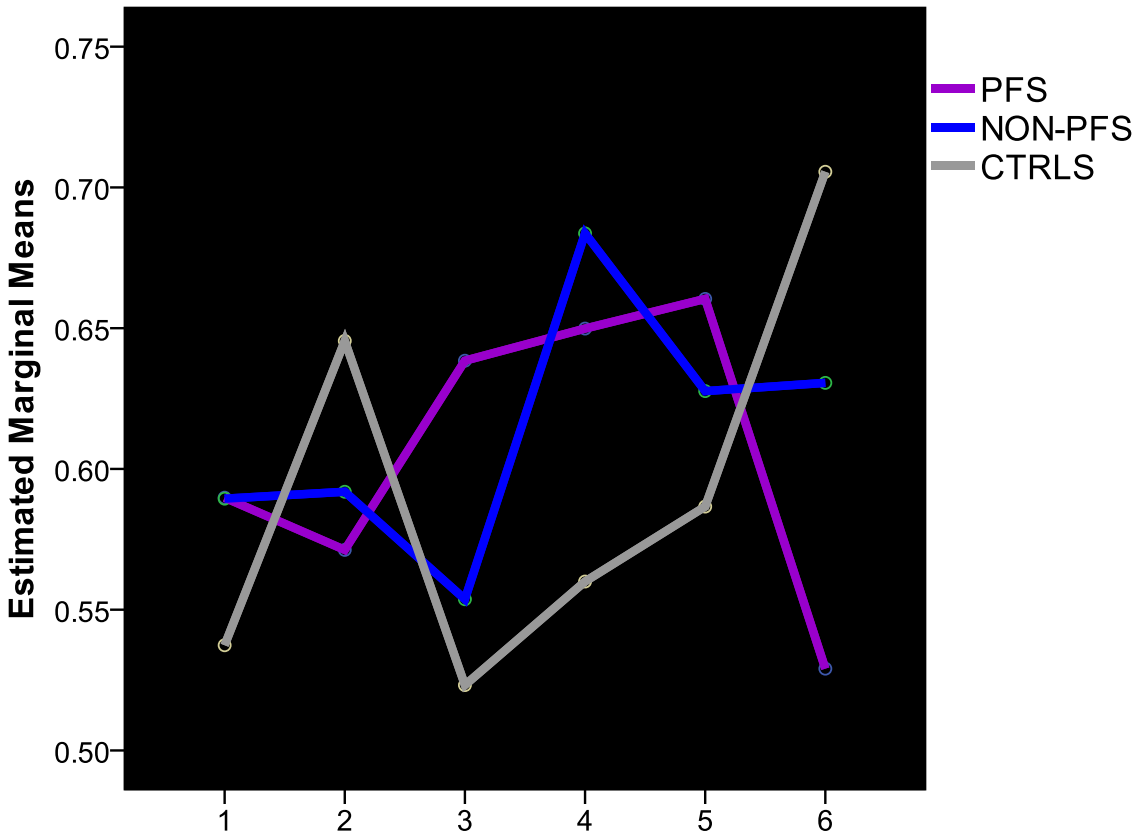
Figure 6.1 Familiarization trials in the immediate condition



A similar repeated measures analysis with novelty preference proportions in the 6 trials as the repeated measures and group as a between subjects factor revealed no main effect of novelty preference suggesting that there was no incremental linear increase in novelty preference across the 6 novelty preference trials (see Figure 6.2). None of the interaction terms were found to be

significant and novelty preference proportions were not found to be affected by age and group membership.

Figure 6.2 Novelty preference trials in the immediate condition



6.3.1.3. Do the groups show familiarization?

The mean and standard error of total familiarization times in the recognition memory task for the three groups are described in *Table 6.1*. A repeated measures analysis with proportion of time attending to the to-be-remembered face during the 5 familiarization trials and group as a between subject's factor revealed a main effect of change in familiarization proportions from trial to trial (F

(4, 76) = 9.036, p<0.001). The interactions between age, group and familiarization trajectories were not found to be significant. Mirroring the familiarization pattern obtained in our novelty preference trials, age was found to have a significant impact on the familiarization proportion averaged across all trials (F (1, 79) =9.305, p=0.003).

Table 6.2 Mean MRI hippocampal and BV measurements of the 3 groups at baseline

	PFS	NON-PFS	CTRLS
BV	1204709 (31616. 25) (n=25)	1249965 (41250.15) (n=30)	1296353 (40721.07) (n=8)
Mean HPCR	16.54 (0.29)	16.05 (0.43)	15.8 (0.38)
AI	0.01 (0.02) (n=25)	0.06 (0.02) (n=30)	0.02 (0.03) (n=8)
Mean T_2	123.43 (1.09) (n=21)	123.61 (1.82) (n=22)	117.16 (2.08) (n=4)
Mean MD	10215.08 (87.6) (n=21)	10201. 29 (182.94) (n=21)	9713.17 (199.62) (n=5)
Mean FA	1296702 (44482.96) (n=21)	1257881 (56607.66) (n=21)	1223467 (41589.41) (n=5)

6.3.1.4. Do the groups recognize the to-be remembered face following a 5 minute delay?

Table 6.1 contains the means and standard errors for the total looking time across both trials, the proportion of novelty preference in the first test trial and the proportion of attendance to novelty across both trials for the three groups. The one sample t-tests revealed that only the control group

showed a preference for the novel face following the 5 minute delay. This was true of both the first trial (t (25) = 2.367, p=0.026) and, the novelty preference proportion averaged across both test trials (t (25) = 2.860, p=0.008). The two patient groups revealed no preference for the novel face as evidenced both by a lack of recognition in the first test trial and the average of the two test trials.

6.3.1.5. Are the groups different in the VPC task?

The MANOVA conducted to compare the performance of the three groups across all VPC derived dependent variables with age, gender and cognition as covariates revealed a trend for a main effect of age (F (6, 60)= 2.057, p=0.072). Specifically age was found to have an effect on the total familiarization proportion in the no delay paradigm (F (1, 65) = 4.143, p=0.046) as well as the total familiarization time (F (1, 65) = 3.765, p=0.057) and the total looking time in the test trial (F (1, 65) = 6.694, p=0.012). There was a trend for Group to have an effect on the total novelty preference proportion (F (2, 65) = 2.514, p=0.089). Bonferroni post hoc corrections revealed that this was driven by a difference in novelty preference between the non-PFS (M=0.667) and the control group (M=0.576) (p=0.087).

The separate MANOVA comparing the performance of the PFS and the control group revealed a trend for a main effect of cognition (F (6, 40) =2.065, p=0.079). The test of between subjects effects revealed that age was having an effect on total looking time (F (1, 45) = 5.014, p=0.030). Cognition was found to have an effect on total novelty preference proportion in the immediate paradigm (F (1, 45) = 7.091, p=0.011) and total looking time during test trials (F (1, 45) = 5.758, p=0.021). Group was found to have an effect on the novelty proportion averaged across both test trials (F (1, 45) = 6.544, p=0.014) and the first trial novelty proportion (F (1, 45) = 8.054, p=0.007).

