Hippocampal Functioning in Adolescents with Congenital Hypothyroidism

by

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Abstract

Congenital hypothyroidism (CH) is a pediatric endocrine disorder caused by an insufficiency of thyroid hormone. Despite treatment following newborn screening, CH is associated with persisting memory weaknesses. Given animal research has shown thyroid hormone plays a crucial role in the development of the hippocampus, a brain structure required for normal declarative memory, it is possible that altered hippocampally-dependent processes underlie the memory weakness associated with CH. Previous studies of individuals with CH have found reduced memory abilities and left hippocampal volumes but no study has thoroughly assessed memory abilities or how the hippocampus functions to support memory. Thus, the present study compared individuals with CH and typically developing adolescents using clinical memory tests and two associative memory tasks shown in adults to activate the hippocampus during functional magnetic resonance imaging (fMRI). Results indicated groups did not differ in memory accuracy on clinical measures or either fMRI task. However, fMRI revealed hippocampal activation differed between the groups when performing the associative memory tasks. The first
task utilized a visuospatial paired associates novelty detection paradigm to show the CH group increased activation relative to controls in left hippocampus and recruited right hippocampus when controls did not. Since previous research suggested the left hippocampus and verbal memory were more vulnerable to the effects of CH, the second task utilized a verbal paired associates paradigm to demonstrate that when making old and new judgments about associations versus items, the CH group increased activation relative to controls in left hippocampus. Further investigation revealed that when recognizing old associations versus items, the CH group had increased bilateral posterior hippocampal activation whereas controls showed increased activation in right anterior hippocampus, a distinction noted in previous research with this paradigm which suggests individuals with CH may retrieve associations in a less flexible manner than controls. In addition, worse memory performance and increased hippocampal activation, particularly on the left, was predicted by severity of hypothyroidism experienced early in life. In conclusion, these studies demonstrate that early thyroid hormone insufficiency associated with CH alters the functioning of the hippocampus and engenders use of compensatory mechanisms to support associative memory functions.
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List of Abbreviations

C=Control task
CA=Corne Ammonis
CANTAB=Cambridge Automated Neuropsychological Battery
CH=Congenital Hypothyroidism
CMS=Children’s Memory Scale
DG=Dentate Gyrus
EPSP=Excitatory Post-Synaptic Potential
fMRI=Functional Magnetic Resonance Imaging
IP=Intact Pairs
IQ=Intelligence Quotient
LTP=Long-Term Potentiation
MARINA-Masks for Region of Interest Analysis
MTL=Medial Temporal Lobe
NEPSY=A Developmental NEuroPSYchological Assessment
NI=New Items
NP=New Pairs
ONI=One Old and One New Item
RI=Rearranged Items
ROCF=Rey-Osterrieth Complex Figure
RP=Rearranged Pairs
SPM=Statistical Parametric Mapping
SPSS=Statistical Package for the Social Sciences
SS=Scaled Score
T3=Triiodothyronine
T4=Thyroxine
TD=Typically Developing
TH=Thyroid Hormone
TOMAL=Test of Memory and Learning
TSH=Thyroid Stimulating Hormone
WASI=Wechsler Abbreviated Scale of Intelligence
WRAML=Wide Range of Assessment of Learning and Memory
Chapter 1
Introduction

Congenital hypothyroidism (CH) is a pediatric endocrine disorder caused by a lack of endogenously produced thyroid hormone (TH). TH is involved in controlling brain development and is especially important for the development of the hippocampus (Gilbert & Sui, 2006; Zoeller & Rovet, 2004), a brain structure required for normal declarative memory abilities (Eichenbaum, 2001). Despite newborn screening and treatment early in life, previous studies have shown that CH is associated with hippocampal volume reductions and memory weaknesses (Rovet, 1999; Rovet, 2002; Wheeler & Rovet, 2007; Wheeler, Willoughby, McAndrews, & Rovet, 2011). Although the role of the hippocampus in memory has been extensively studied in adults, much less is known about how the hippocampus functions following a disruption early in development. Since the development of the brain is quite protracted, early insult to the brain can result in different functional consequences than when damage occurs in adulthood (de Haan, Mishkin, Baldeweg, & Vargha-Khadem, 2006; Dennis, 2000; Hessen, Nestvold, & Anderson, 2007). Therefore, investigating the consequences of early damage to the hippocampus can provide novel information about hippocampal function and the ability of the structure to adapt to early challenges.

Previous studies of individuals with CH have found that areas of memory particularly affected are their verbal-associative recall and spatial learning abilities (Rovet, 1999; Rovet, 2002), which are known to rely on the hippocampus. In contrast, their working memory, which involves primarily extra-hippocampal structures (Eichenbaum, 2001; Mayes, Montaldi, & Migo, 2007; Milner, Corkin, & Teuber, 1968; Vargha-Khadem, Gadian, & Watkins, 1997) is preserved (Hepworth, Pang, & Rovet, 2006; Rovet, 1999). However, the scope of previous studies of
individuals with CH has been relatively limited and how the hippocampus functions to support memory has not been explored. Thus, the present studies were designed to 1) utilize neuropsychological memory tests to characterize and quantify the memory weaknesses in CH, 2) use functional magnetic resonance imaging (fMRI) to determine how the hippocampus functions during memory tasks previously shown to selectively engage the hippocampus in healthy adults and 3) determine if memory performance or hippocampal functioning is predicted by the severity of CH experienced by each individual. Through these studies, the impact of CH and lack of TH early in development on the subsequent functioning of the hippocampus is explored.

In order to provide context for the present studies, I first provide background information about the hippocampus and memory to provide a basis for understanding the current theories about the role and functioning of the hippocampus in memory processes. Next, I give a brief overview on the condition of CH and how TH, or lack thereof, affects brain development and the hippocampus in particular. Finally, I review and critique previously published studies concerning the memory abilities of children and adults with CH as a lead into the research questions to be addressed in this dissertation.

The research described herein is comprised of three studies presented in journal manuscript format. Briefly, results of the first study indicated that clinical memory measures revealed no significant differences between the CH and typically developing (TD) groups in any domain tested (Chapter 2). The second study investigated hippocampal activation during a visuospatial paired associates task and found that successful performance of the task was related to increased hippocampal activation in CH relative to controls (Chapter 3). Since previous studies of CH have shown that the left hippocampus and verbal memory may be more vulnerable to a lack of TH early in life (Wheeler et al., 2011), the third study investigated hippocampal activation...
during a verbal paired associates task and found that hippocampal activation differed in CH relative to controls (Chapter 4). In addition, these studies demonstrated that longer duration and increased severity of CH was associated with weaker memory abilities and altered hippocampal activation signifying the importance of early diagnosis and treatment. Together the results of these three studies indicate that early developmental disruption as a result of CH leads to subsequently altered hippocampal functioning.

1.1 The Hippocampus and Memory

1.1.1 The Role of the Hippocampus

The hippocampus is a seahorse-shaped structure located deep within the medial temporal lobes (MTL) of the brain, which is within the inferomedial aspect of each hemisphere (Duvernoy, 2005). The link between the hippocampus and memory has gained widespread acceptance over the years following Scoville and Milner's (1957) famous case of patient H.M. who presented with profound amnesia after bilateral removal of his MTLs including the hippocampus (Scoville & Milner, 1957). More recently, research in the field of memory research has focused on elucidating the role of the hippocampus and other MTL structures in different types of memory functions. Both patients who sustained hippocampal damage and fMRI studies of intact adults have demonstrated that the MTLs are crucial for declarative memory, which is memory that can be consciously recalled such as facts and events (Eichenbaum, 2001; Mayes et al., 2007; Milner et al., 1968; Milner, 2005; Montaldi & Mayes, 2010; Squire, Stark, & Clark, 2004; Vargha-Khadem et al., 1997). Within the declarative memory domain, researchers have explored the functional heterogeneity within the MTLs in terms of their relative contributions to different types of memory processes.
In particular, the division of labour within the MTLs has been extensively studied in recognition memory. The current major theoretical accounts propose that recognizing previously encountered stimuli can rely on two distinct processes: recollection and familiarity. Recollection is accompanied by knowledge of associated contextual information such as where or when a stimulus was presented. In contrast, knowledge of familiarity with an item is not accompanied by any contextual information (Eichenbaum, Yonelinas, & Ranganath, 2007; Yonelinas, 2002). Recollection occurs similarly to free recall as an ‘all-or-none’ phenomena in which the initial encoding event is re-experienced whereas familiarity occurs in a more continuous fashion and recognition is based on strength of the memory signal (Yonelinas, 2002). Additionally, behavioural research has provided evidence that recollection and familiarity are dissociable brain processes. For example, recollection has been shown to be a slower process than familiarity, analyses of confidence ratings in recollection and familiarity produce different receiver operating characteristics (ROCs), distinct electrophysiological correlates are associated with each, and recollection and familiarity are differentially affected by encoding and retrieval manipulations (Yonelinas, 2002). Thus there is evidence to suggest that recollection and familiarity are functionally dissociable processes.

Since these two distinct processes are thought to underlie recognition, researchers have investigated if the neural substrates of these processes differ. The most widely accepted theories propose that the hippocampus supports recollection but not familiarity and the parahippocampal cortex contributes to recollection by providing contextual information. In contrast, the perirhinal cortex is necessary for familiarity (Bowles et al., 2010; Eichenbaum et al., 2007; Mayes et al., 2007; Yonelinas, 2002). It has been proposed that during encoding, information about individual stimuli is processed by the perirhinal cortex and contextual information by the parahippocampal cortex (Eichenbaum et al., 2007). Information from both structures converges in the
hippocampus where the associations between items and their context are bound into non-overlapping representations of similar inputs (i.e. pattern separation) (Diana, Yonelinas, & Ranganath, 2007; Eichenbaum et al., 2007; Montaldi & Mayes, 2010). Then, when a previously encountered stimulus is presented, familiarity with the item is signaled by suppression of activity within the perirhinal cortex. This familiarity signal may also trigger recovery of associated information in the hippocampus (i.e. pattern completion processes) and reactivation of contextual associations within the parahippocampal cortex thus eliciting recollection (Eichenbaum et al., 2007).

Patients with lesions to MTL structures have provided evidence of a double dissociation between recollection and familiarity (Bowles et al., 2010). Those with damage to the hippocampus show impaired recall and recollection whereas item familiarity remains intact (Bowles et al., 2010; Mayes et al., 2004; Mayes et al., 2007; Quamme, Yonelinas, & Norman, 2007; Vargha-Khadem et al., 1997). Conversely, one patient NB who underwent a resection that included perirhinal cortex but left the hippocampus intact, had impaired familiarity but preserved recollection (Bowles et al., 2007). Thus, results from lesion studies support the dissociation between recollection and familiarity and the specificity of MTL structures to each of these processes.

In order to investigate the distinction between recollection and familiarity in the healthy hippocampus, fMRI paradigms contrasting associative versus item recognition memory have been utilized (Yonelinas, 2002). For example, in an fMRI study using word pairs, adult participants showed greater hippocampal activation when recollecting whether words had been previously paired together than when they responded on the basis of familiarity with words, irrespective of pairings (Giovanello, Schnyer, & Verfaellie, 2004). A similar pattern of hippocampal selectivity for recollection of associations rather than familiarity with items was
demonstrated by Kohler et al. (2005). This study reported activation in the hippocampus for novel spatial configurations and novel object pairings and activation in other MTL areas for novel items (Kohler, Danckert, Gati, & Menon, 2005). Thus in both examples, the division of labour within the MTL was observed with a preferential and selective role for the hippocampus in associative memory.

Taken together, the extant literature provides extensive evidence that the MTL structures are heterogeneous in their support of recollection and familiarity and thus for associative and item information. Specifically, the hippocampus is preferentially involved in recollecting the associative components of episodes and the neocortical MTL structures are involved with item familiarity.

A contrasting view has been proposed that suggests the hippocampus is equally involved in all types of declarative memory rather than being more engaged by either associations or individual items or by recollection or familiarity (Squire, Wixted, & Clark, 2007). Specifically, the proponents of this view claim that the greater hippocampal activation observed for recollection over familiarity-based memory is related to the strength of the memory, with the hippocampus more engaged by stronger memories, as opposed to a division of labour between MTL structures (Kirwan, Wixted, & Squire, 2008; Squire et al., 2004; Squire et al., 2007). Although this view has been challenged by recent research demonstrating that hippocampal engagement at retrieval is related to recollection not strength of familiarity (Cohn, Moscovitch, Lahat, & McAndrews, 2009), some researchers maintain that it is the methodology used in many studies that produces strength of memory confounds and creates the apparent division of labour within the MTLs observed in other studies (Wixted, Mickes, & Squire, 2010). Thus, while the division of labour within the MTLs remains an open area of research and there is continued debate about what
mediates hippocampal involvement in memory processes, there is a consensus that the hippocampus is involved with recollective processes.

