The Effect of Fetal Valproate Exposure on Memory Function and Hippocampal Structure in School-Aged Children

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Submitted in fulfilment of the requirements of the degree of

Doctor of Psychology (Clinical Neuropsychology)

School of Psychology and Psychiatry,

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<td>Brain-derived neurotrophic factor</td>
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<td>LTD</td>
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<td>MRI</td>
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<td>mRNA</td>
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<td>Methylene tetrahydrofolate reductase</td>
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DECLARATION

I, Sarah Barton, declare that the Doctor of Psychology (Clinical Neuropsychology) thesis entitled, “The Effect of Fetal Valproate Exposure on Memory Function and Hippocampal Structure in School-Aged Children” contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person, except where due reference is given in the text.

Signature: __________________________

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ABSTRACT

Women with epilepsy are usually advised to continue taking anti-epileptic drugs (AEDs) throughout pregnancy due to the dangers that uncontrolled seizures can pose for both mother and fetus. Compared to other AEDs, sodium valproate (VPA) has been associated with greater risk of birth defects and cognitive impairment. Previous research suggests that VPA impacts on the development of intellectual abilities, however the long-term effect of fetal VPA exposure on other cognitive skills has been poorly characterised. Further, it is unknown whether abnormalities in brain structure underlie these cognitive deficits. This research aimed to investigate memory functioning and hippocampal structure in school-aged children who were exposed to VPA in the intrauterine environment. One hundred and five prospectively recruited school-aged children (aged six to eight years) exposed to AEDs in utero participated in the study. The sample included 26 children exposed to VPA monotherapy and 15 exposed to VPA polytherapy. All children underwent a neuropsychological assessment that included measures of memory. MRI scans were also collected from 14 children exposed to VPA monotherapy. Results from neuropsychological measures showed that verbal memory skills were negatively affected by exposure to VPA, in a dose-dependent manner. Conversely, non-verbal memory abilities were developmentally appropriate. Language impairment appeared to underlie the reduced performance on some verbal memory measures, except for on a measure of retroactive interference. Analyses of the imaging data did not reveal differences between left and right hippocampal volumes (after controlling for head size) in children exposed to VPA monotherapy. VPA dose was not correlated with hippocampal volume; however there was a significant relationship between hippocampal volume and associative learning ability. These data provide evidence of memory impairment.
in school-age children exposed to VPA, adding additional weight to the argument that VPA places children at risk of poorer developmental outcomes. The results suggest that these deficits in memory functioning may be partly mediated by abnormal hippocampal development; however studies with larger sample sizes and the inclusion of controls are warranted. The findings suggest that the prescription of VPA to women of childbearing age should be carefully considered in terms of risks and benefits. Women taking VPA should be supported throughout pregnancy and their children monitored from an early age. Future research is required to improve detection of children at risk and develop effective intervention strategies.
CHAPTER 1. INTRODUCTION

Epilepsy is a relatively common neurological disorder that is estimated to affect 5–10 people per 1000 (Duncan et al., 2004). Around 25% of these individuals will be women of childbearing age (Duncan et al., 2004). It is estimated that 3-5 births per thousand will be to women with epilepsy (Yerby, 2000). The majority of women with epilepsy will have a normal pregnancy and birth; however these women do face additional obstetric risks. Most women continue taking anti-epileptic drugs (AEDs) throughout their pregnancy because of the increased risk to the fetus and mother of death or injury from recurrent seizures.

AEDs act on the central nervous system and concerns have been raised about how exposure to these medications in utero might affect development of the unborn child. The long-term consequences of exposure are emerging as a significant clinical management dilemma, with the likelihood that some AEDs affect brain development and subsequent neuropsychological outcomes. The primary goal of this thesis was to examine the long-term effects of fetal AED exposure on memory and its underlying neural basis.

1.1. EPILEPSY

Epilepsy is a brain disorder characterised by transient paroxysmal events (seizures) generated by abnormal electrical discharges in the brain. In many cases, seizures can be adequately controlled by medications, although surgery may be considered if seizures are resistant to treatment.
Epilepsy has multiple etiologies, which may include congenital CNS lesions, infection, brain tumours, substance use, head trauma, cerebrovascular disease and metabolic disorder. There are over 40 different epilepsy syndromes, each with unique seizure types, age of onset, EEG findings, treatment and prognosis (Arndt, Stodgell, & Rodier, 2005). Epilepsy syndromes can be divided by their presumptive cause. Idiopathic epilepsies are generally believed to arise from genetic abnormalities. Symptomatic epilepsies are those which can be linked to a specific focal lesion or metabolic issue within the brain. Cryptogenic epilepsies have an unknown cause.

The most common classification system for seizures was established by the International League Against Epilepsy (ILAE) in 1981 (Panayiotopoulos, 2010). Seizures are principally divided by whether the source of the seizure is localised (focal or partial seizures) or distributed (generalised seizures). Partial seizures are further classified on the basis of whether consciousness is unaffected (i.e. a simple partial seizure) or impaired (i.e. a complex partial seizure). A partial seizure can also progress to generalised convulsions in a process known as secondary generalisation. Generalized seizures involve both hemispheres and may be convulsive or non-convulsive. They are divided according to the effect on the body and all involve loss of consciousness. Generalised seizure types include absence, myoclonic, clonic, tonic, tonic-clonic, and atonic seizures.

1.2. ANTI-EPILEPTIC DRUGS (AEDs)

AEDs are a diverse group of pharmaceuticals used in the treatment of seizure disorders. The medication aims to suppress the rapid and excessive firing of neurons that initiate a seizure. These medications are not solely taken to treat epilepsy; AEDs are also increasingly being
used in the treatment of mood disorders (e.g. bipolar disorder), as well as migraine, movement disorders, myotonia and neuropathic pain (Kaindl et al., 2006).

The earliest AEDs included primidone, phenytoin and phenobarbital. In the 1970’s, valproate and carbamazepine were approved and remain in frequent use today as treatment options. Since the 1990’s many newer AEDs have emerged which claim to have fewer side effects. These include ethosuximide, gabapentin, lamotrigine, levetiracetam, tiagabine, topiramate, oxcarbazepine, vigabatrin and zonisamide. AEDs generally aim to inhibit neuronal bursting and seizure spread, however each AED has a unique mechanism of action. Subsequently, certain AEDs may be more effective for different epilepsy types.

1.2.1. Valproate (VPA)

Valproic acid (VPA) and its salt, sodium valproate, have effective anticonvulsant and mood-stabilising properties. VPA is widely accepted as a drug of first choice for patients with generalised onset seizures (Marson et al., 2007b). VPA does not appear to have a single mechanism of action and exerts effects on both inhibitory and excitatory neurotransmitter systems in the brain (Kaindl et al., 2006). GABA is the major inhibitory neurotransmitter and represents a well-known target for VPA. VPA acts to increase regional GABA concentrations by promoting its synthesis and inhibiting breakdown (Loscher, 2002). It has been hypothesised that this may help inhibit seizure generation and propagation. Glutamate is the main excitatory neurotransmitter and is also affected by VPA. VPA has been shown to suppress glutamate-mediated excitation, which may mediate its anticonvulsant properties (Loscher, 2002). VPA may also affect voltage-dependent sodium channels. The multiple
mechanisms of action of VPA may be the basis for its high level of efficacy in seizure control (Marson et al., 2007b).

1.3. EPILEPSY AND PREGNANCY

The majority of women with epilepsy will experience a normal pregnancy, however compared to the general population these women may be more likely to experience difficulties in relation to both conception and pregnancy. Women with epilepsy experience higher rates of reproductive and endocrine disorders, including menstrual abnormalities, infertility, polycystic ovary syndrome and sexual dysfunction (Morrell, 2003). Past reviews have stated that the relative risk for pregnancy complications in women with epilepsy is slight; standing approximately 1.5 to 3 times the risk of women without epilepsy for complications including toxemia, bleeding, placental abruption, and premature birth (Hiilesmaa, 1992). The American Academy of Neurology recently advised that there is a moderately increased risk of caesarean but insufficient evidence for preeclampsia, pregnancy-related hypertension or spontaneous abortion (Harden, Hopp, et al., 2009).

It is generally agreed that avoidance of seizures during pregnancy is important to maintain the physical wellbeing of the mother and fetus. First trimester seizures are associated with higher malformation rates (12.3%) compared with children exposed to maternal seizures at other times (4%; Lindhout, Meinardi, Meijer, & Nau, 1992). Generalized tonic–clonic seizures can cause hypoxia and acidosis in both mother and fetus, as well as injury from blunt trauma (Stumpf & Frost, 1978; Yerby, 2000). Fetal intracranial hemorrhage, miscarriage, and stillbirth have also been reported (Pennell, 2003). The effects of non-convulsive seizures are not as clear within the literature.
Clinicians generally endeavour to prescribe an AED that is most likely to produce seizure control with good tolerability at the lowest dose (Morrell, 1998). Because of the risks associated with seizures, women are recommended to continue their AEDs during pregnancy. However this places these women in a difficult position because some AEDs are known to cross the placenta and act as teratogens (Harden, Pennell, et al., 2009; Lindhout & Omtzigt, 1992). This places the fetus at an elevated risk of developing birth defects.

By the time women present at the clinic after conception, it may be too late to make changes to AED treatment regimes. Yerby (2000) notes that the ideal situation would be to withdraw the patient from medications prior to conception, however acknowledges that for most women, this is not a realistic option. As major malformations arise from early exposure to AEDs, there is no benefit in changing to another medication after conception and the risk for teratogenicity is increased by exposure to a second agent (Morrell, 1998). The situation is further complicated by the risk of poorer seizure control following the medication change. Similarly, sudden withdrawal from AEDs may present a greater risk to the developing fetus if uncontrolled seizures in result (Duncan et al., 2004).

### 1.3.1. Changes in Seizure Frequency

During pregnancy, seizure frequency increases in approximately one-third of women with epilepsy (Morrell, 1998). However seizure freedom for at least 9 months prior to pregnancy is probably associated with a high likelihood (84–92%) of remaining seizure-free during pregnancy (Harden, Hopp, et al., 2009). The increase in seizures has been reported to occur mostly during the first trimester or evenly throughout pregnancy (Pennell, 2003). This is partly considered to be a consequence of physiological changes that occur during pregnancy.
which alters the pharmacokinetics of AEDs. Additional factors may include hormonal and metabolic changes, sleep deprivation and medication compliance (Morrell, 1998; Yerby, 2000).

Plasma concentrations of AEDs decline in pregnancy and a number of potential mechanisms have been postulated. Pennell (2003) and Yerby (2000) concisely summarise these: there are changes in drug distribution due to increases in body water content and extracellular fluid; renal clearance rates and hepatic enzyme activity increases which can cause serum levels of AEDs to fall; conversely, a reduction in serum albumin concentration leads to reduced protein binding and a relative increase in the free fraction of AEDs.

Variations in seizure frequency across the menstrual cycle suggests that hormones may have an anti-epileptic effect or interact with AED levels (Morrell, 1998). This is consistent with the findings that some AEDs may reduce levels of sex steroids and reduce the efficacy of hormonal contraceptives (Yerby, 2000). Pregnancy is a time of substantial hormonal fluctuation, which may also interfere with AED efficacy and contribute to increased seizure frequency.

1.4. IMPACT OF AEDS ON COGNITION

AEDs produce changes in the excitation of the central nervous system, which can lead to disturbances in cognition. The most prevalent cognitive side effects experienced by patients taking AEDs are sedation, somnolence, distractibility, insomnia and dizziness (Ortinski & Meador, 2004). Carbamazepine, phenytoin and VPA have also been associated with modest effects on attention, motor speed, and memory. The most profound impairments in cognitive performance have been observed with phenobarbital treatment (Meador et al., 1993; Meador,
Loring, & Allen, 1991; Meador et al., 1995). In general, studies of the newer AEDs suggest they have considerably fewer cognitive and behavioural side effects for patients (Hermann, Meador, Gaillard, & Cramer, 2010; Meador, Gilliam, Kanner, & Pellock, 2001). These studies illustrate that treatment with AEDs have the potential to impact on an individual’s brain function. This issue is particularly significant for women taking AEDs during pregnancy, as they are also exposing their fetus to these medications. Researchers are only just beginning to understand how fetal development is disrupted by exposure to AEDs. The mechanisms underlying brain dysfunction in women directly taking AEDs are expected to be completely different to the mechanisms by which AEDs affect fetal development, where exposure occurs in the intrauterine environment.

1.5. EVIDENCE FROM ANIMAL MODELS OF AED EXPOSURE

Evidence from experimental animal models suggests that exposure to AEDs in the prenatal environment affects brain development on a variety of levels. Gestational or early postnatal exposure to AEDs appears to affects the development of cells within the brain, with disrupted proliferation, differentiation, migration and maturation of glia and neurons (Fennrich, Ray, Nau, & Schlosshauer, 1998; Festag, Viertel, Steinberg, & Sehner, 2007; Finnell, Waes, Eudy, & Rosenquist, 2002; Laeng et al., 2004; Ohmori et al., 2001; Snow, Hartle, & Ivanco, 2008; Yu et al., 2009). Electrophysiological changes in the areas of neuronal connectivity, excitability and synaptic plasticity have also been documented (Rinaldi, Kulangara, Antoniello, & Markram, 2007; Rinaldi, Perrodin, & Markram, 2008; Rinaldi, Silberberg, & Markram, 2008; Zhang, Yu, Xiao, & Ruan, 2003). When administered postnatally, a range of AEDs have been demonstrated to cause widespread apoptosis throughout the brain and reduce the expression of neurotrophic factors (Bittigau et al., 2002; Kim, Kondratyev, &
Exposure to AEDs early in life has been associated with poor neuronal cell membrane integrity and reduced synthesis of myelin (Patsalos & Wiggins, 1982; Vorhees, Rauch, & Hitzemann, 1990). There is also evidence that prenatal exposure to AEDs alters the activity of various neurotransmitters (Dufour-Rainfray et al., 2010; Kuwagata, Ogawa, Shioda, & Nagata, 2009; Nakasato et al., 2008; N. Narita, Kato, Tazoe, & Miyazaki, 2002).

Animal studies have also reported changes in brain structure in response to gestational or early postnatal AED exposure. Reductions in brain weight have been observed in response to VPA (Bittigau et al., 2002; Thurston et al., 1981), phenytoin (McCartney, Scinto, Wang, & Altan, 1999), and phenobarbital exposure (Diaz, Schain, & Bailey, 1977; Schain & Watanabe, 1975). Exposure to AEDs has also been associated with regional brain changes in areas including the hippocampus, cortex, brainstem, cerebellum (Frisch et al., 2009; Hatta et al., 1999; Ingram, Peckham, Tisdale, & Rodier, 2000; Manent, Jorquera, Franco, et al., 2007; Manent, Jorquera, Mazzucchelli, et al., 2007; McCartney et al., 1999; Ohmori et al., 2001; Rodier, Ingram, Tisdale, Nelson, & Romano, 1996; Singh, 2010). Rats exposed prenatally to AEDs also demonstrate impairments on a range of behavioural tests suggesting brain dysfunction may also be occurring in other regions. Animals have demonstrated dose-dependent performance deficits on tasks involving spatial learning and working memory, startle response, locomotor activity, exploratory behaviour and negative geotaxis (Dufour-Rainfray et al., 2010; McCartney et al., 1999; T. Schneider & Przewlocki, 2005; Wagner, Reuhl, Cheh, McRae, & Halladay, 2006).
1.6. MEMORY AND THE HIPPOCAMPUS

It is evident from the animal studies described above that the brain can be adversely affected by prenatal AED exposure. This has significant implications for human fetal development, as the physical structure of the brain subserves cognitive functioning. It further suggests that fundamental cognitive skills have the potential to be affected by fetal AED exposure. This thesis will focus on memory function.

Memory is a complex system with many subcomponents and numerous theoretical models have been proposed over the years to explain how it functions. This thesis will adopt a model of memory functioning informed by cognitive neuropsychology studies. Much of the knowledge about memory in this viewpoint comes from historical case studies of patients with circumscribed brain injuries, such as the infamous H.M. who developed a severe amnesia following a bilateral medial temporal love resection (Kolb & Whishaw, 2008).

1.6.1. Types of Memory

According to neuropsychological models, memory can be broken down into short-term and long-term components. These components can be broken down even further, as will be illustrated in the following section.

1.6.1.1. Short-Term Memory

When information is first received from the sensory systems, it is held in a short-term store of memory. It has been commonly cited that this store has a capacity of 7 ± 2 items (Miller, 1956). This short-term memory store is usually conceptualised as working memory, which is
actually a more complex construct (Engle, Tuholski, Laughlin, & Conway, 1999). Working memory is the ability to actively hold and manipulate information in the mind. It underlies our ability to perform complex tasks such as reasoning, comprehension and learning. Baddeley and Hitch (1974) proposed the most popular model of working memory, which is comprised of a supervisory central executive which controls the flow of information to and from the three slave systems. The central executive is responsible for binding information, coordinating the slave systems, directing attention to relevant information and inhibiting irrelevant information. Working memory shows a strong connection to executive functioning, but short-term memory does not (Engle et al., 1999). The prefrontal cortex has been identified as an neuroanatomical region strongly implicated in working memory (Kolb & Whishaw, 2008).

1.6.1.2. Long-Term Memory

Studies of H.M. led researchers to believe that the type of information that the brain can store in long-term memory can be divided into two broad categories. Anderson’s (1976) cognitive model divides long-term memory into explicit and implicit memories. This view of memory function is widely accepted by modern neuropsychologists.

- **Explicit/Declarative Memory**

  Declarative (or explicit) memories are able to be consciously recalled, such as facts and events. Declarative memories can be further divided into episodic and semantic. Episodic memories are those derived from personal experience and relate to specific events in one’s past, such as a holiday to Vietnam. Semantic memory refers to general
knowledge about the world that is independent of personal experience, such as types of fruit or the population of Australia. The processing of explicit memories occurs in a “top-down” manner, whereby the information is actively manipulated by higher cortical processes in order to store it. The structures in the temporal lobe of the brain are considered largely responsible for processing these types of memory.

- Implicit/Procedural Memory

On the other hand, procedural (or implicit) memories are those that are learned from experience without conscious awareness. Procedural memory allows us to learn and perform activities, such as writing or riding a bike, without requiring conscious thought. Implicit memories are subject to “bottom-up” processing, meaning the information is received and stored in a passive manner. The striatum and basal ganglia are considered to be the key anatomical structures in this circuit.

1.6.2. Stages of Memory

It is generally the accepted view within neuropsychology that the formation of declarative memories involves three stages: encoding, storage and retrieval (Rapp, 2001). Encoding refers to the process of changing stimulus information to a useable form. Storage is linked to the process of consolidation whereby memory traces are formed. Retrieval processes are responsible for bringing information out of storage. An encoding deficit, such as is commonly seen in Alzheimer’s dementia, manifests as rapid forgetting due to an inability to move information into storage. Conversely, in the instance of a retrieval deficit, memory traces are intact within storage but are unable to be retrieved autonomously. Retrieval deficits
can be partially overcome by providing recognition prompts which assist in providing a cue to locate the information.

1.6.3. Development of Memory Skills in Childhood

Learning and memory skills are of central importance to the acquisition of cognitive, educational and social skills in childhood. These skills undergo rapid development at school-age. Individual differences in memory performance develop in childhood and are relatively unaffected by differences in educational experience (W. Schneider, Knopf, & Stefanek, 2002). Studies have shown that there is an initial growth spurt in declarative memory skills at around 7-8 years, with a second spurt at 12-13 years (V. A. Anderson & Lajoie, 1996). These age ranges are considered to be consistent with maturation of the prefrontal areas and ongoing myelination. Anderson and Lajoie (1996) showed that between the ages of seven and thirteen years, there are improvements in the capacity to register new information and in the efficiency of memory and learning skills. This was evident in both verbal and spatial domains. Conversely, long term memory capacity did not greatly change with age, but was able to be extended with the implementation of effective strategies.

Knowledge rather than intellectual ability has been argued to exert the greatest influence on memory development (Allexander & Schwanenflugel, 1994; Bjorklund & Schneider, 1996; Gaultney, Bjorklund, & Schneider, 1992). Comparisons between expert and novice children have shown that individual differences in IQ did not account for variance in recall measures when expertise was controlled (Gaultney et al., 1992; W. Schneider, 1996). What children know influences how they encode and interpret information and how efficiently they store and retrieve it. Older children generally know more than younger children, and so have better memory performance.
Strategy use also develops rapidly during school aged years. Memory strategies are effortful processes that improve memory efficiency, such as rehearsal, organisation and elaboration. Strategies are not observed in children younger than 5 or 6 but longitudinal studies have shown that children acquire memory strategies very rapidly (Bjorklund, Schneider, Cassel, & Ashley, 1994; W. Schneider, 2001). Age related improvements in the frequency of use and quality of strategies play a large role in memory development between preschool and adolescence (W. Schneider, 2001). Along with the use of more efficient strategies, Sigelman and Rider (2008) suggest that older children are also able to process information faster, hold more information in working memory, have a better understanding of memory (metamemory) and have a greater knowledge base which improves their ability to learn and remember.

### 1.6.4. The Hippocampus

The hippocampus is a structure located in the medial temporal lobe of the brain. It has a distinctive curved ‘S’ shape which has been likened to a ram’s horn (Cornu Ammonis). The hippocampal formation comprises four CA fields (CA1-4), the dentate gyrus and the subiculum (y Cajal, Swanson, & Swanson, 1995). The hippocampus is considered to play an important role in learning and memory. Long term potentiation (LTP) and long term depression (LTD) are mechanisms of synaptic plasticity thought to mediate learning in the hippocampus. Electrical stimulation techniques have demonstrated that inducing LTP in the hippocampus of rabbits before training in a classical conditioning paradigm increased the rate at which animals learned the conditioning task (Berger, 1984). In CA1 region of the hippocampus, LTP and LTD are dependent on NMDA receptors.
The hippocampus has been proposed as a structure critical to the formation of declarative memories. Damage can result in problems encoding new memories and retrieving stored memories. Evidence suggests that the hippocampus plays also an important role in the formation of associations between novel stimuli. Lesions to the hippocampus has been shown to disrupt associative memory in monkeys (Mahut, Zola-Morgan, & Moss, 1982). Further, rats with lesions to the hippocampus or fornix have difficulty learning associative memory tasks (Brasted, Bussey, Murray, & Wise, 2003; Sziklas, Lebel, & Petrides, 1998). In humans, patients with temporal lobe lesions that involve the hippocampus exhibit deficits on conditional associative memory tasks (Petrides, 1985). PET studies in humans have also demonstrated hippocampal and parahippocampal activation during associative learning (Henke, Buck, Weber, & Wieser, 1997). The hippocampus is also involved in dealing with interference phenomena. Monkeys and rats with hippocampal ablations show abnormal sensitivity to pro- and retroactive interference (Mahut et al., 1982; Winocur, 1979). Patients with temporal lobe epilepsy are highly susceptible to retroactive interference (Giovagnoli & Avanzini, 1999; Saling, 2009). Consequently, a drop off in recall across an interference condition can be considered be an indicator of hippocampal memory function.

1.6.5. Material Specificity

The theory of material specificity generally postulates that verbal memory processes are assumed by the left (dominant for language) hippocampus and non-verbal memory processes are undertaken by the right (non-dominant) hippocampus. Milner (1970) was the first to suggest the left and right temporal lobes processed different sorts of information based on her postoperative observations of intractable epilepsy. Studies of temporal lobe epilepsy have continued to produce consistent evidence to support this hypothesis. Lee (1989) documented
that patients who underwent left temporal lobectomy exhibited reduced performance on verbal memory tests. Early lesion studies also demonstrated that right sided excisions disrupt non-verbal memory (Jones-Gotman, 1986; M. L. Smith & Milner, 1981). Material specificity has been observed in Alzheimer’s disease, with left hippocampal volumes predicting verbal recall and right hippocampal volumes predicting spatial recall (Cahn et al., 1998; de Toledo-Morrell et al., 2000). Laterisation of memory skills also exists in normal adults without neurological illness. In functional MRI (fMRI) studies, verbal encoding produces left-lateralised activation of the medial temporal lobe, whereas face or pattern encoding activates the right medial temporal lobe (Golby et al., 2001; Kelley et al., 1998). It should be noted however, that the material specific relationship is not exclusive (Saling, 2009). In particular, demonstrating an association between the right hippocampus and non-verbal memory measures has been more variable. This is potentially due to the poor sensitivity of non-verbal encoding tasks and it has been suggested that the laterisation of encoding processes is instead determined by the verbalisability of stimuli (Golby et al., 2001).

Material specificity has not been as clearly illustrated in children and research has been limited to cohorts with temporal lobe epilepsy. Findings from some studies appear consistent with the adult literature. Language dominance is established by 4 years of age, so other cognitive functions may also be lateralised at this time (Kimura, 1963). For example, Jambaqué et al. (2007) found that children with left sided temporal foci had poor verbal memory and children with right temporal foci had poor visual memory. Similarly lateralised patterns in children with temporal lobe epilepsy have been also reported by other authors across a range of verbal and spatial memory tasks (M. Cohen, 1992; Gleissner et al., 2002). However other studies have suggested the material specific effect is not as striking in childhood and is only observed for visually based material. This may reflect the younger brain’s greater capacity to overcome functional losses with synaptic plasticity. Mabbott and
Smith (2003) demonstrated that children with right temporal lesions had difficulty learning spatial information, but children with left temporal lesions performed equivalent to controls on verbal memory tasks. Hammond, Hepworth and Smith (2002) also reported similar restrictions of material specificity to non-verbal material in the right temporal lobe. No material specific effects were observed for verbal information. Other reports of children with left and right temporal lobe epilepsy being equally impaired on verbal and visual memory tests has led to alternative arguments that there may be a more global representation of memory in children (Adams, Beardsworth, Oxbury, & Oxbury, 1990).

1.6.6. Language and Verbal Memory

Language skills and verbal memory function are highly interrelated. The storage of verbal memory is dependent on the ability to recognise the linguistic elements of a word. Conversely language is dependent verbal memory in that new vocabulary is acquired by associating a word with its meaning (Crosson, 1992). Language can subserve memory; for example in the act of comprehension, one must understand what is being said before the information can be stored. Expressive language skills mediate our ability to recite information after it has been retrieved from memory stores. Sometimes language and memory converge in a function, such as in confrontational naming when information is retrieved from semantic storage.

Language function can be a robust predictor of verbal learning and memory performance. This has consistently been shown in samples of epilepsy patients (Hermann, Seidenberg, Haltiner, & Wyler, 1992; Hermann, Wyler, Steenman, & Richey, 1988). Language functions such as object naming and comprehension have also been related to memory function following blunt head injury (Crosson, Cooper, Lincoln, Bauer, & Velozo, 1993). Saling
(2009) reported that while language ability is a predictor of list learning ability in left temporal lobe epilepsy, language is unrelated to the magnitude of retroactive interference. The material specific effects within the hippocampus also appear related to the side of language dominance. Lateralisation of fMRI activation in the medial temporal lobe during a verbal encoding task was positively related to language dominance, whereas lateralisation during a face encoding task was negatively related to language dominance (Weber, Fliessbach, Lange, Kügler, & Elger, 2007).

1.7. OVERVIEW OF THESIS

Following this introductory chapter, Chapter 2 of this thesis presents a review of the current literature examining cognitive outcomes in children exposed to AEDs in utero. The evidence associated with prenatal exposure to VPA, carbamazepine, phenytoin, phenobarbital and newer AEDs are described. The review highlights the methodological shortcomings of past studies and concludes by promoting the need for methodologically robust studies that characterise cognitive function in this group of children in more detail.

Chapter 3 reviews the findings related to brain changes resulting from fetal VPA exposure. The review draws on evidence from animal studies to illustrate that VPA has the potential to disrupt multiple processes critical to normal brain development. This chapter emphasises the need to investigate how brain development is altered in children exposed to VPA and speculates that neuroimaging studies might inform the biological basis for decrements in their cognitive abilities.
Chapter 4 provides the rationale for the studies contained in this thesis and states the aims and hypotheses. The primary aim of this thesis is to investigate the effect of fetal VPA on memory functioning and brain development.

Chapter 5 describes in detail the methodology of the two studies that comprise this thesis. The first study involved 105 school-aged children exposed to a variety of AEDs. Children completed a neuropsychological assessment that included verbal and non-verbal memory tasks. In the second study, a subset of 14 children prenatally exposed to VPA monotherapy participated in neuroimaging and computerised cognitive tasks. Hippocampal structure and functional correlates of memory were examined.

Chapter 6 presents the results of the two studies. The first study evaluated the impact of fetal VPA exposure on memory functioning. The performances of children exposed to VPA on the neuropsychological measures of memory were compared to the performance of children exposed to other AEDs. The effect of VPA dose was also examined. In the second study, the relationship between performance of VPA-exposed children on tasks sensitive to hippocampal functioning and VPA dose was explored. Hippocampal volumes were calculated by manual segmentation and within group comparisons were conducted.

Chapter 7 comprises an integrative discussion of the results of the two studies. An overview of the results is presented and the findings are related to past research. The possible mechanisms by which fetal VPA exposure might give rise to cognitive impairments are discussed. The limitations of the study are considered, as are the implications of the findings for clinical practise and research. Finally, potential directions for future research are raised and the thesis is concluded.
CHAPTER 2.  COGNITIVE AND BEHAVIOURAL OUTCOMES FOLLOWING AED EXPOSURE IN PREGNANCY

2.1. BACKGROUND

Recent estimates in the U.S. population indicate that 3-5 births per thousand will be to women with epilepsy (Harden, Meador, et al., 2009). Similarly, within Australia a significant number of women with epilepsy treated with anti-epileptic drugs (AEDs) become pregnant each year. Although a relationship between fetal exposure to AEDs and birth defects has been recognised, AEDs are continued throughout pregnancy in the majority of affected women because of the increased risk to fetus and mother of death or injury from recurrent seizures.

The evidence from animal models presented in Chapter 1 indicates that fetal exposure to AEDs affects brain development. Consistent with the idea that brain structure supports cognitive function, these animals also demonstrate consequential abnormalities in behaviour. This chapter reviews what is currently known about the impact of fetal AED exposure on human development. Information generated by international pregnancy registers has assisted greatly in understanding the impact of AED exposure on immediate birth outcomes, however the longer term impact on children remains insufficiently understood (Tomson et al., 2004; Vajda et al., 2007). Specifically, there is a lack of methodologically robust studies examining cognitive outcomes in children exposed to these medications and little is known about the specific cognitive domains affected. The shortcomings of previous work will be described.
2.2. NEURODEVELOPMENTAL DEFECTS IN EXPOSED CHILDREN

The establishment of pregnancy registries has enabled immediate birth outcomes following AED exposure to be studied and documented internationally (Tomson et al., 2007). Prominent registers exist in North America, the UK, Europe and Australia (Holmes, Wyszynski, & Lieberman, 2004; Morrow et al., 2006; Tomson et al., 2004; Vajda et al., 2007). Women treated with AEDs during their pregnancy have a higher incidence than the general population of an adverse outcome, including miscarriages and stillbirth, congenital malformations both major and minor, prematurity and intrauterine growth retardation. Major malformations of the central nervous system include neural tube defects such as spina bifida, hydrocephaly and microcephaly (Rosa, 1991; Tomson et al., 2004; Vajda et al., 2007). Unlike other teratogens where the effects on brain development relate to the timing of exposure, AEDs are typically maintained throughout pregnancy and therefore potentially have an effect upon all aspects of brain development.

Children exposed to AEDs in utero exhibit higher rates of developmental problems. Dean et al. (2002) reported that congenital malformations occurred in 14% of exposed pregnancies (compared with 5% of non-exposed siblings) and developmental delay occurred in 24% of exposed children (compared with 11% of non-exposed siblings). Higher rates of speech and motor delays following AED exposure have also been confirmed by other studies (Mawer, Clayton-Smith, Coyle, & Kini, 2002). Exposed children also had much higher frequencies of facial dysmorphism and medical problems compared to non-exposed siblings (Dean et al., 2002; Viinikainen et al., 2006).

There have been multiple descriptions in the literature of specific ‘fetal anticonvulsant syndromes’ resulting from valproate, carbamazepine or phenytoin exposure in utero (Christianson, Chester, & Kromberg, 1994; DiLiberti, Farndon, Dennis, & Curry, 1984;
Hanson, 1976; Hiilesmaa, Teramo, Granström, & Bardy, 1981; Ornoy & Cohen, 1996; Wide, Winbladh, Tomson, Sars-Zimmer, & Berggren, 2000). The most prominent features of these syndromes are facial dysmorphism, congenital malformations and developmental delay, with certain malformations specific to each medication (Clayton-Smith & Donnai, 1995; Dean, Moore, & Turnpenny, 2000; Moore et al., 2000). Diagnostic criteria proposed by Dean et al (2000) includes (1) a history of in utero AED exposure, (2) presence of characteristic facial appearance, (3) presence of at least one of the following: (a) evidence of neonatal withdrawal, (b) compatible malformation, (c) compatible childhood medical problem, (d) compatible developmental history, (e) compatible behavioural problem and (4) negative findings on investigations of alternative aetiologies.

Of the individual AEDs, women who take VPA appear to be at the greatest risk of experiencing poor immediate birth outcomes. Studies have reported higher rates of adverse outcomes and fetal malformations following exposure to VPA compared to carbamazepine, lamotrigine and phenytoin (Meador et al., 2006; Wide, Winbladh, & Källén, 2004). One study by Vajda and colleagues (2004) reported a malformation rate of 16.0% with VPA compared with 2.4% for other AEDs. In terms of timing, it is generally accepted that exposure to VPA in the first trimester places the fetus at the greatest risk of neurodevelopmental problems (Morrell, 2003). Spina bifida has been associated with a significantly higher average daily doses of VPA in the first trimester (Omtzigt, Los, et al., 1992). The use of VPA in the first trimester has also been associated with higher rates of other malformations (Jentink et al., 2010; Vajda et al., 2004). VPA also appears to exhibit a dose dependent effect, with higher doses associated with steeply increasing malformation rates (Vajda & Eadie, 2005; Wide et al., 2004). The incidence of abnormality with VPA doses ≥ 1100 mg was 30.2% compared to 3.2% with doses <1100 mg (Vajda et al., 2004). However, it is also important to keep in mind that other AEDs also become more detrimental
to fetal development as dose increases. A recent study from the EURAP pregnancy register reported that dose-dependent relationships with congenital malformations were not only observed for VPA, but also for carbamazepine, lamotrigine and phenobarbital, albeit at lower rates (Tomson et al., 2011). Rates were lowest for doses of less than 300mg/day of lamotrigine and less than 400mg/day of carbamazepine.

Treatment with multiple AEDs (polytherapy) during pregnancy has frequently been reported to carry a higher risk of birth abnormalities compared to monotherapy treatments (Tomson et al., 2007; Vajda & Eadie, 2005). A review of existing studies showed that in 10 of 14 studies, the relative risk for malformations in AED polytherapy compared with monotherapy was above 1.0 (Vajda et al., 2010). Recommendations from the American Academy of Neurology purport that AED polytherapy should be avoided during pregnancy if possible, particularly during the first trimester, to minimise the risk of defects (Harden, Meador, et al., 2009). Despite this, more recent evidence suggests it may be the specific combination of drugs used in polytherapy which increases the risk of malformation. Findings from within Australia suggest it is the presence of VPA in a treatment regime which may act as the major determinant in the development of malformations rather than exposure to multiple medications (Vajda et al., 2010). Vajda et al. (2010) found the rate of fetal malformations was 7.26% for VPA polytherapy and 17.09% for VPA monotherapy, yielding an relative risk of 0.39. A potential protective effect of lamotrigine when used in a polytherapy regime was also suggested. The authors hypothesised that the teratogenic effect of VPA may be dose-dependent; however they lacked data to support this claim. Even more recently, a study noted that the risk of malformations among lamotrigine and carbamazepine polytherapy was only higher than the corresponding monotherapy group when VPA was included (Holmes, Mittendorf, Shen, Smith, & Hernandez-Diaz, 2011).
2.3. BEHAVIOURAL DIFFICULTIES IN EXPOSED CHILDREN

Prenatal exposure to AEDs has been associated with behavioural problems (Dean et al., 2002). In a sample of children diagnosed with fetal anticonvulsant syndromes, 81% of mothers reported behavioural problems (Moore et al., 2000). The most commonly reported behavioural problems were hyperactivity or poor concentration and 7% of the sample had been diagnosed with Attention Deficit Hyperactivity Disorder. Similar trend level differences have been detected in results from behavioural questionnaires, with children exposed to AEDs scoring higher on domains measuring social problems and inattention (Viinikainen et al., 2006). VPA exposure in utero has also associated with high levels of parental stress induced by maladaptive behaviour (Vinten et al., 2009). Children in the study were poorer for daily living skills and skills relating to socialisation. No differences on measures of psychiatric or psychological dysfunction have been detected to date (Steinhausen, Lösche, Koch, & Helge, 1994).