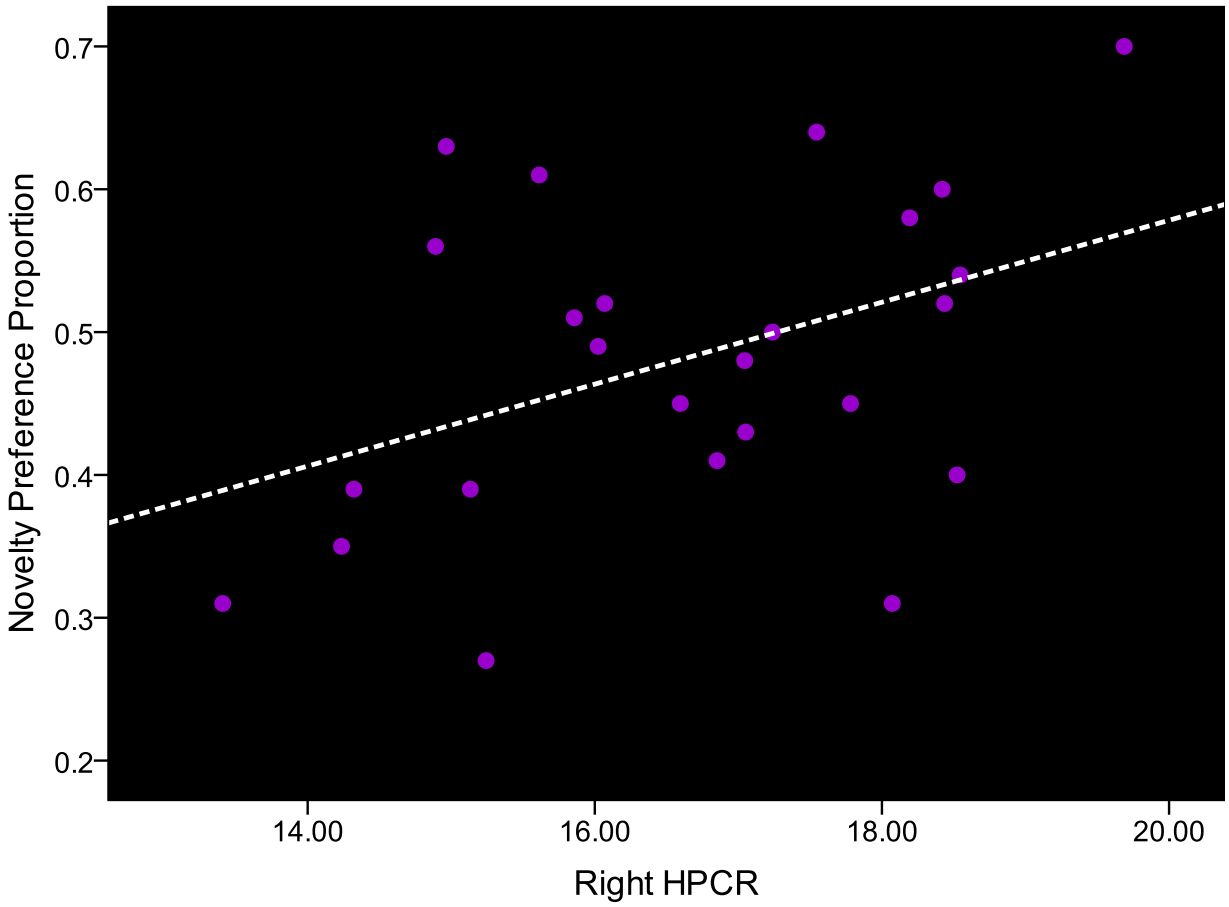
6.3.1.6. Correlative analysis

We observed no correlation between the average proportion of time spent familiarizing with a face and novelty preference in the immediate novelty preference paradigm. Moreover, the novelty preference average obtained in the no delay paradigm was not predictive of the novelty preference proportion evinced in the 5 minute delay paradigm. For the 5 minute delay paradigm, total familiarization time was found to be predictive of novelty preference averaged across both trials in the non-PFS group ($r=0.482$, $p=0.005$), and, novelty preference as evinced in the first test trial in the control group ($r=0.410$, $p=0.037$).

Spearman's Rho correlations revealed that language scores were positively correlated with overall novelty preference in the memory paradigm in the non-PFS group ($r=0.402$, $p=0.034$). None of the memory measures provided by the CMS were correlated with novelty preference as evinced in the no delay and the 5 minute delay paradigm. However, this latter finding only applies to the 8 patients from the non-PFS groups which were tested on both the VPC and the CMS.

No association between days elapsed from CSE and performance on the VPC task was revealed in the two patient groups. In the PFS group alone, mean hippocampal ratio was positively correlated with novelty preference proportion across both memory test trials ($r=0.384$, $p=0.058$). This was shown to be driven by a positive correlation between the right hippocampal ratio and novelty preference ($r=0.418$, $p=0.038$) (see Figure 6.3) as no such correlation was obtained for the left hippocampal ratio and novelty preference ($r=0.278$, $p=0.178$). Controlling for age had no effect on the relationship between the right hippocampal ratio and novelty preference ($r=0.468$, $p=0.021$). There was also a significant partial correlation between BV and novelty preference proportion in the 5 minute delay paradigm controlled for age in the non-PFS group ($r=0.385$, $p=0.043$) and a trend for a positive correlation between BV and novelty preference in the first test trial ($r=0.350$, $p=0.063$).

Figure 6.3 The Relationship between right HPCR and novelty preference following a delay in the PFS group



6.3.2. Follow-up results

6.3.2.1. Description of the patient sample tested at follow-up

Thirty nine patients were seen a mean of 12 months (range: 6 -21 months) following their first assessment (see *Table 6.3*). Twenty one patients had been classified as PFS at baseline and the remaining 18 as non-PFS. The two patient groups were found to be different in age (p=0.032) and gender ratio (p=0.026) with the non-PFS group containing more males and being significantly older than the PFS group. As expected the PFS group was found to be better than the non-PFS group on

cognitive (p=0.033) and language (p=0.056) measures. No other differences between the two groups were observed. *Table 6.4* contains the means for the MR measurements in the two patient groups.

6.3.2.2. Are the groups able to show novelty preference without a delay?

In line with the baseline results, both patient groups revealed a preference for the novel face in the no delay paradigm (p<0.018). A repeated measures analysis with the proportion of time attending to the familiar face during the 6 familiarization trials and group as a between subjects factor revealed a main effect of familiarization (F (5, 31) =3.336, p=0.016) establishing that all children were spending less and less time looking at the familiar face during the familiarization trials. There was also an interaction between group and familiarization (F (5, 31) = 2.673, p=0.040). A similar repeated measures analysis with novelty preference proportions in the 6 trials as the repeated measures and group as a between subjects factor revealed no main effect of novelty preference suggesting that there was no incremental linear increase in novelty preference across the 6 novelty preference trials. None of the interaction terms were found to be significant and novelty preference proportions were not found to be affected by age and group membership.