1.1.2 Development of Memory Abilities

The development of memory abilities has been proposed to reflect the increasing functional distinction of structures within the MTLs (de Haan et al., 2006) as the abilities underlying semantic and familiarity-based memory develop earlier than the recollective abilities underlying episodic memory that rely on the hippocampus. Evidence suggests that within the first months of life, the MTLs support novelty preferences/familiarity-based recognition and by 1 year of age begin to support recall. At 3-4 years of age, relational memory abilities begin to emerge (de Haan et al., 2006; Richmond & Nelson, 2007) and there is further improvement in episodic memory abilities paralleling improvement in the ability to bind and recall relational information between the ages of 4 and 6 years (Sluzenski, Newcombe, & Kovacs, 2006). Associative memory improves between the ages of 6 and 10 years whereas the flexible use of associative memory improves between 8 and 10 years of age (Townsend, Richmond, Vogel-Farley, & Thomas, 2010). Source memory also shows gains until at least the age of 10 years (Richmond & Nelson, 2007). Also, recollection following elaborative encoding improves from childhood to adolescence whereas familiarity does not show similar gains (Ghetti & Angelini, 2008). The improvement in recollective and episodic memory abilities through later childhood and adolescence parallels development of the hippocampus (de Haan et al., 2006; Richmond & Nelson, 2007) and its connections with other brain structures, particularly as myelination increases substantially after birth and throughout adolescence (Arnold & Trojanowski, 1996; Seress, Ábrahám, Tornóczky, & Kosztolányi, 2001).
Although a plethora of fMRI studies exists with adults, much less is known about hippocampal functioning before the brain fully matures into its adult form as the imaging data with children and adolescents are limited to few studies. One developmental study of the episodic memory network showed an age-related change in fMRI activation during encoding (Chiu, Schmithorst, Brown, Holland, & Dunn, 2006). Specifically, prior to age 10, performance on a subsequent memory test was predicted by activity within the left posterior MTL, whereas older children also activated left anterior MTL, including the hippocampus, and left prefrontal cortex. These results suggest that beginning at age 10, children engage a similar network as adults during the encoding of episodic memory (Chiu et al., 2006). A second developmental study found that between the ages of 8 and 24 years, the changes in brain activity that occurred within the episodic memory network corresponded with increased recollection accuracy. Specifically, while activation in both hippocampal and prefrontal areas predicted subsequent memory for studied scenes, activity within prefrontal areas, not MTLs, was correlated with age (Ofen, Ka, Sokol-Hessner, Kiml, & Whitfield-Gabrieli, 2007). However, a more recent study by Ghetti, DeMaster, Yonelinas, & Bunge (2010) used a subsequent memory paradigm to investigate activation during incidental encoding of associative information and found differences within the MTLs across the same age range (Ghetti et al., 2010). In adults and 14 year olds, subsequent recollection of associative information was associated with activation of the hippocampus and posterior parahippocampal gyrus. Although these same regions were also activated in 8 year olds, they were involved with both subsequently remembered associative and item information. Interestingly, although 10 and 11 year olds activated these regions as well, their pattern of activation was less reliable than that of either the older or younger children (Ghetti et al., 2010). Thus, results from this study suggest that between the ages of 8 and 14 years, the hippocampus becomes increasingly specialized for the processing of associative information that will be later recollected.
In sum, both Chiu et al. (2006) and Ghetti et al. (2010) found changes in recruitment of the hippocampus between the ages of 8 and 14 years of age. Although Ofen et al. (2007) had dissimilar results, this study did not look specifically at the same time period but rather looked at trends across all participants in a broader age range (8-24 years). As such, it is difficult to determine whether or not the same effect would be observed within this age range in the study. In addition, different paradigms and participant instructions were used across the studies and thus it is possible that differences in encoding or retrieval conditions may have impacted the results observed. Another consideration is that these three studies only assessed activation during encoding and, as such, it is not known if there are differences across development at retrieval. Clearly additional research is needed in order to determine how the hippocampus and other MTL structures support different memory processes throughout development.

1.1.3 Hippocampal Functioning Following Early Damage

Relative to other brain structures, the hippocampus is particularly vulnerable to damage during the perinatal period and childhood (Curtis, Zhuang, Townsend, Hu, & Nelson, 2006) and the functional consequences of brain damage can differ depending on the age and developmental stage at which the insult occurs (de Haan et al., 2006; Dennis, 2000; Hessen et al., 2007). For example the patient Jon, who suffered hippocampal damage from hypoxic-ischemic attacks shortly after birth and then a febrile convulsion around the age of four years, had similar memory difficulties in adulthood as other adults with hippocampal damage (e.g. episodic memory difficulties in the presence of preserved semantic abilities). However, activity within the hippocampus differed from patients with adult onset temporal lobe epilepsy during memory retrieval (Addis, Moscovitch, & McAndrews, 2007; Maguire, Vargha-Khadem, & Mishkin, 2001).
Studies addressing the lateralization of hippocampal function have also revealed differences between adults and children. Traditionally in patients with hippocampal damage, damage to the left hippocampus has been associated with verbal memory deficits (Frisk & Milner, 1990; Milner, 1968a) and the right with visuospatial (Milner, 1968b; Smith & Milner, 1981). For example, adults with temporal lobe epilepsy have demonstrated differential material specific deficits in remembering, with right temporal lobe epilepsy patients showing a deficit for face photos and left temporal lobe epilepsy patients showing a deficit for words (Moscovitch & McAndrews, 2002). Of note, meta-analyses of studies of patients with epilepsy have revealed that the overall effect is stronger and more consistent for verbal memory deficits following left resections than for visual memory and resections on the right (Lee, Yip, & Jones-Gotman, 2002). In contrast, children with epilepsy may not have similar lateralization of memory abilities (Smith, 2010). One study showed children with right-sided temporal lobe epilepsy demonstrate a deficit in face recognition however, children with a left-sided focus did not differ from controls on a verbal associative memory test (Gonzalez, Anderson, Wood, Mitchell, & Harvey, 2007). Similarly, learning spatial information was more difficult for children with right-sided foci than for children with left sided foci or controls, in contrast to learning verbal information for which no group had more difficulty acquiring the task than controls (Hepworth & Smith, 2002). Another study indicated no differences between children with left and right sided foci or site of seizure (i.e. frontal or temporal) for either face recognition or word recall (Smith, 2010). Therefore, the strong pattern of lateralization observed for verbal material to the left hippocampus observed in adults appears to be disrupted in children with epilepsy.

In addition, the long-term outcome of hippocampal damage to memory abilities may differ between children and adults with epilepsy. In one study by Gleissner, Sassen, Schramm, Elger, & Helmstaedter (2005), children who received left hippocampal resections recovered verbal
learning to pre-operative baseline at one-year follow-up whereas adults did not. Children with right hippocampal resections had improvement in the visual memory in the year following surgery whereas it deteriorated in the adults (Gleissner et al., 2005). Overall, these results suggest that the link between brain and behaviour may differ if the disruption of the hippocampus occurs at different times throughout the lifespan.

Nevertheless, only a few studies to date have used fMRI to identify the functional consequences of damage to the hippocampus early in life on its functioning during childhood and adolescence and these studies have reported inconsistent findings (e.g. Chiu, 2009; Curtis et al., 2006; Gimenez et al., 2005; Maguire et al., 2001; Maheu, 2008; Sowell et al., 2007). For example, children prenatally exposed to alcohol did not activate their hippocampus on verbal paired associates tasks (Sowell et al., 2007) whereas children with mild traumatic brain injury activated their right hippocampus to a lesser degree than controls on a similar task (Chiu, 2009). Studies of adolescents born preterm show mixed results reflecting no difference from controls in hippocampal activation during delayed match and nonmatch to sample tasks (Curtis et al., 2006) but increased activation of the right hippocampus relative to controls during a face-name encoding task (Gimenez et al., 2005). Similarly, adolescents with Cushing syndrome showed greater right anterior hippocampal activation than controls during successful face encoding (Maheu, 2008). Together, this relatively sparse literature supports the hypothesis that early hippocampal insult impacts its functioning subsequently; however the nature of these changes is unclear given the heterogeneity of tasks and types of damage.

Based on the current neuroimaging literature, no consensus exists about the direction of change with studies demonstrating both more and less hippocampal activation relative to controls. From the current research, it is not clear if the critical factor(s) mediating these differences between
studies are the nature of the task, cause of the hippocampal damage, age at test or insult, or the performance accuracy and memory abilities of the population studied. In addition, the lack of consistent methodologies may have influenced results and rendered comparison between studies specious. Clearly, more research on memory-impaired populations using well designed and executed hippocampally-sensitive memory tasks is needed in order to fully understand the consequences of hippocampal damage and the link between structure, function, and behaviour.

A further outstanding issue concerns the fact that since the underlying etiology of hippocampal abnormality differs between an acquired injury and a disrupted developmental process, there may be different consequences and implications for the structure and network. In the case of an acquired injury, damage occurs as a result of an external influence to an initially normally developing structure and consequent reorganization of an originally normal brain and network can occur. In the case of a disrupted developmental process, the hippocampus undergoes suboptimal development from the beginning and the original organization of the brain and network is abnormal. Although understanding the consequences of an atypically developing hippocampus will further elucidate how the brain organizes rather than reorganizes itself, all fMRI studies of the hippocampus in children and adolescents to date have involved only cases with damage or injury to the hippocampus acquired from environmental factors (Chiu, 2009; Curtis et al., 2006; Gimenez et al., 2005; Maguire et al., 2001; Sowell et al., 2007) or the result of an ongoing condition (Maheu, 2008). Thus, the consequence of an endogenous but relatively circumscribed disruption to the development of the hippocampus and how it impacts the subsequent functioning of the hippocampus remain unknown.
1.2 Congenital Hypothyroidism

1.2.1 Description of the Condition

Congenital hypothyroidism (CH) is a pediatric endocrine disorder that results from insufficient endogenous production of thyroid hormone (TH) at the level of the thyroid (primary CH) or as a result of insufficient production of factors that stimulate TH production. Recent estimates indicate CH affects approximately 1 in 2500 newborns in the United States (Harris & Pass, 2007), a rate that exceeds previous estimates of about 1 in 4000 newborns (LaFranchi, 1999; LaFranchi & Austin, 2007).

CH can be caused by a problem at the level of the thyroid gland or the central nervous system. CH of thyroidal origin is not a homogeneous disease as different genetic defects can produce a primary CH condition. In 15% of cases, CH is caused by an error in a mechanism required for TH synthesis in an intact thyroid gland and is thus referred to as dyshormonogenesis. This etiology typically follows a recessive pattern of inheritance and is often familial (Kopp, 2002; Rovet & Brown, 2007). The remaining 85% of thyroidal cases are thought to be sporadic and arise from thyroid dysgenesis due to a defect in gland organogenesis. Thyroid dysgenesis includes a total absence of the thyroid gland, termed athyreosis, or a gland located in an unusual position, called thyroid ectopy. The latter is caused by failure of the thyroid primordium to descend along the normal pathway; consequently, the vestigial thyroid is typically found in the dorsum of the tongue and is also termed a lingual thyroid. An ectopic thyroid usually has some initial function but this eventually stops sometime during early infancy (De Felice & Di Lauro, 2004). In a small number of cases, CH can also arise from thyroid hypoplasia or a hemithyroid (Rovet & Daneman, 2003). Central forms of CH are relatively rare and affect only about 1 in 25,000 to 100,000 neonates. These etiologies, termed secondary and tertiary CH, typically result
from problems at the level of the pituitary or the hypothalamus respectively (LaFranchi, 1999; Rovet & Brown, 2007). Since the thyroid gland, TH synthesis and regulatory mechanisms of the fetus begin to develop in utero, TH insufficiency as a result of CH can begin during gestation (LaFranchi & Austin, 2007; Rovet & Daneman, 2003).

During fetal development, maternal transfer of thyroxine (T4) may partially compensate for a lack of TH prior to birth (LaFranchi & Austin, 2007; Vulsma, Gons, & De Vijlder, 1989). However, this transfer is usually not sufficient to fully compensate for all effects of gestational hypothyroidism (Sack, Kaiserman, & Siebner, 1993), particularly in those children with missing glands. For children with a dysfunctional or ectopic gland, the TH deficiency is usually not as severe and begins after birth since their thyroid gland may function minimally during gestation and after birth. Regardless, in all cases, the hypothyroid period continues until postnatal hormone replacement therapy normalizes TH levels (Rovet & Daneman, 2003).