2.4. ASSOCIATION WITH AUTISM

Exposure to AEDs appears to be a risk factor for the development of autism (Bescoby-Chambers, Forster, & Bates, 2001; Dean et al., 2002). In fact, an experimental animal model of autism frequently used in research involves prenatal exposure of animals to VPA (Ogawa, Kuwagata, & Shioda, 2009; T. Schneider & Przewlocki, 2005). This model is discussed further in Chapter 3. There have been three published studies examining the development of autistic traits following AED exposure in utero. In a study of 57 exposed children, Moore et al. (2000) reported that four children (7%) had a diagnosis of autism. Another study has reported the prevalence rate of autism to be 1.9-4.6% in children exposed to AEDs, compared to 0.25% in the general population (Rasalam et al., 2005). VPA in particular has been
associated with higher rates of autism, with 5 of 56 (8.9%) children exposed to VPA monotherapy and 9 of 77 (11.7%) children exposed to VPA polytherapy meeting the diagnostic criteria (Rasalam et al., 2005). Similar figures have been reported by Bromley et al. (2008), where 6.3% of the children exposed to VPA monotherapy had features of autism. This figure was seven times higher than the control group (0.9%) and higher than the reported incidence of 6 per 1,000 children in the general population.

2.5. PSYCHOMOTOR DIFFICULTIES IN EXPOSED CHILDREN

Psychomotor skills are also reduced in the children of women with epilepsy. Motor functioning has been demonstrated to be reduced in a range of studies assessing children aged from 1.5 to 6 years (Hirano, Fujioka, Okada, Iwasa, & Kaneko, 2004; Parisi et al., 2003; Steinhausen et al., 1994; Wide et al., 2000). Carbamazepine exposure has demonstrated negligible effects on motor functioning (Leavitt, Yerby, Robinson, Sells, & Erickson, 1992; Ornoy & Cohen, 1996; Rovet et al., 1995) and locomotor development (Hiilesmaa et al., 1981; Wide et al., 2000). In a prospective longitudinal study, children did not exhibit differences in psychomotor development at 9 months of age compared to controls, however subtle reductions were noted at a 4.5 year follow up (Hiilesmaa et al., 1981; Wide et al., 2000).

2.6. EDUCATIONAL OUTCOMES FOLLOWING AED EXPOSURE

AED exposure has been associated with an increased risk of learning difficulties and exposed children are more likely to require additional educational support. In a sample of school-aged children, those exposed to VPA monotherapy had a far higher odds ratio (3.4) for receiving
additional educational supports compared to those exposed to carbamazepine (.26) (Adab, Jacoby, Smith, & Chadwick, 2001). In another study, 62% of children exposed to VPA required educational support compared with 15% in carbamazepine-exposed and nonexposed groups (Viinikainen et al., 2006). Learning disorders are also more prevalent in children with fetal valproate syndrome (Moore et al., 2000). Carbamazepine exposure does not appear to have a significant impact on educational achievement (Adab et al., 2001; Van der Pol, Hadders-Algra, Huisjes, & Touwen, 1991). At 16 years of age, children exposed to carbamazepine in utero did not display an increased risk of leaving school without a final grade (Forsberg, Wide, & Källén, 2010). Children exposed to phenobarbital are more likely to attend a special development school because of learning difficulties and demonstrate poorer academic achievement (Dessens, 2000; Van der Pol et al., 1991). Higher rates of learning disorders have also been recorded following phenytoin and phenytoin with phenobarbital polytherapy (Dessens, 2000). Polytherapy has also been associated with educational difficulties. Children are more likely to require educational assistance and have lower levels of achievement on formal educational tests (Adab et al., 2001; Dessens, 2000; Van der Pol et al., 1991). Children exposed to polytherapy including VPA had higher odds ratios (2.51) for additional educational needs compared with those unexposed of (1.51) (Adab et al., 2001). In another retrospective study, adolescent children exposed to polytherapy had an increased risk of not receiving a final grade at school (Forsberg et al., 2010). This suggests that AED exposure can have long-standing effects on cognitive development.

2.7. IMPACT OF AED EXPOSURE ON COGNITIVE OUTCOMES

In recent years, studies have begun examining the longer-term consequences of exposure to AEDs in utero. Since the 1990’s, there has been a growing body of literature documenting a
negative impact of prenatal AED exposure on the development of a range of cognitive skills (Dean et al., 2002; Eriksson et al., 2005; Kantola-Sorsa, Gaily, Isoaho, & Korkman, 2007; Koch et al., 1999; Leavitt et al., 1992; Lösche, Steinhäuser, Koch, & Helge, 1994; Ornoy & Cohen, 1996; Scolnik et al., 1994; Thomas, Sukumaran, Lukose, George, & Sarma, 2007; Vanoverloop, Schnell, Harvey, & Holmes, 1992). When this thesis was initially proposed, research in this area was still limited. However, during the time course of this project there has been a significant increase in the number of publications from a US/UK research group on cognitive outcomes (Bromley, Baker, & Meador, 2009; M. J. Cohen et al., 2011; Kimford J. Meador et al., 2011; Meador et al., 2009; K. J. Meador et al., 2011). There have also been recent publications from other investigators working within the Australian research group that this study formed part of (Nadebaum et al., 2011a, 2011b). Despite these gradual advances in our understanding of cognitive sequelae for these children, many studies have remained focused on the development of broader intellectual skills. There are few studies that examine whether and to what extent more specific cognitive abilities are impacted. Learning and memory in particular are poorly characterised. These key skills undergo rapid development in childhood and are critical for the acquisition of skills and knowledge.

Early studies examined outcomes in the context of general AED exposure; however more recent research has focused on differentiating the effects of specific drugs. There is also some suggestion that AEDs have a dose-dependent effect, with more significant cognitive impairments associated with higher AED doses (Gaily et al., 2004; Meador et al., 2009; Thomas et al., 2007). The most frequently studied AEDs are valproate, carbamazepine, phenytoin and phenobarbital. The literature pertaining to these individual AEDs will be discussed, emphasising VPA which is reported to have the most significant impact on long term development. There are also issues surrounding polytherapy regimes, which have been reported as more detrimental than monotherapy treatment. Newer AEDs have been purported
to pose fewer risks to cognitive outcome and the limited evidence to date appears to support this.

2.7.1. Valproate

There is a growing body of literature associating prenatal VPA exposure with prominent negative cognitive sequelae. However much of the evidence supporting this relationship has only emerged in the past decade. A summary of the characteristics of the most prominent studies examining cognitive outcomes following fetal VPA exposure is presented in Table 2.1. Results from a number of studies suggest that children exposed to VPA exhibit impairments in general intellectual abilities. The most commonly used measure of intellectual functioning is IQ (or full scale IQ; FSIQ), which is sometimes able to be split in terms of verbal (VIQ) and performance (PIQ) based skills (Wechsler, 2003). A recent meta-analysis of seven studies found significantly reduced VIQ (88.3), PIQ (93.7) and FSIQ (83.9) in children exposed to VPA, compared to unexposed controls (Banach, Boskovic, Einarson, & Koren, 2010). Deficits have recently been detected in children as young as two years, with reduced performances on measures of infant mental development (Bromley et al., 2010; Cummings, Stewart, Stevenson, Morrow, & Nelson, 2011). In a study by Meador et al. (2009), three year-old VPA-exposed children displayed significantly lower IQ scores on the Differential Ability Scales (6-9 points) than children who had been exposed to other AEDs. Similar impairments have also been observed in slightly older children (aged 6-13 years) with exposure to VPA monotherapy being associated with higher prevalence rates of “low IQ” (< 80) and “exceptionally low IQ” (<70) compared to children exposed to carbamazepine or controls (Eriksson et al., 2005). On average, children exposed to VPA have significantly lower IQs than children exposed to lamotrigine, phenytoin and carbamazepine (Eriksson et al., 2005;
Meador et al., 2009). Intellectual ability in group of children also falls below age-appropriate levels established in typically developing children (Nadebaum et al., 2011a).

Verbal skills may be particularly at risk in children whose mothers took AEDs. There have been reasonably consistent reports of specific reductions in verbal IQ scores in school-aged children exposed to VPA (Adab, Kini, et al., 2004; Eriksson et al., 2005; Gaily et al., 2004; Vinten et al., 2005). The association between VPA use and verbal intellectual skills appears to be dose dependent (Meador et al., 2009). Studies have reported negative associations between VPA dose and verbal IQ scores, with doses above 800 mg associated with greater score decrements (Adab, Kini, et al., 2004; Koch et al., 1999). Most recently, Meador et al. (2011) found that the verbal abilities abilities of children exposed to VPA in utero fell significantly below their non-verbal abilities. While this differentiation was also observed in other drug groups, the effect was most pronounced following VPA exposure. VPA-exposed school-aged children have also shown reductions on an index of verbal intelligence measuring verbal conceptualisation, stored knowledge and oral expression (Nadebaum et al., 2011a). Conversely, performance was preserved on the non-verbal intelligence index of perceptual reasoning. Children exposed to VPA also perform below age-appropriate levels on measures of core language ability (Nadebaum et al., 2011b). This is consistent with studies that have found a higher prevalence of speech delay following VPA exposure compared to other AEDs or controls (Dean et al., 2002). Only one study has failed to find a difference between the intellectual or language skills of children exposed to VPA monotherapy and other AEDs, however the authors acknowledge the small sample size and low doses within their group (Thomas et al., 2007). Interestingly though, this study did find that children generally exposed to AEDs scored significantly lower than unexposed children on subtests of information, vocabulary, digit span and other language tasks.
Up until now, intellectual outcomes have been the major focus of studies examining long-term cognitive outcomes. Relatively little work has been undertaken to characterise the cognitive impairments exhibited by VPA-exposed children in terms of broader neuropsychological domains. There is subtle evidence to suggest that attention, working memory and learning may be affected in this group. In one of the more comprehensive studies by Eriksson and colleagues (2005), children aged 6-13 years who were exposed to VPA performed significantly lower on a digit symbol coding task than children exposed to carbamazepine or controls. This task requires working memory and psychomotor control. In the same study, VPA exposure was associated with poorer performance on a memory for faces task relative to carbamazepine exposure, and poorer performance on a list learning task relative to children of women with epilepsy who did not take medication. Memory impairments on the Rivermead Behavioural Memory Test for Children at 5-16 years of age have also been documented (Vinten et al., 2005). Another study found that VPA exposure has a significant effect on domains of attention and memory, with specific decrements on tasks of complex attention and sentence repetition (Kantola-Sorsa et al., 2007). Children exposed to VPA also display reduced performance on working memory tasks relative to population norms (Nadebaum et al., 2011a). Significant differences have been detected in cognitive fluency and originality between children exposed to VPA and children exposed to lamotrigine and carbamazepine (McVearry, Gaillard, VanMeter, & Meador, 2009). It is possible that this may reflect a degree of executive dysfunction. Despite these reports of cognitive dysfunction in other domains, with strong indication particularly towards memory and learning abilities, studies are yet to investigate this further to fully delineate the nature of the impairment.
Table 2.1.

*Characteristics and Findings of Studies Examining Cognitive Outcomes Following Fetal Valproate Exposure in Monotherapy*

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Recruitment Source</th>
<th>Age range</th>
<th>Group Size</th>
<th>Sample Size</th>
<th>Measures</th>
<th>Cognitive abilities reported as impaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromley, et al., 2010</td>
<td>Prospective</td>
<td>Regional antenatal clinics</td>
<td>&lt; 2 years</td>
<td>42</td>
<td>167</td>
<td>Griffiths Mental Development Scales</td>
<td>Global development</td>
</tr>
<tr>
<td>Cummings, et al., 2011</td>
<td>Prospective</td>
<td>UK Epilepsy and Pregnancy Register</td>
<td>9–60 months</td>
<td>23</td>
<td>210</td>
<td>BSID or the Griffiths Mental Development Scales</td>
<td>Global development</td>
</tr>
<tr>
<td>Meador et al., 2009</td>
<td>Prospective</td>
<td>US &amp; UK epilepsy clinics</td>
<td>3 years</td>
<td>53</td>
<td>258</td>
<td>BSID or Differential Ability Scales</td>
<td>IQ</td>
</tr>
<tr>
<td>Meador et al., 2011</td>
<td>Prospective</td>
<td>US &amp; UK epilepsy clinics</td>
<td>3 years</td>
<td>48</td>
<td>216</td>
<td>Differential Ability Scales, Preschool Language Scale, Peabody Picture Vocabulary Test and Developmental Test of Visual-Motor Integration</td>
<td>IQ, verbal &amp; non-verbal abilities</td>
</tr>
<tr>
<td>Gaily et al., 2004</td>
<td>Prospective</td>
<td>Regional hospital</td>
<td>5-9 years</td>
<td>13</td>
<td>137</td>
<td>WISC-R, WPPSI-R</td>
<td>VIQ</td>
</tr>
<tr>
<td>Kantola-Sorsa, Gaily, Isoaho, &amp; Korkman, 2007</td>
<td>Prospective</td>
<td>Regional hospital</td>
<td>5-11 years</td>
<td>8</td>
<td>154</td>
<td>NEPSY</td>
<td>Attention &amp; sentence repetition</td>
</tr>
<tr>
<td>Thomas, Sukumaran, Lukose, George, &amp; Sarma, 2007</td>
<td>Retrospective</td>
<td>Kerala pregnancy register</td>
<td>6 years</td>
<td>12</td>
<td>71</td>
<td>WISC-Indian, Malayam language test</td>
<td>IQ &amp; language</td>
</tr>
<tr>
<td>Nadebaum, et al., 2011a</td>
<td>Prospective</td>
<td>Australian Pregnancy Register</td>
<td>6-8 years</td>
<td>23</td>
<td>57</td>
<td>WISC-IV</td>
<td>IQ, verbal comprehension &amp; working memory</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Location</td>
<td>Age Range</td>
<td>N</td>
<td>Test(s)</td>
<td>Domain</td>
<td></td>
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<tr>
<td>Nadebaum, et al., 2011b</td>
<td>Prospective</td>
<td>Australian</td>
<td>6-8 years</td>
<td>23</td>
<td>102</td>
<td>CELF-4</td>
<td></td>
</tr>
<tr>
<td>Eriksson et al., 2005</td>
<td>Prospective</td>
<td>Community</td>
<td>6-13 years</td>
<td>13</td>
<td>39</td>
<td>WISC-III, NEPSY</td>
<td></td>
</tr>
<tr>
<td>Adab et al., 2004</td>
<td>Retrospective</td>
<td>Epilepsy clinics</td>
<td>6-16 years</td>
<td>41</td>
<td>249</td>
<td>WISC-III</td>
<td></td>
</tr>
<tr>
<td>Vinten et al., 2005</td>
<td>Retrospective</td>
<td>Epilepsy clinics</td>
<td>6-16 years</td>
<td>42</td>
<td>249</td>
<td>WISC, RBMT</td>
<td></td>
</tr>
</tbody>
</table>

1. $N$ of children exposed to valproate monotherapy
2. Total $N$ of study; children of mothers with epilepsy
3. BSID = Bayley Scales of Infant Development; CELF = Clinical Evaluation of Language Fundamentals; IQ = Intelligence Quotient; NEPSY = A Developmental Neuropsychological Assessment; RBMT = Rivermead Behavioural Memory Test; VIQ = Verbal Intelligence Quotient; WAIS = Wechsler Adult Intelligence Scale; WISC = Wechsler Intelligence Scale for Children; WPPSI = Wechsler Preschool and Primary Scale of Intelligence.
2.7.2. Carbamazepine

In comparison to VPA, there are fewer studies suggesting that carbamazepine has detrimental effects on cognitive development. Some reviews have even concluded that carbamazepine “probably” does not increase the risk of poor cognitive outcome (Harden, Meador, et al., 2009). Despite this, there have still been some reports of developmental or language delay, with rates ranging from 8% to 20% (Cummings et al., 2011; Dean et al., 2002; Ornoy & Cohen, 1996; Scolnik et al., 1994). Carbamazepine exposure has also been associated with higher rates of autism spectrum disorder compared to unexposed children (Rasalam et al., 2005). This supports the idea that carbamazepine does have the potential to disrupt normal development.

Findings have been slightly varied in regards to carbamazepine’s impact on cognitive development. Many studies have suggested that the performance of children exposed to carbamazepine does not differ from unexposed children (Adab, Kini, et al., 2004; Bromley et al., 2010; Eriksson et al., 2005; Gaily et al., 2004; Hiilesmaa et al., 1981; Leavitt et al., 1992; Scolnik et al., 1994; Wide et al., 2000). However other studies, particularly those that have been published more recently, have detected poorer performances relative to control samples. Ornoy and Cohen (1996) were the first to demonstrate that children exposed to carbamazepine monotherapy scored lower on mental and cognitive scales than unexposed children. Other prospective studies have also found similar reductions on mental development scales in children aged seven years and under (Cummings et al., 2011; Rovet et al., 1995). Carbamazepine exposed children also show declines in motor functioning and adaptive functioning at three years of age (M. J. Cohen et al., 2011). In Ornoy and Cohen’s (1996) study, level of impairment was not associated with carbamazepine dose; however more recent studies indicate a relationship may exist specifically with verbal skills. At 3 years of age,
carbamazepine dose was negatively associated with verbal performance and motor functioning (M. J. Cohen et al., 2011; K. J. Meador et al., 2011). Banach and colleagues’ (2010) meta-analysis showed that exposure to carbamazepine was not associated with changes in FSIQ or VIQ, but did find reductions in PIQ. Interestingly, the only other study to report PIQ deficits was by Thomas and colleagues (2007), who reported that PIQ was reduced following general AED exposure with a specific deficit on a digit symbol coding task. In another study that used children of women with epilepsy who did not take medication as a comparison group, children exposed to carbamazepine performed more poorly on a list learning task (Eriksson et al., 2005). Conversely, studies have reported that the language skills of carbamazepine exposed children are equivalent to unexposed, typically developing children (Nadebaum et al., 2011b; Rovet et al., 1995; Scolnik et al., 1994).

### 2.7.3. Phenobarbital

Studies of cognition in children exposed to phenobarbital in utero have provided mixed results. Some studies have reported that the performance of children exposed to phenobarbital does not differ significantly from controls (Dean et al., 2002; Leavitt et al., 1992). However Thomas (2007) found that children exposed to phenobarbital were the only AED group to perform more poorly on a measure of intelligence. Further, verbal IQ deficits have detected in a sample of adult men exposed to phenobarbital and gestational exposure in the third trimester was a predictor of effect on intelligence (Reinisch, Sanders, Mortensen, & Rubin, 1995). A dose effect of phenobarbital was documented in this study. In adults who were exposed as children, deficits in verbal abstract reasoning and attention have also been detected (Dessens, 2000). Language skills within this group of children have been reported as equivalent to other AED exposure group (Thomas et al., 2007).
2.7.4. Phenytoin

Research has also produced conflicting findings about fetal phenytoin exposure. Several studies have failed to find any differences between exposed children and controls on developmental measures (Adab, Kini, et al., 2004; Koch et al., 1999; Leavitt et al., 1992; Thomas et al., 2007; Vinten et al., 2009; Wide et al., 2000). Studies have reported the intellectual ability of children exposed to phenytoin is not comparable to the deficits exhibited by VPA-exposed children and a dose effect is absent (Meador et al., 2009; K. J. Meador et al., 2011). However other authors have reported elevated rates of developmental delay (Dean et al., 2002) as well as lower scores on intelligence tests (Scolnik et al., 1994; Vanoverloop et al., 1992). In one such study, children exposed to phenytoin had a mean global IQ 10 points lower than age matched controls and scored lower on a language development task (Scolnik et al., 1994). This is consistent with other studies demonstrating deficits in intellectual ability and language (Rovet et al., 1995). Another study found lower verbal abilities when compared to non-verbal abilities (K. J. Meador et al., 2011). Vanoverloop et al., (1992) demonstrated a slightly different pattern of impairments. FSIQ scores in phenytoin-exposed children were reduced relative to controls. There was no difference in VIQ scores but PIQ deficits were exhibited as well as impairments in visuo motor integration skills.

2.7.5. Other AEDs

Other AEDs have received far less attention and their effects on cognitive development are unclear. Studies are beginning to emerge on lamotrigine exposure, which generally has not been associated with significant deficit. Verbal abilities have been shown to be lower than non-verbal abilities in 3 year old children exposed to lamotrigine, however a dose effect was
not detected (K. J. Meador et al., 2011). Further, when compared to VPA and carbamazepine, in utero exposure to lamotrigine did not have a detrimental effect on child neurodevelopment (Cummings et al., 2011). Lamotrigine-exposed children demonstrated comparable performance on a mental development and language scales to unexposed control children (Bromley et al., 2010; Nadebaum et al., 2011b).

One study has examined the effects of prenatal exposure to primidone, finding a negative correlation between children’s IQ scores and dose (Koch et al., 1999). However this AED has now largely fallen into disuse. Little has been published about the effects of newer AEDs. The only study to investigate levetiracetam exposure to date has shown positive results. Children exposed to levetiracetam obtained higher developmental scores than children exposed to VPA and performed at a comparable level to controls (Shallcross et al., 2011).

The effects of other newer AEDs such as topiramate, gabapentin and vigabatrin are unknown.

2.7.6. Polytherapy

There is evidence to suggest that exposure to a polytherapy treatment regime in utero may have a greater negative impact on cognitive development compared to monotherapy exposure (Gaily et al., 2004; Koch et al., 1999; Leavitt et al., 1992; Lösche et al., 1994; Nadebaum et al., 2011a, 2011b; Thomas et al., 2007; Van der Pol et al., 1991). The characteristics of studies examining polytherapy exposure are summarised in Table 2.2. Along with a higher risk of fetal malformations, children exposed to multiple AEDs achieve lower scores on measures of general intellectual ability compared to their age-matched counterparts or children exposed to monotherapy regimes (Eriksson et al., 2005; Koch et al., 1999; Lösche et al., 1994; Nadebaum et al., 2011a; Thomas et al., 2007). In a longitudinal study, infants exposed to polytherapy with low developmental quotient at one year of age continued to have
low scores on outcome measures at six years (Thomas et al., 2007). However, a Cochrane review notes that while polytherapy exposure has been consistently associated with lower developmental scores at younger ages, studies in school-aged children are less consistent (Adab, Tudur, Vinten, Williamson, & Winterbottom, 2004). Wide, et al. (2002) did not detect differences in global development scores of 4.5 year old children. Similarly, Adab, Kini, et al. (2004) did not find a significant effect of polytherapy on IQ scores in children aged 6-16 years. A possible reason for these inconsistencies may be related to the composition of the polytherapy group, which is discussed later in this section.

In terms of other cognitive domains, verbal skills appear to be sensitive to polytherapy exposure, with significant differences reported between the verbal IQ scores of polytherapy exposed and unexposed children (Gaily et al., 2004; Nadebaum et al., 2011a). Polytherapy has also been associated with poorer performances on other verbal tasks that tap language, comprehension and psycholinguistic abilities (Leonard, Andermann, Ptito, & Schopflocher, 1997; Löshe et al., 1994; Nadebaum et al., 2011b; Thomas et al., 2007). Further, children exposed to polytherapy may be more distractible (Leonard et al., 1997) and demonstrate reduced memory skills (Vinten et al., 2005). Dessens (2000) reported persisting deficits into adulthood following polytherapy exposure, with poorer performances in verbal abstract reasoning and selective attention. Children exposed to polytherapy also have shown decrement on tasks of attention, sentence repetition and motor functioning (Kantola-Sorsa et al., 2007). This represented more widespread cognitive change relative to children exposed to monotherapy, whom only demonstrated changes on tasks of attention.

Despite these findings, the ability to draw clear conclusions on the effects of polytherapy on fetal development has been hindered by differences in drug combinations between studies. In general, older studies have pooled mothers taking polytherapy and have failed to indicate the
degree of homo- or hetero-geneity of AED use within the group. Specific drugs, drug doses or combinations may be more important determinants of outcome than polytherapy itself. It has recently been suggested by Vajda, et al. (2010) that VPA is the primary contributor to birth defects when included in a polytherapy regime and there is emerging evidence to suggest this also applies to cognitive outcomes. Studies have shown that children exposed to VPA in a polytherapy regime perform more poorly than children exposed to polytherapy without VPA on measures of intellect and language (Nadebaum, et al., 2011a, 2011b). In further support, children exposed to polytherapy including VPA scored more poorly on an adaptive behaviour measure than children exposed to polytherapy that did not include VPA (Vinten et al., 2009). It has been speculated that high-dose VPA exposure rather than polytherapy was responsible for the poorer cognitive outcomes observed in a VPA-polytherapy group (Nadebaum et al., 2011a). Finally, polytherapy may exert a specific effect on mental processing speed that is independent of the effects of VPA inclusion (Nadebaum et al., 2011a).
Table 2.2.

**Characteristics of Studies Examining Cognitive Outcomes Following Fetal Polytherapy Anti-Epileptic Drug Exposure**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Recruitment Source</th>
<th>Age range</th>
<th>Group Size¹</th>
<th>Sample Size²</th>
<th>Measures³</th>
<th>Cognitive abilities reported as impaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leavitt, Yerby, Robinson, Sells, &amp; Erickson, 1992</td>
<td>Prospective</td>
<td>Regional clinic</td>
<td>1 year</td>
<td>Not reported</td>
<td>43</td>
<td>BSID</td>
<td>MDI</td>
</tr>
<tr>
<td>Lösche, Steinhausen, Koch, &amp; Helge, 1994</td>
<td>Prospective</td>
<td>Regional hospital</td>
<td>15 months</td>
<td>26 (?)</td>
<td>52</td>
<td>BSID or WPPSI</td>
<td>IQ</td>
</tr>
<tr>
<td>Wide, Henning, Tomson, &amp; Winbladh, 2002</td>
<td>Prospective</td>
<td>Regional hospital</td>
<td>4.5 years</td>
<td>9 (2)</td>
<td>67</td>
<td>Griffiths' test</td>
<td>No effect on global development</td>
</tr>
<tr>
<td>Gaily et al., 2004</td>
<td>Prospective</td>
<td>Regional hospital</td>
<td>5-9 years</td>
<td>30 (17)</td>
<td>137</td>
<td>WISC-R, WPPSI-R</td>
<td>VIQ</td>
</tr>
<tr>
<td>Kantola-Sorsa, Gaily, Isoaho, &amp; Korkman, 2007</td>
<td>Prospective</td>
<td>Regional hospital</td>
<td>5-11 years</td>
<td>24 (14)</td>
<td>154</td>
<td>NEPSY</td>
<td>Attention &amp; sentence repetition</td>
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<tr>
<td>Thomas, Sukumaran, Lukose, George, &amp; Sarma, 2007</td>
<td>Retrospective</td>
<td>Kerala pregnancy register</td>
<td>6 years</td>
<td>23 (7)</td>
<td>71</td>
<td>WISC-Indian, Malayam language test</td>
<td>IQ &amp; language</td>
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<td>Unclear</td>
<td>Regional clinic</td>
<td>6 years</td>
<td>12 (0)</td>
<td>61</td>
<td>Dutch test of reading, arithmetic, spelling</td>
<td>Reading, arithmetic &amp; spelling</td>
</tr>
<tr>
<td>Study</td>
<td>Type</td>
<td>Location</td>
<td>Age Range</td>
<td>Sample Size</td>
<td>Tests Administered</td>
<td>Findings</td>
<td></td>
</tr>
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<td>------------------------------</td>
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<tr>
<td>Nadebaum, et al., 2011a</td>
<td>Prospective</td>
<td>Australian Pregnancy Register</td>
<td>6-8 years</td>
<td>15 (15)</td>
<td>WISC-IV</td>
<td>IQ, verbal comprehension &amp; working memory</td>
<td></td>
</tr>
<tr>
<td>Nadebaum, et al., 2011b</td>
<td>Prospective</td>
<td>Australian Pregnancy Register</td>
<td>6-8 years</td>
<td>15 (15)</td>
<td>CELF-4</td>
<td>Language</td>
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</tr>
<tr>
<td>Adab et al., 2004</td>
<td>Retrospective</td>
<td>Epilepsy clinics</td>
<td>6-16 years</td>
<td>52 (29)</td>
<td>WISC-III</td>
<td>No effect on IQ</td>
<td></td>
</tr>
<tr>
<td>Vinten et al., 2005</td>
<td>Retrospective</td>
<td>Epilepsy clinics</td>
<td>6-16 years</td>
<td>36 (?)</td>
<td>WISC, RBMT</td>
<td>VIQ</td>
<td></td>
</tr>
<tr>
<td>Koch et al., 1999</td>
<td>Prospective</td>
<td>Regional hospital</td>
<td>10-19 years</td>
<td>23 (?)</td>
<td>Hamburg-Wechsler Intelligence Test</td>
<td>IQ</td>
<td></td>
</tr>
</tbody>
</table>

1. *N* of children exposed to polytherapy (*N* of children exposed to valproate polytherapy, where specified)

2. Total *N* of study; children of mothers with epilepsy

3. BSID = Bayley Scales of Infant Development; CELF = Clinical Evaluation of Language Fundamentals; IQ = Intelligence Quotient; MDI = Mental Development Index; NEPSY = A Developmental Neuropsychological Assessment; RBMT = Rivermead Behavioural Memory Test; VIQ = Verbal Intelligence Quotient; WAIS = Wechsler Adult Intelligence Scale; WISC = Wechsler Intelligence Scale for Children; WPPSI = Wechsler Preschool and Primary Scale of Intelligence.
2.8. METHODOLOGICAL SHORTCOMINGS OF PREVIOUS STUDIES

Our ability to draw firm conclusions about developmental outcomes in children exposed in utero to AEDs has been undermined by a number of methodological shortcomings. There is a significant degree of inconsistency within the literature in part due to a wide degree of homogeneity in terms of control group, type of AED studied, sample size, included age groups and measured outcomes. There are also many important confounding factors in the relationship between prenatal AED exposure and cognitive outcome which have not adequately been taken into account by previous studies. The methodological difficulties have been reviewed in detail in the literature, but will be briefly summarised and elaborated on here (Adab, Tudur, et al., 2004; Nicolai, Vles, & Aldenkamp, 2008).

2.8.1. Study Design Issues

Many previous attempts to examine cognitive outcomes in AED exposed children have employed retrospective study designs e.g. (Adab et al., 2001; Adab, Kini et al., 2004; Dean et al., 2002; Moore et al., 2000; Rasalam et al., 2005). While studies of this nature are cost effective in regards to time and finances, they risk introducing potential error due to confounding and bias. Recall bias is of particular concern as these studies rely on the memory capabilities of participating mothers to provide medical information and medication data and their recollections may not always be accurate.

While randomised controlled studies would provide the most reliable forms of scientific evidence, their use in this population is limited by ethical considerations. As such, prospective longitudinal research would allow the most valid interpretation of outcomes. This approach would enable assessment of mothers at baseline to ascertain drug intake and
assessment of cognition over time. While there have already been a number of prospective case-controlled and population-based studies in school-aged children, more are required (Eriksson et al., 2005; Gaily et al., 2004; Kantola-Sorsa et al., 2007; Meador et al., 2009; Nadebaum et al., 2011a). It is important that all future studies adopt a prospective study design in order to add legitimate findings to this body of literature.

2.8.2. Sample Recruitment Bias

Concerns have been raised that the samples of previous studies have been poorly representative of the general population. Recruitment strategies have varied across studies with some participants sourced from specialised epilepsy or teratogen clinics (Adab, Tudur, et al., 2004; Meador et al., 2009; K. J. Meador et al., 2011; Ornoy & Cohen, 1996; Rovet et al., 1995). Further, studies have failed to provide information about the proportion of eligible women that participated. It is also possible that mothers with children more disadvantaged may be drawn to the study. As a consequence of these factors, samples could have included disproportionate numbers of severe cases which is not representative of the general population of ‘mothers with epilepsy’. Ideally, women would be recruited before conception through population-based epilepsy registers to avoid selection bias.

2.8.3. Selecting an Appropriate Comparison Group

There is also the issue of the most appropriate comparison or control group for AED exposed children. In the majority of cases, exposed children have been compared to the children of women without epilepsy. However, this makes it difficult to differentiate the effects of the mother’s epilepsy on the child’s development from the effects of the AEDs. The issues
relating to maternal epilepsy are discussed in detail in the following section, but include the effects of seizures during pregnancy and genetic risk of the child also developing epilepsy. Another approach has been comparisons with women with epilepsy who did not take AEDs (Eriksson et al., 2005). There is another issue here, in that women who did not need to take AEDs may not have comparable types of epilepsy or of equal severity to women who required AEDs. Others have included a comparison group including children born to fathers with epilepsy to control for genetic epilepsy influence but still these children were not exposed in utero to epilepsy-related factors such as seizures (Durner, Greenberg, & Delgado-Escueta, 1992). Nicolai et al. (2009) suggested that mothers without epilepsy but matched in SES may form the most appropriate control group.

2.8.4. Poor Control of Confounding Factors

There are multiple factors which can influence development in this cohort, which may interact or have a cumulative effect, making it difficult to dissociate the effects of AED exposure on cognitive outcomes. These confounding factors have not been well controlled in previous studies and include the following:

2.8.4.1. Epilepsy Related Factors

One major potential confounding factor is the effect of maternal epilepsy on fetal development. Epilepsy etiology and seizure type are both possible contributors, as is seizure frequency during pregnancy. Some studies have suggested that maternal seizures have a negative impact on the cognitive development of offspring (Hirano et al., 2004; Koch et al., 1999; Leonard et al., 1997; Lösche et al., 1994), whereas others have not (Gaily et al., 2004;
Koch et al., 1999; Ornoy & Cohen, 1996; Scolnik et al., 1994; Thomas et al., 2007). One study noted that children of mothers with idiopathic generalised epilepsy performed significantly better than a partial epilepsy subgroup (Eriksson et al., 2005; Kantola-Sorsa et al., 2007). The inconsistent findings between studies may reflect the heterogeneity of seizure types and etiologies in their samples. Some studies have suggested that primary generalised epilepsy and tonic-clonic seizure types may be associated with greater risks to child development (Adab, Kini, et al., 2004; Granström & Gaily, 1992; Koch et al., 1999; LaJoie & Moshé, 2004).

Seizure type can also dictate which AED is prescribed, which may bias the composition of some samples. For example, VPA is generally a drug of first choice for patients with generalised onset seizures and is better tolerated than topiramate and more efficacious than lamotrigine (Ikonomidou et al., 2000). However studies have shown that for partial onset epilepsy, carbamazepine and phenytoin provide better control than VPA (Hill, Wlodarczyk, Palacios, & Finnell, 2010; P. G. Williams & Hersh, 1997).

There are also genetically based confounds which need to be taken into account. As some epilepsy syndromes have a heritable component, a proportion of children of women with epilepsy can be expected to also develop the disorder. The presence of epilepsy in children participating in a study could potentially impact on brain functioning and confound the results. Ideally, these children should be excluded from samples.

2.8.4.2. Pregnancy Factors

The accurate documentation of AED type and dose is critical. Many studies have collected this information retrospectively, and as such may be subject to recall bias. The period of
exposure to the medication can also influence different developmental processes in different trimesters, so there is a need to record dates of prescription changes. Medical complications during pregnancy, such as pre-eclampsia, bleeding, prematurity and caesarean birth also have the potential to impact on child development and must be controlled. Lifestyle factors during pregnancy such as caffeine use, alcohol intake, smoking, substance use or folic acid supplementation should all be documented and also taken into account during analyses. These variables may in part be associated with socio-economic factors, as lower classes may be less inclined to take folic acid supplements and use intoxicants during pregnancy (Nicolai et al., 2008).