6.3.2.3. Do the groups show familiarization?

The mean and standard error of total familiarization times for the two groups are described in *Table 6.3*. A repeated measures analysis with proportion of time attending to the to-be-remembered face during the 5 familiarization trials and group as a between subject's factor revealed a main effect of change in familiarization proportions from trial to trial (F (4, 32) = 3.397, p=0.020). The test of within subjects contrasts revealed a linear relationship between trial and mean familiarization time (F (1, 35) = 10.576, p=0.003). The interactions between age, group and familiarization trajectories were not found to be significant.

Table 6.3 The performance of the two patient groups on the VPC task at follow-up

	PFS	Non-PFS
N	21	18
Male	6 (29%)	12 (66.7%)
Age at test (months)	34.52 (2.41)	56.5 (9.18)
Time from 1st assessment (months)	12.24 (0.72)	11.22 (0.51)
Mean familiarization proportion (immediate)	0.65 (0.03)	0.68 (0.04)
Mean novelty proportion (immediate)	0.64 (0.02)	0.57 (0.028)
Mean familiarization looking time (delayed)	28.03 (1.73)	29.92 (2.32)
Mean looking time during test (sec)	12.29 (0.95)	11.17 (1.1)
Mean novelty preference proportion (test)	0.49 (0.02)	0.53 (0.04)
Mean novelty preference proportion on 1st trial (test)	0.59 (0.04)	0.53 (0.070)
Cognition	95.05 (2.83)	82.94 (4.88)
Language	94.76 (4.63)	81.63 (4.71)

6.3.2.4. Do the groups recognize the to-be remembered face following a 5 minute delay?

The non-PFS group doesn't show any sign of recognition for the to-be-remembered face either via preference for the novel face on the first trial (p=0.653) or via preference for the novel face as averaged for both test trials (p=0.533). The PFS group does show a preference for the novel face on

the first memory trial (t (19) = 2.087, p=0.051) but not on the overall novelty preference proportion (p=0.610). A repeated measures analysis looking at changes in novelty preference from baseline to follow-up revealed no main effects for any of the two measures suggesting that the individuals from the patient groups that were seen on both occasions did not change from time point 1 to time point 2.

6.3.2.5. Are the groups different in the VPC task?

The MANOVA conducted to compare the performance of the three groups across all VPC derived dependent variables with age, gender and cognition as covariates revealed a main effect of age (F (6, 43)= 2.646, p=0.028) and group (F (12, 88) =2.330, p=0.012). Specifically age was found to have an effect on the total familiarization proportion in the no delay paradigm (F (1, 48) = 6.038, p=0.018) as well as novelty percentage on the first memory trial (F (1, 48) = 10.521, p=0.002) and the total looking time in the test trial (F (1, 48) = 8.007, p=0.007). Cognition was found to have an effect on the novelty preference proportion evinced on the first memory trial (F (1, 48) = 4.153, p=0.024). Group was found to have an effect on all VPC derived measures apart from total familiarization time in the familiarization paradigm and total looking time in the memory paradigm. Bonferroni post hoc corrections revealed that the PFS group had a higher novelty preference proportion from controls (p=0.039) in the immediate condition. There was also a trend for a difference between the two in the average novelty proportion in the memory trials with the control group obtaining a higher average than the PFS group (p=0.099). The non-PFS group was revealed to obtain lower means from controls in total familiarization time in the no-delay paradigm (p=0.052) as well as in the overall novelty preference proportion (p=0.058) and the first trial novelty proportion (p=0.029) in the memory trials. The two patient groups were found to be different on the novelty preference

proportion exhibited in the first memory trial with the PFS group obtaining a higher average (p=0.037).

Table 6.4 Mean MRI hippocampal and BV measurements of the 2 patient groups at follow-up

	PFS	NON-PFS
BV	1334197 (53977.27) (n=10)	1182311 (70526.25) (n=11)
Mean HPCR	16.83 (0.46) (n=10)	17.09 (0.77) (n=11)
AI	0.06 (0.02) (n=14)	0.10 (0.04) (n=12)
Mean T_2	120.81 (1.30) (n=14)	122.99 (2.02) (n=12)
Mean MD	12822.14 (3284.2) (n=7)	9901.1 (138.8) (n=9)
Mean FA	1191005 (25795.33) (n=7)	1221852 (45046.29) (n=9)

The separate MANOVA comparing the performance of the PFS and the control group revealed a trend for a main effect of group (F (6, 34) =2.202, p=0.067). Namely, group was found to have an effect on average familiarization (p=0.011) and novelty preference (p=0.023) proportions evinced in the no delay paradigm. These differences were driven by a higher familiarization average in the

control group and a higher novelty preference average in the PFS group. No other differences between the two groups were obtained.