If CH is left untreated or inadequately managed, the resulting impairments are severe (Mendorla et al., 1988; Rovet & Daneman, 2003). In the past, a condition known as cretinism, which involved extensive brain damage and mental retardation, resulted from CH (Berenbaum, 1999; Eayrs, 1959; LaFranchi & Austin, 2007; Rovet, 2002). Cretinism arose because treatment with TH was typically delayed since the outward symptoms of CH can take months to appear. Consequently, a clinical diagnosis occurred well past the critical period to prevent irreversible severe brain damage. However, since the advent of newborn screening for CH, infants can now be identified with CH soon after birth and, if treated shortly thereafter, their physical and mental development are minimally affected (Derksen-Lubsen & Verkerk, 1996; Dubuis et al., 1996; Dussault, 1999; Rovet, 2002). In the few cases with late or inadequately treated CH, mental
retardation, cognitive deficits, and an increased risk for psychopathology and problematic social
skills can result (Mendorla et al., 1988; Rovet & Brown, 2007).

Despite improved outcome following newborn screening and early and adequate treatment
(Berenbaum, 1999; Morreale de Escobar, Obregon, & Escobar del Rey, 1987), the brief period of
hypothyroidism that children with early-treated CH experience is not without impact. Studies of
these children have revealed weaker neurodevelopmental skills relative to normally developing
peers (Dugbartey, 1998; Rovet, 1999). Indeed, as early as age two weaknesses are evident and
include reduced perceptual-motor, visuospatial, and language skills (Rovet, Ehrlich, & Sorbara,
1987). Although the children with CH identified by screening obtain intelligence quotients (IQ)
scores within the normal range (Salerno et al., 1999), their scores are typically about 6 points
lower than controls and their low scores reflect initial CH severity (Derksen-Lubsen & Verkerk,
1996; Rovet, 2005; Rovet, 2005; Tillotson, Fuggle, Smith, Ades, & Grant, 1994). Attention
(Kooistra, van der Meere, & Kalverboer, 1996), perceptual sensitivity, and memory abilities are
also particular areas of weakness in this population (Rovet & Hepworth, 2001b). School
performance is affected in children with CH, especially in the area of arithmetic (Rovet &
Ehrlich, 2000). Mild hearing loss occurs in approximately 20% of CH children and may be
associated with a delayed ability to learn to read (Debruyne, Vanderschueren-Lodeweyckx, &
Bastijns, 1983; Rovet, Walker, Bliss, Buchanan, & Ehrlich, 1996). In addition, children with CH
show weaker colour vision abilities than controls, particularly those that rely on blue-yellow
processing streams, and this may affect their ability to perform cognitive tasks that rely on colour
as an important feature (Borkowski, Westall, & Rovet, 2005).

Among adolescents with CH, intellectual functioning remains within the normal range, but they
fail to match the intellectual gains that their peers make and thus they tend to fall progressively
behind their peers with age (Rovet, 1999). Adolescents with CH continue to show relatively poorer performance in language, fine motor skills, and some aspects of attention and memory (Rovet, 1999). In addition, weaknesses are observed in visuospatial processing on tasks that rely on the dorsal route or ‘where’ pathway but not on the ventral route or ‘what’ pathway (Leneman, Buchanan, & Rovet, 2001). As adults, individuals with CH continue to perform below sibling controls on indices of intellectual, motor, and school-associated functioning (Oerbeck, Sundet, Kase, & Heyerdahl, 2003). Furthermore, adults with CH are less likely to have completed high school than their siblings without CH (Oerbeck et al., 2003). Adults with CH are also more likely to have lower health-related quality of life and report more difficulties with cognitive functioning, sleeping, pain, daily activities, vitality, aggressiveness, and depressive moods compared with healthy adults (van der Sluijs Veer, Kempers, Last, Vulsma, & Grootenhuis, 2008). In addition, adults with CH reported lower self-esteem, delayed social development, and poorer sociodemographical outcomes and self-esteem relative to the general population (van der Sluijs Veer et al., 2008).

Because different neuroanatomic structures require TH at different times during development, the precise time and severity of the TH deficit have different influences on the brain (Bernal & Nunez, 1995) and thus treatment factors can affect the nature and severity of the effects of CH (Rovet & Erlich, 1995; Salerno et al., 1999; Song, Daneman, & Rovet, 2001). Generally, early treatment and high doses of TH are associated with better outcomes for intellectual ability (Rovet & Erlich, 1995) and psychomotor development (Bongers-Schokking, Koot, Wiersma, Verkerk, & de Muinck Keizer-Schrama, 2000). Mental development is also affected by later serum levels of T4 such that higher serum T4 levels during the first two years of life are related to better intellectual outcome at ages two and six (Heyerdahl, Kase, & Lie, 1991). Studies have also found that earlier normalization of thyroid stimulating hormone (TSH) is associated with better
outcome in memory, attention, arithmetic, and learning abilities (Song et al., 2001). Among adolescents, spatial and verbal abilities are correlated with the duration and severity of hypothyroidism prior to diagnosis in infancy (Rovet, 1999). Studies of specific attentional skills have revealed that the abilities to inhibit and focus attention are most affected by the severity of hypothyroidism and length of hypothyroid period, whereas the ability to sustain attention was not (Rovet & Hepworth, 2001a). As adults, motor skills are associated with the initial severity of CH and performance on cognitive tasks is associated with treatment factors. This dissociation suggests an earlier role for TH in the development of motor skills and a postnatal role for the involvement of TH in developing cognitive abilities (Oerbeck et al., 2003). Overall the literature suggests that both the severity and duration of hypothyroidism that those with CH experience early in life impacts subsequent abilities later in life.

Taken together, research on CH following diagnosis by newborn screening and early treatment suggests that TH is crucial for normal cognitive development and even a transient lack of TH during the perinatal period may not be without consequence.

### 1.2.2 Mechanisms of Thyroid Hormone Action

TH is essential for normal development and functioning of the mammalian central nervous system (Bernal & Nunez, 1995; Nunez, Celia, Nga, & Forrest, 2008). The two main forms of TH are 3,5,3’-triiodothyronine (T3), which contains three iodide residues, and tetra-iodothyronine or thyroxine (T4), which has four iodide residues. T4, which is the prohormone, is much more abundantly produced by the thyroid than T3 and is converted to T3, the bioactive form of TH, locally within the brain (Bernal & Nunez, 1995).

TH acts as a maturation factor that regulates neurodevelopment through its involvement in controlling the timing of different developmental processes. Specifically, TH differentially
regulates protein transcription via its action on a variety of nuclear TH receptors (Anderson, 2001; Oppenheimer & Schwartz, 1997; Thompson & Potter, 2000) and these actions differ in both gene expression and rate of cell differentiation (Bernal & Nunez, 1995). In addition, TH may have an effect on protein formation at the post-transcriptional level by regulating the stability of transcribed gene products or the alternative splicing of the products (Bernal & Nunez, 1995; Nunez et al., 2008). TH may also have an effect on neurotropins, which are responsible for the survival and differentiation of neuronal populations, and their receptors (Bernal & Nunez, 1995).

TH availability is regulated by endogenous mechanisms that control the hypothalamic-pituitary-thyroid axis and thus TH production by the thyroid gland. In this pathway, the paraventricular nucleus (PVN) of the hypothalamus releases thyrotropin-releasing hormone (TRH) either in response to signals from higher brain centres or as a response to low TH levels (Fliers, Alkemade, & Wiersinga, 2001). The TRH released then stimulates the pituitary gland to produce thyroid-stimulating hormone (TSH) (Wells & Murphy, 2003), which acts in turn on the thyroid gland to regulate the production of the two THs, T3 and T4 (Hendrick, Altshuler, & Whybrow, 1998). T3 and T4 exert a negative feedback influence on the pituitary and hypothalamus to inhibit excess TH production in the thyroid (Hendrick et al., 1998). In the brain, TH action is regulated through the differential spatial and temporal expression of the different TH receptors and deiodonase enzymes that can activate or inactivate THs (Bradley, Towle, & Young, 1992; Oppenheimer & Schwartz, 1997). This allows TH to have different functional effects in different brain areas at different times, thus creating different critical periods for the involvement of TH (Bradley et al., 1992; Nunez et al., 2008).
Hypothyroidism during early development has a devastating impact on the developing brain resulting in cretinism and mental retardation (Nunez et al., 2008). However, even a transient lack of TH during a circumscribed period of development can result in permanent changes in the structure and function of the central nervous system (Oppenheimer & Schwartz, 1997; Thompson & Potter, 2000) since TH is involved with many developmental processes including neuronal cell differentiation and migration, axonal and dendritic outgrowth, synaptogenesis, myelination, neurotransmitter synthesis, neuronal cell death, electric activity and gene expression (Bernal & Nunez, 1995; Thompson & Potter, 2000). The specific effects resulting from TH deficiency in CH are dependent on the severity of the deficiency, when the deficiency occurred and the critical period of development taking place (Black, 1998; Bradley et al., 1992; Forrest, Reh, & Rüsch, 2002). The timing requirements for TH are specific to each cell and tissue type within the brain (Smith, Evans, Costall, & Smythe, 2002). Appropriate levels of TH must be present within the critical periods within each brain area, since a return to normal TH levels after these periods is insufficient for the recovery of normal function (Forrest et al., 2002).

1.2.3 Thyroid Hormone and the Hippocampus

Within the brain, TH has been shown to affect cell acquisition and maturation in areas that have significant postnatal neurogenesis, which includes the hippocampus (Rami, Rabie, & Patel, 1986). The hippocampus is a bilaminar structure consisting of the dentate gyrus (DG) and the four subfields of the cornu Ammonis (CA1-4, also referred to as Ammon’s horn) (Duvernoy, 2005). Although each of the subfields is comprised of the same layers, the relative density and composition of each layer within each field is not uniform and the different subregions develop at different rates (Rami, Patel, & Rabié, 1986). Thus, there are differences in the sensitivity of the various fields to environmental factors and different subregions can be affected with
differential severity (Duvernoy, 2005). The different sensitivity of the subregions can alter the normal interactions and connections between them leading to permanent functional impairments (Rami, Rabie et al., 1986).

Structural studies of rodent models of CH show that a lack of TH during gestation results in fewer mature glial cells within area CA1 of the hippocampus and impaired migration of cells to this region (Martínez-Galán et al., 1997). Also, TH deficiency results in a quantitative difference in cell acquisition within the DG, and thus its overall size (Rami, Rabie et al., 1986). Morphological alterations are also observed within the granule cells of the DG and the pyramidal cells of Ammon’s horn in the form of reduced arborization of dendritic fields, due to impairment of the maturation processes (Rami, Patel et al., 1986). The alterations to pyramidal cell maturation depend on the location of the cell within the CA fields, with CA 3 and 4 experiencing the greatest degree of delayed maturation and CA1 the least (Rami, Patel et al., 1986). The differential effect of TH deficiency on the specific hippocampal subfields causes distortion of the normal developmental gradient and the relationships between the subregions. Consequently, this may disrupt the normal developmental trajectory of the hippocampus, leading to the permanent functional impairments seen after transient developmental hypothyroidism (Rami, Patel et al., 1986).

To investigate the impact of a lack of TH during early development to the functioning of the hippocampus, Gilbert and colleagues (2003, 2004 & 2006) induced transient hypothyroidism in rats during the perinatal period and then studied the effects of this TH deficiency on different subregions of the hippocampus when the rats were adults. Results indicated that this transient hypothyroidism during early development resulted in altered synaptic transmission and plasticity within the DG, characterized by reductions in excitatory post-synaptic potential (EPSP) slopes.
and population spike amplitudes (Gilbert & Paczkowski, 2003). In a subsequent study of the DG, the alterations in synaptic transmission and the long-term potentiation (LTP) that underlies synaptic plasticity and thought to be a mechanism for learning and memory, were demonstrated to reflect the severity of hypothyroidism the animal experienced (Gilbert & Sui, 2006). Additionally within the DG, more activation at the synapse was required to produce the same level of firing in the rats exposed to insufficient TH as in control animals (Gilbert & Sui, 2006). Furthermore, induction of LTP in the animals that experienced TH insufficiency led to an uncoupling of the synaptic and somatic components of the compound field potential such that there was impairment in the LTP of the EPSP but an increase in the population spike (Gilbert & Sui, 2006). In short, TH insufficiency resulted in DG neurons that were less responsive to input and not as likely to fire from the same level of input as in a control animal and, once the input was sufficient to cause the cell to fire, the output was larger.