2.8.4.3. Environmental Factors

In terms of environmental factors, there is a critical need to control for socioeconomic status (SES). Research shows that SES is associated with a wide array of health, cognitive, and emotional outcomes in children, with the effects beginning prior to birth and continuing into adulthood (Bradley & Corwyn, 2002). Poverty and low SES are associated with lower scores on tests of cognitive function, lower levels of school achievement and increased levels of socioemotional problems (McLoyd, 1998). While maternal education appears to make some contribution to a child’s IQ, family income and poverty status have been reported as more powerful predictors in some studies (The Infant Health and Development Program, 1990). The family environment may also contribute to cognitive outcome. Children from a lower SES environment may differ in the level of cognitive stimulation that they receive at home (McLoyd, 1998). The provision of learning, academic and language stimulation influences intellectual development. The children of women with epilepsy have also faced with additional psychosocial issues relating to having a mother with a chronic illness. They appear
more vulnerable to the effects of environmental disadvantage than control children, but they also showed longer-lasting effects of environmental support. (Titze et al., 2008)

2.8.4.4. Parental Factors

There is also a need to control for parental IQ and education. The heritability of cognitive ability in childhood is well established (Bradley & Corwyn, 2002; Plomin & Spinath, 2002). Genetic factors account for around 50% of IQ variance (K. J. Meador et al., 2011). One of the most significant predictors of a child’s IQ is the IQ of the mother. Maternal education and SES, often substituted for IQ correlate at a lower level with child’s IQ (Meador, Baker, Cohen, Gaily, & Westerveld, 2007). The correlation for parental and child IQs is 0.42 (Kaufman, 1990; cited in Meador et al., 2007). SES also interacts with the heritability of IQ; with environment accounting for a significantly higher proportion of variance in impoverished families, and the opposite existing in affluent families (Pohl-Guimaraes, Krahe, & Medina, 2011). In samples of women with epilepsy, low maternal IQ, maternal education, and AED exposure were associated with significant impairment of intellectual and language functions for children at six years of age (Thomas et al., 2007).

Furthermore, there is suggestion in the literature that women who take VPA through pregnancy may be more likely to have lower IQ scores or lower levels of education than women taking other AEDs or controls (Eriksson et al., 2005; Gaily et al., 2004; Koch et al., 1999). Studies have shown that children’s IQs were significantly related to maternal IQs among children exposed to carbamazepine, lamotrigine, or phenytoin but not among those exposed to VPA (Meador et al., 2009). Thus, women taking VPA may represent a fundamentally different population from women taking other types of AED.
2.8.5. Sample Size and Power

Previous studies have been limited by small sample sizes. A Cochrane review found that only seven prospective studies contained more than 50 children exposed to AEDs (Adab, Tudur, et al., 2004). In many studies, there have been only small numbers of women taking certain drugs in monotherapy and high variability in polytherapy regimes. Frequently, monotherapy and polytherapy groups have been pooled together to increase power. Results have also been reported in terms of effects of AED exposure in general. As such, the effect of each specific drug has been difficult to isolate and this has limited the ability to make clinical interpretations based on the data.

2.8.6. Age at Assessment

A significant proportion of studies examining cognitive outcomes following AED exposure have focused on infant and preschool ages (see Table 2.1). While the early identification of impairments is critical to being able to implement appropriate interventions, these studies are limited in their ability to contribute to our understanding of the cognitive functioning and prospects of these children. In infancy and early childhood, cognitive skills are undergoing a greater velocity of change than at any other stage (G. P. Aylward, 1997). Subsequently, assessments of cognitive ability at this age can be unreliable. Lack of attainment on a cognitive measure may be for a number of reasons; the skill might be emergent, latent, delayed, deficient or disordered (G. P. Aylward, 2009). Further, performance on assessments of developmental ability at a younger age may not be representative of intellectual ability. There is significant argument within the literature over whether intellect can be adequately
predicted from scores on developmental scales in infancy (Blaga et al., 2009; Sternberg, Bundy, & Grigorenko, 2001). Aylward (2009) proposes that developmental and cognitive abilities merge into intelligence and IQ testing should be reserved for children aged 3 and over.

There has been a comparable lack of studies examining cognitive outcomes in school aged children. The capacity to learn, process information and problem solve only exist at simple levels in young infants and are subsequently difficult to measure. At school age, these skills develop and children’s thinking begins to become more complex and systematic. Problem solving abilities improve but children are generally limited to thinking in terms of concrete and tangible concepts (CliffsNotes.com, 2011). School aged children are also better at remembering than younger children. Investigations of outcomes in school-aged children will enable specific cognitive skills such as attention, memory and executive functioning to be characterised in detail. Further, if impairments exist in this cohort of children, they may only begin to emerge at this age when children are placed in situations that require higher cognitive processing demands.

### 2.8.7. Issues with Outcome Measures

There has been wide variability in the tools used to measure cognitive outcomes, partly due to the fact that there is little agreement about the best measures to use and at which age to follow children up at. Tests for assessing cognition not only vary in the particular domains that they measure but also in their subscales. It is also important to acknowledge that in many cases where lower scores have been reported on formal assessment tools, scores have still fallen with the age-expected average range. Some outcome variables have been poorly
defined in previous studies with ambiguity in the way variables such as ‘learning difficulties’ and ‘additional educational support’ have been operationalised.

Infant and preschool developmental tests typically sample areas of motor functioning, language, cognition and adaptive/social skills (G. P. Aylward, 1997). However, there is a lack of a “gold standard” for assessments in children within this young age range (G. P. Aylward, 1997). Tests vary in terms of the final index measure, which makes comparisons between studies difficult (G. P. Aylward, 2009). Assessment of school aged children has generally focused on broad IQ measures rather than specific cognitive domains. There is a significant lack of understanding about how skills such as learning and memory might be affected by fetal AED exposure. While the characterisation of the various cognitive domains is an obvious goal for future studies, analyses of tools with many domains and subtests must be interpreted with caution to avoid multiple comparisons and inflating the Type I error rate. It is vital that future studies also implement standardised assessment tools that have appropriate norms.

2.9. CONCLUSION

Due to the high adverse risk associated with seizures during pregnancy, women will continue to take AEDs and more children will be born exposed to these drugs. Therefore it is important that we understand not only the immediate risks, but also the long term outcomes these children may be challenged with. Fetal AED exposure, particularly to VPA or polytherapy, is associated with elevated rates of birth defects and behavioural problems such as autism. There is also strong evidence to suggest that VPA and polytherapy exposure increase the risk of cognitive impairments. Verbal abilities appear to be particularly
vulnerable to these medications. There are conflicting results following carbamazepine, phenobarbital and phenytoin exposure, however these drugs appear to carry fewer risks.

Previous studies have tended to focus on broader measures of cognition such as IQ rather than specific cognitive domains. Memory and learning skills have been poorly characterised. These skills are critical to the progression of cognitive development in childhood as they enable the acquisition of skills and knowledge. The impact of AED exposure at school age, when thinking skills become more complex, requires further investigation. In the early school years, children must adapt to the new educational environment which emphasises learning and memory function. As the cognitive skills of children this age continue to evolve, deficits in specific cognitive skills may become apparent. If impairments in learning and memory are induced by fetal AED exposure, they have the potential to have a longstanding impact on cognitive, educational, social outcomes which could extend into adulthood.

Finally, although previous studies in this area have helped shed some light on longer-term outcomes for these children, a number of methodological limitations have hindered the ability to make clinical inferences about each drug. Future studies must be prospective in design, well controlled in terms of variables and have sufficient power to detect meaningful differences. Studies should also incorporate standardised neuropsychological measures to help elucidate the specific cognitive domains these AEDs impact on. Characterising neuropsychological functioning in these children will be critical to the development of preventative care strategies.
CHAPTER 3. **THE EFFECT OF FETAL VALPROATE EXPOSURE ON BRAIN DEVELOPMENT**

### 3.1. BACKGROUND

The long term cognitive impairments observed in children exposed to AEDs *in utero* are assumed to be primarily related to exposure to the medication. The deficits are also assumed to be mediated by the effects of AEDs on brain development. This chapter will provide evidence to support the contention that brain development is affected by exposure to specific AEDs in the intrauterine environment.

AEDs are classified as teratogenic agents. Teratogens are environmental substances that are capable of inducing birth defects. Thalidomide, a sedative first synthesised in the 1950’s, was the first drug to dramatically illustrate the link between drugs and fetal abnormalities. It was completely withdrawn from the market after enormous increases in congenital malformations were documented in children (Lenz & Knapp, 1962). Ethanol is another example of a teratogen. Mothers who consume excessive alcohol during pregnancy place their children at risk of developing a characteristic ‘fetal alcohol syndrome’ (Jones & Smith, 1973). While the effects of some teratogens on brain development relate to the timing of exposure, AEDs are typically maintained throughout pregnancy. Subsequently, these medications potentially affect all aspects of brain development.

Recent years have seen a rise in the number of studies documenting problems following with sodium valproate (VPA) exposure. As reviewed in Chapter 2, there are higher rates of fetal malformations and poorer intellectual outcomes following prenatal VPA exposure. The discovery that VPA exposure can influence the development of cognitive skills strongly
suggests that underlying changes in the brain might occur in the absence of major malformations. Little research has been conducted into how fetal VPA exposure affects human brain development. Despite this, the effects of VPA have been widely documented in experimental animal models and the findings may help inform the fetal AED exposure literature.

This review describes what is currently known about the effects of VPA exposure on brain development. The key processes and timing of normal brain development will be presented before evaluating the evidence for the impact of VPA, drawing on studies in animal models and the limited research in humans. In animals, VPA has been shown to alter neuronal development and produce changes in cell morphology. The effects on neurotransmitter systems and synaptic plasticity are also discussed. Fetal VPA exposure in animals and humans produces changes in a number of brain regions. The generalisability of animal model findings to human fetal exposure is discussed and the potential mechanisms that may underlie cognitive deficits in VPA-exposed children are considered.

3.2. THE TIMELINE OF BRAIN DEVELOPMENT

Human gestation typically lasts 40 weeks and is divided into three trimesters (see Table 3.1.). Brain development is an ongoing dynamic process which begins in the third gestational week and continues through adolescence (Stiles & Jernigan, 2010). The structures of the central nervous system (CNS) are established through a sequence of developmental processes including gastrulation, proliferation, migration, differentiation, synaptogenesis and apoptosis. Disruption of these processes can give rise to structural malformations and abnormal neuronal circuitry which may impact on typical cognitive development.
The early stages of human brain development start with a process known as gastrulation in which the embryo is transformed into a three-layered structure and stem cells are produced. Neural progenitor cells emerge from this process, which are capable of generating all the cells which will eventually form the brain and CNS. Between Embryonic Day (E) 20 to 28 (E20-28), the neural tube develops. The neural tube is formed along the axis of the developing embryo by the neural progenitor cells. This is the earliest structural precursor to the brain and spinal cord. The anterior neuropore closes first from E24-26, followed by the posterior neuropore from E25-28 (Rice & Barone, 2000). Failure of the neural tube to close produces birth defects such as anencephaly and spina bifida.
3.2.1. **Anatomical Development**

From gestational week 9, the cortex begins to take on its characteristic shape, beginning with the formation of the longitudinal fissure. Primary sulci develop between week 14 and 26 and continues in a sequential manner through primary, secondary then tertiary branches up until the end of gestation (Stiles & Jernigan, 2010). Development of the hippocampus is important to establish the neural networks responsible for learning and memory. At 13 to 14 weeks' gestation, the hippocampus is unfolded and located on the medial surface of the temporal lobe. At 15 to 16 weeks, the dentate gyrus and cornu ammonis start to infold. By 18 to 20 weeks, the dentate gyrus and cornu ammonis have folded into the temporal lobe (Kier, Kim, Fulbright, & Bronen, 1997). At this stage, the hippocampus has acquired most of the characteristics observed in the adult hippocampus; however the hippocampal sulcus continues to fuse until the 30th week (Okada et al., 2003). In the prenatal period, the dentate gyrus displays ongoing proliferation of granule cells in the hippocampal formation (Rice & Barone, 2000).

3.2.2. **Key Developmental Processes**

After gastrulation is complete (around E28), the neural progenitor cells undergo extensive division in a process known as proliferation. This process builds a pool of progenitor cells which will support the later production of neurons. Disordered proliferation can result in macrocephaly or microcephaly (Schaefer, Sheth, & Bodensteiner, 1994). From E42 to E108, neural progenitors alter their division to generate neurons, in a process known as neurogenesis (Stiles & Jernigan, 2010).
Neurons produced in the centre of the brain through neurogenesis migrate outwards to the developing cortex with the assistance of radial glial cells. Migration is at its peak between weeks 10 to 21 gestation (Schaefer et al., 1994). Earlier migrating neurons form the deepest layers of cortex with successively migrating neurons forming the outer layers to produce a final six-layered cortical tissue. If migration is interrupted, neurons may not reach their intended final location within the cortex. Disorders such as schizencephaly, lissencephaly and corpus callosum dysgenesis can arise (Schaefer et al., 1994).

As the young neurons migrate, they begin to differentiate into more specialised cell types. Through differentiation, the neuron takes on particular phenotypic characteristics such as shape, size, polarity, and expression of neurotransmitters and receptors (Rice & Barone, 2000). Differentiation works in conjunction with migration to ensure the correct population of cells reaches its target population.

Organisation of the connections within the cortex begins between weeks 22-26 (6 months) gestation and extends several years postnatally (Schaefer et al., 1994). Neurons that have reached their final position in the cortex extend axons and dendrites to form connections, in a process known as synaptogenesis. Synaptogenesis allows neurons to communicate with each other via synapses and is critical for forming the basic functional circuits of the brain.

Neurogenesis produces around twice as many neurons in the developing brain as is required (Ikonomidou & Turski, 2010). Therefore, apoptosis, or programmed cell death, is also an important adaptive process during prenatal development (Buss, Sun, & Oppenheim, 2006). The death of certain cells is necessary to correct for proliferative and migratory errors and to eliminate transient cell populations. This promotes effective functional circuits within the brain (Buss et al., 2006). Apoptosis generally occurs alongside synaptogenesis; from the sixth month of gestation (weeks 22-26) to several years after birth (Ikonomidou & Turski, 2010).
Disturbances in developmental processes at this time can trigger the deletion of large numbers of neurons from the brain.

In the postnatal period, neurogenesis only continues within the olfactory bulb and hippocampus (Stiles & Jernigan, 2010). After birth, the proliferation and migration of glial precursors takes over as the driving developmental force within the brain, promoting ongoing myelination. There is also substantial pruning of synaptic connections, which aims to promote efficient connectivity between neurons.

### 3.2.3. Comparisons of Brain Development between Species

Although obvious differences exist between the brains of humans and other mammals, authors have suggested that it is possible to compare brain development between species (Ikonomidou & Turski, 2010). Although mammals differ in their gestational time, some anatomical features appear similar and the sequence of brain development events is comparable (Bayer, Altman, Russo, & Zhang, 1993; Rice & Barone, 2000). This suggests it may be possible to extrapolate findings from animal models of fetal drug exposure to human children. Despite this, there are some important considerations to take into account when comparing brain development across species.

Comparisons between species must take into account when the “brain growth spurt” occurs, which refers to a transient phase of rapid growth (Dobbing & Sands, 1979). In humans, this growth spurt corresponds with the period of synaptogenesis, beginning in the third trimester of pregnancy and reaching a peak around the time of birth. Conversely, the peak occurs after birth in rats between Postnatal Days (P) 7 to 10 (P7-P10). Therefore, it has been suggested
that to model human prenatal exposure to AEDs in rodents, exposure should occur prior to P10 (Ikonomidou & Turski, 2010).

The typical gestational period for rats is around 22 days, whereas human gestation lasts for around 280 days. Therefore, the fetal development of rats occurs over a much shorter time span relative to humans. In rats, neural tube formation is complete at E10.5-11, compared to E26-28 in humans. Disruption of neural tube closure on E12 in rodents with VPA produces symptoms consistent with autism, which is discussed in more detail later. The critical window to produce this phenotype is narrow as behavioural alterations were not as prominent following exposure on E7, E9.5 or E15 (Rout & Clausen, 2009).

Neurogenesis and migration begin in the second week of gestation, whereas in humans this begins following neural tube closure, in the fifth week (Rice & Barone, 2000). In rat models, the hippocampus develops between E17-20 (Bayer et al., 1993). Rat pups are born when neurogenesis of the granule cell populations in the olfactory bulb, hippocampus and cerebellum is just beginning; with most neurons generated by P21 days. In contrast, when humans are born the bulk of the granular neurons have already been generated (Bayer et al., 1993). The vulnerable period in which drug-induced apoptosis can occur exists in the first two postnatal weeks of life, whereas in humans stretches from the sixth month of gestation to several years after birth (Ikonomidou & Turski, 2010). Therefore there is a significant distinction between chronological age and relative developmental age.

### 3.3. VALPROATE METABOLISM BETWEEN SPECIES

While effective at controlling seizures during pregnancy, VPA can also be teratogenic to the fetus, evidently more so than other AEDs. Therefore, there is strong motivation to understand
the mechanisms that underlie VPA’s effects on fetal brain development. VPA’s anticonvulsant properties are mediated by its effects on the GABAergic and glutamatergic neurotransmitter systems (Kaindl et al., 2006; Loscher, 2002). In targeting these systems, the balance of synaptic inhibition and excitation is altered. However changes in neurotransmitters are unlikely to be solely accountable for the long-term cognitive difficulties observed in these children. A review by Loscher (2002) on the pharmacology of VPA notes that no single mechanism of action that can completely account for the numerous effects of the drug on neuronal tissue and its broad clinical activity in epilepsy. Therefore examining the mechanisms by which VPA exerts its anticonvulsant effects may not be particularly useful in attempting to elucidate the mechanisms underlying cognitive outcomes. The findings from animal models of exposure may be more beneficial, however some of the differences in VPA metabolism between species must be considered before these findings are discussed.

VPA is known to cross the placenta. Studies in rodent models have shown that VPA can be detected in the amniotic fluid. While concentrations in the amniotic fluid are much lower than in maternal blood serum, VPA remains in the amniotic fluid for longer (Omtzigt, Nau, Los, Pijpers, & Lindhout, 1992). VPA can also be detected in fetal blood serum. In catheterised pregnant rhesus monkeys, VPA crossed the placenta and reached higher concentrations in the fetal blood than maternal blood (Dickinson et al., 1980). Similarly, VPA concentrations in mouse embryos exceed corresponding free maternal plasma levels (Nau & Scott, 1987). In humans, the free concentrations of VPA in fetal blood at birth exceed levels detected in maternal blood (Dickinson, Harland, Lynn, Smith, & Gerber, 1979; Nau, Helge, & Luck, 1984). Therefore, at the time of birth, and potentially earlier developmental periods, the fetus is exposed to higher VPA concentrations than the mother. It is assumed that this exposure to VPA mediates the final phenotype of fetally exposed children.
Animal studies have shown that VPA is effective at controlling seizures at doses of 100-200 mg/kg (Otsuki, Morimoto, Sato, Yamada, & Kuroda, 1998). The corresponding dose for seizure control in humans is 10-60mg/kg (Drug Information Portal, 2009). VPA metabolism differs between species with humans processing VPA at a higher rate. Consistently, the dose of VPA required to exert teratogenic effects in experimental animal models is about 10 times higher than the usual therapeutic doses in humans (Nau & Hendrickx, 1987). Between species comparisons have also shown that the concentrations of unbound VPA in fetal serum are significantly higher in animal models (mouse < rat < monkey) compared to humans (Hendrickx et al., 1988). This is probably due to differences in placental transfer of the drug. It suggests that human embryo is exposed to lower levels of VPA than other species; however, since fetal VPA concentrations have only been measured following birth, direct comparisons of exposure levels between humans and experimental animals are not possible (Hendrickx et al., 1988).

3.4. THE IMPACT OF VALPROATE ON BRAIN DEVELOPMENT

Findings from in vivo and in vitro studies illustrate that VPA disrupts a number of crucial processes in neuronal development. There is evidence to suggest it affects neuronal proliferation and differentiation, promotes neurodegeneration and influences levels of neuroprotective factors. Defects in the cellular and molecular mechanisms of brain development have the potential to cause cognitive, motor, and intellectual disability (Schaefer et al., 1994).
3.4.1. Neuronal Proliferation

VPA has been shown to inhibit neuronal proliferation in cell cultures at therapeutically relevant doses. VPA inhibits proliferation of neuroblastoma and C6 glioma cells by 50% at concentrations of 0.5 and 1.0 mM respectively (Martin & Regan, 1991; Regan, 1985). Regan (1985) reported that these concentrations were within the range of therapeutically effective VPA plasma levels (0.07-1.1 mM). VPA at 1.0 mM reduces the proliferation of C6 glioma cells by blocking cells in the G1 phase of mitosis (Bacon et al., 1998; Martin & Regan, 1991).

VPA also inhibits proliferation in hippocampal neural progenitor cells. This has been demonstrated with 1mM VPA in vitro in embryonic and adult progenitor cell cultures (Hsieh, Nakashima, Kuwabara, Mejia, & Gage, 2004; Yu et al., 2009). An in vivo study has also shown that VPA (300mg/kg) decreases granule cell proliferation in the dentate gyrus of adult rats (Hsieh et al., 2004). Treatment of rats with 300 mg/kg VPA twice daily by intraperitoneal injection also reduced cell proliferation in the sub granular zone of the dentate gyrus within the hippocampus (Umka et al., 2010).

3.4.2. Cell Differentiation

VPA has been described as “embryotoxic” as it inhibits the differentiation of embryonic stem cells into endothelial cells (Festag et al., 2007). However VPA appears to promote differentiation of neuronal progenitor cells into neurons. Continued exposure to 1mM VPA induced differentiation in neuroblastoma and glioma cell cultures (Regan, 1985). It has been suggested that a blockade in the G1 cycle causes cells to exit and differentiate (Martin & Regan, 1991). Laeng et al. (2004) demonstrated a similar induction of differentiation with 0.5 mM VPA in rat embryonic cortical and striatal stem cell cultures and human fetal forebrain
stem cell cultures. Increased neuronal differentiation has also been demonstrated in embryonic hippocampal cell cultures, along with increases in proneural transcription factor expression and neuronal cell numbers (Yu et al., 2009). However the promotion of neuronal differentiation in the hippocampus by VPA is also associated with a suppression of oligodendrocyte and astrocyte differentiation (Hsieh et al., 2004).

3.4.3. Neurodegeneration

Gestational and early postnatal exposure to VPA causes widespread and dose-dependent apoptotic neurodegeneration in the developing rat brain in doses relevant for anticonvulsant control in humans. Administration of 50-400mg/kg of VPA to seven-day-old rats induced degeneration in multiple regions including the hippocampus, caudate, thalamus, striatum, and cerebral cortex (Bittigau et al., 2002; Kim et al., 2007). This is similar to the widespread neuronal death seen following prenatal ethanol exposure (Ikonomidou et al., 2000). Early VPA (400mg/kg) treatment on postnatal day 14 treatment caused significant increases in apoptosis in granule cells of the hippocampus and cerebellum (Yochum, Bhattacharya, Patti, Mirochnitchenko, & Wagner, 2010). Further evidence for increased apoptosis comes from the use of the TUNEL assay, used for detecting DNA fragmentation resulting from apoptotic signalling cascades. Prenatal exposure to VPA (pregnant dam received 100 mg/kg/day) caused a significant increase in the density of TUNEL-positive cells in hippocampal slices of mice prenatally exposed to VPA (Manent, Jorquera, Mazzucchelli, et al., 2007). Another study demonstrated that animals exposed to a single dose of 400 mg/kg VPA on postnatal day 14 had up to a 30-fold increase in the number of TUNEL-positive cells in the external granule cell layer of the cerebellum and a 10-fold increase in TUNEL-positive cells in the dentate gyrus of the hippocampus (Yochum, Dowling, Reuhl, Wagner, & Ming, 2008). These
studies suggest that apoptotic neurodegeneration may be one mechanism that underlies the cognitive impairments seen in human children prenatally exposed to VPA.

3.4.4. Neurotrophins

VPA exposure also affects the expression of neurotrophins, which suggests that the normal neuroprotective responses of the developing brain may be impaired. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and neurotrophic factor-3 (NT-3) promote the growth and maintenance of selected populations of neuronal cells. Neurotrophins also mediate the effects of experience on the brain and environmental deprivation or enrichment can markedly affect BDNF levels (McLoyd, 1998). BDNF has also been implicated in learning and memory processes, with high levels of BDNF immunoreactivity detected in the hippocampus and cerebral cortex (Plomin & Spinath, 2002). Subsequently, BDNF levels have been thought to reflect the degree of brain plasticity.

Studies have used adult animal exposure models and no studies could be found where neurotrophin levels have been examined following fetal VPA exposure. PCR analyses demonstrated that long term treatment of seven day old rats with VPA (100mg/kg) reduced BDNF and NT-3 mRNA expression in the developing brain (Shi et al., 2010). Normally, these neurotrophic factors stimulate neurite outgrowth in the developing nervous system. Findings are variable within the hippocampus. Twice-daily VPA treatment (300 mg/kg) of adult rats has also been associated with reductions in BDNF within the hippocampus (Umka et al., 2010). However converse findings have also been reported, with increases in levels of BDNF documented following chronic ingestion of 20 gm/kg VPA (Einat et al., 2003; Hao et al., 2004). In mice pups exposed in utero (on E11) to 800 mg/kg of VPA, there was lower cortical expression of BDNF mRNA in somatosensory cortex but no effect in hippocampal
subregions (Roullet, Wollaston, Decatanzaro, & Foster, 2010). BDNF is also highly implicated in development and function of serotonergic neurons (Titze et al., 2008), suggesting there may be abnormal development within this population of neurons following VPA exposure.

3.4.5. Neurogenesis

Neurogenesis is a process that starts when proliferating neuronal progenitor cells begin to express neuronal markers (Chen, Cai, Cao, Zhang, & Li, 2009). Ongoing neurogenesis through adulthood occurs within the hippocampus, where new neurons must be formed to support learning processes. Bromodeoxyuridine (BrdU) is a biomarker that is commonly used to detect proliferating cells in living tissues and indicates enhanced neuronal growth. The ERK (extracellular signal-regulated kinase) pathway is used by neurotrophic factors to regulate neurogenesis, neurite outgrowth, and neuronal survival. Chronic treatment of rats with VPA (20mg/kg) activated the ERK pathway and resulted in a significant increase in the number of BrdU-labeled cells in the hippocampus and some regions of entorhinal cortex (Hao et al., 2004). Specifically, neurogenesis was enhanced in the dentate gyrus, the hilius and CA3 regions of the hippocampus. Most recently, Shi et al. (2010) also reported increased neurogenesis in the hippocampus of rats intraperitoneally administered with 100 mg/kg VPA. However, converse findings have also been reported, with changes in hippocampal BrdU labelling following phenobarbital and clonazepam but not VPA (120mg/kg) administration (Chen et al., 2009). In animals exposed prenatally to VPA (pregnant dams administered 100 mg/kg/day), BrdU-labelled neurons were significantly increased in the stratum oriens of the hippocampus, with a parallel reduction in the percentage of cells within the strata pyramidal and radiatum (Manent, Jorquera, Mazzucchelli, et al., 2007). The authors suggested that in
VPA-exposed animals, pyramidal neurons fail to migrate correctly and remain in an ectopic position in the adult hippocampus.

3.5. EFFECTS ON CELL MORPHOLOGY AND STRUCTURE

The treatment of pregnant rats with VPA has been demonstrated to cause morphological and structural changes in the neurons and astrocytes of the offspring. Changes in morphology may impact on the integrity of synaptic connections and normal synaptic transmission. Early studies report that fetal exposure to high doses of VPA (9 times the therapeutic dose) is associated with 10-20% reductions in brain subcellular protein synthesis, including the synthesis of myelin (Patsalos & Wiggins, 1982). Further, prenatal VPA exposure to 200mg/kg on days 7-18 of gestation disrupted cell membrane order in the hippocampus and cortex of rat offspring (Vorhees, Rauch, & Hitzemann, 1991).

The most recent immunohistochemistry studies by Li et al. (2009) showed that the offspring of rats administered a single intraperitoneal injection of 600 mg/kg VPA during pregnancy changes the morphology of PV- and BDNF-immunoreactive neurons. PV-immunoreactive interneurons in the amygdala, prefrontal cortex and hippocampus changed in shape and size of the soma and in density and length of the processes. It was suggested that these shape changes may cause the interneurons to weaken their inhibitory connections to pyramidal neurons. In addition, there were fewer BDNF-immunoreactive neurons in the sensory cortex and the soma of the neurons were smaller and the processes were shorter. Fetal VPA (800 mg/kg on E11) exposure leads to reduced expression of the synaptic adhesion molecule Neuroligin-3 in hippocampal subregions (Kolozsi, Mackenzie, Roullet, Decatanzaro, & Foster, 2009). Neuroligins play an important role in synaptic maturation and contribute to forming synapses.
Findings suggest that fetal VPA exposure causes abnormal dendritic pruning to occur. In a study examining layer II motor cortical neurons in rats prenatally exposed to 350mg/kg VPA on E12.5, dendritic arborisation was more complex in apical dendrites of pyramidal cells (Snow et al., 2008). Long term administration of VPA was also associated with nonspecific changes in Purkinje cell parikarya and their dendritic processes in rat cerebellar cortex (Sobaniec-Lotowska, 2001).

In a study by Fennrich et al. (1998), exposure of cultured rat hippocampal slices to VPA impeded regular formation of the pyramidal cell layer in a dose-dependent manner. Radial astrocyte orientation was affected, even with low doses of VPA, suggesting abnormal cell migration. Prolonged application of VPA administered intragastrically to rats has also been associated with astroglial abnormalities in the pyramidal layer of the hippocampal gyrus and temporal cortex (Sobaniec-Lotowska, 2003). This study also noted that these changes “co-existed with distinct damage to neurons and structural elements of the blood brain barrier”.

3.6. DISRUPTION TO NEUROCHEMISTRY

At a molecular level, rats prenatally exposed to VPA display alterations in neurotransmitter levels and abnormal migration of the neurons which synthesise them. Variations in neurotransmitter levels may alter the efficacy of synaptic communication. Using in vivo microdialysis and high-performance liquid chromatography HPLC, it has been shown that serotonin levels in the frontal cortex of rats exposed prenatally to VPA (800 mg/kg on E9) is significantly higher than in control rats (Tsujino et al., 2007). With the same exposure conditions, the basal level of dopamine in the frontal cortex also appears to be significantly higher and was further exaggerated by swim stress (Nakasato et al., 2008).
Rats exposed to 800mg/kg of VPA on gestational day 11 (E11) demonstrated abnormal migration of catecholamine and serotonin neurons at E16 (Kuwagata et al., 2009; Ogawa et al., 2009). In another study, exposure to the same dose of VPA on E9 was associated with a shift in the distribution of serotonergic neurons later in life on postnatal day 50 (P50; Miyazaki, Narita, & Narita, 2005). Neurons shifted to a more caudal location in the dorsal raphe nucleus which was thought to reflect abnormal serotonergic neuronal differentiation and migration. In addition, Miyazaki et al. (2005) found that administration of VPA to neuronal cultures impeded maturation of serotonergic neurons and the expression of serotonin related genes.

There have been variable findings with regard to serotonin levels within the hippocampus. At E9, exposure to 600 mg/kg VPA resulted in a 46% decrease in hippocampal serotonin levels at P50 (Dufour-Rainfray et al., 2010). In this study, there was no reduction in cortical or cerebellum serotonin levels and there was no change in serotonin transporter density. These findings appear to conflict with earlier research from Narita et al. (2002), in which increased levels of hippocampal and cerebellar serotonin and frontal cortex dopamine were reported. Dufour-Rainfray (2010) hypothesised that the difference may be related to variation in experimental processes and that reduced serotonin levels may explain the altered social behaviours seen in these animals.

Prenatal exposure to VPA also appears to lead to decreased activity of the striatal-enkephalergic system. Enkephalins are an opioid, distributed throughout brain regions involved in processing emotional information. Fetal exposure to a dose of 600mg/kg VPA on E12.5 has been associated with decreased proenkephalin mRNA expression in the dorsal striatum and the nucleus accumbens (T. Schneider, Ziòkowski, Gieryk, Tyminska, & Przewocki, 2007).
3.7. DISRUPTION TO SYNAPTIC PLASTICITY

Long term potentiation (LTP) and long term depression (LTD) are mechanisms of synaptic plasticity thought to mediate learning in the hippocampus. Inducing LTP in the hippocampus of rabbits with electrical stimulation techniques, prior to training in a classical conditioning paradigm, increased the rate at which animals learned the task (Berger, 1984). Studies have demonstrated that these cellular mechanisms are disrupted by postnatal and prenatal VPA exposure, which may have implications for learning and synaptic plasticity.

Perfusion of hippocampal CA1 tissue with VPA reduces neuronal excitability (Yong, Zhang, Wang, & Ruan, 2009). In the CA1 region of the hippocampus, LTP and LTD are dependent on NMDA receptors. Lee (1996) was able to demonstrate that VPA inhibits NMDA receptor mediated LTP in rat hippocampal slices. Using intracellular recording techniques in rat amygdaloid slices, VPA suppressed synaptic responses mediated by NMDA receptors in a dose-dependent manner (Gean, Huang, Hung, & Tsai, 1994). Hence, the reduction of NMDA receptor mediated responses may contribute to impairment of synaptic plasticity.

The dampening effect on synaptic plasticity processes is more significant for exposure in the uterine environment compared to postnatal exposure via breastmilk. Chronic VPA exposure (300mg/kg) in the prenatal period more seriously impaired LTP and LTD levels recorded in hippocampal slices, compared to exposure during the lactation period (Zhang et al., 2003). More recently, 600mg/kg of VPA administered prenatally on E12 has been demonstrated to increase polysialic acid (PSA) expression in the hippocampus (Natori, Kodaira, Hirasawa, Gao, & Nagai, 2008). PSA promotes NMDA-R dependent LTP processes in the CA1 region of hippocampal cultures. Natori et al. (2008) noted that VPA stimulates neurite elongation and branching in cultured neurons and speculated that excess PSA may cause abnormal neuronal circuits in the hippocampus.
Conversely, it has also been demonstrated that cortical LTP between pyramidal neurons is enhanced by prenatal exposure to VPA and is associated with an overexpression of NMDA receptor subunits. In a study by Rinaldi et al. (2007), fetal VPA exposure (500 mg/kg on E11.5) significantly enhanced NMDA receptor-mediated transmission and caused increased plasticity in the neocortex. Fetal exposure to VPA also alters regional cortical connectivity following a single injection of VPA to pregnant rats. Embryonic exposure to VPA enhances local recurrent connectivity formed by neocortical pyramidal neurons, with weaker excitatory synaptic responses (Rinaldi, Silberberg, et al., 2008). In this study, Rinaldi et al. (2008) also observed a reduction in intrinsic excitability with a diminished number of synaptic contacts in layer 5 pyramidal neurons of the somatosensory cortex. Differences in connectivity have also been detected in the prefrontal cortex. Higher numbers of connections between pyramidal neurons and neighbouring neurons have been detected, with these connections displaying enhanced LTP (Rinaldi, Perrodin, et al., 2008). Finally, in vitro single cell electrophysiological recordings have demonstrated hyperreactivity to electrical stimulation in the amygdala. The amygdale of rats exposed to 500mg/kg of VPA on E12.5 was also hyperreactive to electrical stimulation and displayed boosted synaptic plasticity and reduced inhibition (Markram, Rinaldi, Mendola, Sandi, & Markram, 2008).

### 3.8. EVIDENCE FOR STRUCTURAL BRAIN CHANGES

There is surmounting evidence that prenatal VPA exposure affects brain development on a microscopic level. However macroscopic changes in brain structure tend to provide a better indication of functional outcome. This is illustrated by brain injury studies, in which regional abnormalities detected with neuroimaging correlate with cognitive deficits. Although it has
been an area of lesser focus, research does suggest that whole-brain and regional changes are evident at a macroscopic level following prenatal VPA exposure.

Findings from animal studies indicate there are global changes in brain weight. In rats, gestational and early postnatal exposure to VPA reduces brain weight (Thurston et al., 1981). Early postnatal treatment with VPA resulted in a decrease of hemispheric weight of 15% compared with controls (Bittigau et al., 2002). In terms of regional changes, treatment with VPA 800mg/kg on gestational day 9 or 11 induced hypoplasia of the cortical plate and an abnormally running nerve tract in the pons (Kuwagata et al., 2009). In the cerebellum, prenatally exposed rats (600 mg/kg of VPA on E12.5) exhibited smaller cerebellar volumes compared to control animals, both in the hemispheres and vermis. In the vermis, reductions in volume were greater in the posterior lobe than the anterior lobe and there were significantly fewer Pukinje cells (Ingram et al., 2000). Reductions in brain weight were minor in comparison to the reduction in cerebellar volume. The volume of the nucleus interpositus (corresponding to the globose and emboliform nuclei in humans) was also reduced but not the fastigial or dentate nuclei (Rodier, Ingram, Tisdale, & Croog, 1997).