6.3.2.6. Correlative analysis

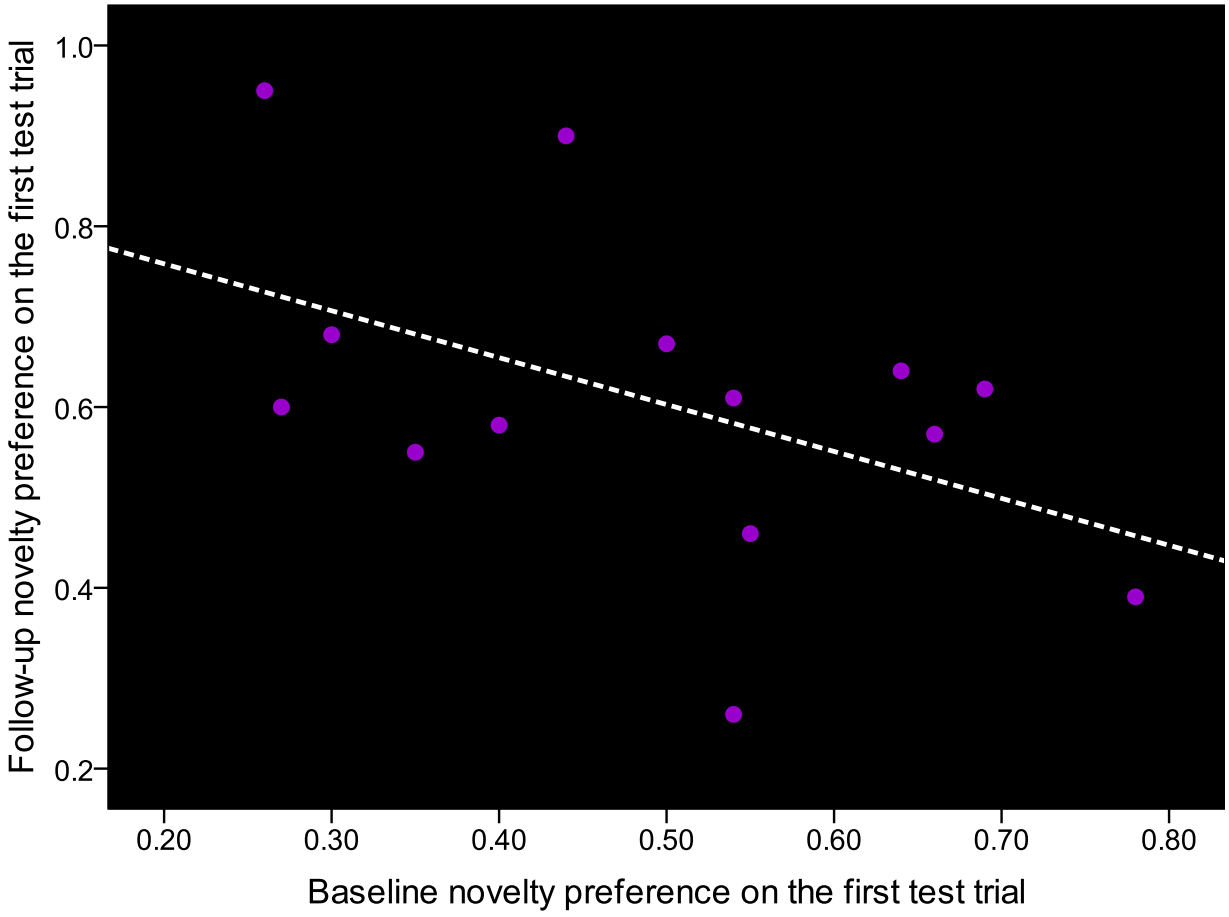
We observed no correlation between the average proportion of time spent familiarizing with a face and novelty preference in the immediate novelty preference paradigm. Moreover, the novelty preference average obtained in the no delay paradigm was not predictive of the novelty preference proportion evinced in the 5 minute delay paradigm. Moreover, we observed no association between total familiarization time and recognition memory measures in any of the groups. Interestingly, partial correlations controlling for age revealed that novelty preference on the first memory trial at baseline was negatively correlated with novelty preference on the first memory trial at follow up in the PFS group ($r=-0.717$, $p=0.006$) (see Figure 6.4) and showed a similar trend in the non-PFS group ($r=-0.664$, $P=0.073$). No other correlations between baseline and follow-up VPC measures were found to be significant.

Spearman's Rho correlations revealed a trend for a positive association between language scores and overall novelty preference in the memory paradigm in the PFS group ($r=0.456$, $p=0.076$). Novelty preference in the no delay and the delay paradigm at baseline was not found to be predictive of cognition a year onwards as has been suggested by other studies.

At follow-up, we observed correlations between MR measures and performance on the VPC task in the PFS group only. Namely, we found that the asymmetry index in this group was positively correlated with the novelty preference on the first novel look ($r=0.661$, $p=0.010$). This relationship persisted even after partially controlling for cognition ($r=0.655$, $p=0.021$). Mean T_2 values were also found to be positively correlated with novelty preference on the first memory trial ($r=0.535$,

p=0.049). After adjusting for age this relationship persisted as a trend (p=0.058). Finally, mean FA values revealed a trend for a negative correlation with recognition memory on the first trial (r=-0.678, p=0.094) and a positive correlation with mean novelty preference in the immediate paradigm(r=0.836, p=0.038). After adjusting for age only the latter relationship persisted as a trend (p=0.069).

Figure 6.4 The relationship between baseline and follow-up novelty preference



6.4 Discussion

This is the first study to prospectively study recognition memory processes in a paediatric cohort following CSE. The design of the current study places it in a unique position in the literature to answer both clinically and theoretically driven questions. Firstly, the current study has shown that a mean of 43 days following PFS, children perform differently from normal controls on a recognition memory test. The difference between the two groups cannot be attributed to an inability of the PFS group to attend to novelty as their novelty preferences are present and indistinguishable from that of controls in the immediate condition. Importantly, novelty preferences in the memory test in this group were found to be correlated with a larger right hippocampal to BV ratio. Secondly, the non-PFS patients also make evident an overall preference for the novel faces in the immediate condition. This is an important finding which suggests that novelty preference is present even in children with a wide spectrum of neurological and neuropsychological impairments. Thirdly, a year onwards the children with PFS show some signs of recognition memory in the form of a novelty preference in the first test trial. Their performance on this trial was found to be positively correlated with a bigger right than left hippocampus attesting to the special role of the right hippocampus in face memory. Finally, baseline novelty preference indices were not predictive of follow-up cognitive scores as has been shown in previous studies. Correlations between language and performance on the recognition memory test were obtained for both groups, albeit, at different time points. In what will follow, I will discuss these findings in turn and attempt to place them within the existing literature landscape.

Baseline Results

In line with our hypothesis, we observed that the PFS group revealed isolated memory impairments relative to normally developing controls. However, contrary to our hypothesis the non-PFS group

also made evident a preference for novelty in the immediate condition which was found to be superior to that of normal controls after controlling for age, gender and cognition differences between the groups. This is surprising given the overall impaired profile of this group and points to the nearly automatic tendency of biological organisms to attend to novelty. The superiority over controls might be due to differences in look patterns which have been described in the literature. For example, shift gazers, i.e. children which have been shown to shift from one picture to the other more frequently, and, short lookers have been found to be more efficient information collectors (Rose and Jankowski, 2004). Therefore, it is possible that control children shifted their gaze more often than the non-PFS children and as a result have lower novelty preference means. Further analysis of the data is required to answer this question.

The absence of a statistical difference between the non-PFS and the control group in their recognition memory performance does not theoretically translate into a superior performance of the non-PFS group in comparison with the PFS group. The novelty preference as indicated by both recognition memory indices was found to be 0.51 in the non-PFS group. This proportion reflects an almost split profile between attendance to the familiar and attendance to the novel face, i.e. no preference at all. The PFS group, on the other hand, obtained a mean of 0.48 for the novelty preference averaged across both trials and 0.44 for the novelty preference on the first trial alone. The PFS group profile is, thus, more in line with a familiarity preference though the one sample t-test did not reach statistical significance. It has been suggested that a familiarity preference represents the insufficient coding of the original stimulus and as such can be classified as a weaker form of recognition memory (Pascalis & de-Haan, 2003).

All groups demonstrated normal familiarization patterns in both paradigms with a decreasing attention to the familiarisation stimulus from trial to trial. Moreover, there were no interactions between group and task pointing to similar familiarization trajectories followed in all groups. However, the non-PFS group was found to spend overall less time looking at the familiarization faces than controls did in the immediate paradigm. This, however, did not seem to have an impact on their overall novelty preference in the immediate paradigm as the non-PFS children were found to achieve the highest novelty preference proportions from the three groups. Interestingly, the three groups did not reveal an upward linear trend in their novelty preferences in the immediate paradigm. This is quite surprising as one would expect that the more the children familiarized themselves with the repeated face the more interested they would become in the novel one. By contrast, the pattern observed in this study is a propensity for novelty preference to drop in the final trials. This could possibly reflect waning interest in the activity at hand which would not be contradicted from the familiarization trajectory.