Similarly, a lack of TH during development resulted in alterations in the functioning of area CA1. However, since the DG has a relatively more protracted development, the impact of perinatal hypothyroidism to area CA1 was not as severe. For example at baseline, the population spike reduction in the DG that was also observed in area CA1 (Gilbert, 2004) was not similarly accompanied by a reduction in the EPSP in area CA1. In addition following induction of LTP, although in both the DG and CA1 there was a decoupling of the synaptic and somatic components of the compound field potential such that the population spike was enhanced in the absence of enhanced EPSP, there was no reduction from baseline in the LTP of the EPSP in area CA1 as there was within the DG (Gilbert, 2004). These results indicate that in both the DG and area CA1, induction of LTP alters the functioning of neurons within these regions. Potential mechanisms that have been proposed to underlie the increase in population spike seen in both the DG and CA1 include changes in cellular excitability, GABA-ergic interneuron inhibition,
presynaptic calcium function, or synaptic function as a result of the effect of a lack of TH (Gilbert, 2004; Gilbert & Sui, 2006).

Behaviourally, hypothyroidism during early rodent development affects their learning and memory abilities as adults. In one early study, rats that had their thyroid glands removed at birth performed worse (longer time, more errors) on a T-maze task than both controls and thyroidectomized rats treated with TH replacement (Eayrs, 1959). Subsequently, it was demonstrated that even a brief period of neonatal hypothyroidism resulted in irreversible impairment in maze learning in adult rats (Akaike, Kato, Ohno, & Kobayashi, 1991). In addition, animals deprived of maternal TH in utero showed prolonged latencies to acquire a water-maze task (Opazo et al., 2008). Similarly, performance on a water maze was found to be affected in animals that experienced a hypothyroid period early in life (Gilbert & Sui, 2006). In addition, a dose-dependent effect was observed in this study with the most severely hypothyroid animals never being able to acquire the task and the less severely affected animals taking significantly more time than controls to learn the maze (Gilbert & Sui, 2006). Another study varied the length of the hypothyroid period that animals were exposed to by beginning different groups of animals on TH replacement therapy at different times after birth (MacNabb, Orsquo, Hare, Cleary, & Georgopoulos, 2000). The results of this study indicated that animals without TH replacement therapy or who received TH late took longer to acquire a response alternation discrimination task and exhibited more perseverative behaviour than did animals that received early treatment (MacNabb et al., 2000). Thus the results from animal behavioural studies consistently indicate that hypothyroidism results in impaired learning and memory as adults and that the degree of impairment reflects the length and severity of the hypothyroidism experienced.
Taken together, the evidence from animal studies of early TH deficiency signifies that the hippocampus and memory abilities are both susceptible to the effects of hypothyroidism during the perinatal period. The severity of the impairments in memory is related to the severity of the hypothyroidism, and the effects cannot be fully reversed with a subsequent return to normal TH levels. Extrapolating from this research, it is not surprising that memory weaknesses have been observed in children with CH. In addition, the animal research suggests that the memory weaknesses associated with CH likely arise from the effect of a lack of TH early in life on hippocampal structure and function and will reflect the severity of hypothyroidism each individual experienced.

1.2.4 Congenital Hypothyroidism, Memory, and the Hippocampus

Surprisingly, only a few studies assessing memory abilities in individuals with CH have been published to date. The first conducted by Rovet, Ehrlich, & Sorbara (1992) did not find any differences between the CH group and sibling controls on a memory scale when the children were 4 years of age (Rovet et al., 1992). However, they did differ on the same scale at age 6 (Rovet, unpublished observation). In addition, a follow-up study of the same cohort at adolescence revealed weaker memory in the adolescents with CH relative to controls (Rovet, 1999). Another study of a different group of children with CH revealed that the severity and duration of the hypothyroid period experienced by each child predicted their memory scores such that those with a longer and more severe hypothyroidism had lower memory scores (Song et al., 2001). Additional analyses of these same children versus healthy controls revealed weaknesses in memory for word pairs and spatial locations but not for stories or faces (Rovet, 2002). Memory differences between CH and a sibling control group in early adulthood were also noted and reflect poorer performance on tasks of verbal list learning, remembering stories, and
continuous monitoring of visual information for previously seen items (Oerbeck, Sundet, Kase, & Heyerdahl, 2005). Overall, research to date suggests that some memory abilities are affected by CH and the weaknesses noted seem to persist throughout the lifespan.

Since measures reflecting new learning and memory are affected but other memory abilities such as working memory have been shown to be spared (Hepworth et al., 2006; Rovet, 2002) and in light of the extensive animal literature demonstrating hippocampal abnormalities, we hypothesized that hippocampal dysfunction may underlie the memory weaknesses observed in CH. Thus, a study utilizing structural MRI to investigate hippocampal volumes was conducted in our lab and showed that adolescents with CH had reduced left hippocampal volumes relative to controls. In addition, volume gains in both left and right hippocampi observed in the control group over the course of adolescence were strikingly absent within the CH group, indicating that the CH hippocampus may fail to thrive during this period (Wheeler & Rovet, 2007; Wheeler et al., 2011).

Although some evidence suggests that children with CH have altered hippocampal functioning, the nature and specificity of their memory weaknesses and how their hippocampi function during memory processes had not been previously elucidated. No previously published studies have thoroughly assessed memory abilities to allow the determination of the specificity or severity of the weaknesses. For example, some studies (e.g. Oerbeck et al., 2005; Rovet, 1999; Rovet, 2002) failed to include scaled scores of the subtests reported or provide a clinical interpretation thus, it is not clear if the weaknesses in the memory performance of individuals with CH fell within a clinically significant range. Another study (Song et al., 2001) contained no control group for comparison and scores for the children with CH were within the normative average range. In addition, although there is some evidence to suggest that verbal memory abilities may
be more affected (Oerbeck et al., 2005; Wheeler et al., 2011) none of the studies systematically or thoroughly assessed a broad range of memory abilities in order to determine if all memory is affected or if there were particular domains of strength and weakness. For example, no study has assessed working memory versus MTL-related processes within both verbal and visuospatial domains within one cohort and thus it remains unknown if one of these areas is more predominantly affected and the severity of any such weakness/deficit. In addition, no studies have utilized functional neuroimaging to investigate how the hippocampus functions to support memory processes. Therefore, based on the previous literature, the exact nature and severity of the memory weaknesses noted in CH and how the hippocampus functions in early-treated CH is not known.

Thus, the present research utilized two approaches to investigate hippocampal functioning in adolescents who were diagnosed and treated for CH. First, performance on a variety of clinical neuropsychological tests was assessed in order to determine if CH was associated with specific memory weaknesses/deficits on tests that reflect hippocampal integrity or are more general to all memory abilities. Second, fMRI was utilized to show how the hippocampus functions during memory tasks in these adolescents.

1.3 Rationale for Proposed Research

1.3.1 Congenital Hypothyroidism and the Functioning of the Hippocampus

Previous literature has suggested that CH is associated with weaker memory performance relative to controls (Oerbeck et al., 2005; Rovet, 1999), which may be more prevalent within the verbal domain (Oerbeck et al., 2005; Wheeler et al., 2011). However, studies to date have not fully elucidated the nature or severity of the weaknesses. In addition, although the animal
literature demonstrates TH is necessary for adequate hippocampal development and functioning (Gilbert & Sui, 2006; Zoeller & Rovet, 2004) and work from our lab has demonstrated that adolescents with CH have reduced left hippocampal volumes relative to controls (Wheeler & Rovet, 2007; Wheeler et al., 2011), no previous study has yet investigated how the hippocampus functions to support memory in CH. Thus, in order to better understand the consequences of CH to the memory abilities and hippocampal functioning, I constructed a set of three studies which were administered concurrently to TD and CH groups. These studies explored the nature and severity of the memory weaknesses associated with CH and investigated how the hippocampus functions to support memory abilities in CH. The studies were conducted over two days for each participant. On day one, a battery of clinical neuropsychological tests primarily focusing on memory abilities was administered and, on day two, fMRI memory tasks which have been previously shown in the adult literature to specifically evoke hippocampal activation were utilized to investigate hippocampal functioning.

### 1.3.2 Research Questions

1. **Are there specific memory weaknesses in individuals with CH?**

   To describe memory abilities of the CH versus TD samples recruited for these studies (Chapter 2), a neuropsychological test battery that assessed different types of memory skills within both verbal and visuospatial domains was utilized. In addition, the relationship between early disease and treatment factors in the CH group and memory abilities at adolescence was assessed.

2. **Does the hippocampus function differently during associative memory in individuals with CH?**

   To investigate how the hippocampus functions to support associative memory in individuals with CH relative to their TD peers, fMRI was utilized to investigate
hippocampal activation during both a visuospatial and a verbal paired associates task (Chapters 3 & 4 respectively).

3. Does the severity of CH predict hippocampal functioning?

To investigate the relationship between CH severity and hippocampal functioning (Chapter 3 & 4), hippocampal activation during both fMRI tasks in the adolescents with CH was correlated with TH hormone levels obtained at diagnosis.

1.3.3 Hypotheses

1. Similar to previous reports, adolescents with CH in the present studies will show weaker, but not impaired memory performance relative to their TD peers. These differences will predominate on tasks that are known to rely on the hippocampus and reflect severity of CH.

2. Adolescents with CH will show altered hippocampal engagement (as indicated by fMRI activation) relative to their TD peers on both visuospatial and verbal memory tasks reflecting compensatory activation.

3. The severity of hypothyroidism experienced early in life by individuals with CH will predict the degree to which hippocampal activation differs from their TD peers.
Chapter 2
Memory Abilities of Adolescents with Congenital Hypothyroidism Relative to their Typically Developing Peers

Congenital hypothyroidism (CH) is a pediatric endocrine disorder caused by insufficient endogenously produced thyroid hormone (TH) (LaFranchi, 1999). Until recently, CH was the leading cause of mental retardation. However, with the advent of newborn screening, early detection has allowed much earlier treatment with TH replacement than before. Nevertheless, this has not ameliorated all of the cognitive effects of the condition as children with CH diagnosed by newborn screening still show persisting weaknesses in some cognitive abilities, and in particular memory (Rovet, 1999; Rovet, 2002). These weaknesses are the result of the period of hypothyroidism that is experienced from the onset of hypothyroidism until treatment takes effect. Given that TH plays a crucial role in the development of the hippocampus (Zoeller & Rovet, 2004), a brain structure required for some memory abilities (Eichenbaum, 2001), it has been suggested that the transient hypothyroid period experienced by newborns with CH has an impact on the developing hippocampus and this may underlie their persisting memory weaknesses. Indeed, research with rodents has shown that transient TH insufficiency during late gestation and early life, the period of time during which children with CH are TH deficient, results in permanent alterations to the structure and function of the hippocampus in addition to learning and memory problems (Gilbert, 2004; Gilbert & Sui, 2006).

The role of the hippocampus in human memory has been investigated primarily with adult populations, both with and without hippocampal damage. The extant research has shown that the hippocampus is crucial for episodic memory as opposed to semantic or working memory (Eichenbaum, 2001; Mayes et al., 2007; Milner et al., 1968; Milner, 2005; Montaldi & Mayes, 2010; Vargha-Khadem et al., 1997). More specifically, the hippocampus is preferentially
involved with memory requiring recall/recollection that is accompanied by knowledge of associated contextual information encoded during the initial episode, rather than familiarity with an item, which is not associated with other information or context (Eichenbaum, 2001; Mayes et al., 2007; Montaldi & Mayes, 2010).

Previous investigations of children, adolescents, and adults with CH have found reduced memory abilities (Oerbeck et al., 2005; Rovet, 1999; Rovet, 2002), however the recollection/familiarity distinction or the specificity of memory weaknesses to tasks that rely on the hippocampus has not been directly studied. The first study of memory abilities in children with CH was conducted by Rovet, Ehrlich, & Sorbara (1992). Results indicated that at 4 years of age there were no differences between the CH group and sibling controls on any memory measure (Rovet et al., 1992). However, a follow-up of the same children at 6 years of age showed weaknesses on the same memory tests (Rovet, unpublished observation). Similarly, a follow-up study of the same cohort at adolescence revealed weaker memory in the children with CH relative to controls for a rote memory measure (Rovet, 1999). Another study of a different group of children with CH revealed that the severity and duration of the hypothyroid period experienced by each child predicted their memory scores such that cases with longer and more severe hypothyroidism had poorer memory performance (Song et al., 2001). Additional comparisons of these children with controls revealed weaknesses in memory for word pairs and spatial locations but not for stories or faces (Rovet, 2002). Memory weaknesses relative to sibling controls have also been observed in CH in early adulthood, especially on tasks of verbal list learning, remembering stories, and continuous monitoring of visual information for previously seen items (Oerbeck et al., 2005). In addition, recent work in our lab has found that in addition to reduced verbal memory abilities, left hippocampal volumes are smaller in CH and both hippocampi fail demonstrate the growth observed in controls through adolescence (Wheeler & Rovet, 2007; Wheeler et al., 2011). The
extant literature is therefore concordant with animal research signifying that CH is associated with reduced memory abilities that may reflect abnormal hippocampal development. However, the studies to date on this population have involved relatively limited neuropsychological batteries and not extensively tested memory functioning. Thus, this research has not been able to fully characterize the nature or specificity of memory weakness associated with CH and it remains unclear if memory weaknesses reflect specific dysfunction of the hippocampus or if verbal and visuospatial memory domains are equally affected.