Abnormalities have also been observed in motor neurons of the cranial motor nuclei. In a study by Rodier et al. (1996), pregnant rats receiving 350mg/kg of VPA bore offspring with a reduced number of motor neurons in the cranial motor nuclei V (trigeminal), XII (hypoglossal), VI (abducens) and III (oculomotor). Shortening of the region caudal to the facial nucleus and lengthening of the region caudal to the facial nucleus has also been demonstrated (Rodier et al., 1997). The mesencephalic nucleus of trigeminal, dorsal motor nucleus of the vagus and locus ceruleus were not affected, even though these nuclei form during the same period (Rodier et al., 1996). This led the authors to speculate that VPA is selective in its effects or that some early-forming cell groups recover more than others. Early
prenatal VPA exposure within the period corresponding to the third trimester of human pregnancy has also been shown to disrupt processing of sensory information in primary visual cortex. Using optic imaging techniques, ferrets exposed to 200mg/kg VPA between P10 and P30 displayed decreased contrast of orientation maps and reduced orientation selectivity (Pohl-Guimaraes et al., 2011).

The hippocampus is a region frequently reported as vulnerable to changes in the intrauterine environment. In line with this, VPA treatment has also been associated with structural abnormalities in the hippocampus. Fetal exposure to VPA at doses of 100mg/kg/day induced dysplastic lesions in hippocampus and cortex of rats (Manent, Jorquera, Mazzucchelli, et al., 2007). In this study, VPA was associated with increased levels of neuronal cell death and disturbed migration of pyramidal neurons from the hippocampus. Manent et al. (2007) noted that the offspring of rats exposed to carbamazepine did not exhibit these dysplasias. MRI analyses in rats have revealed a trend towards increased hippocampal volumes with “medium” doses (500 mg/kg) of prenatal VPA when sustained across the entire pregnancy, where as “high” doses (825 mg/kg) of prenatal VPA were associated with decreased cortical and brainstem volumes (Frisch et al., 2009).

3.9. EVIDENCE FROM BEHAVIOURAL STUDIES

Prenatal exposure to VPA also reduces activity in rats on a range of behavioural tests, which suggests brain dysfunction may occur in other regions. In a wide investigation by Schneider and Przewlocki (2005), the offspring of pregnant dams given 600mg/kg of VPA in a single dose on gestational day 12.5 demonstrated lower sensitivity to pain and higher sensitivity to non-painful stimuli, diminished acoustic prepulse inhibition, locomotor hyperactivity and repetitive behaviour. They also showed delayed maturation, lower body weight and delayed
motor development. Other studies have demonstrated abnormally high and longer lasting fear memories, altered exploratory movement and lower tendencies to initiate social interactions in rodents prenatally exposed to VPA (Dufour-Rainfray et al., 2010; Markram et al., 2008; M. Narita et al., 2010).

Studies have also utilised behavioural tasks known to rely upon integrity of the hippocampus, striatum and cerebellum. Some reductions are dose dependent whereas others are only seen in groups exposed to a high VPA dose (Vorhees, 1987). Animals with lesions of the hippocampus show impaired performance in their ability to swim to a hidden platform in the water maze task (Morris, Garrud, Rawlins, & O'Keefe, 1982). Consistent with the studies presented in earlier sections documenting anatomical and cellular changes in the hippocampus, 500-600 mg/kg VPA has been shown to impair spatial learning following both prenatal and postnatal (Wagner et al., 2006; Wu & Wang, 2002). Previous studies have established that hippocampal but not cortical volume is correlated with maze learning success (Niessen et al., 2005). Therefore, authors have postulated that there may be damage to the hippocampus following both pre- and post-natal VPA treatments. In a recent rodent study by Frisch et al. (2009), high doses of prenatal VPA above 100ug/ml induced decrements in general activity and deficits in learning and memory in a watermaze. Prenatal exposure to 500 mg/kg of VPA led to improved watermaze performance, which was also associated with larger hippocampal volumes. Prenatal exposure to 825 mg/kg of VPA however, was associated with poorer watermaze performance and reduced cerebral volumes. Another study demonstrated that while maze performance in terms of correct and incorrect choices did not differ between VPA-exposed animals (800 mg/kg on E9) and controls, “achievement of learning” was impaired (M. Narita et al., 2010).
The motor behaviours of mid-air righting, surface righting and negative geotaxis are delayed in VPA-exposed rats (Wagner et al., 2006). These behaviours have all been linked to cerebellar activity. The deficit in the appearance of these behaviours is consistent with earlier presented studies suggesting VPA causes cerebellar disruption. In Wagner’s (2006) study, while skills such as surface righting, geotactic response did eventually develop to the level exhibited by control mice, they failed to develop spatial learning in the water maze and deficits in grip strength. Prenatal exposure to VPA (600 mg/kg on E12) also alters both acquisition and reversal of discriminative eyeblink conditioning (Stanton, Peloso, Brown, & Rodier, 2007). VPA rats showed faster eyeblink conditioning, which is somewhat contradictory as literature suggests this response is usually impaired following cerebellar injury. The authors suggested the finding may be due to the timing of insult during the first trimester, followed by compensatory changes in these areas. Stanton’s (2007) exposed animals also showed impaired reversal learning but the evidence was considered inconclusive with respect to potential functional targeting by VPA of hippocampal or prefrontal regions.

3.10. CORRELATES BETWEEN ANIMAL AND HUMAN MODELS OF VALPROATE EXPOSURE

This section will examine how the findings from animal models of VPA exposure compare to human models. Failure of neural tube closure has been linked to major birth abnormalities such as anencephaly, encephalocele and spina bifida (Verity & Firth, 2003). There is strong evidence to suggest that VPA disrupts neural tube closure, however the mechanism is unknown. Exposure of both animals and humans to VPA around the time of neural tube closure (E9-11.5 in rodents; E20-28 in humans) is associated with a higher rate of neural tube
defects (Morrell, 2003). In humans, spina bifida has been associated with a significantly higher average daily doses of VPA in the first trimester (Omtzigt, Los, et al., 1992).

Despite these similarities, there is a degree of variation in the types of neural tube defects typically produced by VPA exposure across species. VPA only causes neural tube defects in humans, mice, and hamsters. Further, the dose of VPA required to exert teratogenic effects in experimental animal models is about 10 times higher than the usual therapeutic doses in humans. (Nau & Hendrickx, 1987). In early mice studies, VPA was only known to induce exencephaly, a defect of the anterior neural tube (Nau & Hendrickx, 1987). This only partially mimics the defects produced in humans, in which spina bifida is most commonly observed. A later study was able to induce spina bifida in mice, but it required three administrations of high dose VPA (Ehlers, Stürje, Merker, & Nau, 1992). This suggests that animals may be more resistant to VPA in comparison to humans (Ornoy, 2009).

Prenatal exposure to VPA during neural tube closure is gaining acceptance as a suitable means of experimentally inducing autism in rats. The discovery of this model was made based on observations that VPA was a risk factor for autism (Christianson et al., 1994; P. G. Williams & Hersh, 1997). The drug induces morphological, social and developmental deviations in rats which are deemed consistent with autism in humans (T. Schneider & Przewlocki, 2005). It has been extrapolated from studies of autism following thalidomide exposure, that autism in humans might emerge from VPA exposure at E20-24, during early neural tube development (Arndt et al., 2005). There appears to be a narrow critical window in which VPA might produce symptoms of autism. An animal study showed that VPA exposure on embryonic day 12 (E12) showed the most significant changes in behaviour compared to E7, E9.5 and E15 (Rout & Clausen, 2009). This is consistent with the period of neural tube
closure in rodents. It is unknown whether this is also the timing for human embryos, where neural tube closure occurs early in the first trimester of human pregnancy.

Schneider (2005) observed from the literature that offspring of rats injected with VPA on the 12.5th day of gestation showed brain abnormalities resembling those found in studies of autistic patients. It was subsequently proposed that VPA administration of VPA on 12.5 might induce behavioural changes that correspond to those frequently seen in autism. In confirmation, the offspring rats demonstrated altered nociception, diminished acoustic prepulse inhibition, locomotor hyperactivity and lower exploratory behaviour and diminished social behaviour. Given the apparent support in the literature for the validity of the VPA induced animal model of autism in being able to reproduce the behavioural disturbances seen in autistic patients, some of the findings from animal models may also be generalisable to children exposed to VPA as part of an epileptic treatment regime.

While the animal model of autism assumes a causative relationship between VPA exposure and autistic-like behaviours, a direct relationship does not exist in children exposed to VPA. Nevertheless, as discussed in Chapter 2, VPA exposure does appear to be a risk factor for the development of an autism spectrum disorder (Bescoby-Chambers et al., 2001). This illustrates that the relationship between VPA exposure and behaviour is more complex in children and other factors must also contribute to developmental outcomes.

### 3.11. EVIDENCE FROM HUMAN STUDIES

There are currently only two studies examining neuroanatomical changes in humans following prenatal AED exposure. In a case study, VPA treatment during pregnancy led to temporal lobe atrophy in the infant (Pardal-Fernández, Carrascosa-Romero, Rodriguez-
Vazquez, Marco-Giner, & Martinez-Gutierrez). Ikonomidou et al (2007) utilised voxel based morphometry in the only MRI neuroimaging study. Compared to age-matched unexposed adults, there were significant regional decreases of grey matter volumes in the lentiform nucleus and hypothalamus of adults exposed to AEDs. It was speculated that this may partly represent apoptotic neurodegeneration induced by AED exposure (Bittigau et al., 2002). No regional differences in white matter volumes were found. Despite this, the sample in the study was reasonably small (n=18) and was highly heterogeneous with respect to maternal AED use (phenytoin, carbamazepine, valproate, primidone). Only four subjects had been exposed to AED monotherapy and ten to polytherapy, therefore the study lacked power to differentiate the effects of each drug. Further, only four participants were exposed to VPA (in monotherapy and polytherapy) in utero. Clearly this highlights a critical area for future study, as each AED may affect brain development uniquely.

Interestingly, another recent study examining data of 900,000 births found that the birth-weight-adjusted head circumference of children exposed to VPA in monotherapy was reduced (Almgren, Källén, & Lavebratt, 2009). AED polytherapy was associated with an increased risk of microcephaly, but not VPA in monotherapy. This has potential implications for brain size, which may also be smaller following VPA exposure.

3.12. CONCLUSION

VPA is commonly taken by women with epilepsy throughout pregnancy; however there is an established risk that this medication can have a teratogenic effect on fetal development. While there is clear evidence that VPA can cause major malformations of the CNS and cognitive impairments, little is known about how brain development is affected in the absence of major malformations.
Evidence from cell culture and adult animal treatment models suggests the fundamental processes of neuronal development are adversely affected by VPA. Studies have shown that VPA has the ability to cross the placenta and attains equivalent or higher concentrations in fetal blood than in maternal blood (Dickinson et al., 1979; Nau et al., 1984). Therefore, it could be expected that similar disruptions in neuronal development may be observed following fetal exposure to VPA in humans.

Many studies have focused on the validity of using fetal VPA exposure as an animal model of autism and have failed to consider the implications of the findings for AED research. Evidence from behavioural studies in these animals suggests VPA causes dysfunction in the hippocampus, cerebellum and striatum. Further, structural changes have also been documented in the hippocampus, cerebellum and brainstem. These regions are consistent with the areas of the brain where increased apoptosis has been documented following gestational and early postnatal exposure. These findings suggest that these brain regions are specifically vulnerable to VPA exposure and children exposed to may also display abnormalities in these regions. There is an obvious gap in the literature regarding how the structure of the human brain is affected by fetal VPA exposure and there is opportunity for investigation with neuroimaging. Given that children exposed to VPA tend to exhibit the most significant cognitive impairments, they may be expected to show the greatest degree of brain abnormality or dysfunction.

Animal studies have established that there are alterations in synaptic plasticity following fetal VPA exposure and neurogenesis is reduced in the hippocampus. These findings have significant implications for the learning and memory skills of children exposed to VPA. The cognitive profile of children exposed to VPA has been poorly defined beyond reduced intellectual skills and language impairment. However, there is emerging evidence to suggest
poorer performance on memory tasks (Kantola-Sorsa et al., 2007; Vinten et al., 2005), which supports the idea of hippocampal dysfunction in these children.

Evidence from the animal models suggests there is a degree of VPA dose dependency against dysfunction in cellular processes (Bittigau et al., 2002) and behavioural impairment (T. Schneider & Przewlocki, 2005). However, it is difficult to compare the doses provided to animals to those effective for seizure control in humans. Neuropsychological studies have identified a dose relationship between VPA and cognitive skills, suggesting there is a potential for VPA’s impact on brain development to show a similar relationship. Whether a dose dependent relationship exists with cellular mechanisms in humans is an issue which is yet to be established. By identifying doses that carry a higher risk of adverse outcome, clinical management of pregnant women with epilepsy can be better managed.

In conclusion, women taking VPA during pregnancy are placed in a difficult position where the risks of teratogenicity are known, however the factors that contribute to the child's brain development are not. By investigating how brain development is altered by VPA exposure, the mechanisms underlying poor cognitive development can be understood. Understanding this relationship has major implications for the clinical management of women with epilepsy and for the creation of prevention and educational strategies for their children.
CHAPTER 4. RATIONALE, AIMS AND HYPOTHESES

This chapter will present the rationale and primary arguments that justify the two studies contained within this thesis. The aims and hypotheses that are to be evaluated will also be described. This chapter will highlight the clinical significance the findings of this thesis may have.

4.1. STUDY RATIONALE

The evidence reviewed in the preceding chapters provides strong support for the idea that fetal VPA exposure can impact on the development of cognitive skills. However, previous work has primarily focused on the broad characterisation of intellectual skills. The influence of VPA exposure on the development of other cognitive skills such as learning and memory has not been adequately explored. There are emerging reports in the literature that prenatal exposure to VPA may impair learning and memory skills in children, however little work has been done to characterise the impairment. There are four primary arguments which justify the studies contained in this thesis.

4.1.1. Memory is Poorly Characterised in Children Exposed to VPA

Only three studies have attempted to examine the performance of children exposed to VPA on measures of memory. In a retrospective study by Vinten et al. (2005), children exposed to VPA displayed impairments in everyday memory skills on the Rivermead Behavioural
Memory Test for Children. Eriksson and colleagues (2005) demonstrated that children exposed to VPA performed more poorly on a memory for faces task compared to children exposed to carbamazepine. In the same study, VPA exposure was associated with poorer performance on a list learning task relative to children of women with epilepsy who did not take medication. The other study that detected deficits on measures of memory following VPA exposure was conducted by Kantola-Sorsa and colleagues (2007). In this study, VPA-exposed children scored lower on tasks of sentence repetition and auditory memory than an unexposed control group, suggesting a weakness in working memory. Despite these findings, studies have failed to establish the pattern of memory deficit and suggest how this might fit into our current understanding of memory functioning. The studies to date have also been small or retrospective, limiting the ability to control for variables which may also influence cognitive outcomes, such as family demographics, seizures during pregnancy or birth complications.

4.1.2. Hippocampal Abnormalities Occur in Animal Models of VPA Exposure

The neurobiological mechanisms underlying the cognitive deficits in children exposed to AEDs are poorly understood, however behavioural studies in animal models suggest the fundamental processes of learning in the hippocampus may be disturbed. Prenatal exposure of rodents to VPA is associated with reduced spatial learning skills and longer avoidance latencies on passive avoidance tasks (Wagner et al., 2006). In another study, female animals exposed to VPA made more errors on a maze task (Vorhees, 1987). High doses of prenatal VPA above 100ug/ml has been shown to induce decrements in learning and long-term memory in a watermaze (Frisch et al., 2009). Further, animals exposed to VPA have difficulty learning a radial maze despite making the same number of correct choices as
control animals (M. Narita et al., 2010). These tasks are known to involve the hippocampus, suggesting brain dysfunction occurs in this region.

There is also evidence that VPA exposure can also cause cellular abnormalities and disrupt synaptic plasticity in the hippocampus. Fetal exposure to VPA at doses of 100mg/kg/day induces dysplastic lesions in hippocampus and cortex of rats (Manent, Jorquera, Mazzucchelli, et al., 2007). VPA exposure has also been associated with increased levels of neuronal cell death, disturbed migration of pyramidal neurons and disrupted cell membrane order in the hippocampus (Bittigau et al., 2002; Manent, Jorquera, Mazzucchelli, et al., 2007; Vorhees et al., 1991). Chronic VPA exposure (300mg/kg) in the prenatal period seriously impairs LTP and LTD recorded in hippocampal slices (Zhang et al., 2003). Prenatal VPA exposure has also been demonstrated to increase polysialic acid expression in the hippocampus, which may cause abnormal neuronal circuits (Natori et al., 2008).

In rodents, it has been demonstrated that hippocampal volume is correlated with maze learning success (Niessen et al., 2005). Frisch et al. (2009) showed that medium prenatal doses of VPA led to improved watermaze performance, which was also associated with larger hippocampal volumes. High doses of VPA led to poorer performance and reduced cerebral and brainstem volumes. Associations of a similar direction between hippocampal volumes and performance on memory have tasks been documented in pathological conditions in humans. Generally, smaller hippocampal volumes have been linked with poorer performance on memory tasks. Preterm children have significantly smaller hippocampal volumes bilaterally and show specific deficits in certain aspects of everyday memory (Isaacs et al., 2000). Left hippocampal volumes are main predictors for verbal memory function in elderly women and adolescents born preterm (Giménez et al., 2004; Ystad et al., 2009). Positive correlations between hippocampal volume and memory performance have been frequently
reported in Alzheimer’s patients (review; Van Petten, 2004). It is unknown whether a similar relationship exists between hippocampal volume and memory performance in children exposed to VPA. The literature suggests children exposed to AEDs have a vulnerability in their language skills. In line with the above evidence and the theory of material specificity, it raises question whether the left hippocampus might be more affected relative to the right.

4.1.3. Human Brain Structure may be Altered Following Fetal VPA Exposure

There is a substantial lack of studies examining how fetal exposure to VPA might affect brain structure in human offspring and whether these deviations in development are reflected in aspects of their cognitive functioning. In a case study, VPA treatment during pregnancy led to temporal lobe atrophy in the infant (Pardal-Fernández et al.). In the only neuroimaging study to date, Ikonomidou et al (2007) found significant regional decreases of grey matter volumes in the lentiform nucleus and hypothalamus of adults exposed to AEDs prenatally. Studies are yet to examine whether hippocampal dysfunction might also occur in children prenatally exposed to VPA.

4.1.4. Measures of Hippocampal Memory Functioning have not been Examined in Children Exposed to VPA

Lesion studies have illustrated that the hippocampus contributes to the learning of associations between novel stimuli (Petrides, 1985). Higher susceptibility to retroactive interference is also an indicator of hippocampal dysfunction (Giovagnoli & Avanzini, 1999; Saling, 2009). Given the suggestions from both the animal and human exposure literature that the development of the hippocampus is affected by fetal VPA, deficits in tasks tapping
hippocampal function may be expected. Associative learning abilities and retroactive interference have not yet been examined in children exposed to VPA.

4.2. AIMS AND HYPOTHESES

This thesis had two broad aims which contained a number of specific goals:

1. *To examine memory outcomes in children prenatally exposed to AEDs.*

   Specifically, this thesis aimed to characterise the memory profile of children exposed to VPA *in utero* at school age. This thesis aimed to determine if any detected memory impairment is primary in nature or secondary to impairment in other cognitive domains. Finally, this thesis aimed to examine the relationship between VPA dose and performance. A range of potentially confounding background factors were examined to identify predictors of outcome.

2. *To understand the role of AEDs on development of the brain systems important for memory function.*

   In particular, this thesis aimed to examine whether prenatal VPA exposure impacts on hippocampal volume in human offspring. This study also aimed to assess if a dose-dependent relationship existed with hippocampal volume in this cohort. This thesis aimed to examine associative memory skills in children exposed to VPA and whether performance is related to hippocampal volume or VPA dose.
There were two principle hypotheses each of which included subsidiary predictions.

1. Children exposed to VPA will demonstrate a poorer performance on all neuropsychological memory tasks (verbal, non-verbal, associative learning) compared to typically developing children and children exposed to other AEDs.
   
   a. It was expected that the performance of VPA-exposed children on memory tasks will be associated with VPA dose.

   b. After controlling for potentially confounding background variables, it was hypothesised that VPA dose will be the most significant predictor of memory performance.

2. Children exposed to VPA will display a difference between left and right hippocampal volumes;

   a. It was expected that VPA dose will exert a dose-dependent effect on hippocampal volume.

   b. It was predicted that hippocampal volume would be positively associated with associative learning performance in children exposed to VPA.
4.3. CLINICAL SIGNIFICANCE

The studies in this thesis will contribute to the growing body of literature dedicated to understanding fetal outcomes following exposure to AEDs in utero. The studies in this thesis will be clinically significant as they are the first to specifically investigate whether memory difficulties exist in this cohort of vulnerable children. Further, it will establish whether the problems experienced by these children are attributable to a primary memory impairment or other confounding factors. The findings of these studies will be of high importance in identifying and characterising the adverse impact that VPA exposure has on cognitive development in school-aged children. This thesis will also undertake the first study internationally to examine brain structure in a group of children exposed to a single medication. The findings of this study will help to elucidate the underlying mechanisms for cognitive changes in VPA-exposed children.

The outcomes of these studies will have major implications for the creation of prevention, developmental surveillance and educational strategies. After characterising the cognitive impairments of exposed children, early interventions can be designed to target areas of difficulty and minimise the impact of the impairment on later development. The study findings will also have a major impact upon the clinical management of women receiving VPA. Treating doctors will be in a more informed position about the impact of certain medication regimes on the developing fetus and in some situations may be able to alter their prescriptions accordingly. Further, the findings will also inform women with epilepsy, providing an evidence base to inform their decision making and facilitate their understanding of risks and possible outcomes. Finally, this thesis will also inform our general understanding about the sensitivity of the developing fetus to anomalies in the intrauterine environment.
CHAPTER 5. METHODOLOGY

This thesis comprises two studies that were conducted in chronological order. The first study focused on memory outcomes following AED exposure. Memory outcomes were examined in a group of 105 children exposed to different AEDs by means of neuropsychological assessment. From this cohort, a smaller number of children were selected to participate in the second, neuroimaging study. The neuroimaging study involved 14 children exposed to VPA monotherapy who underwent a MRI scan and additional computerised cognitive tasks. This study had a specific focus on hippocampal volumes and correlates of associative learning. The current chapter will present the methodology used in these studies.

5.1. PARTICIPANTS

Recruitment of women with epilepsy and their children took place through the Australian Pregnancy Register of Antiepileptic Drugs for Women with Epilepsy and Allied Conditions (APR). The APR is a voluntary register which was established in 1999 to document the incidence of adverse fetal outcomes in a cohort of women taking AEDs through pregnancy. It is a centralised, observational study that enrols women prospectively. Women enrolled in the APR are of varying demographic characteristics (i.e. age, ethnicity, socioeconomic background, urban region), and the majority were taking AEDs in the context of epilepsy treatment (Vajda et al., 2007). In the original study conducted by the APR examining birth outcomes, women with epilepsy completed four telephone interviews over the course of their
pregnancy and after birth with study nurses (Vajda, O'Brien, Hitchcock, Graham, & Lander, 2003).

Children between the ages of six and eight participated in the memory outcomes study. This age range was selected to capture early school-age functioning. Children below the age of six years old may not have fully developed the cognitive skills required to complete the study tasks and may not have been able to complete the imaging procedure without sedation (Hallowell, Stewart, de Amorim e Silva, & Ditchfield, 2008). It is generally assumed in the literature that cognitive assessment at the age of six years or older is predictive of school performance and cognition as an adult (Eriksson & Kalviainen, 2010). An upper limit of eight years old was set to contain the amount of variability in the sample with respect to level of cognitive ability, which increases exponentially as schooling progresses. From this sample, women who took VPA monotherapy were invited to participate in a follow-up MRI study. Some children who participated early on in the memory outcomes study later participated in the neuroimaging study, so there was a wider age range in these children; from six to ten years old.

Children were excluded from the study if they had been diagnosed with major malformations or neurological disorders, including epilepsy. Previous studies have included these children in their sample, which could be a source of confound as these conditions may impact on cognitive development (Adab, Kini, et al., 2004; Dean et al., 2002; Gaily et al., 2004; Meador et al., 2009). Children were also excluded if their parents declined to participate for any reason, including if they would prefer not to know about their children’s performance on cognitive testing, or about brain abnormalities which may be detected on neuroimaging.
5.1.1. Recruitment

Women enrolled onto the APR in the first four years of its inception were recruited to the study. These women often had not been in contact with the APR since the last research interview was conducted by the study nurse when the child was aged one year. During the time line of the current project, their children were aged between six and ten years old. A summary of the recruitment process is presented in Figure 5.1.

Between 1999 and 2002, there were 402 live births to women enrolled on the APR. Twenty-one of these children were excluded on the basis of birth defects. Women living in the states of Victoria (VIC), New South Wales (NSW) and Queensland (QLD) were invited to participate due to higher enrolment numbers. Between November 2007 and September 2010, 176 women with children aged six to eight years were contacted and invited to participate in the initial memory outcomes study. Women with epilepsy who did not take medication were also contacted to take part in a larger ongoing study. Other women enrolled on the APR were not contacted because their child had not yet entered the eligible age range or attempts to contact them by telephone were unsuccessful.

Women in the current study were initially contacted by a study researcher by telephone and asked if they would be willing to receive an information package. They were sent an explanatory statement and consent forms by mail. If written consent was not returned in two weeks, a follow up phone call was made. Women were given an opportunity to discuss the project with the researcher and raise any concerns. Verbal consent was often provided during these discussions. Written consent was obtained from all women prior to participating in the study, either before or at the first assessment.

There were 21 women who declined to participate. The most commonly provided reasons for declining were an inability to meet the time commitment (33%), concerns about their child’s
ability to cope with testing (29%), and maternal illness (14%). Twelve women consented but researchers were unable to visit their geographical location. Forty women expressed interest but did not provide written consent prior to cessation of the study. The final sample comprised 99 women with 111 children. Of these, six children were excluded; two had developed epilepsy, two had malformations detected since birth, and two were not exposed to AEDs. The results of 105 children were included in the final analysis. All assessments were conducted blind to drug exposure status. To ensure this information remained unknown to researchers, drug exposure details were only collected from medical records following participation in the cognitive study.

Following participation in the cognitive study, twenty-four women were invited to participate in the neuroimaging study. Two women were unable to be contacted by telephone to receive an invitation. Selection was based on VPA monotherapy treatment, which was revealed once participation in the cognitive study was complete. Six women declined to participate, expressing concerns about their child undergoing the MRI procedure (66%) or they were unable to travel to Melbourne (33%). Five women verbally expressed interest but did not return their consent prior to cessation of the study. Thirteen women with 17 children participated in the study. Two children refused the MRI scan and another child was excluded on the basis of updated drug exposure information, which changed from VPA monotherapy to VPA polytherapy during data quality control checks with medical records. A total of 14 children were included in the final data analysis.
Figure 5.1. Recruitment Process for Memory Outcome and Neuroimaging Studies
5.1.2. Control Sample

It was initially proposed that a control group would also participate in this study. A sample size of 30 children aged six to eight years was sought. The control group was intended to comprise healthy mothers without epilepsy and their offspring, matched to study participants on parental socioeconomic status; maternal age, IQ and alcohol and smoking history; and age, sex and birth order of the child. Women who had taken an AED in pregnancy, children who had been diagnosed with epilepsy or had other pre-existing neurological disorders and major congenital malformations were to be excluded.

Multiple modes were used in an attempt to recruit this typically developing sample to this study. Women with epilepsy recruited to the study were encouraged to invite a friend with a similarly aged child to participate. Pregnancy Register updates sent to epilepsy publications also invited eligible women to participate as controls. Advertising flyers and pamphlets were produced and displayed at the participating institutions (Monash University, Royal Children’s Hospital, Murdoch Childrens Research Institute). Advertising material was also circulated through the institutions’ email bulletins. Community-run child care facilities were also contacted and sent advertising material to display. It was anticipated that after the first contact from interested parties, additional information about the study would be sent. No response was received utilising these modes.

As a secondary measure, council run Maternal Child and Health Centres were approached to assist with recruitment. This was approved by the Department of Education and Early Childhood Development Early Childhood Research Committee. Fifteen inner suburban municipalities of Melbourne were selected to be targeted. Each municipality had at least five individual Maternal Child and Health centres. A researcher liaised with each municipality’s Maternal Child and Health coordinator to attend their regular team meeting attended by all
the Maternal Child and Health nurses. In these meetings, the researcher gave a brief presentation on the study and distributed advertising material for the nurses to display in their individual Centres. This approach was also unsuccessful as no interested parties contacted the research team.

In light of the unsuccessful recruitment strategies, attempts were made to obtain pre-existing control data from other studies within the Royal Children’s Hospital. In particular, 14 structural imaging scans was sought to act as a comparison group for the VPA-exposed children within the current study. Studies that included children aged six to eight years and who met the exclusion criteria were identified. These studies had conducted MRI scans with comparable sequence parameters to the current study. It was planned that these studies would be supplied with a list of ideal age/gender combinations for the 14 VPA-exposed children. MRI received from these studies would be stripped of all identifying data, other than age and gender information. While chief investigators on these other projects consented to the provision of MRI data, this was not approved by the ethics board. Given the timeline of the thesis, further attempts to obtain control data were not made. Despite extensive literature searches, no studies could be found which provided normative data for brain variables within the appropriate age range and which were acquired on a comparable MRI scanner.

5.2. PROCEDURES

The study was coordinated from the Southern Clinical School at Monash University until the end of 2008 and then the Murdoch Childrens Research Institute. The study was approved by ethics review boards of both coordinating institutions (Monash University and Royal Children’s Hospital, Australia).
The cognitive assessments were generally conducted at Monash Medical Centre or the Royal Children’s Hospital; however some women were unable to travel due to work commitments or medical related issues. In these instances, assessments were conducted at the child’s school or in the family home. Interstate assessments were conducted at rental apartments, the child’s school or in the family home. All MRI scans were conducted at the Royal Children’s Hospital, Melbourne.

The cognitive tasks that are the focus of this research were administered in the context of a larger battery that forms part of an ongoing study. The cognitive assessments were conducted in an appointment lasting five hours including breaks; however parents were also given the option to conduct the assessment over two sessions. All children completed a fixed order battery of neuropsychological tests that included measures of interest to this study. Mothers completed a brief intellectual assessment. These tasks were administered by an appropriately qualified researcher who was blind to drug exposure details. Mothers also completed a questionnaire to provide additional information about their family and child. Information on maternal epilepsy, pregnancy and medical history was obtained from prospectively collected records by an independent researcher.

One child had completed a psychological assessment in the 12 months prior to participating in the study. Three had completed a language assessment with a speech pathologist within 12 months prior to participation. In these instances, consent was sought from parents to obtain the scores from the clinician to avoid practice effects and prevent interference with clinical treatment. All mothers were provided with verbal feedback outlining their child’s performance approximately two weeks following the assessment. If concerns were raised by the findings of the assessment, this was communicated by the researcher and recommendations were provided. Soon afterwards, mothers were sent a short report outlining the neuropsychology results.
In the follow-up imaging study, which targeted children exposed to VPA monotherapy, families attended the Royal Children’s Hospital on one or two occasions, depending on time availability of the families. Families from VIC, NSW and QLD attended the hospital. All children, except one, underwent a mock MRI scan with trained staff at the Royal Children’s Hospital prior to their MRI scan. Participation in a mock MRI scan has been associated with higher success rates for completing MRI scans in children and reduces anxiety (De Amorim e Silva, Mackenzie, Hallowell, Stewart, & Ditchfield, 2006; Hallowell et al., 2008). The mock scan lasted approximately 30 minutes. The MRI scan was not attempted if the child was unable to complete the mock scan, due to distress or excessive movement. One child did not pass their mock scan and the child who did not receive a mock scan did not participate in the MRI scan.

The MRI scanning procedure lasted up to 60 minutes and included sequences that are part of a larger ongoing study. The scans were reviewed by a radiologist. No clinically significant findings were detected on any participating child’s scans. A copy of the MRI report was sent to the child’s family.

The subset of children who underwent MRI also completed additional computerised tasks that were not part of the previous cognitive assessment. These children also completed a fixed-order battery of computerised tests lasting approximately 30 minutes. The software was run on a PaceBlade Tablet PC with a touch-sensitive screen. Participants sat at a comfortable height approximately 50cm from the screen.

5.3. MEASURES

As discussed in Chapter 2, previous studies have failed to adequately control for maternal factors (other than those that are AED related) that could contribute to poorer cognitive
outcomes in children. As such, this study sought to obtain accurate measures of pregnancy and medical variables which could also influence development. Measures of maternal intellectual ability and family SES were also collected as these factors have been correlated with childhood abilities.

In light of the literature reviewed earlier suggesting that mesial temporal memory function may be compromised by fetal VPA exposure, a targeted neuropsychological assessment was prepared to administer to participating children. The primary goal was to use measures with known sensitivity to hippocampal function, but also to examine aspects of memory which may be affected due to verbal impairments in affected children. Accordingly, measures of intellectual ability and core language skills were also obtained to enable discrete impairments to be identified. Memory abilities identified as important to measure were verbal learning, recall and recognition, non-verbal/visual learning and recall, retroactive interference, associative learning ability and working memory capacity. A summary of the variables collected in the child assessment and the corresponding cognitive skills intended to be measured is presented in Table 5.1.

5.3.1. Prospective Information

Information on drug exposure, pregnancy and medical history was obtained from prospectively collected records from the APR. Information was not available for two children, and this information was collected retrospectively from mothers over the telephone.

The following information was obtained from all women:

i. Demographic details of the parents;
ii. Family socioeconomic status (SES) was rated according to the ANU-4 Scale (Almgren et al., 2009). The mean SES score on this scale in the Australian population is 47±22.5.

iii. Epilepsy history of the mother, including age of onset of seizure disorder, seizure type and frequency, both habitual and during pregnancy;

iv. History of previous pregnancy and outcomes;

v. Information on AEDs taken during pregnancy, including dosage levels, dates of medication changes, duration of exposure of fetus during pregnancy;

vi. Child's exposure to other potential teratogens during pregnancy, including tobacco, alcohol, marijuana, caffeine and other medications;

vii. Date of birth of child, birth weight, birth term, duration of breast feeding, birthing complications, perinatal history of child including any serious illnesses, and congenital malformations.

5.3.2. Maternal Measures

5.3.2.1. Wechsler Abbreviated Scale of Intelligence (WASI)

The Wechsler Abbreviated Scale of Intelligence (WASI) was used to assess the intellectual ability of mothers recruited to the study (Wechsler, 1999). The WASI is a short measure derived from the Wechsler Adult Intelligence Scale (WAIS-III) and is used to estimate intellectual ability in individuals aged 6 to 89 years. The two-subtest was administered, which includes the subtests of Vocabulary and Matrix Reasoning. This form took approximately 15 minutes to administer. The WASI yields an estimated Full-Scale IQ score (100±15).
5.3.3. Child Measures

The following section describes the neuropsychological tests that were administered to participating children.

5.3.3.1. Wechsler Intelligence Scale for Children 4th Edition (WISC-IV)

Intellectual ability of the participating children was assessed using Wechsler Intelligence Scale for Children 4th Edition (WISC-IV), an intelligence test for children between the ages of 6 and 16 (Wechsler, 2003). The test comprises ten core subtests which generate a Full Scale score (FSIQ) and four composite scores known as indices: Verbal Comprehension (VCI), Perceptual Organizational (POI), Processing Speed (PSI) and Working Memory (WMI). Each of the ten core subtests is given equal weighting towards the FSIQ. The VCI comprises the subtests of Vocabulary, Similarities and Comprehension. The subtests of the POI are Block Design, Matrix Reasoning and Picture Concepts. The PSI comprises the subtests of Coding and Symbol Search. WMI consists of the Digit Span and Letter-Number Sequencing subtests. The ten core subtests of the WISC-IV were administered to participating children in approximately 90 minutes. The Full Scale IQ score (FSIQ; 100±15) was the primary outcome measure.

5.3.3.2. Clinical Evaluation of Language Fundamentals – Fourth Edition (CELF-4)

Children’s language skills were measured using the Clinical Evaluation of Language Fundamentals – Fourth Edition – Australian standardisation (CELF-4; Banach et al., 2010).
This test is considered the ‘gold standard’ to assess language skills in children aged 5 through 21 years. The CELF has four core subtests measuring expressive and receptive language skills: Concepts and Following Directions, Word Structure, Recalling Sentences, and Formulated Sentences. These subtests take 30-60 minutes in total to administer. Scores on these tasks were summed to produce a measure of general language ability termed the Core Language score index (100±15).