As anticipated from the current results, the average novelty preference exhibited in the immediate condition was not predictive of novelty preference during the test trials. This was also true of control children who did make evident a novelty preference response following a delay. However, as the two time points probably engage slightly different processes this is not altogether surprising. On the other hand, total familiarization time was found to positively correlate with measures of recognition memory in the non-PFS and the control group suggesting that the more time you spend encoding a stimulus the more likely it becomes to recognize it later.

In the group analysis we carried out, cognition was not found to have an effect on performance. The absence of a correlation was probably driven by the fact that even children with lower cognitive composites/IQs (non-PFS group) were similar with the other two groups on a number of different

measures. However, when we looked at the PFS and the control group separately, cognition was found to affect novelty preference proportions in the immediate paradigm and total looking times during the test trials. Moreover, language scores were found to be positively correlated with novelty preference proportions evinced in the memory trials by the non-PFS group. In the literature, novelty preferences have been repeatedly shown to be predictive of future developmental outcome. The current study adds to the literature by demonstrating that novelty preferences also correlate with current cognitive and language measures highlighting their importance as a tool in studying many facets of development.

However, memory measures derived from the CMS which included visual delayed and visual immediate indices were not found to correlate with performance on the VPC task. This is surprising but not an altogether unexpected result given previous reports of impaired performance on the VPC task in the presence of unimpaired performance on other tasks of recognition memory (Adlam et al., 2009; Kipp et al., 2010;(Munoz et al., 2010)). Previous reports have reported correlations between novelty preferences and memory outcomes a few years onwards (Thompson et al., 1991; Rose & Feldman, 1995). However, none of these studies have looked at the correlation between the two concurrently, something that the current study was able to do because of the wide age range of participants. Unfortunately, the small number of patients (n=8) who undertook both the VPC and the CMS does not facilitate a definitive conclusion regarding this matter.

The relationship between the right hippocampal ratio and the average novelty preference proportion in recognition memory trials observed in the PFS group corroborates previous findings of a material specific dichotomy between the left and right hippocampal roles (Nolan et al. 2004, Mabbot & Smith, 2003). Moreover the current findings suggest that such a dichotomy appears early on during development as the current PFS group was tested at a mean of 23.4 months with ages ranging from

9 to 55 months. Moreover, controlling for age didn't make any difference to the correlation suggesting that this relationship may be evident in younger ages than 23 months. Recently, Zeamer et al. (2010) suggested that the hippocampus is not necessary for unimpaired performance on the VPC task (incorporating delays as long as 2 minutes) prior to the age of 18 months in macaque monkeys. The current study seems to suggest that at delays of 5 minutes the hippocampus is necessary for recognition memory from an early age. Unfortunately given the small sample size and the clinical definition of PFS (children aged older than 6 months) the existence and calculation of a cut-off point is not rendered possible in this study.

The absence of a correlation between hippocampal measures and recognition memory in the other two groups is a bit surprising. In their study, Kipp et al. (2010) observed a relationship between recognition memory and hippocampal volumes when they collapsed these for the FS group and controls. This suggests that they did not observe any correlations when the groups were analysed separately. As the FS group in that study was made up of 13 children and the control group of 14 children possibly the lack of a correlation was due to lack of statistical power. In the current study, volumetric correlations were performed with 25 PFS patients, 29 non-PFS patients and 8 control children. Therefore, it is possible that in the case of the control group the small sample size was responsible for the lack of a correlation. In the case of the non-PFS group the lack of a correlation between hippocampal measures and recognition measures may be related to the positive correlation between BV and these measures. Possibly in this group the greater variability in BV overrides any potential variability in hippocampal volumes and contributes to their memory performance.

Follow-up Results

Akin to the baseline results both patient groups exhibited novelty preferences in the immediate paradigm. However, contrary to the baseline results the PFS group was found to show a preference for the novel face on the first trial following the 5 minute delay. Moreover, PFS patients were no longer different to controls in recognition memory measures. Whereas the repeated measures test did not reveal a main effect for a change in recognition performance from baseline to follow-up in the two groups, partial correlations did demonstrate that the children who were most likely to show a familiarity preference at baseline were the ones most likely to show a novelty preference on the first recognition memory trial. This was especially true of the PFS children who became better from baseline to follow-up at recognizing the familiar face as evidenced by their significant novelty preference. As not many children had been found to show a novelty preference at baseline the above relationship was mostly driven by children who had shown familiarity in the first trial which was converted into a novelty response in the follow-up as can be seen from the figure presented.

This finding extends previous suggestions that familiarity is a weaker form of recognition memory. As such it is not surprising that familiarity preferences can be more easily converted into novelty preferences at follow-up in this cohort. However, the question remains as to what changed from baseline to follow-up which has resulted in a change in performance in the PFS group. One argument would be that the seizure had an effect on performance at baseline which led to weakened representations of the memorandum. The fact that the exact number of days from CSE did not correlate with performance on this task does not make this scenario less likely. It is possible that functional interference may last for a number of days after the seizure without its magnitude being related to the exact number of days elapsed from the seizure.

The other alternative would be that age was a factor in the change in performance witnessed from baseline to follow-up. However, an effect of age was not observed either in the baseline or the

follow-up MANOVA which compared the PFS and the control group making age a more unlikely candidate. Moreover, the non-PFS group did not show a similar difference in performance from the baseline to the follow-up assessment. The latter finding, however, begs the question of whether the absence of amelioration in the non-PFS group renders the argument regarding the effect of the seizure mute. However, the non-PFS group is a non-uniform group with a history of neurological abnormalities and a number of individuals with recurring seizures. Therefore, such factors may have hindered their improvement on the VPC task since CSE.

A final alternative we need to consider would be the possibility of a test-retest effect seeing as the controls were only seen once. However, this is highly unlikely for three reasons. Firstly, this is a task where no verbal instructions are given to the participant as in many cases the participant has not even developed language yet. Therefore, the participant is left to gaze freely and is never given an aim for the task at hand. Therefore, the VPC is not like traditional standardized experiments where practice effects may arise. Secondly, the children were seen approximately a year onwards and as the majority of them were infants it is very unlikely that they even remembered the VPC task. Finally, we made sure that children were tested on a different set of faces at baseline and follow-up to avoid memory for the stimuli in the older children participating in our study.

In the current study we didn't observe any correlations between baseline VPC measures and follow-up cognitive scores. Previous studies have shown that novelty preference indices can predict later IQ. However, previous studies have tended to study very homogeneous groups both in terms of age and clinical profile. The same cannot be argued for the current cohort, which included a large age range and children with different etiological profiles. Moreover, such studies have been designed to answer these questions and therefore may contain more novelty preference problems.