Therefore, the purpose of the present study was to utilize a neuropsychological test battery to assess a variety of memory abilities in a group of adolescents with CH and their typically developing (TD) peers. It was hypothesized that if the memory weaknesses in CH are specific to the hippocampus, individuals with CH would show deficits or weaknesses on tasks shown to be affected in other populations with hippocampal damage such as those requiring episodic learning, long-term memory or recollection/recognition of associative information rather than short-term or working memory (Eichenbaum, 2001; Mayes et al., 2007; Milner et al., 1968; Milner, 2005; Montaldi & Mayes, 2010; Vargha-Khadem et al., 1997). Thus, subtests from the neuropsychological test battery were grouped according to the memory domain that each assessed in order to determine if memory weaknesses associated with CH are specific to a particular domain or vary in degree across the domains. Memory tests were divided into four domains based on material utilized (i.e. verbal or visuospatial) and the degree to which the hippocampus is involved in the task (i.e. working memory or episodic learning and retention). It was hypothesized that if memory weaknesses associated with CH result from hippocampal dysfunction, adolescents with CH would show reduced abilities relative to their peers on episodic learning and retention measures, but not in working memory. In addition, it was hypothesized
that within the CH group, poor memory performance would be predicted by more severe and longer duration of hypothyroidism experienced early in life.

2.1 Method

2.1.1 Participants

The study cohort of 10 to 14-year old participants was mainly ascertained from existing databases of participants that included both CH and typically developing (TD) adolescents. Additional CH participants were recruited through the Division of Endocrinology at the Hospital for Sick Children (SickKids) while additional TD participants were ascertained via posters placed within the hospital. General information about the participant and family background was collected through the Developmental NEuroPSYchological Assessment (NEPSY) history questionnaire (Korkman, Kirk, & Kemp, 1998). Exclusionary criteria for both groups included exposure to alcohol or other teratogens during pregnancy, preterm birth, head injury, or a debilitating or chronic medical condition.

The CH group originally consisted of 18 adolescents. However, one female was eliminated from analyses after she was diagnosed with pseudoparathyroidism on the basis of the MRI scan acquired during a portion of the larger study. Thus, the final CH sample consisted of 17 participants with a mean age of 12.8 years (range 10.8-14.3) and they included 7 females and 10 males. All children except two were identified by the Ontario newborn screening program, which uses a thyroid stimulating hormone (TSH) test. The two exceptions were a boy who was diagnosed clinically at 62 days of age because his newborn sample was not delivered to the provincial laboratory by the courier and a boy born abroad. All of the Canadian-born children had their CH diagnosed by staff endocrinologists at SickKids with the majority (12/17) first being treated within the first 15 days of life (mean age = 18.1 days). Three children with
borderline TH levels were not treated until their T4 levels declined; two were at one month of age and the third was unknown as he was diagnosed abroad. On the basis of technetium scans conducted at the initial diagnostic visit, 11 children were deemed to have ectopic glands, 2 had athyrosis, 2 had dyshormonogenesis and 2 had unknown etiologies, one because parents refused the scan and the other, because the child born abroad was not assessed in this way. Medical chart review revealed that the CH group had a mean screening TSH level of 143.3 ± 66.8 nmol/L (N=11) and the values at diagnosis were 400.8 ± 302.1 nmol/L (N=15) for TSH, 8.3 ± 4.6 pmol/L (N=14) for free thyroxine (T4), and 47.3 ± 38.9 nmol/L (N=13) for total T4 levels, all of which are well outside of the normal range for newborns (Rose & Brown, 2006). The average mean starting dose of L-T4 was 9.98 ± 1.13 μg/kg/day (N=13).

TD participants included children with a normal newborn screen and no evidence of later thyroid or other medical disorders. Any child with a history of learning disabilities or known neurological or psychiatric disorders was excluded. Of the 21 participants who initially met criteria for inclusion, one was eliminated due to a periventricular cyst that was found during the MRI scan and considered significant by the neuroradiologist, and four were eliminated after participating in the neuropsychological assessment as their IQ scores were within the superior range (i.e. >130) and thus were not representative of a normal control sample. Thus, the final TD control sample consisted of 16 adolescents with a mean age of 12.2 years (range 10.6-14.0) and included 7 females and 9 males.

All procedures were approved by the research ethics board at SickKids and the University of Toronto.
2.1.2 Procedures

Most participants underwent two days of testing as part of a larger study. On the first day, they received an extensive battery of clinical tests that included a brief test of general intelligence and clinical tests of memory abilities. On the second day, some participants were involved in functional neuroimaging studies of the hippocampus and memory, which are not reported presently.

Participants were given a gift certificate to use for lunch and/or a snack break, a certificate of participation in the study, a letter to use for mandatory high school volunteer hours, and, if they participated in the functional imaging portion of the study, a CD with a structural picture of their own brain. All parents/caregivers were compensated for parking and/or travel expenses and, within two months of testing, received a clinical report of their child’s neuropsychological testing results. Neuroradiological reports for children who underwent scanning were sent to the participant’s physician.

2.1.3 Neuropsychological Assessment

Clinical neuropsychological tests evaluating general intelligence (IQ) and multiple aspects of memory were administered to all participants. IQ levels were ascertained from the Vocabulary and Matrix Reasoning subtests of the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). Memory was assessed with the Test of Learning and Memory (TOMAL) (Reynolds & Bigler, 1994), and selective subtests from the Children’s Memory Scale (CMS) (Cohen, 1997), Wide Range of Assessment of Learning and Memory (WRAML) (Adams & Sheslow, 2001), and Cambridge Automated Neuropsychological Battery (CANTAB) (Cambridge Cognition Limited, 2005). Participants also completed the Rey-Osterrieth Complex Figure (ROCF) test (Osterrieth, 1994; Rey, 1942; Strauss, Sherman, & Spreen, 2006).
In order to determine if the memory weaknesses associated with CH were specific to a particular domain, the subtests were separated by the type of stimuli utilized (i.e. verbal versus visuospatial). Additionally, tests were grouped based on those emphasizing working memory demands versus those requiring learning and retention of information, in order to determine if individuals with CH would show greater difficulty with tasks that were more hippocampally-dependent.

2.1.3.1 Verbal Working Memory Measures

In order to assess verbal working memory, Digits Forward and Backward from the TOMAL were administered in addition to the Verbal Working Memory subtest from the WRAML.

TOMAL Digits Forward required participants to repeat sequences of numbers of increasing length which were spoken by the examiner. TOMAL Digits Backward required participants to recite the sequences of numbers spoken by the examiner in reverse order.

The WRAML Verbal Working Memory, which was administered in order to assess complex verbal working memory abilities, required participants to listen to lists of animals and non-animals that increased in length. There were three levels, two of which were completed by participants determinate on their age. Level A (ages 9-13 years) required participants to name animals first followed by non-animals. Level B (ages 9-13 years and 14 years-adult) required participants to name animals first in the order of increasing size followed by all non-animals. Level C (ages 14 years-adult) required participants to name in order of increasing size, the animals first, followed by the non-animals.
2.1.3.2 Visuospatial Working Memory Measures

To assess visuospatial working memory, Visual Sequential Memory from the TOMAL and Spatial Span and Spatial Working Memory from the CANTAB were administered.

TOMAL Visual Sequential Memory required participants to study a sequence of abstract designs and then immediately point to the designs in the order presented.

CANTAB Spatial Span required participants to observe and then touch the sequence of squares in the order they changed colour. Sequences progressively increased in length as the trials progressed. CANTAB Spatial Working Memory required participants to find hidden tokens in an array of boxes. In each stage, the number of hidden tokens and boxes increased and, in order to be successful, participants had to remember which boxes have previously held tokens and were therefore already empty.

2.1.3.3 Verbal Episodic Learning and Retention Measures

In order to assess verbal episodic learning and retention, Stories from the CMS as well as Word Selective Reminding, Object Recall and Paired Recall from the TOMAL were administered.

CMS Stories required participants to listen to two stories and, immediately following each, repeat the story verbatim. Then, following a 30 minute delay, they were asked to tell the story again. Subsequently, a series of ‘yes’ or ‘no’ questions probing for story details was administered. Scores derived from Stories included Immediate and Delayed Recall and Recognition, which indicated the amount of story details recalled. Also calculated were Thematic Scores for the Immediate and Delayed Recall, which indicated the amount of ‘gist’ information recalled.
TOMAL Word Selective Reminding required participants to listen to a list of words and then repeat the list. Forgotten words were reminded by the examiner and participants were asked to repeat the entire list again until successfully repeating the entire list twice or eight trials were completed. Then, following a 30 minute delay, participants were asked to repeat the list.

TOMAL Object Recall required participants to study an array of simple line drawings while they were named by the examiner. Then, the card was removed from sight and participants were asked to name all objects on the card. The process was repeated until all objects were successfully named or 5 trials were completed. TOMAL Paired Recall required participants to listen to a list of word pairs and then recall the second word when the first word is read. Errors were corrected by the examiner and the procedure continued until all word pairings were recalled or 4 trials were completed.

2.1.3.4 Visuospatial Episodic Learning and Retention Measures

The assessment of visuospatial episodic learning and retention included the Dot Locations and Faces subtests from the CMS as well as the Visual Selective Reminding and Facial Memory from the TOMAL, the Delayed Match to Sample and Paired Associates Learning subtests from the CANTAB, and the ROCF.

CMS Dot Locations required participants to learn the location of dots in a grid by observing a card indicating their location and then placing dots in a grid in the same locations. Following three trials, a different array of dots was presented and participants then had to indicate the location of the new dots in the array. Subsequently, participants were asked to indicate the configuration of the original array once more. Then, following a 30 minute delay, participants indicated where the original dots were in the grid. Scores derived from this subtest included a measure of learning, memory after both the short and long delay, and a total score. CMS Faces
required participants to study a series of faces presented sequentially. A second group of faces (half from the studied set and half new) were then presented sequentially and participants indicated if each face was previously seen. Following a 30 minute delay, another group of faces (half from the studied set and half new) were shown and participants indicated if each face was previously seen.

TOMAL Visual Selective Reminding required participants to watch a sequence in which the examiner pointed to a series of dots on a card. Participants were then asked to point to the same dots and dots that were missed were reminded. The participants were then asked to repeat the sequence until they were able to produce the correct sequence twice in a row or until eight trials were completed. Then following a 30 minute delay, participants were asked to reproduce the sequence. TOMAL Facial Memory required the participants on each trial to study a group of faces and then identify the faces from another group that contained lures. Then, following a 30 minute delay, participants were asked to identify previously seen faces from another group.

CANTAB Delayed Matching to Sample required participants to study a complex abstract figure in the centre of the screen and then following a delay select the figure from a group. CANTAB Paired Associates Learning required participants to observe boxes ‘open’ to display contents. Then, each figure was displayed in the centre of the screen and participants indicated in which box each figure was previously shown.

For the ROCF, participants were required to copy a complex figure and then draw the figure from memory following a 30 minute delay.
2.1.4 Statistical Analyses

All scores from the CMS, TOMAL, and WRAML were converted into age-corrected scaled scores with a mean of 10 and a standard deviation of 3. All scores from the CANTAB were converted into z-scores utilizing normative data included in the software, with the exception of the Delayed Match to Sample which is presented as raw percentage correct (no normative data was available for this subtest). The ROCF score is presented as the raw score derived from the 36-point scoring system described by Strauss et al. (2006).

SPSS 17.0 was used for all analyses. A t-test was performed to investigate group differences in age and IQ. Chi-square analysis was used to determine if the sex distribution differed between groups. In order to account for multiple comparisons, MANOVAs were utilized to investigate group differences for all scores derived from each test battery within each of the four memory domains. Within the CH group, Pearson’s r was used to quantify correlations between TH variables and memory performance (significance level of p<0.05). Participants with missing data from their medical records were excluded from the relevant correlational analyses.