5.3.3.3. Developmental Neuropsychological Assessment (NEPSY-II)

The NEPSY-II is a collection of neuropsychological tests, that is frequently using in clinical settings used to assess neuropsychological development in children aged 3 to 16 years (Korkman, Kirk, & Kemp, 2007). The second edition was published in 2007 and improved on the original edition by extending age norms, adding subtests and eliminating domain-level scores. There are 32 subtests and four delayed tasks. It provides comprehensive assessment over six functional domains: Attention/Executive Functions, Language, Sensorimotor Functions, Visuospatial Processing, Memory and Learning and Social Perception. Each NEPSY test is freestanding and scaled scores are based on the standardisation of over 1,000 children tested throughout the United States (10±3). Three subtests from the NEPSY-II were administered to children in our sample.

5.3.3.3.1. List Memory and List Memory Delay

The List Memory subtest is designed to assess verbal learning and memory, rate of learning and the role of interference in recall for verbal material. It also assesses rote learning of unorganised information. The child is read a list of 15 words five times and recalls them after
each presentation. This is followed by an interference trial and an immediate recall trial. A delay condition is administered 25-35 minutes following completion of the subtest to assess long-term memory for words. The task takes around 8 minutes to administer.

The scaled score for List Memory was used (10±3), which combines the scores from the learning and delay subtests. Normative data are available for children aged 7-12 years. Six process scores can also be calculated; however these were not utilised in our analysis. For the purposes of our analyses, we calculated two additional variables. Learning ability was represented by the sum of total correct responses across the five learning trials ($\sum_{T1-T5}$). A measure to represent the Interference effect was calculated ($\frac{T5-T7}{T5}$) and expressed as a percentage. The raw score from List Memory Delay subtest was used as a measure of delayed recall.

5.3.3.3.2. **Narrative Memory**

Narrative Memory assesses memory for organised verbal material under free recall, cued recall and recognition conditions. It requires the ability to listen attentively, comprehend what is heard, organise and retrieve the information. The child listens to a story and is then asked to repeat the story. The child is then asked two types of questions (cuing and forced choice recognition) questions to elicit missing details from their recall of the story. Cuing serves to prompt recall and demonstrates encoding versus memory search capacities. Recognition indicates the degree to which an encoding versus retrieval deficit is present. The subtest takes approximately seven minutes to administer.

The raw score under the Free Recall condition was used to provide a primary measure of encoding prose. The corresponding scaled score (10±3) was another outcome measure.
Normative data are available for children aged 5-12 years. The subtest provides a percentile rank for the recognition trial, however only the raw recognition score was utilised.

5.3.3.3.3. **Memory for Designs & Memory for Designs Delay**

Memory for Designs is a visual learning task designed to measure a child’s memory for visual details and spatial locations. The child is shown a 4x4 grid with four to ten designs on a page, which is then removed from view. The complexity of the grid varies depending on whether the child is aged above or below seven years. The child selects the designs from a set of cards and places the cards on a grid in the same location as previously shown. This is repeated over four trials with an increasing number of designs. A delayed task is administered 15-25 minutes later to assess long-term visuospatial memory. This subtest takes approximately 13 minutes to administer.

The raw and scaled scores for Memory for Designs and Memory for Designs Delay were used. The first raw score sums learning across trials and represents the child’s overall ability to learn the location of novel visual details. The delayed raw score measures long-term storage and retrieval of visual information. Normative data are available for children aged 3-16 years to produce two corresponding scaled scores (10±3). There are also process scores which break performance down into recall for content and spatial locations; however these were considered too specific and were not incorporated into our analysis.
5.3.3.4. The Cambridge Neuropsychological Test Automated Battery (CANTAB)

The Cambridge Neuropsychological Test Automated Battery (CANTAB) is a computer-based cognitive assessment system originally developed at the University of Cambridge in the 1980’s (Sahakian & Owen, 1992). It comprises a collection of neuropsychological tasks that are administered to subjects using a touch screen computer. The 22 subtests of the CANTAB examine various areas of cognitive function, including visual memory and learning, working memory and executive function, attention and reaction time, semantic/verbal memory and decision making and response control. It also has basic screening tests. Studies have validated the use of the CANTAB in children between five and twelve years of age (Luciana & Nelson, 2002). It aims to be language independent through the use of non-verbal stimuli. Only children who underwent MRI were administered the CANTAB. The children completed a screening task and two cognitive tasks from this battery.

5.3.3.4.1. Motor Screening Task

The Motor Screening task screens for visual, movement and comprehension difficulties. The task involves pressing a series of flashing crosses in different locations on the screen. It is administered at the beginning of the test battery to familiarise the participant with the computer and the touch screen.

The Motor Screening task takes 3 minutes to complete. If the participant is unable to follow the requirements of the test it is unlikely they will be able to complete other tests successfully. All children participating in the study passed this introductory screening task.
5.3.3.4.2. **Paired Associative Learning (PAL) Task**

The Paired Associative Learning (PAL) task assesses visual memory and learning. The task is sensitive to changes in medial temporal lobe functioning and taps aspects of memory that represent fundamental functions of the hippocampus. This task has been shown to be a predictor of memory dysfunction in early Alzheimer’s dementia and patients with temporal lobe excisions (De Jager, Milwain, & Budge, 2002; Fowler, Saling, Conway, Semple, & Louis, 2002; Owen, Sahakian, Semple, Polkey, & Robbins, 1995). The task takes approximately 10 minutes to complete.

In the task, six to eight boxes are displayed on the screen and are opened in a randomised order. One or more of them will contain a pattern. The patterns shown in the boxes are then displayed in the middle of the screen, one at a time, and the subject must touch the box where the pattern was originally located. There are eight stages, each increasing in difficulty with more patterns. If the subject makes an error, the patterns are represented to remind the subject of their locations. When the subject gets all the locations correct they proceed to the next stage. Each stage may have up to 10 trials in total. If the subject cannot complete a trial correctly in this time, the test is discontinued. All children in our sample were able to complete the task.

Numerous outcome measures were produced including total errors for each level of difficulty, total number of trials for each level of difficulty and stages completed. The “first trial memory score” represents the number of patterns correctly located after the first presentation summed across the stages completed. The “mean trials to success” and “mean errors to success” scores are the total number of trials/errors for all stages and then divided by the number of stages. Standardised Z-scores (0±1) were generated by the CANTAB program based on a normative sample of children.
5.3.3.4.3. **Spatial Working Memory (SWM) Task**

The Spatial Working Memory (SWM) subtest assesses the subject’s ability to retain spatial information and manipulate information in working memory. It is a self-ordered task which also assesses strategy use and is a sensitive measure of executive functioning (Robbins et al., 1998). SWM seems particularly sensitive to frontal lobe damage, with deficits relating to inefficient searching strategies (Owen et al., 1995). Patients with temporal lobe damage show deficits at the most challenging level of difficulty and strategy was not impaired (Owen et al., 1995). It takes around 8 minutes to complete.

The test begins with a number of boxes shown on the screen. The subject must touch each box in turn until one opens with a blue token inside. When a blue token has been found, the subject uses it to fill up an empty column on the right hand side of the screen. The subject must then begin a new search for the next blue token. It may be in any of the boxes that have so far been empty. This is repeated until a blue token has been found in every box on the screen. Touching any box in which a blue token has already been found is an error. The number of boxes is gradually increased from three to eight. The colour and position of the boxes is changed from trial to trial to discourage the use of stereotyped search strategies.

The primary outcome measures were between errors (i.e. revisiting a box which has already been found to contain a token), within errors (i.e. revisiting a box already found to be empty) and total errors. A measure of strategy is obtained by counting the number of times the subject began a new search with the same box. Standardised Z-scores (0±1) were generated by the CANTAB program according to in-build child norms.
5.3.3.5. The Rey Complex Figure (RCF)

The Rey Complex Figure was designed by Rey (1941) and elaborated by Osterrieth (2010). It assesses visuospatial constructional ability, visual memory and organisation skills. The RCF was originally designed for adults but has been successfully used with school-aged children. The task is appropriate for children aged 6 years and older, and the normative data indicates it is sensitive to developmental changes (V Anderson, Lajoie, & Bell, 1996).

The task requires the participant to copy a complex abstract figure and then draw the figure from memory after a 3 minute delay. This provides a measure of incidental learning. The measures of performance that are typically derived include a copy score, which reflects the accuracy of the original copy and is a measure of visual-constructional ability as well as a delayed-recall score, which assess amount of information retained over time. Different coloured pens were used to record the child’s strategy. Scoring criteria are based on Osterreith’s (2010) 36-point system, which breaks the figure down into 18 elements. Each element is awarded up to 2 points based on depending on accuracy, distortion, and location of each element. The delayed recall score was the primary outcome measure.
### Table 5.1.

**List of Variables from Child Neuropsychological Assessment**

<table>
<thead>
<tr>
<th>Test/Task</th>
<th>Variables</th>
<th>Cognitive Skill</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WISC-IV</strong></td>
<td>Full Scale IQ (FSIQ)</td>
<td>Intellectual Ability</td>
</tr>
<tr>
<td><strong>CELF-4</strong></td>
<td>Core Language</td>
<td>Language</td>
</tr>
<tr>
<td><strong>NEPSY-II</strong></td>
<td>Narrative Memory</td>
<td>Verbal learning (prose)</td>
</tr>
<tr>
<td></td>
<td>Narrative Memory Free Recall (SS)</td>
<td>Verbal learning</td>
</tr>
<tr>
<td></td>
<td>Narrative Memory Free Recall (RS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Narrative Memory Recognition (RS)</td>
<td>Verbal recognition</td>
</tr>
<tr>
<td></td>
<td>List Memory Learning (\sum T1-T5)</td>
<td>Verbal learning</td>
</tr>
<tr>
<td></td>
<td>List Memory Interference Effect (\frac{T5-T7}{T5}) x100</td>
<td>Retroactive interference</td>
</tr>
<tr>
<td></td>
<td>List Memory Delay (RS)</td>
<td>Verbal delayed recall</td>
</tr>
<tr>
<td><strong>Memory for Designs</strong></td>
<td>Memory for Designs (SS)</td>
<td>Non-verbal learning</td>
</tr>
<tr>
<td></td>
<td>Memory for Designs (RS)</td>
<td></td>
</tr>
<tr>
<td><strong>Memory for Designs Delay</strong></td>
<td>Memory for Designs Delay (SS)</td>
<td>Non-verbal delayed recall</td>
</tr>
<tr>
<td></td>
<td>Memory for Designs Delay (RS)</td>
<td></td>
</tr>
</tbody>
</table>

**Rey Complex Figure**  
Recall  
Non-verbal delayed recall

**CANTAB**  
Paired Associative Learning (PAL)  
First Trial Memory Score  
Associative learning  
Mean Trials to Success  
Mean Errors to Success  
Total Errors  
Total Trials  
Spatial Working Memory (SWM)  
Between Errors  
Working memory  
Strategy  
Total Errors  
Within Errors

RS = Raw score; SS = Scaled score

*Normative data were only available for ages 7 and above.
5.4. MRI SCANNING

5.4.1. Image Acquisition

All scans were acquired on a 3T Siemens Trio at the Royal Children’s Hospital, Melbourne, Australia. High resolution T1-weighted magnetisation-prepared rapid acquisition with gradient echo (MP-RAGE) MRI scans [TR 1900ms, TE 2.63ms, flip angle 9°, field of view 25cm] comprising 208 contiguous slices of 0.8mm thickness and 0.8mm x 0.8mm pixel dimension was acquired in each VPA-exposed child.

5.4.2. Image Processing

Image processing was conducted on a Dell Desktop PC with dual 1.86 GHz Intel processors and 2GB RAM running the Linux Ubuntu 10.10 operating system. DICOM format images from the scanner were converted to NIfTI-1 (.nii) format using the dcm2nii tool (MRIcron; http://www.cabiatl.com/mricro/mricron/index.html). The FSL-4.1 software package (FMRIB; http://www.fmrib.ox.ac.uk/fsl/) was used to reorient the axes of images.

5.4.3. Hippocampal Segmentation

To begin with, NIfTI-1 format images were converted to MINC format using the nii2mnc tool (Montreal Neurological Institute; http://www.bic.mni.mcgill.ca/). The hippocampus was manually segmented using a paintbrush method in the neuroimaging tool 'Display' from the Montreal Neurological Institute. Manual tracing has been shown to have high precision and reproducibility (Jack, Bentley, Twomey, & Zinsmeister, 1990). The segmenting was conducted by one rater who had prior training in hippocampal segmentation.
Well-defined anatomical landmarks were used to guide segmentation, as described by Jack et al. (1989). The hippocampal formation included the subiculum, Ammon’s horn (CA1 through CA4, including the region within the posterior uncus), the dentate gyrus, the alveus and the fimbria. Hippocampal boundaries were the choroid fissure, temporal horn, undersurface of the subiculum and uncal cistern (Jack, Petersen, O’Brien, & Tangalos, 1992). Decisions regarding boundaries were guided by reference to Naidich’s (1987) neuroanatomical atlas of the hippocampus as observed with MRI. On the most anterior slice, distinguishing the pes hippocampus from the uncinate gyrus required arbitrary judgements about borders. The pes hippocampus was recognised by its “undulating contour” and also by the fact that the alveus forms a thin bright line along its superior margin, where it meets the underlying amygdal (Jack et al., 1992). The posterior boundary was defined by the oblique coronal plane intersecting the posterior commissure on the midline sagittal slice (Jack et al., 1989).

Once the hippocampus had been defined on serial sections, a three dimensional binary MINC image was created. The volume of voxels in cubic centimeters was calculated with the mincstats tool (Montreal Neurological Institute; http://www.bic.mni.mcgill.ca/). Measures of left HCV, right HCV and total HCV were computed.

### 5.4.4. Reliability

Six hippocampi were randomly selected from the total 28 (21% of sample) to calculate intra-rater reliability. This provides a measure of the rater’s consistency in segmenting. These images were analysed at two separate time points, 8 weeks apart, by the same rater. Voxel-wise comparisons were conducted to generate Cohen’s kappa coefficient. This coefficient is a popular and useful reliability and agreement index frequently used in observer performance studies (Willoughby, Sheard, Nash, & Rovet, 2008). The mean kappa coefficient within our
sample was 0.91 (.02); range 0.88-0.92. According to Landis and Koch’s scales, this suggests near perfect agreement across ratings. Another rater appropriately trained in hippocampal segmentation was not available during the timeline of the project to enable inter-rater reliability analysis.

5.4.5. **Intracranial Volumes**

Intracranial volumes (ICVs) were calculated using the SPM8 package (Functional Imaging Laboratories, Institute of Neurology, London) running in Matlab 7.11 2010b (Mathworks, Massachusetts). The registration of the NIfTI-1 format images to SPM8’s T1 template was checked using the ‘Check Reg’ function and manually altering the image alignment as necessary. SPM8 was used to segment the three tissue subtypes (grey matter, white matter and cerebrospinal fluid). Matlab was used to calculate the number of voxels in each segmented image. The number of voxels was then multiplied by the voxel size to give volume in cubic centimetres. The volumes of the three tissues were then summed to provide a measure of ICV.

5.5. **DESIGN**

A prospective and cross-sectional design was adopted in these studies. This represents an improvement on many previous studies in this field, which have employed retrospective designs and may therefore include higher rates of bias. The memory outcomes study involved between-group comparisons in the group of children exposed to AEDs. The imaging study involved within-group comparisons of MRI variables.
The neuropsychological and computerised tests selected in these studies allowed a more detailed examination of memory functioning than has been previously reported. By including (where available) measures with standardised scaled scores for the Australian population (i.e. WISC-IV, CELF-4), some clinically relevant interpretations could be made regarding the performance of exposed children relative to their peers. These studies also improved on past research by collecting important medical variables to explore their impact on cognitive development.

5.6. STATISTICAL PROCEDURES

5.6.1. Preparation of Data

SPSS Statistics version 17.0 (SPSS Inc.) was used for all data analysis. As described previously, six children were excluded from the neuropsychological memory outcomes study; two had developed epilepsy, two had malformations detected since birth, and two were not exposed to AEDs. The results of 105 children were included analysis of the memory outcomes study. One child was unable to complete the cognitive tasks due to developmental delay; therefore scores were imputed at three standard deviations below the mean. Of the exposed children who were recruited to the neuroimaging study, two children refused the MRI scan and another child was excluded on the basis of updated drug exposure information (changed from VPA monotherapy to VPA polytherapy). A total of 14 children were included in the final data analysis for the imaging study.

Preliminary data analysis was conducted to assess whether the data set met the assumptions of parametric testing. Tests of normality using the Shapiro-Wilk statistic were conducted to detect any significant deviations from normality. No consequential deviations from normality were detected for any dependent variable within the sample. Homogeneity of variance
between drug exposure groups was evaluated with Levene’s test. No significant violations to the assumption of homogeneity of variance were observed.

5.6.2. Neuropsychological Measures Analysis

The following medical and pregnancy variables were dichotomised (absent/present): polytherapy exposure; generalized epilepsy onset; seizure(s) during pregnancy (any, convulsive and nonconvulsive); use of tobacco, alcohol, caffeine, and marijuana during pregnancy; folic acid consumption (pre-conception and first trimester); prematurity (gestational age < 37 weeks); breastfeeding; and child’s gender. Drug exposure was split into four groups: VPA monotherapy, non-VPA monotherapy, VPA polytherapy, non-VPA polytherapy. The drug exposure groups were compared on demographic, medical and pregnancy variables. Where variables were continuous, analysis of variance (ANOVA) was used to examine differences between groups. Post hoc multiple comparisons were conducted using Tukey’s Honest Significant Difference (HSD) test to control Type I error rates. While the Bonferroni adjustment is generally considered the “gold standard” for controlling multiple comparisons, this statistic was thought to be too stringent for the small sample size in this study. Where variables were categorical, group differences were examined with \( \chi^2 \) (chi-square) and Fisher’s exact tests.

To determine how far children exposed to AEDs deviated from age-appropriate performance on memory measures, a series of one sample t-tests were conducted between the scaled scores and Z-scores for each drug exposure group. For this analysis, scaled scores and normative data for List Memory and Rey Figure Recall were not available for children aged under seven years old (7 years, 0 months; 7:0). Subsequently, a scaled score for List Memory could only be generated for 21 (81%) VPA monotherapy, 34 (76%) non-VPA monotherapy,
10 (67%) VPA polytherapy and 13 (68%) non-VPA polytherapy exposed children. For this same subset of children, normative data were used to calculate standardised Z-scores (0±1) for Rey Figure Recall and List Memory Delay (V Anderson et al., 1996; Bromley et al., 2009).

Differences in test raw scores amongst the drug exposure groups (VPA monotherapy, non-VPA monotherapy, VPA polytherapy and non-VPA polytherapy) were examined using targeted and planned pairwise comparisons derived from the hypotheses. Authors have previously stipulated that by minimising the number of comparisons in an analysis, researchers reduce the risk of type I errors (Ruxton & Beauchamp, 2008). Subsequently, comparisons deemed unrelated to the hypotheses were not performed. Raw scores on memory measures were examined using independent sample t-tests and analysis of covariance (ANCOVA) when including covariates.

Although it has been suggested that the impact of VPA varies depending on the trimester of exposure, it was not known which trimester might specifically influence memory development. Therefore the mean VPA dose across the entire pregnancy was utilised in correlational analyses. Mean VPA dose was calculated by averaging dose over the three trimesters, taking into account dates of prescription changes. Pearson correlations were conducted within the VPA (monotherapy and polytherapy) group to ascertain whether a relationship existed between mean VPA dose across the pregnancy and performance on memory measures. Linear regression was used to examine the relationship between VPA dose and memory scores after adjusting for significant group differences.
5.6.3. Neuroimaging Analysis

Hippocampal volumes were corrected for head size (ICV) using the following equation: \( CV = MV - \text{Grad} \times (\text{ICV}_i - \text{ICV mean}) \); where \( CV \) is the corrected hippocampal volume, \( MV \) is the measured hippocampal volume, \( \text{Grad} \) is the gradient of the regression line between the hippocampal volume and ICV, \( \text{ICV}_i \) is the ICV for that subject, and \( \text{ICV mean} \) is the mean value of that measure for all subjects (Free et al., 1995). Within-group comparisons to examine differences between left and right HCVs were conducted with paired sample \( t \)-tests.

5.6.4. CANTAB Analysis

One sample \( t \)-tests were conducted on Z-scores of PAL and SWM, as exported from the CANTAB to ascertain if the scores of children exposed to VPA-monotherapy deviated from age-appropriate performance. Pearson correlations were conducted to examine the relationship between VPA dose, PAL, SWM and HCV.

5.6.5. Rationale for Selection of Covariates

In the analysis of the memory outcomes study, efforts were made to partial out the effects of other cognitive skills on neuropsychological test performance. Performance on verbal memory tasks is dependent on language skills (Crosson, 1992). Receptive language must be preserved to comprehend verbal material before encoding it. Expressive language skills determine the ability to recite the information retrieved from memory stores. We sought to partial out the effects of language on verbal memory performance by selecting an appropriate covariate. It is commonly assumed that IQ should be used as a covariate for specific measures
of cognitive outcome, however this assumption has been shown to be flawed in some situations (Dennis et al., 2009).

The Verbal Comprehension Index (VCI) of the WISC-IV was also considered as a covariate, however this composite relies more on semantic knowledge rather than language proficiency. In support of this, a study by O'Jile, Schrimsher and O'Bryant (2005) demonstrated that VCI was a poor predictor of performance on a list learning task. The authors argued that the verbal abilities that these tests require are fundamentally different. The Core Language score from the CELF-4 was ultimately considered the most appropriate variable to enter as a covariate as it reflects pure expressive and receptive language function. Consistently from the literature, language function emerged as the most robust predictor of verbal learning and memory performance in a sample of epilepsy patients (Hermann et al., 1992; Hermann et al., 1988).

5.6.6. *A priori* Power Analysis

As assessments were conducted blind to drug exposure group, we were unable to predict the size of drug exposure groups. Therefore, it was difficult to conduct *a priori* power analyses. A previous study by Eriksson (2005) showed a large effect (\(d = 1.15\)) of VPA on a list learning task relative to unexposed children. Based on this effect size, the total sample size required for \(\alpha = .05\) (two-tailed) with power over 80\% in a two-group comparison is \(N=26\) (G*Power 3 program; [http://wwwpsychouni-duesseldorfaapprojectsgpower/](http://wwwpsychouni-duesseldorfaapprojectsgpower/)). For a one-sample \(t\)-test with a medium effect size (\(d=.50\)), a sample size of 34 is required for \(\alpha = .05\) (two-tailed) and power over 80\%. To detect a medium effect size (\(f^2 = 0.15\)) in a multiple regression model with 4 predictor variables, a sample size of 85 provides power of 80\%. Post hoc power tests were conducted and are discussed in Chapter 7.
CHAPTER 6. RESULTS

6.1. THE EFFECT OF FETAL VALPROATE EXPOSURE ON NEUROPSYCHOLOGICAL MEMORY FUNCTIONING

The following section presents the results from the broader neuropsychological study investigating memory outcomes in children exposed prenatally to VPA. The performance of children exposed to VPA is compared to the performance of children exposed to other medications and other factors contributing to memory outcome are examined.

6.1.1. Sample Characteristics

There were 97 participating mothers and 105 children. The sample comprised of 26 children exposed to VPA monotherapy, 45 to non-VPA monotherapy, 15 to VPA in polytherapy and 19 to non-VPA polytherapy. The composition of each exposure group is presented in Table 6.1.
Table 6.1.

*Exposure Details of Sample*

<table>
<thead>
<tr>
<th></th>
<th>Valproate Monotherapy</th>
<th>Non-valproate Monotherapy</th>
<th>Valproate Polytherapy</th>
<th>Non-valproate Polytherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>VPA 26</td>
<td>CBZ 34</td>
<td>VPA+LTG 6</td>
<td>CBZ+LTG 5</td>
</tr>
<tr>
<td></td>
<td>LTG 9</td>
<td>VPA+CBZ 1</td>
<td>VPA+LEV 1</td>
<td>CBZ+PHY 3</td>
</tr>
<tr>
<td></td>
<td>GBP 1</td>
<td>VPA+TGB 1</td>
<td>VPA+CZP+CBZ 2</td>
<td>LTG+PHY 1</td>
</tr>
<tr>
<td></td>
<td>TPM 1</td>
<td>VPA+CZP+LTG 1</td>
<td>VPA+CZP+CBZ+VGB 1</td>
<td>LTG+CLB+PHB 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBZ+CZP+VGB+GBP 1</td>
<td></td>
</tr>
</tbody>
</table>

1. CBZ = Carbamazepine; CLB = Clobazam; CZP = Clonazepam; ESM = Ethosuximide; GBP = Gabapentin; LEV = Levetiracetam; LTG = Lamotrigine; PHY = Phenytoin; PHB = Phenobarbital; TPM = Topiramate; VPA = Valproate; VBG = Vigabatrin.
Demographic, medical and pregnancy information across the drug groups is presented in Table 6.2. Mothers taking VPA in a polytherapy regime exhibited numerous differences from the other women in the study. There was a significant effect of maternal IQ across groups, $F(3,101) = 7.14, p < .001$. Post hoc tests showed that maternal IQ was significantly lower in the VPA polytherapy group ($M = 91.7, SD = 14.8$) compared to the VPA monotherapy ($M = 105.9, SD = 12.8; p = .027$) and non-VPA monotherapy ($M = 108.9, SD = 11.2; p = .002$) groups. There was only a trend level difference with the non-VPA polytherapy group ($M = 102.3, SD = 14.3; p = .082$). Only the group of mothers taking VPA polytherapy contained individuals (3 mothers) with a FSIQ lower than 80. Mean VPA dose was significantly higher in the polytherapy group than the monotherapy group, $t(39) = -2.49, p = .017$. Mothers were taking an average of 961.8 mg/day ($SD = 629.7$) of VPA in the monotherapy group and 1589.0 mg/day ($SD = 986.5$) in the polytherapy group. Significant differences were also detected amongst mean SES ratings, $F(3,97) = 5.00, p < .003$. Post hoc testing revealed that SES ratings were significantly lower in the VPA polytherapy group ($M = 36.8, SD = 21.4$) compared to the VPA monotherapy ($M = 57.3, SD = 22.2; p = .005$) and non-VPA monotherapy ($M = 61.3, SD = 20.6; p < .001$) groups. The mean SES of the non-VPA polytherapy was lower than the VPA polytherapy group, but the difference was not significant ($M = 51.2, SD = 18.2; p = .107$).

In terms of medical and pregnancy variables, when generalised seizures were dichotomised as absent or present, there was significant variation across groups, $\chi^2 (3, N = 105) = 33.46, p < .001$. Overall, women taking VPA were more likely to have a generalised type of epilepsy. The incidence of generalised epilepsy was significantly higher in mothers who took VPA monotherapy than those taking non-VPA monotherapy ($p = .001$) and non-VPA polytherapy ($p < .001$). Similarly, the frequency of generalised type epilepsy was higher in mothers taking
VPA polytherapy than mothers who took non-VPA monotherapy ($p < .001$) and non-VPA polytherapy ($p = .014$).

The occurrence of seizures in pregnancy varied across groups, $\chi^2(3, N = 105) = 13.39, p = .004$). Fisher’s exact test showed women in the VPA polytherapy were more likely to have suffered one or more seizures during pregnancy than the VPA monotherapy group ($p = .001$). Differences were detected in the incidence of nonconvulsive seizures across groups, ($\chi^2(3, N = 105) = 8.79, p = .032$) but not convulsive seizures ($\chi^2(3, N = 105) = 6.65, p = .084$). Women taking VPA in polytherapy were more likely than women taking VPA in monotherapy ($p = .005$) to have one or more nonconvulsive seizure during their pregnancy. Comparisons between the other AED groups did not reveal differences in the incidence of nonconvulsive seizures.

There was also variation in the presence of tobacco use during pregnancy across AED groups, $\chi^2(3, N = 105) = 15.69, p = .001$. Mothers who took VPA polytherapy were more likely to smoke tobacco during pregnancy than mothers who took non-VPA polytherapy ($p = .011$). No differences were found between the VPA monotherapy, VPA polytherapy, non-VPA monotherapy and non-VPA polytherapy groups with respect to the presence of the following dichotomised variables: use of caffeine ($\chi^2(3, N = 105) = 2.51, p = .472$), alcohol ($\chi^2(3, N = 105) = 2.65, p = .450$), marijuana ($\chi^2(3, N = 105) = 2.60, p = .458$), pre-conceptual folic acid suppletionation ($\chi^2(3, N = 105) = 4.79, p = .188$), folic acid suppletionation in the first trimester ($\chi^2(3, N = 105) = 1.50, p = .682$), breastfeeding ($\chi^2(3, N = 105) = 5.18, p = .159$) or prematurity ($\chi^2(3, N = 105) = 4.78, p = .189$).

Comparisons of other pregnancy and child related variables revealed that the four drug exposure groups were equivalent in terms of maternal age at birth ($F(3,101) = 1.01, p = .393$),
child’s birth weight \( (F(3,100) = 0.35, p = .787) \), child’s age at testing \( (F(3,101) = 0.89, p = .451) \) and child’s gender \( (\chi(3) = 1.25, p = .741) \).

However, differences between groups were detected in regards to FSIQ, \( F(3,101) = 8.46, p < .001 \). Children exposed to VPA polytherapy possessed lower FSIQ scores than those exposed to VPA monotherapy \( (p = .007) \), non-VPA monotherapy \( (p < .001) \) and non-VPA polytherapy \( (p = .047) \). There was also an effect of drug group on Core Language scores \( (F(1,101) = 9.20, p < .001) \). Children exposed to VPA polytherapy scored significantly lower than the VPA monotherapy \( (p = .006) \), non-VPA polytherapy \( (p = .002) \) and non-VPA monotherapy groups \( (p < .001) \).
**Table 6.2.**

**Sample Characteristics for Neuropsychological Memory Outcomes Study**

<table>
<thead>
<tr>
<th></th>
<th>Valproate Monotherapy (N = 26)</th>
<th>Non-valproate Monotherapy (N = 45)</th>
<th>Valproate Polytherapy (N = 15)</th>
<th>Non-valproate Polytherapy (N = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal IQ (SD)</td>
<td>105.9 (12.8)</td>
<td>108.9 (11.2)</td>
<td>91.7 (14.8)</td>
<td>102.3 (14.3)</td>
</tr>
<tr>
<td>Mean valproate dose; mg daily (SD)</td>
<td>961.8 (629.7)</td>
<td>-</td>
<td>1589.0 (986.5)</td>
<td>-</td>
</tr>
<tr>
<td>Mean family SESa</td>
<td>57.3 (22.2)</td>
<td>61.3 (20.6)</td>
<td>36.8 (21.4)</td>
<td>51.2 (18.2)</td>
</tr>
<tr>
<td>Mean maternal age; years at birth (SD)</td>
<td>30.5 (5.2)</td>
<td>32.3 (3.5)</td>
<td>31.2 (6.9)</td>
<td>32.4 (4.3)</td>
</tr>
<tr>
<td>Epilepsy type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalised onset; N (%)</td>
<td>21 (80.8)</td>
<td>9 (20.0)</td>
<td>11 (73.3)</td>
<td>5 (26.3)</td>
</tr>
<tr>
<td>Partial onset; N (%)</td>
<td>5 (19.2)</td>
<td>32 (71.1)</td>
<td>4 (26.7)</td>
<td>13 (68.4)</td>
</tr>
<tr>
<td>Unable to classify; N (%)</td>
<td>0 (0.0)</td>
<td>4 (8.9)</td>
<td>0 (0.0)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Seizures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any; N (%)</td>
<td>6 (23.1)</td>
<td>27 (60.0)</td>
<td>3 (20.0)</td>
<td>9 (47.4)</td>
</tr>
<tr>
<td>Convulsive; N (%)</td>
<td>4 (15.4)</td>
<td>6 (13.3)</td>
<td>6 (40.0)</td>
<td>6 (31.6)</td>
</tr>
<tr>
<td>Nonconvulsive; N (%)</td>
<td>4 (15.4)</td>
<td>14 (31.1)</td>
<td>9 (60.0)</td>
<td>6 (31.6)</td>
</tr>
<tr>
<td>Caffeine; N (%)</td>
<td>19 (73.1)</td>
<td>30 (66.7)</td>
<td>12 (80.0)</td>
<td>16 (84.2)</td>
</tr>
<tr>
<td>Tobacco; N (%)</td>
<td>4 (15.4)</td>
<td>1 (2.2)</td>
<td>5 (33.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Alcohol; N (%)</td>
<td>10 (38.5)</td>
<td>19 (42.2)</td>
<td>3 (20.0)</td>
<td>6 (31.6)</td>
</tr>
<tr>
<td>Marijuana; N (%)</td>
<td>1 (3.8)</td>
<td>0 (0.0)</td>
<td>1 (6.7)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Folic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconception; N (%)b</td>
<td>21 (87.5)</td>
<td>31 (73.8)</td>
<td>9 (60.0)</td>
<td>12 (63.2)</td>
</tr>
<tr>
<td>1st trimester; N (%)a</td>
<td>25 (96.2)</td>
<td>44 (97.8)</td>
<td>14 (93.3)</td>
<td>19 (100.0)</td>
</tr>
<tr>
<td>Breastfed; N (%)b</td>
<td>23 (88.5)</td>
<td>38 (86.4)</td>
<td>8 (61.5)</td>
<td>15 (78.9)</td>
</tr>
<tr>
<td>Born premature (&lt; 37 weeks); N (%)</td>
<td>1 (3.8)</td>
<td>1 (2.2)</td>
<td>2 (13.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Birth weight; grams (SD)b</td>
<td>3500.9 (590.0)</td>
<td>3386.8 (480.7)</td>
<td>3383.2 (480.7)</td>
<td>3484.8 (420.1)</td>
</tr>
<tr>
<td>Child’s age at testing; years (SD)</td>
<td>7.4 (0.6)</td>
<td>7.3 (0.6)</td>
<td>7.2 (0.6)</td>
<td>7.5 (0.7)</td>
</tr>
<tr>
<td>Child’s gender; N girls (%)</td>
<td>11 (42.3)</td>
<td>20 (44.4)</td>
<td>7 (46.7)</td>
<td>11 (57.9)</td>
</tr>
<tr>
<td>Child’s IQ (SD)</td>
<td>95.8 (13.3)</td>
<td>101.4 (13.7)</td>
<td>81.0 (17.5)</td>
<td>93.9 (10.6)</td>
</tr>
<tr>
<td>Core Language (SD)</td>
<td>92.5 (16.5)</td>
<td>100.4 (17.4)</td>
<td>85.8 (20.6)</td>
<td>95.6 (12.9)</td>
</tr>
</tbody>
</table>

aSES = socioeconomic status. SES information was missing for three non-VPA monotherapy mothers and one VPA polytherapy mother.

bData on preconception folic acid were missing for two VPA monotherapy mothers and three non-VPA monotherapy mothers. Information on breastfeeding was missing for two VPA polytherapy mothers and one non-VPA monotherapy mother. Birth weight information was missing for 1 non VPA monotherapy mother.
6.1.2. The Impact of AED Exposure on Intellectual and Language Functioning Relative to Age-Appropriate Levels

To determine whether the performance of children exposed to AEDs deviated from age-appropriate levels of intellect and language functioning, one sample t-tests were conducted on index scores from the WISC-IV and CELF-4 for each drug group. Children were compared to a test mean of 100 on IQ and Core Language index scores (Figure 6.1). The FSIQ of children exposed to VPA polytherapy (*t*(14) = -4.21, *p* = .001) and non-VPA polytherapy (*t*(18) = -2.54, *p* = .020) fell significantly below the expected mean. Conversely, children in the VPA monotherapy (*t*(25) = -1.62, *p* = .119) and non-VPA monotherapy (*t*(44) = 0.69, *p* = .496) groups did not deviate from the expected mean. Core Language ability was significantly below the level expected in the VPA monotherapy (*t*(25) = -2.33, *p* = .028) and VPA polytherapy (*t*(14) = -4.63, *p* < .001) groups. Core Language scores for the non-VPA monotherapy (*t*(44) = 0.14, *p* = .893) and non-VPA polytherapy groups (*t*(18) = -1.50, *p* = .151) were consistent with age-appropriate levels.
Figure 6.1. Full Scale IQ and Core Language Scores for children exposed to VPA Monotherapy, Other (non-VPA) Monotherapy, VPA Polytherapy and Other (non-VPA) Polytherapy.