Finally we observed that a bigger right to left hippocampal asymmetry was associated with higher novelty preference proportions on the first memory trial in the PFS group. This confirms our baseline data regarding the special role of the right hippocampus in face recognition memory. We also observed trends for positive correlations between MR measures reflecting hippocampal integrity such as mean FA and mean T_2 relaxation times and the degree of novelty preference evinced at the first memory trial by the PFS group. Such trends would have to be investigated in a larger cohort to determine whether they would persist.

6.5 Conclusions

This is the first study to demonstrate recognition memory deficits in a group of children following CSE. Both the PFS and the non-PFS group were able to demonstrate normal novelty preferences in the context of impaired recognition memory in a task where controls showed evidence of both novelty preference and recognition memory. The PFS group seems to show signs of recognition memory a year following the event pointing to a possible effect of seizure at the baseline assessment, but the non-PFS group doesn't make evident any difference between the two time points. Moreover, the lack of recognition memory may not be attributed to an overall higher difficulty of the memory task as cognition wasn't found to influence recognition memory measures in 3 out of the 4 MANOVAs carried out. Recognition memory measures were correlated with hippocampal measures in the PFS group pointing to the possibility that some children may have subtle hippocampal abnormalities that influence their performance on the VPC task.

Chapter 7 Discussion

The aim of the present thesis has been to investigate the consequences of CSE on neurodevelopmental functions soon after the event and a year onwards. No previous study has looked at the short term effects of CSE (i.e. within a month of the event) using standardized assessments, which places the present study in a unique position to answer clinically and theoretically important questions. In what will follow, I shall try and weave together the results obtained in our four experimental chapters and address our original hypotheses as well as questions that have been raised throughout this thesis. Limitations of the present study will also be introduced and suggestions for future research will be advanced at the end of this section.

7.1. Outcome after Prolonged Febrile Seizures

In the present study, we have shown that children with PFS lag behind normal controls developmentally. Moreover, while there is clear evidence of an improvement in motor, language and recognition memory abilities in their one-year follow-up assessment, deficits are still present in all areas save from motor abilities. Previous reports of impairments in this group during middle childhood raise the possibility that these children will continue to underperform relative to their unaffected peers in the longer term.

Nonetheless, the above findings do not apply to all children following a PFS, with children who are older when they experience a PFS performing on a par with controls in the developmental assessments. This finding suggests that older children may be more resistant to PFS related effects than younger ones. The positive correlation between age and language obtained in the infant population and the all-participants analysis seems to also attest to an increasing resistance to seizure related effects with age. These results go against findings in the animal literature that suggest that the

younger the animal the more resistant it is to functional impairments. However, studies in the human literature have reported that an earlier age of seizure onset is an important factor in the later development of neuropsychological impairments and, therefore, this finding is not altogether surprising (Chang et al., 2001). Moreover, in children with established TLE, an age of onset prior to the age of 1 is a significant predictor of later intellectual dysfunction independent of duration (Cormack et al., 2007).

Notably, an age dependency with performance was not obtained for all the measures assessed in this study. For example, incidental recognition memory performance was compromised in children following PFS regardless of age, which is consistent with findings from developmental amnesia where age at injury is not related to the later expression of episodic memory deficits (Vargha-Khadem et al., 1997). This pattern of results may be related to the presence of differences in *sensitive* periods from one neural system to the next. Sensitive periods are periods during which mechanisms of plasticity can have the largest impact on the developing brain. For example, studies of children with a hearing impairment that are treated for their impairment by the age of six months demonstrate normal language skills at the age of three, whereas children who are treated after the age of six months evince language deficits when tested at the same age (Moeller, Tomblin, Yoshinaga-Itano, Connor, & Jerger, 2007). It may be the case that the functions assessed in this study follow distinct developmental paths which may be affected differently by the presence of a disruptive event such as a CSE during development.

In line with our hypothesis, incidental recognition memory deficits were present in a group of children following PFS. Namely, overall novelty preferences following a delay were lacking in this group close to the event and a year onwards. Nonetheless, these children did make evident a preference for the novel face on the first memory trial in their one year follow-up. Moreover, a

novelty preference was most evident in the children that had revealed a preference for the familiar face in their baseline assessment. This suggests that in some children there is some evidence of recognition memory in the form of a familiarity preference that has been suggested to stem from an incomplete encoding of the memorandum during the familiarization phase. However, children with PFS were not found to be different in total familiarization time from the control group. Two possibilities can be advanced for the presence of a familiarity response in the PFS group; (a) an incomplete encoding of the stimulus given the same amount of familiarization time, (b) a faster decay of the stimulus representation in the PFS group. The latter alternative seems most likely given extensive work in the human and animal literature that has revealed the delay-dependency of visual recognition memory deficits following hippocampal injury (Pascalis et al., 2004).

The effect of PFS on behavioural outcome in our age group is difficult to measure mainly because behaviour has not yet fully manifested itself in a manner that can be easily quantifiable (especially in the younger infants). The infant parental questionnaires employed in this study mainly queried the development of everyday abilities and functions. These questionnaires confirmed our findings for underperformance of the PFS group at both time points. However, we also used the social-emotional questionnaire (SEC) that looks at the infant's capacity to engage with a range of emotions, and, children following PFS were found to be indistinguishable from normal controls in this respect. In the older PFS group, where behaviour was assessed using the strengths and difficulties questionnaire some evidence of behavioural issues emerged, however, the number of completed questionnaires is too small to reach a firm conclusion regarding the behavioural outcome in this group.

What was quite striking, however, was the proportion of parents that reported seeing a behavioural difference in their children that was reported to last a couple of weeks by some. Almost 1 out of 3

parents witnessed a behavioural difference in their child and in sixty percent of cases this consisted of increased irritability. This consensus was arrived to independently by parents as the question was simply: "*Have you noticed any behavioural difference in your child since the seizure?"*. Thus, parents were not guided as to the possible changes expected following a seizure. Other observations included feeding problems, aggression, as well as speech and motor regression. These parental reports suggest that PFS have at the very least a transient effect on behaviour. Moreover, whereas it is quite natural for parents following this event to be worried and very alert to the possibility of behavioural modifications, which in some cases may lead to an overrepresentation of these, the unanimity of their responses makes us confident that they are reporting *real* phenomena.

What cannot be surmised by the above findings is the premorbid intellectual and motor abilities of children prior to their PFS. One possibility would be that these children were performing below normative standards prior to their seizure. According to this theory, their premorbid underperformance would be a sign of a subtle neurological abnormality which subsequently manifested itself as a seizure. Reports of academic underachievement prior to the onset of idiopathic epilepsy in children (Hermann et al., 2006; Berg et al., 2005) seem to support such a view. So, does the finding that children who were judged to be neurologically suspect prior to a febrile seizure were the only children that were associated with intellectual deficits in a population-based US study (Nellson and Ellenberg, 1978). This study is particularly important in this respect for it prospectively assessed children at 4, 8 and 12 months of age for signs of abnormal development. They also used the Bayley Infant Scales of Development (2nd edition) to assess the child at the age of 8 months, however, findings from these assessments were never reported upon. These results would have been very helpful in addressing the current issue.