2.2 Results

2.2.1 Demographics

The mean ages of the CH and TD groups did not differ significantly [t(31)=1.516, p=0.14]. The sex ratio of females to males also did not differ between CH and TD groups [χ²=0.130, p=0.72]. Mean IQ for both CH (105.1±8.0) and TD (109.3±9.3) groups fell within the average range and did not differ significantly [t(31)=0.889, p=0.38].
2.2.2 Memory Abilities

Adolescents with CH did not have significantly weaker performance relative to their TD peers on any of the measures administered within the verbal or visuospatial domain of working memory. Unexpectedly and in contrast to previous reports, there were also no significant differences on any measures of episodic learning and memory retention within either the verbal or visuospatial domain (see Tables 1 and 2). In addition, almost all group averages for all subtests fell within the average range. The only exception was within the visual spatial domain on the CMS Faces Delayed subtest where the CH group scored just below the average range. However, as with all tests administered, there was no significant difference between the groups on this subtest and the mean score for the TD group was similar and at the low end of the average range.

2.2.2.1 Relationships between CH Variables and Memory Abilities

Despite no correlation between any CH variable and general intelligence, correlations between CH variables and measures of memory abilities revealed that greater severity of hypothyroidism early in life and longer duration to treatment was associated with weaker memory performance. Screen TSH value predicted TOMAL Facial Memory scores ($r=-0.602$, $p=0.05$) such that higher TSH level was associated with lower performance for recognizing faces. Longer delay to diagnosis was associated with lower scores on the CANTAB subtests Delay Match to Sample Delay Condition ($r=-0.476$, $p=0.05$), Paired Associates Learning ($r=-0.633$, $p=0.01$), and Spatial Span ($r=-0.530$, $p=0.03$). TH levels at diagnosis also predicted memory performance. Higher TSH at diagnosis associated with lower scores on CMS Faces Immediate ($r=-0.628$, $p=0.01$) and higher total T4 at diagnosis was associated with better performance on CMS Dots Total Score ($r=0.587$, $p=0.03$). In addition, higher initial dose/kg of TH replacement was associated with better scores on CANTAB Delay Match to Sample Delay Condition ($r=0.625$, $p=0.02$).
Interestingly, all correlations were in the visuospatial domain and all but one subtest (CANTAB Spatial Span) reflected episodic learning and memory retention, rather than working memory.

2.3 Discussion

Contrary to our hypothesis, no areas of weaker performance were observed in the CH group relative to their TD peers in any domain tested, including all memory measures. This finding is inconsistent with previous research (Oerbeck et al., 2005; Oerbeck et al., 2003; Rovet & Daneman, 2003; Rovet, 1999; Rovet, 2005), in which CH was associated with slightly lower scores in general intelligence and with weaker learning and memory abilities. However within CH, the impact of hypothyroidism is heterogeneous since each individual experiences a different length and severity of TH insufficiency during late gestation and early life. Results from the cohort in the present study do reflect this heterogeneity as a relationship between severity of hypothyroidism and memory abilities was observed, particularly within the visuospatial domain for episodic learning and memory retention measures that engaged the hippocampus. These correlations indicate that the severity of the TH insufficiency, as measured by increased TSH levels at screen and diagnosis, as well as the delay until the achievement of euthyroidism, as measured by longer duration to diagnosis and lower starting dose, resulted in greater memory difficulties at adolescence. These results emphasize the role that prompt diagnosis and adequate treatment may have in ameliorating the memory problems previously associated with CH.

One limitation with interpreting results from the present study is that the cohort may not adequately reflect the entire CH population. The lack of differences between the CH and TD groups may be reflecting the improved efficacy of the Ontario Newborn Screening Program and the optimal quality of care that the adolescents with CH had received from staff endocrinologists at the Hospital for Sick Children throughout childhood. In addition, the study was conducted for
research purposes and as such the method of recruiting both TD adolescents and adolescence with CH through the hospital may have led to a bias in terms of the participants and their families who agreed to participate in the study. Also, the number of participants recruited was largely driven by the corresponding fMRI study and as such the present analyses may be underpowered as a result of relatively small sample size. In regard to this, a recent structure-function study published by our lab, which included the cohort in the present study as well as CH and TD participants from other studies, did find differences in verbal memory (CMS Stories) in the larger sample (Wheeler et al., 2011). Thus, the lack of group differences in the present study must be interpreted with caution.

A further limitation concerns the CH variables in the study. For example, data were missing for some measures (given the retrospective nature of this portion of the study) from the medical charts. Also, we lacked precise measurement of when normalization of TH levels was achieved for each participant and as such the exact length and severity of each hypothyroid period could not be fully characterized. Also, TH levels at time of assessment, which could have affected cognitive functioning (Rovet, 2002; Song et al., 2001), were not determined. One final limitation is that although the data suggest hypothyroidism early in life may have subtle effects on the memory abilities of adolescents within the CH population, this study did not investigate how the hippocampus or the episodic memory network functions in adolescents with CH relative to their TD peers. As such, it is possible that compensatory brain mechanisms or different neural strategies underlie the normal performance observed in the CH group in the present study. Further study of this cohort of adolescents was undertaken to ascertain if hippocampal function is altered in adolescents with CH despite their normal memory performance and results will be presented subsequently.
In conclusion, results of the present study indicate that adolescents with CH in the current cohort do not have significantly reduced memory abilities relative to their TD peers. However, their memory abilities were predicted by the severity and duration of the period of hypothyroidism experienced during late gestation and early life. Thus, these results underscore the necessity for early and adequate treatment of hypothyroidism to optimize memory abilities of adolescence with CH.
Table 1: Neuropsychological test means (and standard deviations) for TD and CH groups for verbal memory measures

<table>
<thead>
<tr>
<th>Verbal Memory Measures</th>
<th>TD (n=16)</th>
<th>CH (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Memory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOMAL Digits Forward SS</td>
<td>8.00 (2.94)</td>
<td>8.29 (3.36)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TOMAL Digits Backward SS</td>
<td>9.94 (2.05)</td>
<td>10.41 (2.76)</td>
<td>n.s.</td>
</tr>
<tr>
<td>WRAML Verbal Working Memory SS</td>
<td>10.81 (1.05)</td>
<td>10.82 (3.68)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Learning &amp; Retention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMS Stories Immediate SS</td>
<td>12.40 (2.28)</td>
<td>11.06 (2.90)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Stories Delayed SS</td>
<td>12.20 (2.31)</td>
<td>10.94 (3.07)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Stories Delayed Recognition SS</td>
<td>11.88 (3.10)</td>
<td>10.41 (2.62)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Stories Immediate Thematic SS</td>
<td>11.53 (3.29)</td>
<td>10.65 (2.91)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Stories Delayed Thematic SS</td>
<td>10.87 (3.34)</td>
<td>10.29 (2.54)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TOMAL Word Selective Reminding SS</td>
<td>12.19 (1.72)</td>
<td>11.41 (2.96)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TOMAL Word Selective Delayed SS</td>
<td>10.75 (1.24)</td>
<td>10.18 (1.47)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TOMAL Object Recall SS</td>
<td>10.38 (3.48)</td>
<td>9.06 (2.30)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TOMAL Paired Recall SS</td>
<td>12.69 (1.96)</td>
<td>12.82 (2.98)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

[TD=typically developing; CH=congenital hypothyroidism; SS=Scaled Score; CMS=Children’s Memory Scale; TOMAL= Test of Learning and Memory; WRAML= Wide Range of Assessment of Learning and Memory; n.s.=non-significant]
Table 2: Neuropsychological test means (and standard deviations) for TD and CH groups for visuospatial memory measures

<table>
<thead>
<tr>
<th>Visuospatial Memory Measures</th>
<th>TD (n=16)</th>
<th>CH (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Memory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOMAL Visual Sequential Memory SS</td>
<td>11.19 (2.88)</td>
<td>11.35 (1.80)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CANTAB SSP z-score</td>
<td>0.58 (1.02)</td>
<td>0.33 (1.38)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CANTAB SWM z-score</td>
<td>0.00 (0.97)</td>
<td>-0.04 (0.88)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Learning &amp; Retention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMS Dot Locations Learning SS</td>
<td>11.94 (2.32)</td>
<td>12.12 (2.45)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Dot Locations Total Score SS</td>
<td>12.31 (1.92)</td>
<td>12.29 (2.29)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Dot Locations Long Delay SS</td>
<td>12.19 (1.28)</td>
<td>11.82 (2.58)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Dot Locations Short Delay SS</td>
<td>12.20 (1.37)</td>
<td>11.59 (1.70)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Faces Immediate SS</td>
<td>8.50 (4.00)</td>
<td>8.00 (3.04)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CMS Faces Delayed SS</td>
<td>8.56 (4.37)</td>
<td>7.56 (3.97)</td>
<td>C n.s.</td>
</tr>
<tr>
<td>TOMAL Facial Memory SS</td>
<td>10.31 (3.09)</td>
<td>10.47 (2.76)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TOMAL Facial Memory Delayed SS</td>
<td>10.31 (1.89)</td>
<td>11.35 (3.94)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TOMAL Visual Selective Delayed SS</td>
<td>10.25 (0.93)</td>
<td>10.13 (1.09)</td>
<td>n.s.</td>
</tr>
<tr>
<td>ROCF Delayed Raw Score</td>
<td>17.47 (7.79)</td>
<td>17.94 (6.98)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CANTAB DMS Raw Score</td>
<td>82.14 (12.03)</td>
<td>80.39 (11.48)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CANTAB PAL z-score</td>
<td>0.72 (0.42)</td>
<td>0.52 (0.77)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

[TD=typically developing; CH=congenital hypothyroidism; SS=Scaled Score; CMS=Children’s Memory Scale; TOMAL= Test of Learning and Memory; ROCF= Rey-Osterrieth Complex Figure; CANTAB= Cambridge Automated Neuropsychological Battery; SSP= Spatial Span; SWM= Spatial Working Memory; DMS= Delayed Matching to Sample; PAL= Paired Associates Learning; C=clinical range; n.s.=non-significant]
Chapter 3
Visuospatial Associative Memory in Adolescents with Congenital Hypothyroidism

Congenital hypothyroidism (CH) is a pediatric endocrine disorder of newborns caused primarily by a missing, ectopic, or dysfunctional thyroid gland. This leads to an early insufficiency of thyroid hormone (TH) (LaFranchi, 1999), which is necessary for brain development. Although CH is readily treated with replacement TH, clinical diagnosis was previously delayed due to the late appearance of outward symptoms and as a result, CH was a leading cause of mental retardation. However, with the advent of newborn screening, diagnosis and treatment of CH now take place shortly after birth thereby avoiding retardation (Dugbartey, 1998; Rovet, 1999). Nevertheless, affected children still exhibit a variety of subtle cognitive weaknesses (Rovet, 1999; Rovet, 2002) that persist into adulthood (Oerbeck et al., 2005).

Although the results from this set of studies (Chapter 2) was not concordant with previous literature as no areas of memory weakness were observed within the CH group, typically weaknesses are observed among individuals with CH in their learning and memory abilities (Rovet, 2005). These abilities typically fall below peers on standard neuropsychological tests although still within the normal range of population norms (Oerbeck et al., 2005; Rovet, 1999; Rovet et al., 1992). Previous studies have shown that areas particularly affected are their verbal-associative recall and spatial learning abilities (Rovet, 1999; Rovet, 2002), which are known to rely on the hippocampus. In contrast, their working memory, which involves primarily extra-hippocampal structures (Eichenbaum, 2001; Mayes et al., 2007; Milner et al., 1968; Vargha-Khadem et al., 1997) is preserved (Hepworth et al., 2006; Rovet, 1999). These findings are supported by basic research with rodents deprived perinatally of TH and showed significant learning and memory impairments as well as structural and functional abnormalities of the
hippocampus (Gilbert, 2004; Gilbert & Sui, 2006; Rami, Patel et al., 1986; Rami, Rabie et al., 1986). Consequently, these findings led us to speculate that the memory deficits in children with CH may also reflect altered hippocampal functioning (Rovet & Daneman, 2003; Rovet, 1999; Rovet, 2002; Song et al., 2001). Recently, we reported these children show reduced hippocampal volumes and disturbed hippocampal maturation during adolescence and that their hippocampal volumes are correlated with performance on selective memory tasks (Wheeler & Rovet, 2007; Wheeler et al., 2011). However, we do not yet know whether their hippocampi function atypically when supporting memory abilities.