* Statistically significant difference from test mean of 100, $p < .05$.

Error bars represent ±2 standard errors (SE)
6.1.3. The Impact of AED Exposure on Memory Functioning Relative to Age-Appropriate Levels

One sample t-tests were also conducted on memory test scaled scores for each drug group to determine whether performance deviated from the expected mean. Mean scaled scores are presented in Table 6.3. The comparisons are presented in Figure 6.2. As discussed in the methodology (Chapter 5), normative data were not available for children aged below seven years old on some subtests and these data points were subsequently excluded from the following analyses.

On Narrative Memory, children exposed to VPA polytherapy were the only group to perform significantly below the expected mean of 10 ($t(13) = -3.69, p = .003$). Relative to the normative data, children exposed to polytherapy VPA performed 1.0 SD below the test mean. Children exposed to VPA monotherapy ($t(25) = -0.82, p = .422$), non-VPA monotherapy ($t(44) = 1.74, p = .088$) and non-VPA polytherapy ($t(17) = 1.13, p = .274$) did not differ significantly from the mean.

On List Memory, scaled scores were reduced in children exposed to both VPA monotherapy ($t(20) = -2.90, p = .009$) and VPA polytherapy ($t(9) = -2.32, p = .046$). This represents deviations of 0.5 SD and 1.0 SD from the test mean respectively for these groups. Conversely, the groups of children exposed to medications not including VPA performed consistently with the test mean (non-VPA monotherapy $t(33) = 0.80, p = .430$; non-VPA polytherapy $t(12) = -1.25, p = .235$).

In contrast to the above findings, all four drug groups performed at a level consistent with the expected mean of 10 on the non-verbal memory subtests. Mean scaled scores for all groups were equal to the expected mean on Memory for Designs (non-VPA monotherapy $t(25) = 0, p = 1.00$; VPA polytherapy $t(14) = -1.28, p = .221$; non-VPA monotherapy $t(44) = -0.11, p = .
.915; non-VPA polytherapy $t(18) = 0.47, p = .648$ and Memory for Designs Delay (VPA monotherapy $t(25) = -0.22, p = .830$; VPA polytherapy $t(14) = 0, p = 1.00$; non-VPA monotherapy $t(44) = 1.56, p = 0.127$; non-VPA polytherapy $t(18) = 1.05, p = .306$).

Table 6.3.

*Scaled Scores on Memory Measures Following AED Exposure*

<table>
<thead>
<tr>
<th>Scaled Scores</th>
<th>VPA Monotherapy Mean (SD)</th>
<th>N</th>
<th>Non-VPA Monotherapy Mean (SD)</th>
<th>N</th>
<th>VPA Polytherapy Mean (SD)</th>
<th>N</th>
<th>Non-VPA Polytherapy Mean (SD)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrative Memory</td>
<td>9.58 (2.64)</td>
<td>26</td>
<td>10.87 (3.33)</td>
<td>45</td>
<td>7.07 (2.97)*</td>
<td>14</td>
<td>10.83 (3.13)</td>
<td>18</td>
</tr>
<tr>
<td>List Memory</td>
<td>8.38 (2.56)*</td>
<td>21</td>
<td>9.96 (2.79)</td>
<td>34</td>
<td>6.90 (4.23)*</td>
<td>10</td>
<td>9.00 (2.89)</td>
<td>13</td>
</tr>
<tr>
<td>Memory for Designs</td>
<td>10.00 (2.37)</td>
<td>26</td>
<td>10.71 (3.07)</td>
<td>45</td>
<td>9.00 (3.02)</td>
<td>15</td>
<td>10.26 (2.47)</td>
<td>19</td>
</tr>
<tr>
<td>Memory for Designs</td>
<td>9.88 (2.72)</td>
<td>26</td>
<td>10.47 (3.43)</td>
<td>45</td>
<td>10.00 (3.46)</td>
<td>15</td>
<td>10.84 (3.48)</td>
<td>19</td>
</tr>
</tbody>
</table>

*For Narrative Memory, scores were missing for one child exposed to VPA polytherapy and one child exposed to non-VPA polytherapy.*

* Statistically significant difference from test mean of 10, $p < .05$. 
One sample $t$-tests were also conducted on $Z$-scores calculated for children aged over seven years (see Table 6.4). On List Memory Delay, both groups of VPA-exposed children (i.e. monotherapy and polytherapy) performed below the expected mean of 0 (VPA monotherapy $t(20) = -2.81, p = .011$; VPA polytherapy $t(9) = -2.48, p = .035$). Children exposed to the non-VPA therapies performed consistently with the test mean (non-VPA monotherapy $t(33) = -0.96, p = .347$; non-VPA polytherapy $t(11) = -0.12, p = .906$). There was not a significant difference between the expected mean of 0 and performance on the Rey Complex Figure Recall for any of the four drug exposure groups (VPA monotherapy $t(20) = -1.84, p = .080$; VPA polytherapy $t(9) = -1.41, p = .191$; non-VPA monotherapy $t(33) = -0.25, p = .800$; non-VPA polytherapy $t(11) = 0.99, p = .341$).

Table 6.4.

Comparison of $Z$-Scores on Memory Measures (SD) between AED Exposure Groups

<table>
<thead>
<tr>
<th></th>
<th>VPA Monotherapy ($N = 21$)</th>
<th>Non-VPA Monotherapy ($N = 34$)</th>
<th>VPA Polytherapy ($N = 10$)</th>
<th>Non-VPA Polytherapy ($N=12$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>List Memory Delay</td>
<td>-0.54 (0.88) *</td>
<td>-0.17 (1.01)</td>
<td>-1.12 (1.44) *</td>
<td>-0.03 (0.78)</td>
</tr>
<tr>
<td>Rey Complex Figure Recall</td>
<td>0.34 (0.85)</td>
<td>-0.04 (0.91)</td>
<td>-0.57 (1.28)</td>
<td>0.31 (1.08)</td>
</tr>
</tbody>
</table>

* Statistically significant difference from test mean of 0, $p < .05$. 
6.1.4. The Impact of VPA Exposure on Memory Functioning Relative to Other AEDs

Mean raw scores on the memory measures were calculated for each of the four drug exposure groups. The raw scores for each memory measure are presented in Table 6.5. Raw scores were utilised rather than scaled scores to include children outside the range of normative data in the analyses.

6.1.4.1. Comparison of Monotherapy Treatment Regimes

The performance of children exposed to VPA monotherapy was compared to children exposed to non-VPA monotherapy using ANOVA. There was not a significant difference between the raw scores of the two groups on any memory measure; Narrative Memory Free Recall \(t(69) = -1.24, p = .219\), Narrative Memory Recognition \(t(69) = -1.56, p = .122\), List Memory Learning \(t(69) = -1.48, p = .145\), List Memory Interference Effect \(t(69) = 1.50, p = .138\), List Memory Delay \(t(69) = -1.09, p = .278\), Memory for Designs \(t(69) = 0.24, p = .811\), Memory for Designs Delay \(t(69) = -1.06, p = .293\) and Rey Complex Figure Recall \(t(69) = 1.84, p = .071\).
Table 6.5.

**Mean Raw Scores on Memory Measures (SD)**

<table>
<thead>
<tr>
<th></th>
<th>Valproate Monotherapy (N = 26)</th>
<th>Non-valproate Monotherapy (N = 45)</th>
<th>Valproate Polytherapy (N = 15)</th>
<th>Non-valproate Polytherapy (N = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Narrative Memory⁴</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free Recall</td>
<td>9.38 (3.66)</td>
<td>10.71 (4.69)</td>
<td>6.31 (3.15)</td>
<td>10.72 (4.56)*</td>
</tr>
<tr>
<td>Recognition</td>
<td>14.23 (1.45)</td>
<td>14.73 (1.21)</td>
<td>13.46 (1.33)</td>
<td>14.22 (1.44)</td>
</tr>
<tr>
<td><strong>List Memory⁵</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Learning</td>
<td>34.12 (7.92)</td>
<td>37.89 (11.55)</td>
<td>25.79 (13.55)</td>
<td>34.58 (8.71)*</td>
</tr>
<tr>
<td>Interference Effect (%)</td>
<td>24.63 (24.75)</td>
<td>13.40 (33.17)</td>
<td>48.88 (38.34)</td>
<td>25.53 (20.40)*</td>
</tr>
<tr>
<td>List Memory Delay</td>
<td>6.85 (2.38)</td>
<td>7.62 (3.14)</td>
<td>4.86 (3.63)</td>
<td>7.63 (2.65)*</td>
</tr>
<tr>
<td><strong>Memory for Designs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>88.19 (19.65)</td>
<td>86.78 (26.06)</td>
<td>84.29 (19.81)</td>
<td>90.21 (27.11)</td>
</tr>
<tr>
<td>Memory for Designs Delay</td>
<td>25.50 (10.50)</td>
<td>28.38 (11.32)</td>
<td>28.07 (8.97)</td>
<td>28.68 (12.20)</td>
</tr>
<tr>
<td><strong>Rey Complex Figure Recall</strong></td>
<td>12.19 (5.34)</td>
<td>9.88 (4.99)</td>
<td>8.07 (5.40)</td>
<td>10.90 (6.67)</td>
</tr>
</tbody>
</table>

⁴For Narrative Memory, scores were missing for 2 children VPA polytherapy and one child exposed to non-VPA polytherapy.

⁵For List Memory, scores were missing for 1 child exposed to VPA polytherapy.

*Statistically significant group difference, p<.05.
6.1.4.2. Comparison of Polytherapy Treatment Regimes

The VPA polytherapy group differed from the monotherapy group on a number of background variables and children were exposed to a higher dose of VPA. Subsequently, it was not considered appropriate to compare these two groups. To examine the potential effects of high dose VPA while controlling for polytherapy treatment, the raw scores of the group of children exposed to VPA polytherapy were compared to children exposed to non-VPA polytherapy.

Children exposed to VPA polytherapy scored lower than children exposed to non-VPA polytherapy on Narrative Memory Free Recall ($t(29) = 3.01, p = .005$) but there was no difference on scores for Recognition ($t(29) = 1.50, p = .145$). These comparisons are presented in Figure 6.3. On the List Memory task, children exposed to VPA polytherapy demonstrated significantly poorer scores on Learning ($t(31) = 2.27, p = .030$) and Delay ($t(31) = 2.54, p = .016$), and higher percentages for Interference Effect ($t(30) = -2.42, p = .033$) than non-VPA children (see Figure 6.4). Figure 6.5 presents the comparisons between groups on the non-verbal memory measures. There was no difference in performance between the two groups on Memory for Designs ($t(31) = 0.69, p = .494$), Memory for Designs Delay ($t(31) = 0.16, p = .875$) or Rey Complex Figure Recall ($t(31) = 1.30, p = .203$).
* Statistically significant difference between groups, $p < .05$.

Error bars represent ±2 standard errors (SE)

**Figure 6.2.** Comparisons between VPA Polytherapy and Other (non-VPA) Polytherapy on Narrative Memory Variables
* Statistically significant difference between groups, $p < .05$.

Error bars represent ±2 standard errors (SE)

*Figure 6.3. Comparisons between VPA Polytherapy and Other (non-VPA) Polytherapy on List Memory Variables*
Error bars represent ±2 standard errors (SE)

**Figure 6.4.** Comparisons between VPA Polytherapy and Other (non-VPA) Polytherapy on Memory for Designs and Rey Complex Figure
6.1.4.3. The Contribution of Language to Verbal Memory Performance in the VPA Polytherapy Group

As reported earlier, children exposed to VPA polytherapy possessed lower scores on Core Language relative to other exposure groups. Given the VPA polytherapy group also demonstrated lowered performance on the verbal memory measures, it raises the possibility that reduced language ability is contributing to poorer verbal memory performance. A series of comparisons were conducted to investigate this issue.

The comparisons performed in the above section demonstrated that there were significant differences between VPA polytherapy and non-VPA polytherapy groups on the verbal memory variables. A number of ANCOVAs were performed to examine the contribution of language skills to performance on verbal memory tasks. After entering Core Language scores as a covariate, there was no longer a statistically significant difference between children exposed to VPA polytherapy and non-VPA polytherapy on Narrative Memory Free Recall \(F(1,28) = 2.41, p = .132\) or List Memory Learning \(F(1,30) = 0.04, p = .853\), Interference Effect \(F(1,29) = 0.52, p = .478\) and Delay \(F(1,30) = 0.75, p = .395\).

6.1.5. Relationship between VPA Dose and Scores on Memory Measures

To establish whether a dose-dependent relationship exists between mean VPA dose and memory raw scores, correlation analysis was conducted within the pooled sample of monotherapy and polytherapy VPA-exposed children \(N = 41\).

Mean VPA dose was significantly correlated with all variables from the List Learning subtest; Learning \((r = -.42, p = .007)\), Delay \((r = -.45, p = .004)\) and Interference Effect \((r = .56, p < .001)\). On Narrative Memory, Free Recall negatively correlated with VPA \((r = -.39, p\)
= .015) but Recognition did not \((r = -.28, p = .087)\). Rey Complex Figure Recall scores were significantly negatively correlated with VPA dose \((r = -.35, p = .026)\) but Memory for Designs \((r = -.26, p = .111)\) and Memory for Designs Delay \((r = -.10, p = .545)\) were not.

To examine the contribution of language to the relationship between VPA dose and verbal memory functioning, partial correlations were conducted between mean VPA dose and List Learning measures, with Core Language scores entered as a covariate. After controlling for language, there was no longer significant relationships between VPA dose and List Memory Learning \((r = -.23, p = .118)\) and Delay \((r = -.31, p = .056)\), but a relationship persisted with the Interference Effect \((r = .43, p = .007)\).

### 6.1.5.1. Dose relationship within the Monotherapy VPA Group

Given the significant differences in characteristics and the added confound of multiple AED exposure in the polytherapy VPA group, the correlation analysis was repeated within the monotherapy VPA group only.

Significant relationships were identified between the mean VPA dose across the entire pregnancy and the raw scores for all three List Memory variables. VPA dose was negatively correlated with List Memory Learning \((r = -.60, p = .001)\) and Delay \((r = -.60, p = .001)\). A significant positive relationship was identified with Interference Effect \((r = .67, p < .001)\). The relationship between Narrative Memory Free Recall approached but did not reach significance \((r = -.36, p = .074)\), and there was not a significant relationship with Recognition \((r = -.31, p = .127)\). Similarly, there was not a correlation between mean VPA dose and Rey Complex Figure Recall scores \((r = -.32, p = .109)\). Memory for Designs was negatively
correlated with dose \((r = -0.44, p = 0.023)\) but this relationship was not significant for Memory for Designs Delay \((r = -0.37, p = 0.065)\).

To examine the contribution of language to the relationship between VPA dose and verbal memory functioning, partial correlations were conducted between mean VPA dose and List Learning measures, with Core Language scores entered as a covariate. After controlling for language, there were still significant relationships between VPA dose and List Memory Learning \((r = -0.57, p = 0.003)\), the Interference Effect \((r = 0.59, p = 0.002)\) and Delay \((r = -0.56, p = 0.004)\).

### 6.1.6. Predictors of Memory Outcome

Linear regression analysis was conducted in a pooled sample of all children exposed to VPA (monotherapy and polytherapy) to establish the independent contribution of VPA dose on verbal memory performance (i.e. List Memory variables). The analysis also sought to identify other contributors to outcome. VPA dose, polytherapy exposure (dichotomised) and Core Language scores were entered into Model 1. Collinearity was investigated prior to regression analyses. The highest Variance Inflation Factor (VIF) detected was 3.4 for Core Language scores in the model predicting List Memory Interference. The general “rule of thumb” is that a VIF of 10 indicates excessive collinearity (O’Brien, 2007). Background and medical variables identified as differing between groups (i.e. maternal IQ, family SES, tobacco use and seizures during pregnancy) were entered into Model 2. Significant predictors are presented in Table 6.6.

Core Language scores were the only significant predictor of performance on List Memory Learning \((\text{Adjusted } R^2 = 0.34, F(3,34) = 7.46, p = 0.001)\) and Delay \((\text{Adjusted } R^2 = 0.32, F(3,34)\).
= 6.71, \( p = .001 \) raw scores in Model 1. Both mean VPA dose and Core Language scores predicted the Interference Effect (Adjusted \( R^2 = .49, F(3,33) = 12.60, p < .001 \)). When maternal IQ, SES, tobacco use and seizures during pregnancy were controlled for, there was not a significant change to the regression model (Learning \( \Delta R^2 = .08, p = .382 \); Delayed Recall \( \Delta R^2 = .06, p = .565 \); Interference Effect \( \Delta R^2 = .04, p = .654 \)). However, maternal IQ emerged as a significant predictor of List Memory Learning (Adjusted \( R^2 = .35, F(7,30) = 3.85, p = .004 \)). When controlling for group differences, the association between mean VPA dose and List Memory Delay was retained (Adjusted \( R^2 = .30, F(7,30) = 3.22, p = .011 \)). Mean VPA dose and Core Language scores continued to predict the Interference Effect (Adjusted \( R^2 = .47, F(7,29) = 5.50, p < .001 \)).
Table 6.6.

**Predictors of List Memory Scores**

<table>
<thead>
<tr>
<th></th>
<th>List Memory Learning</th>
<th>List Memory Interference Effect (%)</th>
<th>List Memory Delay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B Coefficient (SE)</td>
<td>p</td>
<td>B Coefficient (SE)</td>
</tr>
<tr>
<td>1 (Constant)</td>
<td>16.32 (8.61)</td>
<td>.066</td>
<td>76.47 (22.15)</td>
</tr>
<tr>
<td>Core Language Index</td>
<td>0.22 (0.08)</td>
<td>.013</td>
<td>-0.70 (0.22)</td>
</tr>
<tr>
<td>Mean VPA Dose Whole Pregnancy (mg daily)</td>
<td>-0.003 (0.002)</td>
<td>.221</td>
<td>0.014 (0.005)</td>
</tr>
<tr>
<td>Polytherapy (any trimester)</td>
<td>-4.52 (3.65)</td>
<td>.224</td>
<td>6.20 (9.76)</td>
</tr>
<tr>
<td>2 (Constant)</td>
<td>35.98 (14.67)</td>
<td>.020</td>
<td>111.44 (40.62)</td>
</tr>
<tr>
<td>Core Language Index</td>
<td>0.22 (0.09)</td>
<td>.015</td>
<td>-0.66 (0.23)</td>
</tr>
<tr>
<td>Mean VPA Dose Whole Pregnancy (mg daily)</td>
<td>-0.003 (0.002)</td>
<td>.192</td>
<td>0.015 (0.006)</td>
</tr>
<tr>
<td>Polytherapy (any trimester)</td>
<td>-5.85 (4.42)</td>
<td>.196</td>
<td>4.98 (12.33)</td>
</tr>
<tr>
<td>WASI FSIQ estimate</td>
<td>-0.24 (0.12)</td>
<td>.060</td>
<td>-.43 (.34)</td>
</tr>
<tr>
<td>SES Score</td>
<td>0.10 (0.08)</td>
<td>.194</td>
<td>.13 (.20)</td>
</tr>
<tr>
<td>Tobacco during pregnancy (any trimester)</td>
<td>1.33 (3.81)</td>
<td>.729</td>
<td>-9.30 (10.83)</td>
</tr>
<tr>
<td>Seizures during pregnancy</td>
<td>-0.09 (3.62)</td>
<td>.980</td>
<td>-4.39 (9.61)</td>
</tr>
</tbody>
</table>
6.2. THE EFFECT OF FETAL VALPROATE EXPOSURE ON HIPPOCAMPAL MEMORY SKILLS

This section will present the results from the smaller neuroimaging study which was conducted after the neuropsychological memory outcomes study. Only children exposed to VPA monotherapy were involved in this study. Relationships between VPA dose, hippocampal structure and hippocampal memory function were explored.

6.2.1. Sample Characteristics

Demographics information for the 14 children who completed the neuroimaging protocol and additional computerised testing are presented in Table 6.7. The sample comprised of 14 children exposed to VPA monotherapy. VPA-exposed children were exposed to a mean dose of 863.8mg in monotherapy across the whole pregnancy, with doses ranging from 111.1mg to 1500.0mg. The mean Full Scale IQ of the children in this group was 98 and ranged from 77 to 117.
Table 6.7.

**Sample Characteristics for Neuroimaging Study**

<table>
<thead>
<tr>
<th>Sample Characteristic</th>
<th>VPA Monotherapy (N = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child’s age at scan; years (SD)</td>
<td>8.3 (1.1)</td>
</tr>
<tr>
<td>Child’s gender; N boys (%)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>Child’s handedness; N right (%)</td>
<td>13 (92.9)</td>
</tr>
<tr>
<td>Mean valproate dose taken by mother; mg daily (SD)</td>
<td>863.8 (443.6)</td>
</tr>
<tr>
<td>Child’s Full Scale IQ (SD)</td>
<td>98.0 (11.1)</td>
</tr>
</tbody>
</table>

### 6.2.2. The Impact of VPA-Monotherapy Exposure on Age-Appropriate Performance on Hippocampal Memory Measures

A series of one sample t-tests against a test value of 0 were conducted on Z-scores from the PAL and SWM tasks from the CANTAB to determine how representative the sample was on standard clinical measures (see Table 6.8). Children exposed to VPA performed above the expected mean for SWM Within Errors (t(13) = 6.45, p < .001), indicating that their performance was better than the population average. On other measures of SWM or PAL, Z-scores did not deviate significantly from 0. Statistical analysis confirmed that there were no differences relative to the normative sample on other measures; SWM Between Errors (t(13) = -1.02, p = .328), SWM Strategy (t(13) = 0.18, p = .859), SWM Total Errors (t(13) = -0.82, p = .430), PAL First Trial Memory (t(13) = -0.05, p = .960), PAL Mean Errors to Success (t(13) = 1.15, p = .269), PAL Mean Trials to Success (t(13) = 0.88, p = .393), PAL Total Errors (t(13) = 0.82, p = .428), or PAL Total Trials (t(13) = 0.20, p = .843).
Table 6.8.

**Z-Scores on Cognitive Measures (SD) for VPA Monotherapy**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean Score (SD)</th>
<th>Z-score (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWM Between Errors</td>
<td>49.64 (19.24)</td>
<td>-0.24 (0.87)</td>
</tr>
<tr>
<td>SWM Strategy</td>
<td>35.29 (4.18)</td>
<td>0.05 (0.94)</td>
</tr>
<tr>
<td>SWM Total Errors</td>
<td>49.93 (19.35)</td>
<td>-0.18 (0.84)</td>
</tr>
<tr>
<td>SWM Within Errors</td>
<td>1.00 (1.24)</td>
<td>0.63 (0.36)  **</td>
</tr>
<tr>
<td>PAL First Trial Memory Score</td>
<td>20.29 (3.65)</td>
<td>-0.01 (0.87)</td>
</tr>
<tr>
<td>PAL Mean Errors to Success</td>
<td>1.11 (1.04)</td>
<td>0.16 (0.53)</td>
</tr>
<tr>
<td>PAL Mean Trials to Success</td>
<td>1.46 (0.40)</td>
<td>0.14 (0.59)</td>
</tr>
<tr>
<td>PAL Total Errors</td>
<td>8.86 (8.28)</td>
<td>0.13 (0.61)</td>
</tr>
<tr>
<td>PAL Total Trials</td>
<td>11.64 (3.23)</td>
<td>0.05 (0.90)</td>
</tr>
</tbody>
</table>

**Statistically significant difference, p < .01.**
6.2.3. Evaluation of Hippocampal Volumes (HCVs)

Hippocampal volumes were corrected for head size by correcting for intracranial volume (ICV; see Table 6.9). Paired sample $t$-tests were conducted to evaluate hippocampal symmetry within the VPA monotherapy group. There was not a difference between left and right corrected hippocampal volumes ($t(13) = -1.57, p = .141$).

Table 6.9.

**Hippocampal Volumes of VPA Monotherapy-Exposed Children**

<table>
<thead>
<tr>
<th></th>
<th>VPA-exposed (N = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracranial volume; cm$^3$ ($SD$)</td>
<td>1657.86 (128.97)</td>
</tr>
<tr>
<td>Left hippocampal volume; cm$^3$ ($SD$)</td>
<td>248.22 (34.04)</td>
</tr>
<tr>
<td>Corrected left hippocampal volume; cm$^3$ ($SD$)</td>
<td>252.71 (28.32)</td>
</tr>
<tr>
<td>Right hippocampal volume; cm$^3$ ($SD$)</td>
<td>256.60 (34.13)</td>
</tr>
<tr>
<td>Corrected right hippocampal volume; cm$^3$ ($SD$)</td>
<td>260.41 (28.55)</td>
</tr>
<tr>
<td>Total hippocampal volume; cm$^3$ ($SD$)</td>
<td>504.82 (65.71)</td>
</tr>
<tr>
<td>Corrected total hippocampal volume; cm$^3$ ($SD$)</td>
<td>513.11 (53.82)</td>
</tr>
</tbody>
</table>
6.2.4. Relationship between VPA Dose and Measures of Hippocampal Memory Skills

Correlation analysis was conducted within the VPA-exposed group to examine the relationship between VPA dose and performance on SWM and PAL variables. Mean VPA dose was not significantly correlated with SWM Between Errors ($r = -.17, p = .563$), SWM Strategy ($r = .16, p = .576$), SWM Total Errors ($r = -.18, p = .55$), SWM Within Errors ($r = -.32, p = .267$), PAL First Trial Memory ($r = -.11, p = .715$), PAL Mean Errors to Success ($r = .07, p = .819$), PAL Mean Trials to Success ($r = -.07, p = .807$), PAL Total Errors ($r = .07, p = .819$), or PAL Total Trials ($r = -.07, p = .807$).

6.2.5. Relationship between VPA Dose and HCV

The relationship between mean VPA dose and HCV volumes was examined with correlations. Within the VPA-exposed group, there was not a significant relationship between mean VPA dose and corrected values for left HCV ($r = .09, p = .770$), right HCV ($r = .06, p = .843$) or total HCV ($r = .07, p = .801$).

6.2.6. Relationship between HCV and Measures of Hippocampal Memory

A series of correlations were conducted to investigate the relationship between left and right corrected HCVs and performance on SWM and PAL measures (Figures 6.6 – 6.15). There was not a significant relationship between left corrected HCVs and performance on any of the SWM variables; SWM Between Errors ($r = -.48, p = .083$), SWM Strategy ($r = -.37, p = .194$), SWM Total Errors ($r = -.48, p = .081$), SWM Within Errors ($r = -.05, p = .858$).
Conversely, significant correlations emerged between left HCV and all PAL variables apart from number of stages completed \((r = -.45, p = .106)\). Left HCV was negatively correlated with PAL First Trial Memory scores \((r = -.66, p = .011)\) and positively correlated with PAL Mean Errors to Success \((r = .65, p = .012)\), PAL Mean Trials to Success \((r = .56, p = .037)\), PAL Total Errors \((r = .65, p = .012)\), and PAL Total Trials \((r = .56, p = .037)\).

A similar pattern was noted between right corrected HCVs and cognitive measures. No relationship was detected between right HCV and SWM Between Errors \((r = -.32, p = .270)\), SWM Strategy \((r = -.42, p = .136)\), SWM Total Errors \((r = -.32, p = .267)\) or SWM Within Errors \((r = .01, p = .980)\). Right HCV was negatively correlated with PAL First Trial Memory \((r = -.64, p = .013)\) and positively correlated with PAL Mean Errors to Success \((r = .65, p = .013)\), PAL Mean Trials to Success \((r = .56, p = .038)\), PAL Total Errors \((r = .65, p = .013)\), and PAL Total Trials \((r = .56, p = .038)\). Right HCV was not correlated with PAL Stages \((r = -.44, p = .118)\).
Figure 6.5. Correlations between SWM Between Errors and Corrected Hippocampal Volumes

Figure 6.6. Correlations between SWM Strategy Score and Corrected Hippocampal Volumes
Figure 6.7. Correlations between SWM Total Errors and Corrected Hippocampal Volumes

Figure 6.8. Correlations between SWM Within Errors and Corrected Hippocampal Volumes
**Figure 6.9.** Correlations between PAL First Trial Memory Score and Corrected Hippocampal Volumes

**Figure 6.10.** Correlations between PAL Mean Errors to Success and Corrected Hippocampal Volumes
Figure 6.11. Correlations between PAL Mean Trials to Success and Corrected Hippocampal Volumes

Figure 6.12. Correlations between PAL Stages Completed on the First Trial and Corrected Hippocampal Volumes
Figure 6.13. Correlations between PAL Total Errors and Corrected Hippocampal Volumes

Figure 6.14. Correlations between PAL Total Trials and Corrected Hippocampal Volumes
CHAPTER 7. DISCUSSION

The broad aim of this thesis was to add to our growing understanding of how fetal exposure to AEDs affects cognitive functioning and brain development. While a link has been established between fetal VPA exposure and birth defects, children without abnormalities appear at risk of developing cognitive impairments in the long-term. The initial chapters highlighted that VPA exposure in particular appears to have a more profound impact on the development of cognitive skills than exposure to other AEDs. However, the primary focus of past studies has been on intellectual outcomes and there has been little work to characterise memory functioning in children exposed to VPA. The neurological basis for these deficits is not understood, however findings from animal models suggests brain dysfunction occurs on multiple levels following exposure to VPA. There is experimental evidence to suggest VPA disrupts a number of biological processes in the hippocampus, however it is unknown whether these findings are generalisable to human models of brain development.

This research specifically aimed to improve our understanding of memory functioning in children exposed to VPA and examine potential underlying biological mechanisms. This final chapter will discuss the findings of this research with reference to the proposed hypotheses. Potential mechanisms by which VPA may interrupt brain development will be proposed. The strengths and limitations of the studies will be considered. This chapter will also discuss the clinical implications of the findings and present potential directions for future research.
7.1. OVERVIEW OF FINDINGS

Chapter 6 presented the results of two studies; the first which aimed to characterise memory functioning in school-aged children prenatally exposed to VPA. The following neuroimaging study aimed to examine whether changes to the structure and function of the hippocampus could be detected following in utero exposure to VPA monotherapy. The findings of these studies will be discussed in relation to the main hypotheses of this thesis. Overall, some hypotheses were supported, but some unexpected findings also emerged.

7.1.1. Memory Profile of Children Exposed to VPA

The hypothesis that children exposed to VPA would perform more poorly on memory tasks compared to typically developing children was supported for some measures but not others. The results suggest that fetal VPA exposure does have an adverse impact on learning and memory skills at school-age. Converse to the hypothesis that all memory skills would be affected by VPA exposure, memory skills were differentially impaired along the lines of material specificity. Children exposed to VPA performed lower than expected for their age on verbal memory tasks while non-verbal memory skills followed a developmentally appropriate trajectory. Children exposed to VPA monotherapy and polytherapy performed lower than expected on the List Memory task, whereas only children exposed to VPA polytherapy also performed lower than expected on the Narrative Memory task. Children exposed to VPA performed at an age-appropriate level on Memory for Designs and the Rey Complex Figure. Children exposed to other AEDs performed consistently with age expectations on all measures.
The hypothesis that children exposed to VPA would perform more poorly on memory tasks relative to children exposed to other AEDs was only supported within the polytherapy VPA group. There were no differences in performance between children exposed to VPA in monotherapy and children exposed to other AEDs in monotherapy on Memory for Designs, Rey Complex Figure, List Memory or Narrative Memory; however differences emerged when comparisons were made between children exposed to polytherapy regimes. Children exposed to polytherapy that included VPA performed more poorly on the Narrative Memory and List Memory tasks compared to children exposed to polytherapy that did not include VPA. There was no difference between the polytherapy groups on Memory for Designs or Rey Complex Figure. Mothers taking VPA in a polytherapy regime demonstrated group differences from the other women participating in the study. They had a lower IQ, were of lower SES, and were more likely to smoke tobacco and have seizures during pregnancy.

7.1.2. **Effect of Dose on the Memory Skills of VPA-Exposed Children**

While there was no difference when comparing the performance of VPA monotherapy exposure group and other AED monotherapy exposure group, a relationship between VPA dose and performance was identified on the List Memory task within the monotherapy group. Learning ability, susceptibility to interference and delayed recall were all correlated with mean VPA dose. These relationships withstood controlling for language. This supports the hypothesis that a dose-dependent relationship exists between VPA and memory skills. Of note, retroactive interference is strongly associated with hippocampal functioning. This suggests that the area of the medial temporal lobe may be vulnerable to dysfunction following VPA exposure *in utero*. 
7.1.3. Predictors of Memory Outcome in Children Exposed to VPA

The linear regression analysis showed that language was a significant predictor of performance on all aspects of the List Memory task. VPA dose was also a significant contributor to the interference effect. It was hypothesised that after controlling for potentially confounding background variables, VPA would be the most significant predictor. Controlling for background variables which differed between the monotherapy and polytherapy VPA groups did not improve the amount of variance explained by the model. While VPA did predict the interference effect, language appeared to be the strongest predictor of performance on verbal learning measures. Therefore the hypothesis was not supported. The findings highlight the strong contribution of language to performance on verbal memory measures and suggest that background factors only contribute minimally to a child’s outcome. That VPA dose is only a significant predictor of the interference effect and no other verbal memory measures lends further evidence to suggest medial temporal lobe dysfunction, which has been implicated in managing interference phenomena. Entering maternal IQ, SES, tobacco use and seizures during pregnancy into the regression model did not assist in explaining the variance, suggesting the results primarily reflect the impact of prenatal factors rather than background factors.

7.1.4. Associative Memory Functioning in VPA-Exposed Children

It was hypothesised that children exposed to VPA would demonstrate impairment on an associative learning task compared to typically developing children. This hypothesis was not
supported. Children exposed to VPA monotherapy did not differ in performance in any aspect from age-expected norms. This indicates that associative memory skills were typically developing in this sample. All facets of performance of the VPA monotherapy-exposed group on the spatial working memory task were also comparable to age-expected norms, indicating that these skills were also typically developing. In summary, this suggests that fetal exposure to VPA at the doses present in this sample has not affected the development of associative learning or spatial working memory skills.

7.1.5. Hippocampal Volumes in VPA-Exposed Children

It was hypothesised that children exposed to VPA would display a difference between left and right hippocampal volume. The findings did not support this hypothesis; hippocampal volumes were not statistically different after controlling for head size. Although, there was a trend for the right side to be larger than the left side, the analyses suggest that the VPA-exposed group tend to have symmetrical hippocampi. It was also hypothesised that hippocampal volume would be associated with VPA dose. This hypothesis was not supported. There was not a significant correlation between any corrected HCV measure and mean VPA dose.
7.1.6. Relationship between Hippocampal Volume and Associative Memory Performance

Within the VPA-exposed group, left and right HCVs were correlated with all PAL measures except for number of stages completed. Both left and right corrected HCVs were negatively correlated with PAL First Trial Memory scores and positively correlated with PAL Mean Errors to Success,PAL Mean Trials to Success, PAL Total Errors, and PAL Total Trials. Thus, children with larger hippocampal volumes correctly located fewer patterns on the first trial, made more errors and required more trials to complete the task. While a relationship between these two variables was hypothesised, it was postulated to occur in a different direction. It was hypothesised that hippocampal volume would be negatively associated with associative memory performance in children exposed to VPA; i.e. smaller HCVs would be associated with poorer performance. In fact, a relationship of an opposite nature was observed in the sample. There was not a significant relationship between HCVs and SWM measures.

7.2. RELATIONSHIP TO PAST RESEARCH

The findings of the studies in this thesis will be discussed in relation to past research. The degree to which the neuropsychological and neuroimaging results support their respective bodies of research will be analysed. An integrative discussion with respect to the neuropsychological theories of memory will follow.
7.2.1. Neuropsychological Studies

The findings of the current study suggest that fetal VPA exposure has an adverse impact on learning and memory skills at school-age, particularly with respect to the verbal domain. This is consistent with previous studies which have reported reductions in verbal intellectual abilities in VPA-exposed children (Adab, Kini, et al., 2004; Gaily et al., 2004). Amongst the handful of studies which have included memory tasks in their neuropsychological battery, one study corroborates the current findings by demonstrating that children exposed to VPA monotherapy scored significantly lower than children of women with epilepsy who did not take medications on the List Learning subtest of the NEPSY (Eriksson et al., 2005). Despite this, it is interesting to note that the children exposed to VPA monotherapy in Eriksson’s Finnish study obtained higher scaled scores than the children in the current study (11.0 vs 9.6). The children in Eriksson’s study also had an older mean age of 9.6 years and were exposed to a higher average VPA dose of 1182mg. However of note, their sample size was far smaller (N=9) than the current study, which may explain the difference in findings.