Two findings in the current study seem to run against the view of a premorbid intellectual dysfunction in this group. The first is the finding that the older group is performing in line with normal controls. If a pre-existing neuro-cognitive impairment were the case we wouldn't expect this result. However, it remains plausible that a subtle neurological abnormality may not always express itself as a premorbid neurocognitive impairment, and when it does, it could be a sign of worse future outcome in this subgroup. Secondly, children following PFS seem to get better from baseline to follow-up on a number of functions. Therefore, the seizure must have had at least a temporary effect on their performance. However, the group is still found to underperform relative to normal peers and normative standards a year onwards, which may signal a return to their pre-morbid levels. Yearly follow-up assessments would be required to further track the progress of this group as the long term effects of PFS on children aged 3-6 is lacking.

In closing it must be added that the deficits uncovered in the PFS group are significant but subtle. The overall means of patients were found to be within one standard deviation below the normative mean. However, there were a number of individuals with means that placed them nearly 2 standard deviations below the mean. It may be that these individuals turn out to be at a higher risk of subsequently developing TLE. Assessing this would require following the children's progress in the next couple of years or re-assessing them after sufficient time has elapsed to allow for the possibility of TLE development. Usually a number of years separate PFS and the full expression of TLE.

7.2. Outcome after non-Prolonged Febrile Seizures

In line with our hypothesis, aetiology was found to be the main determinant of outcome as children classified as non-PFS included a large proportion of children with insults to the CNS (34.8%) which were associated with the worse outcomes in this cohort, lying nearly 3 standard deviations away

from the normative mean. Cryptogenic cases were also found to perform poorly whereas idiopathic cases were found to stand apart from the remaining non-PFS cases with overall means in the mid-90s. What sets apart these two epilepsy syndromes save from the better functional outcome of the idiopathic group is difficult to speculate as no visibly detectable brain abnormalities are present in either of them.

The worst outcome was observed in patients with an acute CNS insult. Interestingly, the cognition of these patients (n=2) was found to be much better at their follow-up assessment suggesting that if an acute insult is treated quickly and adequately a return to normal functioning is possible. This would be particularly pertinent for application in developing countries that have reported high proportions of neurological sequelae following acute CNS insults (Molinero et al., 2009; Sadarangani et al., 2008).

An important but not unexpected finding was the relationship between brain abnormalities and neuropsychological performance in the non-PFS group. Namely, brain abnormalities were found to be predictive of later outcome particularly in infants. In the all participant analysis, where older children were also included, antiepileptic medication also came out as a significant predictor independently of scan abnormalities. The effects of antiepileptic medications on intellectual functioning have been demonstrated time and time again and are pretty well established by now(Meador et al., 2007; Meador, Gevins, Leese, Otoul, & Loring, 2010; Loring & Meador, 2001). The lack of this effect in the infant group is probably due to the fact that they may have been receiving antiepileptic medication for a smaller duration than their older counterparts.

Another finding which merits mention is the finding that the non-PFS children who experienced seizures in between the baseline and the follow-up assessment performed worse than the children

who didn't on cognition and language. Specifically, in the infant group using regression analyses the relationship between the occurrence of seizures and language was found to be independent of medication. This suggests that ongoing seizures during infancy may be disruptive for the normal development of intellectual functioning and, in particular, language processes. Again this differential effect may be related to the time windows during which the presence of seizures may have maximal impact on the building of functions.

In contrast to the PFS group, the non-PFS group as a whole does not improve from the baseline to the follow-up assessment. If anything, they seem to get worse though not significantly worse as evidenced by the lack of a change in the paired sample t-test and the repeated measures analysis. Moreover, age at seizure does not seem to affect performance in this group. Therefore CSE in this group does not seem to affect outcome transiently and its effects don't seem to be related to age. This is possibly because originating aetiology may be masking the effects of CSE.

This interpretation is supported by the fact that parents of children in this group (28%) also report behavioural changes in their children following CSE. Irritability is reported in twenty three percent of non-PFS children and other changes include poor concentration, memory and school performance. It is interesting to note that some of the parental observations include severe changes post CSE such as the loss of the ability to fixate on objects, loss of head control ability, slurred speech, and 45 minute hemiparesis. These are all sequelae that are not present in the PFS group, thus, vouching for the possibility that aetiology is driving these changes rather than the seizure itself. Nonetheless, the finding that duration of seizure in this group influences motor outcomes suggests that CSE is playing a part in the establishment of motor impairments. Previous studies in the literature have also reported a relationship between duration and outcome including motor

impairments, though, no study, to the present day has statistically linked the two(Aicardi & Chevrie, 1970).

An interesting result concerning this group was the expression of novelty preferences in the immediate condition. Therefore, despite their gamut of functional impairments these children are still able to attend to novelty. Nevertheless, this ability has been shown to be present in healthy newborns aged 2-3 days old, therefore, it would be expected that despite their high degree of impairments, children that are on average obtaining composite scores around the 80s might be able to evince novelty preferences.

In sum, in line with our hypothesis aetiology does seem to have a considerable impact on outcome following CSE. This is the first study to use standardized neuropsychological measures to describe performance following CSE which was further stratified into respective diagnostic subgroups. Larger studies are needed to be able to compare the different diagnostic subgroups using formal statistics. This is particularly important in the case of idiopathic epilepsy which seems to stand apart from the remaining non-PFS subgroups.

7.3. Does CSE per se have an effect on performance close to the incident?

There are several lines of evidence that suggest that CSE *per se* may be having an effect on performance. Firstly, parents themselves report seeing a difference in their child following the seizure. Some have reported this change to last up to 2-3 weeks following the incident. As we saw parents around that time (around 3 weeks following the event) we cannot know whether in some cases these findings persisted. In their follow-up assessment, parents were not specifically asked this question and, therefore, the longer term progress of these issues cannot be addressed here.

A second indication that a seizure may be having an effect on performance is that the two unaffected PFS twins tested in this study were found to be better than their sibling on most measures. The superiority of unaffected twins over their affected twins has been reported in a previous study investigating the effects of a history of febrile seizures on intelligence and memory(Schiottz-Christensen & Bruhn, 1973). In that study this finding was obtained a year following the event suggesting that this may not be simply a transient phenomenon but a longer term effect of the seizure. Nonetheless, in both the present study and the Schiottz-Christensen and Bruhn (1973) twin study the lower pre-morbid functioning of the affected twin cannot be excluded as a possibility.

The better performance of the PFS group as a whole in their follow-up assessment also supports an effect of seizure on performance at baseline. Again this finding does not exclude a pre-existing abnormality as PFS children are still lagging behind normal controls at follow-up. It does, however, demonstrate that at the very least the seizure is having a temporary effect on these functions. Finally, the effect of duration on motor abilities in the non-PFS group speaks directly to this issue. However, days elapsed from seizure were not found to be predictive of performance. This may be related to the fact that most children were seen around about the same time following the event and, therefore, there was no big variability in the independent variable.