To determine if early life TH insufficiency due to CH affects hippocampal functioning, fMRI and a task specifically known to engage the hippocampus was utilized. In adults, fMRI studies have shown the hippocampus is preferentially involved when remembering associations between items versus the individual items (Giovanello et al., 2004; Kohler et al., 2005; Mayes et al., 2007) and parallel findings have been established regarding deficits for associative rather than item memory in adults with hippocampal damage (Holdstock, Mayes, Gong, Roberts, & Kapur, 2005; Mayes et al., 2004). Because of the paucity of fMRI studies examining children’s medial temporal lobe functions, we modified a paradigm to use in the present study that was developed by Köhler et al (2005) for adults. This task examining novelty effects (i.e. greater activation for new versus previously seen stimuli) demonstrated that hippocampal activation was particular to visuospatial associations (i.e. pairs of items and item-location relations). In contrast, extra-hippocampal areas of the medial temporal lobes were involved with item novelty effects (Kohler et al., 2005). Thus, to investigate the impact of early life TH insufficiency to memory functions supported by the hippocampus, we adapted the associative novelty paradigm of Kohler et al.’s study. Given that both animal literature and the small relevant literature on brain structure and memory performance in CH suggest hippocampal dysfunction, we anticipated that the CH group
would show an altered pattern of activation, although we could not predict exactly how this
difference would be manifested in terms of greater or less activity in hippocampus.

3.1 Method

3.1.1 Participants

Participants ranged in age from 11.0 to 15.5 years. Many were ascertained from existing
databases of participants that included both CH and typically developing (TD) adolescents, while
additional CH participants were recruited through the Division of Endocrinology at the Hospital
for Sick Children (SickKids) and additional TD participants by posters placed within the
hospital. Exclusionary criteria were exposure to alcohol or other teratogens during pregnancy,
preterm birth, head injury, or a debilitating or chronic medical condition. Any participant
wearing braces or having other metal implants was also excluded in order to ensure compatibility
with MRI scanning requirements.

The CH group originally consisted of 17 adolescents (9 females). However, two were eliminated
due to poor compliance/data corruption and one after being diagnosed with
pseudoparathyroidism on the basis of her scans. Thus the final CH sample consisted of 14
participants, one of whom was left handed. All children except two were identified by the
Ontario newborn screening program, which uses a thyroid stimulating hormone (TSH) test; the
two exceptions were a boy, whose sample failed to be delivered to the provincial laboratory by
the courier and he was diagnosed clinically at 62 days of age, and a boy born abroad. The
Canadian-born children were all diagnosed with CH by staff endocrinologists at SickKids and
the majority (9/14) were first treated within the first two weeks of life (mean age = 19.6 days).
Three children with borderline TH levels were not treated until their T4 levels declined; two at
one month of age and the third was unknown as he was diagnosed abroad. On the basis of
technetium scans conducted at the initial diagnostic visit, 8 children had ectopic glands, 2 had athyrosis, and 2 had dyshormonogenesis; etiologies were unknown for two children, one whose parents refused the scan and the other, who was born abroad and so not assessed in this way. Medical chart review revealed that at diagnosis, the CH group had a mean thyroid stimulating hormone (TSH) value of 139.67 mU/L and mean free thyroxine (T4) and total T4 values of were 8.53pmol/L and 44.23nmol/L, which fall well outside of the normal range for newborns (Rose & Brown, 2006). Their mean starting dose of L-T4 was 10.23 μg/kg/day.

The TD sample included children with a normal newborn screen and no evidence of later thyroid or other medical disorders. Any child with a history of learning disabilities or known neurological or psychiatric disorders was excluded. Of the 17 TD participants who originally met criteria, one was eliminated due to a periventricular cyst found on scanning and considered significant by the neuroradiologist and one due to a lack of responses during the fMRI session. Thus the total TD control sample consisted of 15 adolescents, two of whom were left-handed. All procedures were approved by the research ethics board at SickKids and the University of Toronto.

3.1.2 Procedures
All participants underwent two days of testing as part of a larger study. On the first day, they received a battery of clinical tests that included tests of general intelligence and clinical tests of memory abilities (not reported here). On the second day, two fMRI studies were conducted in separate 1-hour sessions, the first involved the visuospatial memory paradigm described presently and the second, a verbal paired associates task, which is the topic of a separate paper. Participants received a gift certificate to use for lunch and/or a snack break and on completion of the scanning, received a CD with a structural picture of their own brain, a certificate of
participation in the study, and a letter to use for mandatory high school volunteer hours. Parents were compensated for parking and/or travel expenses. Neuroradiological reports were sent to the children’s physicians with recommendations for follow-up if necessary.

3.1.2.1 Visuospatial Paired Associates Task

This task, based on a paradigm by Kohler et al. (2005), was redesigned to be child friendly and age appropriate by (a) using animal icons to remind the participants of the task demands, (b) presenting the different trial types as ‘games’ to play, and (c) involving age-appropriate stimuli. The experimental design is illustrated in Figure 1. Stimuli consisting of simple line drawings of objects were selected from a Snodgrass and Vanderwart-like set (Rossion & Pourtois, 2004). All stimuli were programmed for presentation using E-Prime software. For each participant, object stimuli were randomly paired and assigned to one of 12 unique spatial configurations within an invisible 4 X 6 grid. Placements were constrained so that the objects had to be separated by at least one square either up/down or left/right within the grid. Each spatial configuration was used twice to allow the object to be re-paired with a novel partner without also altering its spatial location.

Prior to scanning, participants received extensive practice with each of the ‘games’. First, sample cards were used to illustrate the concept of each game and then participants practiced with the computer generated games, which used materials that would not appear during scanning. Encoding of the stimuli took place prior to functional imaging. During the first encoding session outside of the scanner, participants were presented with 24 pairs of objects in their assigned spatial configurations and instructed to remember both the pairs and their locations. Each object pair was shown for 4 seconds, followed by an inter-trial interval of 3 seconds. The full set of stimuli was shown twice to ensure adequate encoding of the material. Then in the scanner, participants first underwent a structural scan during which time the same
stimuli were presented twice more in order to remind the participants of the list and ensure optimal learning and retention of information. Stimuli were presented via MR-compatible goggles.

The fMRI acquisition occurred during the recognition phase of the task, which was comprised of two retrieval conditions or ‘games’. In the first game, participants had to decide if the displayed objects had been presented together during the study phase or if the pairing was new (Objects condition). The second game required participants to decide if object locations were the same as viewed previously or had been switched (Place condition). Retrieval task conditions were blocked, with each condition appearing once in each of three 10-minute scans. The order of retrieval tasks during blocks and assignment of YES and NO response keys to the first or second button on the response pad were randomly assigned and counterbalanced across participants. Every retrieval block started with an ‘instruction’ screen showing the game logo/cue and response button allocation for 10 sec. This was then followed by 32 trials, 24 of which were previously seen associations requiring a ‘YES’ response and 8 were novel associations requiring a ‘NO’ response. Trials were presented in pseudorandom order and constrained such that novel trials could not be presented successively and no more than 6 old trials were consecutively presented. Retrieval trials lasted 9 seconds and consisted of the presentation of retrieval stimuli for 5 seconds followed by a 4-second fixation icon, which consisted of a small version of the game logo/cue in order to remind participants which retrieval task they were performing.

An important feature of this study design was the manipulation of only one variable at a time such that each retrieval trial involved alteration from the study phase on only one dimension. Either the pairing of the objects or the location of the objects within the pair was altered from the study phase, never both at once (see Figure 1). Another important aspect was that the retrieval tasks differed only as to the aspect of the association to which the participants attended and
responded (Objects versus Place), not the stimuli that were encoded. Thus, memory for object pairings and spatial locations were both assessed and compared using the same encoded material and no new objects were presented during the retrieval phrase.

3.1.3 Data Acquisition and Analysis

Functional data were acquired using echo-planar imaging (EPI) (TR=2sec, 25 slices, 240mm FOV, 64 X 64 matrix, resulting in a voxel size of 3.75 X 3.75 X 5mm) with an 8-channel head coil on the 1.5T Sigma GE SickKids’ research scanner. Slices were acquired in a coronal-oblique orientation perpendicular to the long axis of the hippocampus. In each of the three runs, 304 functional volumes were acquired with the first 3 being dropped from the analyses to allow for signal equilibrium. E-prime software recorded behavioural data (accuracy and reaction time for each trial) for each participant. Correlations between all TH variables and behavioural data were assessed.

All functional imaging data were pre-processed and analyzed using SPM5 (Statistical Parametric Mapping 5; Wellcome Department of Imaging Neuroscience). The pre-processing stream involved extraction of brain from skull and CSF spaces according to established criteria and automated algorithms. Pre-processing included realigning and screening for excessive motion. Movement exceeding ±1mm and/or ±1 degree rotation from baseline was noted for each run for each participant. Runs containing large motion spikes had the relevant scans removed and any run containing excessive motion throughout was discarded from the analyses. Further pre-processing included slice timing correction to the middle slice, coregistering to the participant’s T1-weighted structural image, normalizing using the Montreal Neurological Institute EPI template, resampling at a voxel size of 4 x 4 x 4mm, and smoothing using a Gaussian kernel of 8 mm full-width half maximum. Each stimulus event was modeled by SPM5’s canonical
hemodynamic response function beginning at the onset of each stimulus presentation. Only those trials in which the participant responded correctly were retained for analyses. First-level analyses of individual subjects’ data were processed using a fixed-effects model to assess condition differences (i.e. New>Old) and those contrasts were then entered into second-level random-effects analyses to assess group differences. New>Old contrasts were of interest for both Objects and Place conditions given previous findings showing distinct novelty effects in the hippocampus for these different types of associative information (Kohler et al., 2005).

Since the primary focus of the present study was hippocampal activation, we applied a small volume correction using an ROI mask for the hippocampus from MAsks for Region of INterest Analysis software (MARINA) (Walter et al., 2003) with a threshold of p<0.05 and a minimum of five contiguous voxels. Covariates of no interest entered into the analyses were age and accuracy to remove the effects of individual variation in these parameters from the results. In order to determine the relationship between early TH levels (T4 and TSH at diagnosis) and hippocampal activation, multiple regressions were conducted in SPM5 for both Object and Place New>Old contrasts with the TH variable entered as the predictor of interest and age and accuracy were entered as covariates of no interest.

### 3.2 Results

#### 3.2.1 Demographic and Behavioural Data

Demographic data for both groups presented in Table 3 show CH and TD groups did not differ in age [t(27)=3.594, p=0.07], handedness [χ²=0.299, p=0.58], or sex ratio [χ²=0.042, p=0.84].

Behavioural data also shown in Table 3 reveal no group differences for either Object or Place in accuracy [t(27)=0.687, p=0.50; t(27)=1.303, p=0.20 respectively] or reaction time [t(27)=1.126,
p=0.27; t(27)=0.618, p=0.54 respectively]. After accounting for multiple comparisons, biomedical indices were not correlated with behavioural data in the CH group.

### 3.2.2 fMRI Results

Table 4 shows the peak activated voxels in the hippocampus for each of the planned contrasts. In the Objects condition, the TD group activated the left hippocampus, whereas the CH group activated bilateral hippocampi. Direct comparison of groups revealed the CH group showed greater activation in both left and right hippocampi (see Figure 2). In contrast, the TD group showed no areas of increased activity in the hippocampus relative to CH. In the Place condition, both TD and CH groups activated left and right hippocampi to a greater degree for novel than previously seen spatial configurations. A direct group comparison revealed greater activation of the left hippocampus in CH relative to TD (see Figure 2). Again, there were no areas of increased hippocampal activity in TD relative to CH.

In the CH group, multiple regression analyses using biomedical data as predictors revealed that severity of hypothyroidism at time of diagnosis significantly predicted hippocampal activation for both Objects and Place novelty-related contrasts. TSH at diagnosis was positively correlated with bilateral hippocampal activations for both Object \( [z=2.42, p<0.01 \text{ right}; z=3.40, p<0.001 \text{ left}] \) and Place New>Old contrasts \( [z=2.28, p<0.01 \text{ right}; z=2.60, p<0.01 \text{ left}] \) while free T4 was negatively correlated with New>Old activations in the left hippocampus for the Object condition \( [z=2.00, p<0.05] \). These associations signify that the more severe the child’s hypothyroidism at time of diagnosis in early infancy (as indicated by a high TSH and a low free T4 level), the greater the hippocampal engagement over a decade later on a task designed to specifically evoke hippocampal engagement.
3.3 Discussion

Using an associative novelty task to probe the functional integrity of the hippocampus, we found that adolescents with CH showed significantly increased activation relative to their TD peers. Specifically, we observed that the CH group showed greater activation in both left and right hippocampi for the Objects condition and increased activation in the left hippocampus for the Place condition. These findings therefore indicate that adolescents with CH recruit additional neural resources to perform the task successfully and accomplish this by activating the left hippocampus to a greater degree than their TD peers across conditions and by also increasing the activation within their right hippocampus when their TD peers do not. Of note, increased hippocampal engagement in the CH group was present despite no significant differences in task performance relative to their TD peers and after statistically controlling for accuracy and age in the analyses to eliminate within-group variance due to these parameters.