Eriksson’s study also compared List Memory performance between small groups of children exposed to VPA monotherapy and CBZ monotherapy. As the non-VPA monotherapy group in the present study contained a high proportion of CBZ-exposed children, a degree of concordance might be expected with regard to the findings. However converse to the current results, Eriksson did not detect a difference between VPA and CBZ monotherapy groups. No other comparisons have been previously been made between AED groups on this subtest, however the findings of this thesis seem consistent with the evidence that children exposed to VPA perform more poorly than other AED groups on Memory for Names from the NEPSY, another verbal learning task (Kantola-Sorsa et al., 2007).
The current study detected differences on the Narrative Memory task between children exposed to VPA in polytherapy and other AEDs in polytherapy. The difference was not found between monotherapy groups. Previous studies have not differentiated the performance of VPA-exposed children from other AED groups or controls on this subtest (Eriksson et al., 2005; Kantola-Sorsa et al., 2007). Scaled scores were reported as 8.6-10.0. This may be for a number of reasons. The two previous studies have comprised smaller sample sizes and recruited wider age ranges. Further, the present result may reflect the reduced language functioning in the VPA polytherapy group. This is supported by the fact that after controlling for language skills, only the interference effect was significant. The Narrative Memory task is heavily reliant on language skills to comprehend the verbal story and then express that information. The findings therefore suggest that some memory difficulties associated with VPA exposure may be mediated by language impairment. It is possible that impaired attentional skills also contributed to poorer performance on Narrative Memory. Children exposed to VPA have exhibited decrements on tasks of complex attention and immediate memory (Kantola-Sorsa et al., 2007). Polytherapy and higher doses of VPA have both been identified as factors contributing to poorer cognitive outcomes, therefore the combined presence these factors in the present group may have promoted dysfunction in attention.

There was no difference between groups exposed to monotherapy with and without VPA on verbal or non-verbal memory measures. However, the conclusion that VPA has no greater impact on the development of memory skills than other medications when administered in monotherapy may be pre-emptive. In the initial neuropsychological memory outcomes study, the VPA monotherapy group was exposed to a mean dose of 961.8 mg/day. Previous literature has suggested a number of VPA dose thresholds over which the risk of physical and
cognitive abnormality increases, ranging from 800-1100mg. As a group, the VPA dose children were exposed to in the monotherapy group falls at the brink of these suggested limits, which may explain the lack of effect.

Within the monotherapy VPA group, significant correlations between VPA dose and verbal memory scores were observed. This is consistent with previous reports of a dose-response relationship between VPA monotherapy exposure and verbal intellectual abilities (Adab, Kini, et al., 2004; Gaily et al., 2004; Meador et al., 2009). This is the first study to identify a similar relationship with memory functioning.

Some of the differences on memory tasks that were found when comparing the polytherapy groups were not detected between the monotherapy exposure groups. This potentially supports the suggestion that exposure to a polytherapy treatment regime in utero has a greater negative impact on cognitive development compared to monotherapy treatment (Eriksson et al., 2005; Koch et al., 1999; Lösche et al., 1994; Thomas et al., 2007). However, in the current study there was a higher mean VPA dose in the VPA polytherapy group compared to the VPA monotherapy group. This contrasts to previous studies which had reported lower VPA doses in their polytherapy groups (Gaily et al., 2004). Subsequently, it may be the case that exposure to higher doses of VPA were responsible for poorer performance within the polytherapy group, rather than the polytherapy exposure itself. The results of the regression analysis support this, illustrating that after covarying for polytherapy exposure mean VPA dose still significantly predicted the interference effect. This is consistent with Vajda, et al. (2010), who postulated that it is therapy with VPA, rather than the number of AEDs taken during pregnancy, that is the primary factor associated with the presence of birth malformations in the offspring.
7.2.2. Memory Impairments in Children Exposed to Other Drugs

The current findings highlight the sensitivity of the developing brain to changes in the intrauterine environment and demonstrate that early exposure to AEDs can have long-standing effects on the development of memory and learning skills. This is consistent with literature examining cognitive outcomes following exposure to other substances in utero, which have also detected impairments in memory function.

Much of this work has been conducted in children exposed to illicit drugs in the prenatal period; however studies in this area are often complicated by poly-substance use by mothers. Infants exposed to stimulants demonstrate impairments on visual recognition (Struthers & Hansen, 1992). Methamphetamine-exposed children score lower on measures of visual motor integration, attention, verbal memory and long-term spatial memory (Chang et al., 2004). Amongst other impairments, the acquisition of general knowledge is negatively affected in cocaine-exposed preschool children (Singer et al., 2004). Prenatal alcohol exposure has been associated with deficits in encoding verbal information, with children exhibiting difficulty with learning and recalling words after a delay (Mattson et al., 1996; Willoughby et al., 2008). Children with fetal alcohol syndrome also show spatial recall deficits (Willoughby et al., 2008). There is a dissociation between object and spatial memory in these children, with the latter being specifically impaired (Uecker & Nadel, 1998). Maternal nicotine use appears to be negatively associated with deficits in both visuo-spatial and verbal domains of learning and memory (Cornelius, Ryan, Day, Goldschmidt, & Willford, 2001; Fried, Watkinson, & Gray, 1992; Jacobsen, Slotkin, Westerveld, Mencl, & Pugh, 2005).
Environmental exposure to neurotoxic substances can also affect cognitive development, with deficits that include memory dysfunction. Early exposure to mercury is associated with neuropsychological dysfunction in the domains of language, attention, and memory, and to a lesser extent in visuospatial and motor functions at seven years of age (Grandjean et al., 1997). Arsenic has been associated with poor knowledge acquisition which may indicate long-term memory deficits (Calderon et al., 2001). Lead does not appear to have specific effects on memory following exposure, but can impact IQ and attention (Calderon et al., 2001; Ris, Dietrich, Succop, Berger, & Bornschein, 2004).

7.2.3. Neuroanatomical Studies

The most rapid rate of ICV growth occurs in the first 5 years of life and then the trajectory slows (Sgouros, Goldin, Hockley, Wake, & Natarajan, 1999). Studies in healthy children have reported that between the ages of 5 and 10 years, the average male ICV is 1399 cm$^3$ and the average female ICV is 1240 cm$^3$ (Sgouros et al., 1999). The observation that ICVs are larger in males compared to females has also been reported elsewhere (Willoughby et al., 2008). The mean ICV found in the VPA-monotherapy group was 1658 cm$^3$. The ICVs in the current sample were slightly larger than those found by Sgouros and were not examined with respect to gender differences due to small numbers. While further investigation is required, the current findings insinuate that head size may be larger in children exposed to VPA compared to typically developing children. This conflicts with the previous reports of reductions in mean birth-weight adjusted head circumferences following VPA exposure in monotherapy (Almgren et al., 2009). This previous study included a large sample size of 460 VPA-exposed children and the effect size was noted to be small. Although ICV constitutes a
different measure of head size, Almgren’s (2009) findings suggest that the current sample size of 14 children is probably too small to detect a significant effect, if one exists for ICV. Due to the lack of involvement of controls in this study, it is not possible to comment on whether the HCVs measured in VPA-exposed children are statistically different to those expected in typically developing children. However, it is worth noting that on visual inspection the mean HCVs in the current study (left = 248 cm$^3$; right = 257 cm$^3$) seem reasonably comparable to values that have been reported previously in a normative study of children by (left = 222-261 cm$^3$; right = 240-285 cm$^3$; Pfluger et al., 1999)). The idea that VPA does not affect hippocampal volume would be consistent with the findings of Ikonomidou et al (2007) who only detected reductions of grey matter volumes in the basal ganglia and hypothalamus via VBM analysis. Conversely, if HCVs are preserved, it would conflict with evidence from animal models which supports the idea that hippocampal development is altered by VPA exposure during the prenatal period. Structural changes have been documented in the form of dysplasias and altered hippocampal volumes in experimental animals (Frisch et al., 2009; Manent, Jorquera, Mazzucchelli, et al., 2007). Further, in a human case study, VPA treatment during pregnancy led to temporal lobe atrophy in the infant (Pardal-Fernández et al.).

Even if a control group was included in this study, it is highly probable that the study lacked sufficient power to detect a difference between groups, an issue which is discussed further in limitations. Another consideration is that hippocampal volume may only be affected when children are exposed to higher doses of VPA, in a similar range to doses associated with developmental defects. The dose of VPA that children in the neuroimaging study were exposed to (863.8 mg/day) was even lower than within the larger neuropsychological study
cohort and falls on the threshold of where malformation risk is purported to increase (Vajda et al., 2010). Further, the group of VPA-exposed children participating in the neuroimaging study were functioning in the average range in terms of IQ, suggesting that they represent a close to typically developing sample. Any adverse effects of VPA exposure within the group are probably subtle.

The idea that the hippocampus is not impacted by prenatal VPA exposure appears to conflict with evidence from neuroimaging studies in children prenatally exposed to other drugs. Brain changes have been documented following cocaine, alcohol, methamphetamine, marijuana and nicotine. Significant reductions in volume have been noted in the hippocampus of children prenatally exposed to methamphetamines, which was associated with poorer performance on sustained attention and delayed verbal memory tasks (Chang et al., 2004). Prenatal alcohol exposure has been strongly implicated in damage to the hippocampus in animals, however the evidence is somewhat inconsistent in humans (Berman & Hannigan, 2000). Some studies have reported smaller left hippocampi in children exposed to alcohol (Riikonen, Salonen, Partanen, & Verho, 1999; Willoughby et al., 2008). In contrast, other studies have reported relative sparing of the hippocampus and basal ganglia reductions when whole brain volumes are taken into account (Archibald et al., 2001; Mattson et al., 1996). In addition, dopamine-rich cortical (e.g., frontal cortex) and subcortical (e.g., basal ganglia) fetal brain structures show evidence of vulnerability to intrauterine drug exposure suggesting that during brain development drugs of abuse share a specific profile of developmental neurotoxicity (Derauf, Kekatpure, Neyzi, Lester, & Kosofsky, 2009).

After controlling for ICV, children exposed to VPA monotherapy displayed statistically equivalent left and right HCVs. While hippocampal volume was comparable between
hemispheres, there is evidence to suggest that the development of this region may not be entirely normal in children exposed to VPA. It was originally hypothesised that the presence of asymmetrical hippocampi would reflect underlying damage, such as is seen in Alzheimer's, however it may be the case that asymmetries in the hippocampus represent typical development. Right-greater-than-left laterality effects have been reported in typically developing children for temporal lobe, superior temporal gyrus, amygdala, and hippocampal volumes (Giedd et al., 1996). Similar findings of larger right hippocampal volumes in children have also been documented by other researchers (Pfluger et al., 1999; Szabó et al., 1999; D.K. Thompson et al., 2009). This asymmetry appears to develop in utero. The same asymmetry has also been reliably confirmed in the hippocampus normal adults (Pedraza, Bowers, & Gilmore, 2004). Despite this, the functional significance of this asymmetry appears unknown. Abnormal hippocampal asymmetry is associated with lower IQ (Abernethy, Palaniappan, & Cooke, 2002). Further, preterm infants tend to have less asymmetrical hippocampi than full term infants, but not disproportionally smaller hippocampal volumes (D.K. Thompson et al., 2009; D. K. Thompson et al., 2008). The lack of asymmetry of the hippocampus within the group of children exposed to VPA monotherapy may in fact suggest the drug may have disrupted developmental processes at some level. It is important to note that there was a trend for the right hippocampus to be larger than the left size. While this finding could potentially reflect insignificant power to detect a significant different, without a control group it is still impossible to discern whether this reflects typical asymmetry or a reduction in left volume from normal.

VPA has previously been shown to exert a detrimental dose effect on a number of developmental variables, including malformation rates (Meador et al., 2006; Vajda & Eadie,
2005; Wide et al., 2004), IQ (Gaily et al., 2004; Meador et al., 2009), cell death (Bittigau et al., 2002), synaptic responses (Gean et al., 1994), pyramidal cell layer formation (Fennrich et al., 1998) and animal behaviour (Vorhees, 1987). Conversely in the hippocampus, Frisch (2009) documented increases in rodent hippocampal volumes and improved learning following prenatal exposure to “medium” doses of VPA (500 mg/kg/day) but not “high” doses (825 mg/kg/day). This was the first study to examine the relationship between VPA exposure dose and HCVs in children. The results showed that there was not a significant association between VPA dose and HCV after correcting for head size. Therefore, the current results do not support either broad viewpoint; i.e. that VPA exposure is detrimental or can in some cases be beneficial to brain development. In the present case there was simply no effect. Previous animal models have utilised doses towards the higher end of the therapeutic range for humans, therefore the lack of effect observed in this study may be due to the narrow and relatively low range of VPA doses included in the sample. Therefore while there is some subtle evidence to suggest VPA affects development of the hippocampus, it is unclear whether the effect follows a threshold model, or dose effect model.

7.2.4. Relationship to Neuropsychological Theories of Memory

Neuropsychological theories of memory are based on the stages of encoding, storage and retrieval (Rapp, 2001). Learning of a word list with repetition was reduced in children exposed to VPA. Within the theoretical model, this is most likely to reflect encoding difficulties. As indicated by Kantola-Sorsa et al. (2007), there may also be a component of attentional or working memory difficulty, which is necessary to ensure successful encoding of information. This is further supported by animal work demonstrating poor spatial working
memory performance in animals exposed prenatally to VPA (Wagner et al., 2006). Children exposed to VPA also exhibited reduced recall of verbal information at delay. This is probably a consequence of poor encoding.

Poor performance on the story recall task was limited to the VPA polytherapy group. There was no difference in recognition scores, which could potentially indicate a retrieval difficulty; however it is more likely that their performance reflected their poor language skills. To support this, after controlling for language within the VPA monotherapy group, the significant relationship between VPA dose and Narrative Memory disappeared. Conversely, after controlling for language skills, there was still a significant dose relationship with learning ability, delayed recall and the interference effect on List Memory. This suggests that language is a strong mediator of performance on story recall tasks.

VPA dose was correlated with the measure of interference, even after controlling for language ability. This suggests that the memory impairment cannot be completely explained by language deficits. The selective effect on interference susceptibility implicates the hippocampus as an underlying contributor to the memory impairment. The limited neuroimaging data tends to support this idea, as the typical asymmetry of the hippocampi appeared disrupted; however further research is necessary to establish a true primary memory impairment.

The dissociation between verbal and non-verbal memory skills in VPA-exposed children in the neuropsychological memory outcomes study is relevant to the material specificity model. Verbal memory skills appeared selectively impaired, whereas non-verbal memory followed a developmentally appropriate trajectory. While this is likely to partially reflect the impact of reduced language abilities in this cohort, the selective effect on the interference measure is
relevant to hippocampal models of memory. In the context of the material specificity theory, it suggests that development in the left hippocampus may be selectively targeted by VPA. In fact, it would seem that the dominant hemisphere may be targeted in a number of regions, however the mechanisms by which this occurs are unknown.

Associative learning skills and spatial working memory skills were developmentally appropriate in the small sample of VPA monotherapy exposed children. Further, there was not a significant relationship between VPA dose and performance. This is the first study to specifically examine these skills in children exposed to AEDs in utero. Studies have shown that associative memory tasks are sensitive to temporal lobe dysfunction (De Jager et al., 2002; Fowler et al., 2002; Owen et al., 1995). Preserved frontal lobe functioning is required to generate the strategies necessary to complete the spatial working memory task; however temporal lobe damage can also degrade performance (Owen et al., 1995). The results suggest that the temporal and frontal brain regions are functionally preserved within the current sample of VPA-exposed children. The findings indicate that brain development is tolerant to a certain degree of intrauterine disruption and VPA monotherapy exposure is not always detrimental to a child’s development.

This preservation of function was unexpected in the context of the previous findings from behavioural models in animals, in which performance was reduced on tasks designed to tap these brain regions (T. Schneider & Przewlocki, 2005). It is important to note two factors: 1) the children were exposed to relatively low doses of VPA; and 2) these children had intellectual skills within the average range. This suggests the children participating in the study were functioning in the normally developing range. Subsequently, if brain dysfunction does result from VPA exposure, it is possible the sample may have been exposed to too low a
dose to detect it. The small sample size and subsequent lack of power could also explain the lack of effect and is discussed in study limitations. It may be the case that exposure to levels of VPA higher than the doses included in the current study place a child at higher risk of abnormal associative learning ability.

Although IQ and memory are not linear correlates (Dodrill, 1997; J. M. Williams, 1997), positive relationships between IQ and memory performance have been identified suggesting intelligence may play a mediating role (Ivnik, Smith, Malec, Petersen, & Tangalos, 1995; Rapport et al., 1997). Adults with low-average IQ perform more poorly than individuals with average and high average IQ on memory measures (Rapport et al., 1997). Further, individuals with average and high average IQ showed a steeper learning slope than those with low average IQ. The children in the smaller study examining correlates of hippocampal memory functioning were generally of average IQ and consistent with Rapport’s research, commensurate memory functioning would be expected. It is also possible that a subgroup of children exposed to VPA exists in which lower IQ acts as a coarse marker of more specific difficulties. Children within this group may be more likely to have altered hippocampal function and memory impairments.

Significant correlations were identified between associative memory performance and HCVs. Relationships were not observed between measures of spatial working memory performance and HCVs. This finding is consistent with previous studies which have emphasised the role of the hippocampus in associative learning processes. In rodents, hippocampal volumes have been associated with maze learning performance (Frisch et al., 2009; Niessen et al., 2005). Further, associative learning skills are a predictor of memory dysfunction in patients with temporal lobe pathology (De Jager et al., 2002; Fowler et al., 2002; Owen et al., 1995).
While it was predicted that the correlation between associative memory performance and HCV would be negative, the relationship identified in this study were primarily positive in direction. In other words, larger HCVs were associated with the child making more errors and taking a greater number of trials to complete the associative memory task. Smaller HCVs were associated with better performance. In older adults, it is generally accepted that loss of volume in the hippocampus mediates the memory deficits seen in pathological conditions such as Alzheimer’s (Giménez et al., 2004; Ystad et al., 2009). Similar relationships between small hippocampal volumes and poor memory functioning have been reported in children born with very low birth weight (Isaacs et al., 2000).

However, developmental morphometry studies have suggested that volume decline and cognitive impairment are not invariably linked. There are three main perspectives on the relationship between hippocampal size and memory ability in healthy subjects (Van Petten, 2004). The “neuropsychological” perspective is that a loss of tissue within a structure will lead to a decline in function, such as is seen in aging. The “bigger is better” hypothesis simply postulates that a larger hippocampus should result in stronger memory function. Finally, the “developmental” perspective emphasises a parallel between loss of cortical matter through pruning and improvement in cognitive ability during childhood. A meta-analysis by Van Petten (2004) evaluating these three viewpoints argued that the “bigger is better” hypothesis may not be applicable to normal childhood development. In fact, studies in children and young adults have suggested “smaller is better”, reporting negative correlations between hippocampal volumes and memory performance (Chantôme et al., 1999; Foster et al., 1999; Sowell, Delis, Stiles, & Jernigan, 2001; Yurgelun-Todd, Killgore, & Cintron, 2003). The current finding that smaller HCVs were associated with better performance within
the VPA monotherapy group therefore lends support to the developmental perspective on the volume-memory relationship. This subsequently implies that the characteristics of the sample in this study reflected typical developmental processes.

7.3. THE IMPACT OF TIMING OF VALPROATE EXPOSURE DURING THE PRENATAL PERIOD

The timing of exposure to substances in the intrauterine environment appears to play a significant role in determining outcome. As discussed in Chapters 2 and 3, there is strong evidence to suggest that VPA exposure in the first trimester disrupts neural tube closure (Morrell, 2003). During the second trimester, neurons undergo intensive proliferation and migration. VPA exposure may cause abnormal migration of neurons (Manent, Jorquera, Mazzucchelli, et al., 2007; Miyazaki et al., 2005). An abnormality in migration, potentially in the hippocampus or temporal lobe in general, may underlie the verbal memory and also possibly language impairments seen in the larger neuropsychological study. Despite this, it is unclear why this abnormality would be restricted to the dominant hemisphere of the brain while preserving functioning of the non-dominant (non-verbal memory) hemisphere.

Disruption of developmental processes during the third trimester could also responsible for the cognitive deficits seen in children exposed to VPA. The brain growth spurt period extends from the gestational third trimester to several years after birth. Any compound that interferes with these processes may trigger apoptotic degeneration of neurons (Kaindl et al., 2006). While studies are yet to directly link the regions of apoptosis induced by VPA with behavioural deficits, it seems likely that there will be an association between the two.
Considering that cell death has been reported in regions including the hippocampus by Bittigau (2002), VPA-induced apoptosis within the hippocampus may underlie the memory deficits detected in the current study.

### 7.3.1. A Dual Insult Model?

In light of the findings of the current study, a dual insult hypothesis is proposed as a mechanism for VPA’s action. It is speculated that children exposed to VPA suffer two insults. The first insult appears to target language abilities, evidenced by the strong contribution of language skills to the verbal memory deficit in these children. This could be mediated by dysfunction in the dominant perisylvian region (D. H. Geschwind & Miller, 2001). After removing the effects of language in the analyses, there was a persistent effect on the effect of retroactive interference. This strongly implicates hippocampal dysfunction, suggesting the hippocampus may be vulnerable to a second insult. This idea could be supported by the apparent lack of asymmetry within the hippocampus of children exposed to VPA monotherapy that has been reported in typically developing samples. These two insults probably stem from events in the late second to third trimester of pregnancy, when neurons are forming complex cortical connections and proliferation of neurons in the hippocampus is at a maximum rate. It is unknown whether these insults might occur simultaneously with a common causative factor or in the context of a disrupted cascade of developmental events.

It is still unknown how VPA might target the dominant hemisphere and have a specific effect on language-based skills. Alterations in the typical asymmetry of the perisylvian language regions are associated with developmental disorders of language (D. H. Geschwind & Miller,
Given that the normal asymmetry of the hippocampus is potentially altered by VPA exposure, perhaps cerebral lateralisation is also disrupted in other regions. Extensive work has been conducted by Geschwind and Galaburda (1985) on the developmental mechanisms of cerebral lateralisation. Anatomical asymmetries are visible at 30 weeks gestation in the planum temporale. It has been hypothesised that asymmetry begins to develop earlier, sometime between 6 and 20 weeks gestation, when the developmental axes of the brain are established. The typical asymmetry of the hippocampus has been shown to be established in utero, however it has not yet been established precisely in which trimester (D. K. Thompson et al., 2008). Studies of developmental dyslexia provide a functional example of how disrupted asymmetry can have a functional impact on cognition (Galaburda, 1993). Dyslexia has been associated with disruption of the developmental events that occur between 15 to 25 weeks gestation when neurogenesis and migration are at their peak. The perisylvian region is normally larger on the left than right and in dyslexia this asymmetry is absent. Cortical symmetry has been hypothesised to arise from incomplete pruning of one side as opposed to stunted unilateral growth (Galaburda, 1995). The net result is a bilateral equivalence of the perisylvian region which has been proposed to result from either increased progenitor proliferation or decreased normal apoptosis that normally occurs following migration (D. H. Geschwind & Miller, 2001). Since VPA is known to disrupt numerous developmental processes, it may prevent normal asymmetries from being established in the developing brain, leading to symmetrical hippocampi and potentially abnormal perisylvian functioning.
7.4. OTHER POTENTIAL MECHANISMS OF VALPROATE ACTION

While it is yet to be exactly established how prenatal exposure to VPA produces impairments in cognition and memory, a single mechanism is unlikely to be responsible. Neurodevelopment is a complex dynamic process and disruption to even a single event has the potential to initiate a cascade of changes which ultimately end with abnormal neuronal circuitry within the brain. The final phenotype of these children probably reflects disturbance to a number of developmental processes, which may include disrupted neurotransmitter levels, altered folate metabolism and inhibition of histone deacetylases.

7.4.1. Neurotransmitters

Altered neurotransmitter levels may contribute to poor memory functioning. The serotonergic system of the brain plays a significant role in memory and appears to be altered following VPA exposure (Dufour-Rainfray et al., 2010; Kuwagata et al., 2009; Ogawa et al., 2009). Dopaminergic systems also appear to be disrupted following VPA exposure in utero and may represent another mechanism by which memory function is altered (Nakasato et al., 2008; N. Narita et al., 2002).

7.4.2. Folate Metabolism

Folate is a co-enzyme that plays an important role in CNS functioning and also appears to play a role in memory function (Hassing, Wahlin, Winblad, & Bäckman, 1999). Abnormal folate expression resulting from VPA exposure may interfere with the typical cascade of
developmental processes and alter gene expression (Alonso-Aperte, Ubeda, Achon, Perez-Miguelsanz, & Varela-Moreiras, 1999; Apeland, Mansoor, Strandjord, & Kristensen, 2000). Folate deficiency dramatically reduces the number of proliferating cells in the dentate gyrus of the hippocampus of adult mice (Kruman, Mouton, Emokpae Jr, Cutler, & Mattson, 2005). Inducing folate deficiency in pregnant mice also reduces progenitor cell proliferation in the fetal mouse brain and significantly increases apoptosis (Craciunescu et al., 2004).

7.4.3. Genetic Vulnerabilities

Genetic factors have the potential to have a long-standing impact on cognition development. While the exact genetic mechanisms that underlie poor memory outcomes are unknown, small genetic changes may be responsible for a chain of events which culminate in the final phenotype. If genes involved in cortical development are altered, it may lead to subtle cortical malformations involving neuronal migration or synapse formation, which in turn can lead to abnormal cortical circuits that affect pathways critical for learning (Galaburda, LoTurco, Ramus, Fitch, & Rosen, 2006).

Genetic vulnerabilities have been identified as one mechanism by which VPA causes teratogenicity and also have the potential to impact on memory development (Faiella et al., 2000; Lindhout & Omtzigt, 1992). Studies have revealed that VPA exposure changes the expression profile of many genes that regulate the normal development of the brain. Fetal exposure to AEDs may also be influenced by polymorphisms in drug transporting proteins in the placenta. Genetic variations in the expression and activity of these transport proteins may
influence fetal exposure to AEDs and thus the risk of teratogenicity or cognitive impairment (Atkinson, Brice-Bennett, & D'Souza, 2007).

7.4.4. Inhibition of Histone Deacetylases

VPA may also influence developmental processes in the brain and hippocampus by inhibiting histone deacetylases (HDACs). HDACs are nuclear and cytoplasmic enzymes that act on histones and regulate gene transcription. VPA has been described as a HDAC inhibitor (Yildirim, 2003). HDAC inhibitors are able to interrupt cell cycle, arrest growth and trigger apoptosis (Ornoy, 2009). Interestingly though, if VPA is a HDAC inhibitor it has interesting implications for memory functioning. The formation of long term memory culminates with the regulation of gene expression, which may be mediated by HDAC activity (Vecsey et al., 2007). Studies have shown that HDAC inhibitors actually have the ability to promote memory formation. The HDAC inhibitors trichostatin A and sodium butyrate have been shown to enhance hippocampus-dependent memory and hippocampal synaptic plasticity (Levenson et al., 2004; Stefanko, Barrett, Ly, Reolon, & Wood, 2009; Vecsey et al., 2007). In line with this, VPA promotes the acetylation of histones in the hippocampal cell cultures (Yildirim, 2003) and enhances long-term memory for both acquisition and extinction of fear in rodents (Bredy & Barad, 2008). The effects of VPA as a memory promoter via HDAC inhibition have not been examined in humans or in fetal exposure models. Despite this, as that memory was reduced in the current sample of children exposed to VPA, it suggests that the action of VPA as a HDAC inhibitor may not generalise directly from experimental models.
7.5. STRENGTHS OF STUDY

As discussed in Chapter 2, previous studies examining cognitive outcomes in children exposed to AEDs have been methodologically flawed. Early studies have been retrospective in design and there has also been poor control of confounding factors. They have also been subject to recruitment bias and outcome measures have been poorly defined. This study has attempted to overcome these limitations and possesses a number of strengths over previous research in this area. It is the first study to specifically examine memory functioning in children exposed to AEDs, with a focus on elucidating the memory profile associated with VPA exposure. This study was the first to investigate whether memory difficulties experienced by these children are attributable to primary memory impairment or other confounding factors. It was also the first study internationally to examine brain structure in a group of children exposed to a single medication.

7.5.1. Prospective Design

Many studies to date have employed retrospective study designs (Adab et al., 2001; Adab, Kini, et al., 2004; Dean et al., 2002; Moore et al., 2000; Rasalam et al., 2005). Therefore, this study had advantages over these previous studies by adopting a prospective design. Retrospective cohort studies are typically ranked lower in the hierarchy of evidence than prospective cohort studies as they possess more potential sources of confound and bias. Randomised controlled studies are generally considered to be the most reliable form of scientific evidence, however implementing a study of this design would not be ethically
sound in this cohort of women. Therefore, therefore prospective studies represent the most robust option for examining cognitive outcomes in offspring of women with epilepsy. This is one of the largest prospective studies to date that has specifically examined memory outcomes in children exposed to VPA.

This study also improves on previous prospective studies which have failed to control for many potential sources of confound (Gaily et al., 2004). In the current study, women were enrolled into the APR at conception and medical records were collected throughout their pregnancy and following their birth. This meant comprehensive prospective data was available documenting epilepsy history, pregnancy variables and birth outcomes. Previous retrospective studies collected medical and pregnancy information directly from women many years after the birth of their child, which may have been subsequently subject to recall bias. By collecting information from prospectively collected records, the accuracy of historical and clinical information has been ensured to a greater extent than many previous studies.

7.5.2. Recruitment Strengths

The sample in this study was recruited from a national pregnancy register (i.e. the APR) to achieve a more accurate representation of women with epilepsy in Australia. Some previous studies have recruited women with epilepsy from specialised epilepsy or teratogen clinics (Adab, Tudur, et al., 2004; Meador et al., 2009; K. J. Meador et al., 2011; Ornoy & Cohen, 1996; Rovet et al., 1995). As a consequence of this, it is possible that there was an overrepresentation of severe cases in their samples. Detailed information on this study’s
recruitment procedures and the proportion of women who participated has been provided. This information has not been explicitly reported in previous studies.

The chances that women with disadvantaged children would be drawn to the study was minimised by recruiting from the APR. The majority of women in this study were approached directly by invitation rather than through advertising for interested parties. The limitations associated with the recruitment strategy are addressed in the following section. Only 11% of contacted women declined to participate, and the reasons they provided for this were recorded. Of the women who declined to participate, 29% did so because of concerns about their child’s ability to participate in the study. Further, there was additional anecdotal evidence to suggest that some women who did not return formal consent before the cessation of the study were hesitant about participation due to concerns about their child’s development. This is a good indicator that concern about their child’s abilities was not a primary motivator for participation.

An additional strength of the recruitment strategy employed in this study was the exclusion of children with birth defects or epilepsy. These children have commonly been included in previous samples (Dean et al., 2002; Gaily et al., 2004; Meador et al., 2009; Thomas et al., 2007). These conditions can affect brain development and may influence cognitive outcomes. Their inclusion in previous studies may have resulted in an overestimation of deficit in AED-exposed children.
7.5.3. Neuropsychological Assessment

This is the first study to comprehensively evaluate memory functioning in school-aged children exposed to VPA. Studies investigating cognitive development in children exposed to AEDs have tended to use IQ as their primary outcome variable. Only a handful of studies have included more specific neuropsychological measures examining other cognitive functions (Eriksson et al., 2005; Kantola-Sorsa et al., 2007). The broader research project that this study formed a part of was the first to systematically examine specific domains. Data has been recently published characterising intellectual ability and language skills in the current sample (Nadebaum et al., 2011a, 2011b).

In terms of other assessment issues, compared with previous studies, the tests selected provide a more comprehensive assessment of memory functioning consistent with neuropsychological theory. Tests with normative data were included to allow clinical interpretation of results. Assessments were also conducted blind to drug exposure. While blind assessment was not a unique aspect of this study relative to previous research, it provided a methodological strength by eliminating the risk of assessment bias.

7.5.4. Imaging Technique

This is the first study to specifically examine hippocampal volumes in children exposed to a single AED. The only other neuroimaging study evaluated adults who were exposed to a variety of AEDs in utero (Ikonomidou et al., 2007). Thus the current research allows more accurate conclusions to be drawn about the specific effects of VPA on the developing brain. Manual segmentation was used to examine hippocampal volumes. The previous
neuroimaging study detected basal ganglia changes with voxel-based morphometry (VBM), however the authors acknowledge that this technique is generally used to approach structural brain abnormalities in association with medical conditions (Ikonomidou et al., 2007). The limitations of VBM include potential misregistration with the template due to anatomical abnormalities, difficulty with accurate localisation of regional volumetric differences, reliance on the normal distribution of residuals, poorly understood nature of grey and white matter changes identified (Mechelli, Price, Friston, & Ashburner, 2005). Manual segmentation appears to be more accurate in some samples in detecting small volume changes. Bergouignan et al. (2009) illustrated in a sample of depression patients that standard VBM could not detect group differences in hippocampal volume, however manual segmentation could.

7.6. LIMITATIONS

This study is the first to examine memory functioning of children exposed to VPA in detail and brain structure in the hippocampus. While the study had a number of advantages over previous research, its strengths also introduced inherent weaknesses. As with most research, there are a number of limitations which must be acknowledged. A number of incidental findings also emerged from the study, which complicated interpretation of the results.
7.6.1. Cross-sectional Design

This study adopted a cross-sectional design, which has its own inherent limitations. Cross-sectional studies generally only provide a 'snapshot' of the outcome and the characteristics associated with it, at a specific point in time. Therefore this study can only comment on the memory functioning of children at 6-8 years of age. Longitudinal studies will be important to characterise the developmental trajectory of their impairment.

Children with a previous diagnosis of epilepsy were excluded from the study, however a potential limitation of the cross-sectional design is there may be a contribution of epilepsy in the sample from undiagnosed children. The risk of epilepsy in children of parents with epilepsy is higher than that in the general population (Yerby, 2000). However determining the proportion of children in the current sample that develop epilepsy is impossible without longitudinal follow-up.

Another potential concern is that the results may reflect other baseline characteristics that could impact on the child’s development, such as mother’s epilepsy type. However this issue is difficult to control as maternal seizure type usually dictates which AED is prescribed. Generalised seizures are more commonly treated with VPA whereas partial seizures are commonly prescribed carbamazepine (Marson et al., 2007a, 2007b). Because women were unable to be randomly allocated to a treatment group (ie VPA therapy), the results could reflect the greater proportion of generalised epilepsy within the cohort of women taking VPA.
7.6.2. Sample Recruitment

While efforts were made to reduce bias by sourcing participants from a national register, it is possible that some recruitment degree of bias persisted as the full cohort of eligible children did not participate in the study. The most common reason for this was these mothers were unable to be contacted by telephone or mail. A number of women (12) expressed interest in the study but lived in geographically isolated regions of Australia and were unable to participate. Assessments involving women from NSW and QLD predominately took place in major cities; therefore, the sample is probably more representative of women living in suburban areas rather than rural regions. As previously mentioned, given that some previous studies recruited women from epilepsy and teratogen clinics (Adab, Tudur, et al., 2004; Meador et al., 2009; K. J. Meador et al., 2011; Ornoy & Cohen, 1996; Rovet et al., 1995), it raises concerns about whether women with disadvantaged children were more likely to volunteer to participate. Although this is still always a possibility within the current sample, the recruitment methods attempted to minimise this risk. Permission was not granted by ethics to use the details of women who did not participate (due to refusal or inability to contact) in an analysis to evaluate this issue. Previous studies also have not reported this type of information. If women with disadvantaged children were more likely to participate, the current data would overestimate the occurrence of deficit in this population.

The exclusion of children with birth defects acted as a strength by eliminating the potential influence of these conditions on cognitive development, however it may also have acted as a weakness. These children were excluded in an attempt to isolate the effects of VPA exposure; however it is important to remember that birth defects are a valid component of these
children’s phenotype. By excluding these children, the study may have underestimated the full extent of morbidity.

7.6.3. Testing and Assessment Issues

The NEPSY-II (Korkman et al., 2007) was selected as a test instrument due to its strong psychometric properties and the presence of normative data. However, despite it being a reliable and valid instrument (Schmitt & Wodrich, 2004), a number of issues can be identified with its use. Like the majority of test instruments, the NEPSY-II was standardised in the U.S. population. This poses a limitation as it is unknown how accurately the normative data for the NEPSY-II represents the Australian population. However, studies investigating the NEPSY in other cultures suggest it is unaffected by language and cultural factors (Mulenga, 2001). Further, interpretation of the NEPSY-II memory subtests was limited by the fact that the test does not differentiate the scaled score into components of learning, delayed recall and recognition; instead a total scaled score for all of these skills is provided. Finally, in the revision of the NEPSY, the norms for List Memory were not updated and are subsequently around 10 years older than other norms (Brooks, Sherman, & Strauss, 2010).