The above suggestions are in line with the imaging literature which has found evidence for enlarged hippocampal volumes suggestive of oedema in children studied within 72 hours following the event. Another study has found evidence for hippocampal oedema within the first 48 hours, which is no longer present in children scanned past that time point. It is possible, however, that functional dysfunctions may outlast structural dysfunctions for a number of days following CSE which may have triggered a hierarchic cascade of events outside the hippocampus. For example, in a recent

hypoxia model, the authors demonstrated how initial hippocampal injury subsequently spread outside the hippocampus in a systems consistent manner (Stone et al., 2008).

In sum, in this section, it has been argued that transient seizure related effects are evident in our cohort, which are expressed via functional and behavioural impairments. What is less difficult to determine is whether in some cases these effects may be more than transient phenomena and lead to long lasting effects.

7.4. Relationship between TLE and PFS

The bigger aim of the present project is to assess the relationship between PFS and the development of TLE. To this date, no child that we have assessed has developed TLE. This is not surprising seeing as the mean follow-up time in this study was approximately a year. Retrospective studies have reported a dormant period between PFS and the development of TLE that lasts a number of years. Moreover, in a prospective study that tracked the progress of 24 children following PFS for a mean of 12 years, none of their participants developed TLE. Therefore, it is possible that none of our participants will develop TLE. Nonetheless, the current study remains promising for the investigation of this issue since it has conducted detailed neuropsychological and MR investigations at the presentation of PFS. The detailed investigation of hippocampal and brain abnormalities in our paediatric cohort is the subject matter of Dr Michael Yoong's PhD thesis and, thus, questions related to these aspects of development will be addressed within that thesis.

7.5. The relationship of the hippocampus, brain volume and development following CSE

In the current thesis, we also investigated the relationship between measures of hippocampal integrity, brain volume and neuropsychological performance. Corroborating our results regarding

the relationship between brain abnormalities and performance in the non-PFS group, thirteen out of the seventeen significant correlations observed in our cohort concerned this group. Moreover, almost half of these attested to the relationship between brain volume and functions in this group irrespective of age. This suggests that gross MR measures rather than specific loci within the brain may be more predictive of performance in this group. For example, grey and white matter density measures may be more useful as predictors in this group rather than *a priori* regions of interest. Hippocampal measures were also correlated to performance in the non-PFS group, though, it cannot be presently known whether this is an isolated phenomenon or more brain regions are involved in these correlations. Only a Voxel Based Morphometry (VBM) approach which tests the presence of differences between two populations voxel by voxel across the whole brain correcting for multiple comparisons would be able to address these issues.

In the PFS group, 3 out of the 4 correlations observed between performance on the neurodevelopmental assessments and the MR measures were related to hippocampal integrity. More importantly, in the recognition memory task, hippocampal measures attesting to the preferential role of the right hippocampus in facial recognition memory were observed. The observed relationships solely concerned novelty preferences following a delay, i.e. no correlations were obtained between hippocampal measures and immediate novelty preferences. Therefore, this is the first study to have uncovered incidental recognition memory deficits following PFS which were found to be related to the size of the right hippocampus. Seeing as these relationships persisted after correcting for age and given the young age of our cohort we support the view that given a sufficient delay (e.g. 5 minutes) the hippocampus supports incidental recognition memory from an early age.

Finally, an interesting theoretical implication of the above findings is that material specificity, i.e. the differential roles of the left and right hippocampi, emerges early on during development. The finding

that the left hippocampal ratio was predictive of language abilities in the non-PFS group further supports this view.

7.6. Limitations

The present study is subject to a number of limitations. In what will follow I will expose these limitations and explain their implications for our findings. Moreover, I shall suggest future ways to tackle such limitations.

One of the limitations in the current study is that normal controls were only assessed once. In an ideal situation, control children would have also been assessed longitudinally to provide a benchmark for normal growth during development. However, given the young age range in our paediatric cohort this was not feasible, particularly for the MR investigations. Nonetheless, there are several reasons why this may not have influenced our results. Firstly, children are tested on different items on the Bayley assessment at their one year follow-up. Moreover, the same can be said of the VPC results as we included different memoranda at the baseline and the follow-up assessment. As the majority of children were tested using these tools we are not concerned about a practice effect. Secondly, obtaining scores that are worse from controls at the follow-up assessment suggests an even bigger gap if one believes that a practice effect may have taken place. Finally we also compared our findings with the normative standards provided by the neuropsychological tests. Therefore, our results were explored in two different ways and our present findings on the whole consist of an agreement between the two methods employed.

Another limitation of the present study was that a large proportion of children, especially in the PFS group, did not have English as their first language. This limitation is unavoidable as ethnicity does seem to play a role in the presentation of CSE (Chin et al., 2009). However, the majority of our

regression analyses found no effect of language on performance, even if the dependent variable was language performance itself. Moreover, parental questionnaires which were highly correlated with our neuropsychological results corroborated the presence of deficits in this group. Therefore, we are confident that our findings reflect the presence of true impairments that are not attributable to language issues. Finally, both patient groups manifested motor impairments another indication that these occurred independent of language requirements.

Related to the above is the failure to control for maternal education and socioeconomic status (SES) in our cohort, which has been shown by many studies to be important predictors of function. This issue becomes particularly pertinent given a recent study which suggests that SES affects the risk for PFS and an acute symptomatic seizure independent of ethnicity but not other types of childhood CSE (Chin et al., 2009). Therefore, further investigation of this issue using postcodes to determine SES may be called for. This issue could have been tackled by the recruitment of siblings as a control group. However, as discussed previously in this thesis, using siblings as controls brings forth a different set of problems which are related to the genetic resemblance of the two groups.

7.7. Future Directions

The present study has been important in identifying and characterizing impairments in the PFS as well as the non-PFS groups in the shorter term. Nonetheless, after our one year follow-up results we are still left with some unanswered questions particularly with respect to the further development of the PFS group. There is a lack of studies in the literature looking at the effects of CSE in children between the ages of 3 and 6 years old, and, given the results presented within the present thesis this would be an interesting time window to assess. This age range would also allow for the use of standardized memory assessments in this group which could then be related to their recognition

memory profiles. Moreover, the use of other tests that capitalize on spatial memory abilities (e.g. searching within a sandbox for hidden objects with relative positions) could also be used in an older PFS cohort with a view to identify groups at risk of latter episodic memory impairments.

7.8. Conclusion

In closing, the present thesis set out to investigate the effects of CSE on child development. Children with symptomatic CSE were discovered to have the worst outcome amongst the other groups pointing to aetiology as the main determinant of outcome. Nonetheless, contrary to a widely held belief, PFS were not revealed to be completely innocuous for child development. Subtle deficits across the board were obtained in this group including deficits in visual recognition memory independent of cognition which signal the presence of hippocampal dysfunction. The PFS group does reveal some evidence for improvement from our baseline to our follow-up assessment pointing, thus, to an effect of seizure at baseline.

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