Unfortunately, normative data for all participating children were not available, which undermined the ability to compare the performance of the VPA-exposed group to that expected of typical development. Standard scores for List Memory on the NEPSY-II could only be generated for children over the age of seven. Normative data for the Rey Complex Figure were also only available for children over seven years. This issue could have been
overcome by inclusion of an age-matched control group for all VPA-exposed participants, which is discussed in more detail below.

Further, it should be noted that environmental factors could always have impacted on a child’s testing performance. Although efforts were made to minimise fatigue, given the long testing procedure it is not possible to completely exclude its influence on test performance. Similarly, poor attention and mood could also have impacted on test scores but this was up to the clinical discretion of the assessor to judge.

7.6.4. Demographic Differences

Mothers taking VPA in a polytherapy regime exhibited numerous differences from the other women in the study. Maternal IQ and family SES were significantly lower, they were more likely to have a generalised form of epilepsy and were more likely to smoke tobacco and have seizures during pregnancy. Therefore, it appears that this group of women is fundamentally different from the other participating mothers in the sample. This is consistent with previous studies, which have also found that women who take VPA through pregnancy may be more likely to have lower IQ scores and educational attainment levels than women taking other AEDs (Eriksson et al., 2005; Gaily et al., 2004; Koch et al., 1999). While attempts were made to statistically control for these differences in the analyses, there may be additional unknown variables which separate this group of mothers from mothers taking AEDs. Further, the small sample size did not allow for all factors to be included in analyses.
7.6.5. **Incidental VPA Dose Findings**

All neuropsychological assessments were conducted blind to drug exposure information. While this acted as a strength and prevented biased assessments of children, it also meant AED exposure information was not available until the analysis stage. In the sample, children were incidentally exposed to higher doses in the VPA polytherapy group compared to the VPA monotherapy group. This contrasts significantly to previous studies, in which VPA dose has been higher in the monotherapy group (Gaily et al., 2004).

This issue induced a significant limitation into the analyses. It meant that the independent contributions of polytherapy and high dose VPA could not be disentangled. It is uncertain whether the poorer performance seen in the VPA polytherapy group on memory measures is a reflection of polytherapy treatment or the higher dose of VPA. This point has been contentious in the literature, however it has recently been suggested that VPA is the primary contributor to poor outcome rather than the number of AEDs taken during pregnancy (Holmes et al., 2011; Vajda et al., 2010). In order to differentiate the independent effects of polytherapy and VPA on memory function, future studies should ideally employ a screening procedure in their recruitment to match the doses of VPA doses within the monotherapy and polytherapy groups.

7.6.6. **Lack of Control Group**

A significant limitation in this study was the lack of a control group; however the degree of impact depended on the research question being asked. The broader neuropsychological memory outcomes study sought to establish whether VPA has a greater impact on memory
development than other AEDs. A control group is not particularly useful in this context, which mirrors the absence of a control group in other studies which have posed similar questions (UK research group; Meador et al., 2009; Vinten et al., 2005). In addressing how AED exposed children fare relative to typical development, comparison with normative data has sufficed in the literature, however a control group of unexposed children such as that recruited by Gaily et al. (2004) or Kantola-Sorsa et al. (2007) probably represents the ‘gold standard’.

In the current study, normative data were used in lieu of a control group as a comparison point for VPA-exposed children in the study. This included the neuropsychological memory measures and computerised PAL and SWM tasks. Unfortunately, normative data for some of the neuropsychological measures were absent for some age groups. This restricted the number of data points that were able to be included in the analysis and reduced power. Scores collected from an age-matched control group would have enabled a more in-depth analysis.

For the MRI study, extensive attempts were made to recruit age-matched, typically developing children. Multiple recruitment avenues were pursued, including advertising at the participating institutions, private after-school care programs and maternal and child health centres. Unfortunately, the response rate was very poor. Given the timeline of this project, further attempts to recruit controls were not made. Further, permission to access existing control data from within participating institutions was not able to be gained from ethics. The absence of control data is probably the most significant limitation of this thesis as meaningful comparisons of the HCV data were not able to be made. It therefore remains unknown how the HCVs of VPA-exposed children compare to unexposed children. Control data could have also provided additional information about the relationship between HCV and associative
memory performance in typically developing children, adding support to one of the three main perspectives on the relationship between hippocampal size and memory ability described previously (Van Petten, 2004).

The issue of selecting an appropriate control group for this cohort of women was discussed in Chapter 2 and there is poor consensus amongst investigators. Further, it is questionable whether the normative data from typically developing children represents the most suitable comparison group. For this study, the ideal control group would have been age-matched children of women who had epilepsy but did not take medications, such as recruited by Erikkson and colleagues (2005). These women were recruited to the larger study but were in too few in number to include in the analysis. Test scores from a larger group of these women’s children on the neuropsychological measures would have allowed more valid conclusions to be drawn about development of memory skills in the cohort. Further, brain imaging of this group would have enabled meaningful comparisons of volumetric data.

7.6.7. Sample Size and Power

Power was a significant issue for both the memory outcomes and neuroimaging studies. In the neuropsychological study, this was a consequence of blinded assessments and therefore an inability to selectively recruit mothers taking VPA as part of the broader study. A priori power analyses were unable to be conducted in detail. This was the only neuroimaging study to examine the effects of a single AED in the largest sample to date, however the sample size was small.
The results of post hoc power analyses are presented in Appendix 1. The power analyses showed that for the statistical comparisons of neuropsychological memory variables in the larger cohort of children, power was over 80% to detect a large effect size within monotherapy groups. Power was lower for polytherapy group comparisons (60-80%); however in light of the significant results suggest the effect size is very large. The regression analysis also possessed adequate power (>90% for a large effect size).

The imaging study possessed less power due to the smaller sample size, falling below 80% on associative learning comparisons with normative data. The power of the correlation study is even lower, suggesting the group size was too small to detect an effect if one was present. This is probably one of the most significant limitations of the neuroimaging study. Evidence from past animal studies and the current neuropsychological findings strongly point to disrupted hippocampal structure following VPA exposure and it is hypothesised that with larger sample sizes, developmental abnormalities will be found.

An additional power analysis was conducted to evaluate the power of a comparison between the VPA group and controls, if matched controls had been able to be recruited. The analysis showed that for two groups of N=14, the power of the comparison to detect a difference in hippocampal volumes was only about 50%. This suggests that even if this group had been able to be found, a group size of N=14 would have been too small. Larger groups are clearly required by future studies in this area.
7.6.8. Imaging Issues

Although the intra-rater reliability analysis suggested good agreement across ratings, it is best practice in imaging studies to also calculate a measure of inter-rater reliability. Unfortunately, another appropriately trained rater was not available to perform this analysis. Recruiting additional raters to the reliability analysis is warranted in future studies and highlights another mechanism by which the accuracy of the current study could be improved.

7.7. IMPLICATIONS FOR CLINICAL PRACTICE

The findings of this study raise many implications for clinical treatment of women with epilepsy. This section will discuss the implications relating to the use of VPA during pregnancy in women with epilepsy, the management of women with epilepsy, the management of women taking VPA for conditions other than epilepsy and the management of the children exposed to VPA in utero.

7.7.1. The Use of VPA During Pregnancy

Concerns have rightfully been raised over the accumulating evidence that taking VPA during pregnancy is associated with significant fetal risks. The current consensus within clinical literature is that VPA treatment should be avoided during pregnancy to minimise the risks of physical malformation and reduce the likelihood of poor cognitive outcomes (Harden, Meador, et al., 2009). The findings of the current study indicate that in addition to the intellectual and verbal deficits previously documented, children exposed to VPA are also at
risk of memory impairment, thereby adding further support to recommendations of VPA avoidance during pregnancy, if possible.

Prescription guidelines recommend the use of monotherapy regimes over polytherapy treatments in pregnancy (Harden, Meador, et al., 2009). However, a dose-dependent relationship between VPA and memory functioning was documented within the current monotherapy group, suggesting that monotherapy VPA exposure still carries some degree of risk. In line with this, the findings imply that reducing VPA dose may reduce the risk of adverse outcome in children. Some studies examining malformations have suggested a threshold effect exists, with doses over 800-1100mg associated with higher malformation rates (Vajda et al., 2004). However, the results of the current study suggest the effect might instead be cumulative.

Pregnant women with epilepsy pose a challenge for clinical management as their medications must be carefully balanced to prevent seizures while minimising the risk of teratogenicity. Changes to AED treatment regimes have been associated with increased seizures, so modification of medication during pregnancy must proceed with a great degree of caution. Further, there is not a clear cause and effect relationship between taking VPA and poor outcome; many children are typically developing and only a minority develop problems. Given this delicate balancing act, it may not be useful to consider the prescription of VPA in terms of a threshold model with a “danger dose”. This may promote concern in women who require high doses of VPA to control their seizures. Rather, it might be more constructive to consider VPA dose as a factor that increases risk along a spectrum.

The timing of VPA exposure also has implications for clinical management. First trimester exposure has been associated with neural tube defects whereas cognitive impairments such as
the memory deficits documented in this study probably arise from disruption of processes later in pregnancy, perhaps during the late second or third trimester. Clearly, reducing VPA dose during the first trimester would be ideal to prevent major malformations, however abnormalities may have already developed by the time the pregnancy becomes known to the mother. Many women on VPA within the current sample were prescribed higher doses of VPA after the first trimester had passed, which might impose a greater risk on brain development within later trimesters and consequently on cognitive outcome. To further complicate matters, it may be the case that the dose of VPA that induces malformations is higher or lower than the dose that might produce cognitive impairments. Despite this, it should also be noted that brain development is ongoing throughout pregnancy and abnormality may result from exposure at any time.

There is strong suggestion in the literature that high doses of VPA should be avoided in women of child bearing age (Harden, Meador, et al., 2009). Ideally, alternative AEDs should be taken by these women but this will depend on the epilepsy type and a woman’s individual response to the medication. It may not be possible for some women to completely withdraw from VPA, particularly if they have generalised seizures (Marson et al., 2007b). Further, changing medication will not completely reduce the risk of poor cognitive outcome. Many have suggested carbamazepine and lamotrigine to be preferable alternatives, however these are generally more effective at treating partial and tonic-clonic seizures (Marson et al., 2007a). These AEDs have also been linked to fetal malformation but at much lower rates compared to VPA (Harden, Meador, et al., 2009; Tomson et al., 2011). Carbamazepine appears to have less of an adverse impact on cognitive development. Many studies have suggested that children exposed to carbamazepine do not differ from controls on measures of
development (Adab, Kini, et al., 2004; Almgren et al., 2009; Eriksson et al., 2005; Gaily et al., 2004; Hiilesmaa et al., 1981; Scolnik et al., 1994). However more recent research has presented converse results, with reports of reduced verbal and performance abilities (Banach et al., 2010; K. J. Meador et al., 2011). Less is known about the cognitive functioning of children exposed to lamotrigine, however children appear to have better intellectual outcomes than VPA children and IQ in the average range (Meador et al., 2009). In another study, carbamazepine but not lamotrigine was observed to have a significant detrimental effect on neurodevelopment (Cummings et al., 2011). Little work has been done to characterise functioning in children exposed to newer AEDs, however authors have suggested they may be more favourable outcomes and are less likely to be teratogenic (Palmieri & Canger, 2002).

### 7.7.2. Management of Women with Epilepsy

The findings that women taking VPA in polytherapy were of lower IQ and SES and were more likely to smoke tobacco and have seizures during pregnancy has implications for their clinical management. These women are likely to have higher support needs than other women with epilepsy during their pregnancy. Given their higher likelihood of seizures, women taking VPA in polytherapy may benefit from closer monitoring by their treating doctors during their pregnancy to manage their AEDs and reduce the occurrence of seizures. They may also benefit from increased education about the impact of substance use on their developing fetus. These women will also probably require ongoing support after the birth of their child. If relying on the past research suggesting polytherapy is associated with a higher risk of cognitive impairment, these women will require access to childhood support services.
Further, maternal intelligence and family environment are key factors in children’s intellectual development. Studies have demonstrated that children whose mothers have low IQs respond positively to intensive early interventions and can drastically reduce rates of intellectual disability (Ramey & Ramey, 1994).

Women with epilepsy will highly benefit from pre-pregnancy counselling from their treating doctor, particularly if they are taking VPA. Many women in the study described receiving little information on drug safety during their pregnancy. The potential to add to this knowledge base was a strong motivator for their participation, suggesting that these women are keen to understand the risks associated with taking AEDs in pregnancy. In depth discussions should be held between the treating doctor and patient regarding planning for pregnancies. If women are of child bearing age, it should be emphasised that medication changes ideally must occur prior to conception. Folate supplementation should also be recommended, despite evidence that it does not completely eliminate the risk of birth defects. Women should also be alerted to the cumulative impact that other substances such as cocaine, marijuana and tobacco use might have on development. For women whom which VPA provides the best seizure control, regular developmental follow up of children is recommended.

### 7.7.3. Other Women Taking AEDs

This study also has implications for women taking VPA for other conditions such as mood disorders (e.g. bipolar disorder) and migraine (Kaindl et al., 2006). Although studies have not investigated birth outcomes in women taking VPA for psychiatric conditions, it is possible
that they are at the same risk of adverse effects observed in women with epilepsy. Given that studies of VPA exposure in animal models are not specific to epilepsy status, the same disruptions to developmental brain processes may be expected to occur. Children of women taking VPA for any condition through pregnancy may more likely to experience memory and other cognitive difficulties than the general population and may also benefit from interventions; however whether deficits are also observed outside epilepsy treatment needs to evaluated further.

7.7.4. Management of Children Exposed to AEDs

The outcomes of this study have implications for the management of VPA-exposed children. Characterising the profile of VPA-exposed children is an important step in designing appropriate intervention strategies to improve cognitive functioning. Successful improvements following early intervention programs have been demonstrated in children with fetal alcohol syndrome and low birth weight (Adnams et al., 2007; The Infant Health and Development Program, 1990). In the absence of early intervention, children at risk display a general decline in intellectual development across the first 5 years of life (Guralnick, 1997). Interventions have the potential for long-term impact on cognitive and academic performance which can lead to improvements in functioning that continue through schooling into early adulthood (Campbell, Pungello, Miller-Johnson, Burchinal, & Ramey, 2001; McCarton et al., 1997; McCormick et al., 2006). However, these interventions must begin shortly after birth to have long-term advantages. Beginning interventions at school-age alone has been shown to be less effective than strategies begun in preschool (Campbell & Ramey, 1994).
Developmental surveillance is time and cost intensive, however the importance of regular developmental screening to enable referral to appropriate services has been highlighted by reviews (Squires, Nickel, & Eisert, 1996). Monitoring of VPA-exposed children should begin immediately after birth and persist throughout schooling. The involvement of speech pathology and neuropsychology to evaluate language and cognitive development is strongly indicated within this cohort. Further assessment of other aspects of cognition, behaviour, academic ability and social skills may also be warranted.

An intervention model proposed by Guralnick (1997) highlights the importance of aspects such as resource supports, social supports and the provision of information and services. Early intervention services could include early childhood education, parenting training, family counselling, home visits, health services and medical services. Family support is a crucial aspect of intervention strategies as environment has been proposed as a better long term predictor of outcome than injury severity. Evidence from brain injury studies in children suggest that family environment can significantly modify the impact of an injury, buffering the impact in high-functioning families and exacerbating it in low-functioning families (VA Anderson, Morse, Catroppa, Haritou, & Rosenfeld, 2004; Yeates et al., 1997).

Deficits may begin to emerge at preschool age as cognitive skills become more complex, but they might also be masked by behavioural issues (G. H. Smith, 1993). Home-based learning strategies may be of benefit, however given that each child is likely to experience different difficulties, an individualised approach may be most appropriate. Educational planning should also be implemented. While VPA-exposed children may present with learning and memory difficulties, the current results indicate their problems may be partially be mediated by language deficits. This suggests that these children’s learning might be greatly assisted by
simplifying language and supplementing material with non-verbal information. In school aged children, classroom based strategies addressing classroom environment, curriculum modification and adaptation of learning strategies may be of benefit. Reviews have emphasised the larger role that schools may need to assume in cooperation with social, medical and rehabilitation agencies to benefit at-risk children (G. H. Smith, 1993).

7.8. IMPLICATIONS FOR RESEARCH

The findings of this research also raise some interesting considerations for other research models. The animal model of autism, the theory of vulnerability and our understanding of the brain regions involved in memory processing are discussed.

7.8.1. Animal Model of Autism

VPA exposure has been associated with an increased risk of autistic traits (Bromley et al., 2008). Animal models of autism are based on fetal VPA exposure; however not all children exposed to VPA display autistic traits. Therefore, other factors must contribute to the development of autism following VPA exposure in children. Regardless, if the animal model of autism is accurate, then common findings between children exposed to VPA and children with autism would be expected.

There has been conflicting results regarding hippocampal size in autism. Some neuroimaging studies have found the hippocampus to be relatively smaller in the autistic brain (E. H. Aylward et al., 1999; Herbert et al., 2003). In contrast, other studies have found bilateral
enlargement of the hippocampi in children with autism (Sparks et al., 2002). Another study found that autism was associated with larger right hippocampal volumes relative to controls and larger left hippocampal volumes only in children with autism but without intellectual disability (Schumann et al., 2004). These findings seem consistent with the larger hippocampal volumes reported by Frisch (2009) in VPA-exposed rats. Unfortunately, the lack of a control sample in the current study prevents comment on the comparative size of the HCVs in children exposed to VPA. Despite this, the volumes observed seemed qualitatively consistent with volumes reported in the literature from typically developing children.

The findings of this study suggest that memory deficits in children exposed to VPA are limited to the verbal domain and may reflect poor encoding. No reduction in performance was found on an associative learning task or spatial working memory task. Individuals with autism typically demonstrate what are thought to be executive-based memory deficits (Mechelli et al., 2005). On neuropsychological testing, children with autism show poor memory for complex visual and verbal information and spatial working memory (D. L. Williams, Goldstein, & Minshew, 2006). Immediate recall, associative learning ability, verbal working memory, and recognition memory tend to remain intact (Forsberg et al., 2010; Mechelli et al., 2005; D. L. Williams et al., 2006). The finding that associative learning is preserved is interesting, as it suggests that the larger hippocampal volumes observed in neuroanatomical studies of autism are not detrimental to this learning process. A measure of spatial working memory discriminated most accurately between the autism and normal control groups (D. L. Williams et al., 2006). These differences in memory profile between children exposed to VPA and children diagnosed with autism suggest that additional
developmental processes must be disrupted to lead to the type of autism seen in VPA-exposed children. The mechanisms underlying this however, are unclear.

### 7.8.2. Theory of Vulnerability

The study outcomes are relevant to theories of recovery of function following injury in childhood. There are two general schools of thought; the theory of plasticity and the theory of vulnerability. The theory of plasticity suggests that the greatest potential for recovery occurs early on in development when cortical networks are being established (Ewing-Cobbs, Barnes, & Fletcher, 2003). Recovery is promoted by the reorganisation of neuronal connections around the damaged brain area. However, more recently it has been suggested that plasticity following injury may not necessarily be adaptive and might occur at a cost (i.e. the theory of vulnerability). Dennis (2000) argues that a younger age of onset is associated with a poorer outcome as early injury disrupts the acquisition of basic skills which provide necessary foundations for later development. There may be few observable difficulties in early stages of recovery, however impairments may emerge later in life. Impairments emerge due to underdevelopment of a skill, caused by cortical crowding or damage to the brain region required to support the skill. These children ‘grow into’ these deficits, with new impairments emerging as expected developmental milestones fail to be attained (VA Anderson et al., 2004). For example, studies have shown that executive functioning emerges as a later deficit in children with severe TBI (Ewing-Cobbs et al., 2003). Focal lesions such as congenital dysplasias are generally associated with better outcomes than diffuse lesions such as hypoxic injury (Leth, Toft, Herning, Peitersen, & Lou, 1997; Levin, 2003).
Children exposed to VPA presumably sustain a diffuse injury very early on in development. According to the plasticity theory, if brain regions are damaged by VPA exposure, plasticity processes will attempt to reorganise the neuronal connections. However, the vulnerability hypothesis dictates that this may disrupt normal cortical organisation. Regions which would normally support other cognitive functions later in life are taken over by the reorganised connections. Subsequently, basic cognitive skills may be poorly acquired and provide poor foundations for the development of later cognitive skills. In the case of the current study, it suggests that the reorganisation might in some way weaken the connections normally established in brain regions responsible for learning and memory. As a result, children exposed to VPA may struggle to acquire learning and memory skills, or deficits in this area may emerge later in childhood when these skills become more complex. There are also other cognitive domains which may be affected in this manner in children exposed not only to VPA, but other AEDs as well. It is even possible that these deficits may persist into early adulthood. Therefore, longitudinal studies are imperative to examine the functioning of AED-exposed children later in their development.

7.8.3. Brain Regions Involved in Memory Processes

It is also possible that the memory deficit observed in children exposed to VPA may not be related to dysfunction solely within the hippocampus. Animal lesion studies have showed that the perirhinal cortex also plays a critical role in associative memory (Buckley & Gaffan, 1998; Gaffan, 1994). It has been argued that the perirhinal cortex represents a node within an extensive network that mediates associative learning (Saling, 2009). Some authors have even gone as far to reject there are specifically localised memory processes within the
hippocampus and that the anatomical connections between the structures of the medial
temporal lobe are more important (Buckley & Gaffan, 1998). Given no major volumetric
changes or gross abnormalities were seen in the hippocampi of participating children, it
suggests their memory impairments may stem from dysfunction in other brain regions
important to these processes, for example the perirhinal cortex.

Theories of memory also implicate other regions of the medial temporal lobe such as the
fornix, mammillary bodies, parahippocampal gyrus and entorhinal cortex (Gaffan, 1994;
Kolb & Whishaw, 2008). The frontal lobes of the brain also contribute to memory
functioning, mediating the strategic and organisational aspects of encoding and retrieval
(Stuss & Levine, 2002). Functional MRI studies have also identified activation in the
prefrontal areas, anterior cingulate, parahippocampal areas, thalamus, parietal areas and
cerebellar regions during an associative learning task (Mottaghy et al., 1999). Therefore, it is
also possible that the poorer performance of VPA-exposed children on the memory measures
in this study may be undermined by dysfunction in multiple brain regions or across multiple
networks.

7.9. FUTURE DIRECTIONS

The studies of this thesis have yielded new insights into the impact of prenatal VPA exposure
on brain development; however many questions remain unanswered and there are still
opportunities to improve on past studies in this area. By fully understanding the outcomes for
children exposed to AEDs in utero and the mechanisms by which development is affected,
clinicians will be in a stronger position to assist women with epilepsy and their children.
While some areas for future research have been mentioned in the preceding sections of this chapter, this section will systematically discuss each area that deserves further attention.

### 7.9.1. Improving Study Design

Although this study is one of the larger studies to date examining memory outcomes, additional prospective studies with larger sample sizes to improve power are clearly necessary. The results need to be replicated to confirm the findings and control groups are required to provide an appropriate comparison.

Longitudinal studies are also required to investigate whether cognitive impairments persist into adolescence and adulthood. There is evidence to suggest that AED exposure can have effects on development that continue into adolescence. A recent study by Forsberg (2010) suggested that children exposed to AED polytherapy had poor academic achievement at the age of 16. The monotherapy group was not disadvantaged, but comprised mainly adolescents exposed to carbamazepine or phenytoin. These AEDs are generally considered to have less of an impact on cognitive outcomes and the effect of VPA on long term cognitive functioning needs to be established. If persisting deficits were detected it would have enormous implications for educational and occupational decision making and overall quality of life.

Studies generally rely on prescribed doses of AEDs to measure how much the fetus is exposed to the medication in utero. Despite this, pharmacokinetic studies have suggested that AEDs undergo complex metabolic interactions and the ingested dose may not be equivalent to the actual fetal exposure level. The only accurate way to measure exposure level is through the measurement of blood serum levels, which studies are yet to utilise. It would be beneficial
for pregnancy registers to collect blood serum data throughout the pregnancy of women with epilepsy, perhaps in combination with routine prenatal medical work-ups.

7.9.2. Better Characterisation of Outcomes

As discussed previously, there is a need to further characterise the cognitive outcomes of children exposed to VPA beyond the broad IQ measures reported in past research. While this study contributes to the emerging body of work that is attempting to disentangle specific neuropsychological deficits in this cohort, domains such as attention, working memory, speed of processing, visuoconstruction, language and executive functioning require further exploration. Assessment of adaptive functioning, social functioning and internalising and externalising behaviour may also reveal other areas of difficulty. Further, evaluation of autism traits and attention-deficit symptoms may also assist in delineating the areas of deficit in VPA-exposed children. Standardised, well validated tests with robust normative data should be used in conjunction with serial assessments across time to plot the developmental trajectory of impairments. This approach will enable researchers to differentiate the age at which various impairments emerge. By clearly establishing the pattern of impairments in these children, health professionals will be in a stronger position to implement strategies to minimise the impact on daily functioning. There is also a need to further research the impact of AED timing and dose so clinicians can be better informed about the prescription of AEDs during pregnancy and in an educated position to suggest alternative treatment regimes.
7.9.3. Effects of Polytherapy

Further research is required to differentiate the effects of VPA and polytherapy on cognitive development. This was not possible in this study due to the dose level split between the VPA monotherapy and polytherapy groups. There is growing support for the idea that polytherapy exposure may not place the fetus at higher risk of adverse outcome and it is VPA that is the deterministic factor. However this requires additional investigation. Different combinations of polytherapy also need to be investigated to identify those that may have a synergistic effect on teratogenesis.

In this study, recruitment and neuropsychological assessments of children were conducted by the same researcher. While blinded assessments prevented bias, it also prevented selective recruitment of different drug groups. As such, group sizes were not known until the cessation of the study. Blinded assessment is a strength to retain in future studies, however might be more beneficial to have recruitment and assessment completed by separate researchers. This would enable AED groups to be targeted for recruitment whilst reducing assessment bias.

7.9.4. Effects of Newer AEDs

Investigations into outcomes following fetal AED exposure have followed the historical evolution of AEDs. Early studies focused on the first AEDs, phenytoin and phenobarbital. Numerous studies examining the effects of VPA followed later, however carbamazepine which was released around the same time has been less intensively studied in comparison. Studies are beginning to emerge on lamotrigine, however clear conclusions on its developmental impact are yet to be drawn. Today, increasing numbers of women are being
prescribed newer AEDs for which no evidence exists. While they have been purported to be less teratogenic, there are no studies to support this. This is clearly an area of focus for further studies, however the increasing range of prescribed AEDs will probably pose a challenge to collecting large sample sizes, particularly of polytherapy regimes which may contain diverse combinations of drugs.

7.9.5. Impact of Risk Factors

This study controlled for pregnancy related risk factors which were hypothesised to have the greatest contribution to poor outcome, such as seizures and tobacco use. However, the sample size of this study was not large enough to examine all factors which have the potential to impact on development. There could be other risk factors which were not identified by this study that are detrimental to development. The characterisation of risk factors will assist in identifying children at higher risk of impairment and enable early intervention programs to be implemented.

Studies have indicated that pregnancy related risk factors play the largest role in child development at a younger age and a child’s environment and parenting style may be more important later on in life (Hirano et al., 2004). Children with prenatal exposure to AEDs have also been shown to be more vulnerable to environmental disadvantage (Titze et al., 2008). Therefore, further research should also investigate environmental factors such as attachment, parenting style and stress.

Intellectual ability is heritable (Free et al., 1995), which is why a measure of maternal IQ was collected to control for differences between groups. Obtaining a measure of paternal IQ
would also be beneficial but this information is often methodologically difficult to collect. Mothers may not be in contact with the father, may not wish for the father to be involved or the father may be unwilling to unable to attend. Despite this, future studies can improve on the assessment of IQ by obtaining measures from both parents.

Future studies might also evaluate whether family size contributes to cognitive outcomes. In Eriksson’s study (2005), there was a tendency for IQ to decrease as the number of children in a family increased. This suggests that some mothers might have difficulty coping with several children at once and as a result each child may not have received an equally stimulating environment. Postnatal variables associated with epilepsy should also be recorded. For example, children may be more at risk of injury at a young age if the mother suffers a seizure while holding the child. The psychosocial effects of a child being raised in an environment with an illness should also be explored.

### 7.9.6. Genetic Mechanisms

Additional animal studies are required to explore the potential genetic mechanisms underlying VPA teratogenicity and establish whether genetic variability also contributes to cognitive outcomes. With the advancement of technology, it may soon be possible to use genome sequencing techniques to generate genetic profiles of individuals. Polymorphisms in genes that encode proteins involved in the metabolic breakdown of VPA, drug transfer across the placenta or cortical development may contribute to impairments in cognition. Identifying polymorphisms which are associated with poorer outcomes in children may help identify
those more likely to have poor outcomes and act as a trigger for early implementation of intervention strategies.

7.9.7. Structural and Functional Brain Imaging

This was the first study to investigate neuroanatomical structure in children exposed to a single AED. The effect of fetal exposure to VPA on brain structure and function remains poorly understood and represents an area that can be easily pursued with current neuroimaging techniques. Whole-brain and regional volumetric analyses may assist to identify regions of the brain where subtle anatomical changes exist. The location of these abnormalities may help to explain some of the cognitive difficulties these children experience. For example, is the specific effect of VPA exposure on the development of language and verbal memory skills subserved by structural changes in the dominant perisylvian or perirhinal regions? There is a strong indication to pursue hippocampal volumetry further with the involvement of controls. There also may be differences in hippocampal morphology which could be investigated with spherical harmonic (SPHARM) analysis (Styner, Lieberman, Pantazis, & Gerig, 2004). There is also a need to investigate the effects of other AEDs on brain development. Future studies will require larger sample sizes than the current study and may benefit from the inclusion of lower functioning children, such as those diagnosed with fetal anticonvulsant syndromes, who may display more prominent neuroanatomical changes.

Diffusion tensor imaging (DTI), which maps neural tracts in the brain through measurement of directional water flow, may also be a useful technique to investigate this cohort with.
Studies indicate that measures of diffusivity derived from DTI in the hippocampus can predict memory function (Blatter et al., 1995). Functional imaging studies assessing brain activation may also yield interesting results in exposed children. It may not be a structural abnormality than underlies cognitive impairment, but altered activation in neural networks. Prenatal nicotine exposure has been associated with activation changes in the medial temporal lobe during memory tasks (Jacobsen et al., 2005). Whether a similar phenomenon might exist in children exposed to VPA remains to be established.

7.9.8. Relationship with Autism

Further research is needed to identify the factors that place children at higher risk of developing autism following VPA exposure. While VPA exposure acts in a deterministic manner to induce autistic features in the animal model, the relationship is much more complex in humans. Other factors, which may include genetic factors, must also contribute to the phenotype and need to be identified. There should be studies that compare children who develop autism following VPA exposure to children with autism who were not exposed to AEDs to determine if there are common underlying pathologies; for example, similar alterations in brain structure. Finally, more rigorous assessment of autistic features is required to determine if the autism seen following VPA exposure represents a similar or unique subtype relative to autism within the general population.
7.9.9. Intervention Strategies

Intervention studies are required to determine which strategies optimise cognitive outcomes in AED exposed children, similar to trials that have been conducted in samples of children with fetal alcohol syndrome and low birth weight (Adnams et al., 2007; The Infant Health and Development Program, 1990). Effective intervention programs are likely to involve multi-factorial care with input from medical, psychological and school based support. Programs should begin early and continue throughout development with periodical assessment of functioning to monitor progress.

Education is also an important aspect of prevention strategies and it is unknown the extent to which women with epilepsy understand the risks of AED use during pregnancy. Women who have a good understanding of the risks might be less likely to engage in behaviour which could further jeopardise their child’s development, such as alcohol or marijuana use. Studies are required to establish the level of understanding within this cohort of women and determine the most effective teaching strategies to communicate this information to them.

7.9.10. Other Conditions

Finally, future research needs to examine whether the children of women taking VPA for other reasons are subject to the same risks. Women prescribed VPA for other disorders need to be included in future studies of cognitive outcomes to establish whether the findings in this thesis are generalisable on a broader scale. It may be of benefit to expand recruitment through pregnancy registers to include women taking AEDs for other reasons.
7.10. CONCLUSION

The findings of this research highlight the vulnerability of the developing fetus to agents in the intrauterine environment. Fetal AED exposure is an unavoidable complication of pregnancy for most women with epilepsy. While the majority of their children are born normal, the consequences for those affected by AED exposure have the potential to be long-standing and high impact. Subsequently, there is a strong motivation to understand the difficulties these children face and needs in order to provide them with appropriate care and services.

The studies within this thesis have succeeded in contributing to the growing body of literature informing outcomes of AED exposure in utero. Specifically it has yielded additional knowledge about the effect of VPA on the brain systems important for memory function. It has been established that VPA impacts on intellectual development, however this research demonstrates that VPA has the potential to impact memory and learning skills as well. Verbal memory skills seem specifically impaired, whereas non-verbal memory skills are preserved. The mechanisms underlying this dissociation are still unclear. As expected, higher doses of VPA appeared to be more detrimental to development, which has obvious implications for pregnancy planning in women with epilepsy of child bearing age.

There are indicators throughout the literature that development of the mesial temporal lobe is affected by prenatal VPA exposure. This was the first study to specifically examine correlates of hippocampal functioning in children exposed to VPA. Children exposed to VPA were more susceptible to retroactive interference, an indicator of hippocampal dysfunction. VPA
dose in monotherapy was not correlated with hippocampal size in the group of children in this study, but there was a possible lack of typical asymmetry of the structure. This was unable to be confirmed without involvement of a control group. The lack of asymmetry may be one mechanism which underlies the specific verbal memory dysfunction in this sample. Larger sample sizes in future studies will be critical in confirming the current findings and identifying other regions of neuroanatomical difference in children exposed to AEDs.

The overarching aim of research in this field is to enable health professionals to improve clinical management and quality of life for both mother and child. By characterising the outcomes of children exposed to AEDs and identifying the risk factors which contribute to poor outcome, clinicians will be in a stronger position to identify at-risk children. This information will also inform intervention strategies that are implemented for their children. Further, empowerment of women with epilepsy with knowledge about potential outcomes may help alleviate some of their concerns.

The findings of this thesis do raise some concerns about the current use of VPA, not only in the treatment of epilepsy but also other medical conditions. However, VPA is highly effective in epilepsy and its avoidance by women who rely on it for seizure control has the potential for even more devastating consequences. It is hoped that with the continued emergence of newer AEDs, alternative medications with high efficacy and fewer risks will be identified. Continued investigation into cognitive outcomes following AED exposure will be important to understand the effects of these medications on development.
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## Appendix 1.

Achieved Power for Statistical Analyses (α = .05; two-tailed)

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<td></td>
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<tr>
<td>Correlations</td>
<td></td>
<td>(</td>
<td>r</td>
<td>=.50)</td>
</tr>
<tr>
<td>Monotherapy VPA</td>
<td>(N=26)</td>
<td>(\beta = 0.81)</td>
<td>(\beta = 0.34)</td>
<td>(\beta = 0.08)</td>
</tr>
<tr>
<td>Regression</td>
<td></td>
<td>(f^2=0.35)</td>
<td>(f^2=0.15)</td>
<td>(f^2=0.02)</td>
</tr>
<tr>
<td>All VPA; 7 predictors</td>
<td>(N=41)</td>
<td>(\beta = 0.95)</td>
<td>(\beta = 0.67)</td>
<td>(\beta = 0.14)</td>
</tr>
</tbody>
</table>
### Analysis of CANTAB Variables (i.e. SWM and PAL tasks)

<table>
<thead>
<tr>
<th>One sample $t$-tests</th>
<th>$d=0.8$</th>
<th>$d=0.5$</th>
<th>$d=0.2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPA Monotherapy</td>
<td>$N=14$</td>
<td>$\beta = 0.79$</td>
<td>$\beta = 0.41$</td>
</tr>
</tbody>
</table>

### Analysis of Imaging Variables

| Correlations | $|r|=0.50$ | $|r|=0.30$ | $|r|=0.10$ |
|--------------|-----------|-----------|-----------|
| Monotherapy VPA | $N=14$ | $\beta = 0.65$ | $\beta = 0.30$ | $\beta = 0.10$ |

**Hypothetical $t$-tests**

1. If controls had been able to be recruited