In addition, the severity of TH deficiency at diagnosis predicted the degree of increased activation within the hippocampus in the CH group. Specifically, higher levels of TSH (which occur in response to insufficient TH) were associated with greater bilateral hippocampal activation in both Object and Place conditions. Similarly, lower T4 levels at diagnosis (signifying more severe hypothyroidism) were related to the extent to which the left hippocampus was active during the Object condition. Overall, these results indicate that TH levels early in life when the hippocampus is still undergoing development are critical for hippocampal functioning during adolescence and, if atypical, can lead to permanent alteration of functional integrity of this region.

Our finding of increased recruitment of hippocampal resources in the CH group when successfully performing a task is consistent with findings described for other adult patient
populations with hippocampal damage. For example, patients with mild cognitive impairment exhibit increased recruitment of bilateral hippocampal resources in order to perform a memory task at the same level as controls (Dickerson, Salat, Greve, Chua, RandGiovannetti, Rentz et al., 2005). Similarly, the patient Jon who acquired bilateral hippocampal damage in early life, showed bilateral recruitment of the hippocampus during retrieval when controls only activated the left hippocampus (Maguire et al., 2001). To date, the few studies of hippocampal functioning in children or adolescents who sustained hippocampal damage have provided inconsistent findings. In some studies, increased activation relative to controls was seen (Gimenez et al., 2005; Maheu, 2008) while in others, no differences (Curtis et al., 2006) or decreased or no hippocampal activation were reported (Chiu, 2009; Sowell et al., 2007). Thus while the developmental literature suggests that early hippocampal insult has an impact on subsequent functioning, the nature and cause of these changes remain unclear. Given the relatively few studies conducted to date and a lack of consistent control over extraneous variables across studies, it is not evident whether the nature of the task or stimuli, cause or severity of the hippocampal damage, age at test or insult, or performance accuracy and overall memory abilities of the studied population were the critical factor(s) in influencing the results in these studies.

In addition, different biological processes may be contributing to the different findings across the patient populations studied to date. In terms of the CH population, extensive research on rodent models of TH insufficiency has provided insight as to potential mechanisms underlying altered activity in the hippocampus (e.g. Gilbert, 2004; Gilbert & Paczkowski, 2003; Gilbert & Sui, 2006; Martínez-Galán et al., 1997; Rami, Patel et al., 1986; Rami, Rabie et al., 1986), particularly in the context of memory operations engaged by the task demands in the present study task. According to one current model, associative novelty detection in the hippocampus
has been proposed to operate via a comparator mechanism that identifies a mismatch between expectations based on prior experiences and current sensory information (Kumaran, 2007). Two critical areas for associative novelty detection (i.e. DG and CA1) are known to be affected by the lack of TH in rodent models of CH. Lack of TH during early development results in reduced numbers of cells within the DG (Rami, Rabie et al., 1986) and area CA1 (Martínez-Galán et al., 1997) as a result of impaired migration of cells to these regions (Martínez-Galán et al., 1997; Rami, Rabie et al., 1986). There are also morphological alterations within the granule cells of the DG and the pyramidal cells of Ammon’s horn resulting in reduced arborization of dendritic fields (Rami, Rabie et al., 1986). Thus the animal literature has shown that the structure of the hippocampus is altered by a lack of TH during development in precisely those regions that appear to subserve associative novelty processes. The structural alterations described also have functional consequences. In both the dentate gyrus and area CA1 under baseline conditions and following induction of long-term potentiation, the hippocampal neurons are less responsive to input and not as likely to fire from the same level of input as a control animal. In addition, once the input is sufficient to cause the cell to fire, the output is larger (Gilbert, 2004; Gilbert & Sui, 2006). Thus, these processes may contribute to the increased hippocampal activation observed presently in the CH group and may provide a biological basis for the weaker memory abilities associated with CH. Unfortunately, given the resolution of our fMRI scans, we cannot directly investigate activation by subfield in this study.

The findings from the present study also have implications for understanding memory functioning in CH. Given the CH group showed recruitment of increased resources relative to controls during a relatively easy (by accuracy criteria) associative recognition memory task, it is possible that tasks which challenge hippocampal engagement, or coordination of hippocampal and neocortical networks, to a greater extent may exceed those resources. This may be reflected
in the relative weaknesses and subtle decrease in clinical memory scores previously observed in studies of CH (Oerbeck et al., 2005; Rovet, 1999; Rovet et al., 1992; Rovet, 2002). However, as the present study did not examine fMRI activation in adolescents with CH using more challenging memory tasks, it remains purely speculative as to how the CH hippocampus would function under such circumstances. In addition, the relevance of the type of stimuli used presently is not known and whether results would be similar had other types of memory stimuli been used. A forthcoming paper using a verbal paired associates memory task will address this issue. In addition, since the present study was not designed to investigate activity within the entire episodic memory network, it is not known to what degree differences in other brain regions which may also be affected by early TH insufficiency are contributing to present findings. Furthermore, this study did not investigate if the differences in hippocampal activity are stable or change further with development. Finally, TH levels at time of assessment and scanning, which could have affected cognitive functioning (Rovet, 2002; Song et al., 2001), were not determined.

In conclusion, even though adolescents with CH and TD adolescents performed comparably during a visuospatial associative memory task, they differed in their hippocampal activation patterns such that the CH group showed increased hippocampal activation relative to TD. The present study therefore supports the view that an early TH deficiency at a time when the hippocampus is undergoing critical development has long-lasting effects on subsequent functioning and necessitates the recruitment of additional hippocampal resources. These results indicate that even well-treated CH can impact hippocampal functioning and also demonstrate how a brain structure/network that is compromised early in development compensates in order to maintain performance.
Table 3: Demographic information and task performance

<table>
<thead>
<tr>
<th>Variable</th>
<th>TD (n=15)</th>
<th>CH (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.60 ± 1.32</td>
<td>13.41 ± 0.99</td>
</tr>
<tr>
<td>Male/Female ratio</td>
<td>8/7</td>
<td>8/6</td>
</tr>
<tr>
<td>Handedness Left/Right</td>
<td>2/13</td>
<td>1/13</td>
</tr>
<tr>
<td>Accuracy (%) – Object</td>
<td>81 ± 15</td>
<td>85 ± 13</td>
</tr>
<tr>
<td>– Place</td>
<td>86 ± 13</td>
<td>77 ± 22</td>
</tr>
<tr>
<td>Reaction Time (msec) – Object</td>
<td>1739 ± 234</td>
<td>1644 ± 215</td>
</tr>
<tr>
<td>– Place</td>
<td>1701 ± 273</td>
<td>1645 ± 203</td>
</tr>
</tbody>
</table>

[TD=typically developing; CH=congenital hypothyroidism]
Table 4: Hippocampal activation as a function of group and contrasts (small volume correction p<0.05) with age and accuracy as covariates of no interest

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Left or right hippocampus</th>
<th>Peak Coordinates (MNI)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td><strong>Object (new&gt;old object pairs)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- TD</td>
<td>L</td>
<td>-16</td>
<td>-32</td>
</tr>
<tr>
<td>- CH</td>
<td>L</td>
<td>-20</td>
<td>-32</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>28</td>
<td>-40</td>
</tr>
<tr>
<td>- TD&gt;CH</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CH&gt;TD</td>
<td>L</td>
<td>-28</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>28</td>
<td>-40</td>
</tr>
<tr>
<td><strong>Place (new&gt;old spatial arrangement)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- TD</td>
<td>L</td>
<td>-20</td>
<td>-36</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>24</td>
<td>-28</td>
</tr>
<tr>
<td>- CH</td>
<td>L</td>
<td>-24</td>
<td>-20</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>28</td>
<td>-24</td>
</tr>
<tr>
<td>- TD&gt;CH</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CH&gt;TD</td>
<td>L</td>
<td>-24</td>
<td>-20</td>
</tr>
</tbody>
</table>

[TD=typically developing; CH=congenital hypothyroidism]
Figure 1: Experimental design for encoding and retrieval of object pairs and locations

Encoding

study pairs

Retrieval: Places block

DRAGON AND CASTLE PLACE GAME

YES NO

Instruction screen

Retrieval: Objects block

KING AND QUEEN PAIRS GAME

YES NO

Instruction screen

‘Yes’ response

‘No’ response

‘Yes’ response

‘No’ response
Figure 2: SPM results for the congenital hypothyroid (CH) vs typically developing (TD) contrast. A and B: Object New> Old for left and right hippocampus; C: Place New>Old for left hippocampus
Chapter 4
Hippocampal Functioning and Verbal Associative Memory in Adolescence with Congenital Hypothyroidism

The hippocampus is a critical brain structure for episodic memory (Eichenbaum, 2001). More specifically, the hippocampus has been shown to be preferentially involved with the retrieval of the associations between pieces of information (such as word pairs, object and spatial locations, or items in specific perceptual contexts) as opposed to the individual pieces of information (such as single words, objects alone, or items without context). Paradigms used to evaluate this distinction typically contrast recognition of associations (e.g., are the pairs ‘intact’ or ‘re-arranged’?) versus recognition of items (e.g., are the words themselves ‘old’ or ‘new’?) or interrogate participants’ subjective sense of recollection (e.g., do you ‘remember’ the original context of study?) versus familiarity (e.g., do you ‘know’ this was in the study set but fail to recover any contextual information?). The distinction has been supported in research involving adults with hippocampal damage (Bowles et al., 2007; Bowles et al., 2010; Eichenbaum et al., 2007; Holdstock et al., 2005; Mayes et al., 2004) and in studies using fMRI to assess hippocampal activation in healthy adults (Diana et al., 2007; Eichenbaum et al., 2007; Giovanello et al., 2004; Mayes et al., 2007; Yonelinas, 2002).

In a developmental context, there is evidence that damage to the hippocampus early in life also results in weaker memory performance on tasks requiring the retrieval of associations (de Haan et al., 2006; Vargha-Khadem et al., 1997). Moreover, studies utilizing fMRI paradigms have suggested that early hippocampal damage leads to altered recruitment of hippocampal resources that support associative memory processes later in life. Children and adolescents with hippocampal damage exhibit altered hippocampal activity relative to controls when successfully encoding verbal associations (Chiu, 2009) and word-face associations (Gimenez et al., 2005).
Differences in hippocampal activation have also been observed during learning and cued recall of verbal associations (Sowell et al., 2007). However, these studies have not investigated the impact of early hippocampal damage on its role in associative versus item recognition memory.

One condition that results in atypical hippocampal development is congenital hypothyroidism (CH). CH is a pediatric endocrine disorder in which endogenous production of thyroid hormone (TH) is lacking or insufficient due to an abnormality in thyroid gland development or function. This condition results in a hypothyroid period during late gestation and ensues until replacement therapy is provided and takes effect (Dugbartey, 1998; LaFranchi, 1999; Rovet, 1999). Rodent models have shown that a transient lack of TH during the perinatal period leads to permanent impairments in learning and memory, as well as structural and functional differences in the hippocampus that are not corrected once TH levels subsequently return to normal (Gilbert & Sui, 2006). In patients with CH extending from childhood through adulthood, the early period of hypothyroidism is not without consequence with many individuals exhibiting learning and memory weaknesses that reflect the severity of the hypothyroidism (Oerbeck et al., 2005; Rovet, 1999; Rovet et al., 1992). Moreover, their memory weaknesses are suggestive of abnormal hippocampal development and function (Rovet, 2002) since the affected areas of memory are similar to those observed in other populations with hippocampal damage (Vargha-Khadem et al., 1997). Specifically, children with CH show memory weaknesses on tasks of episodic recall (Rovet & Daneman, 2003; Rovet, 1999; Rovet, 2002; Song et al., 2001) and preserved ability on working memory tasks (Hepworth et al., 2006; Rovet, 1999).

In order to investigate the link between the hippocampus and memory in adolescents with CH more directly, recent work in our lab has focused on utilizing neuroimaging techniques to investigate structure-function relationships between the hippocampus and memory abilities and
to determine how the hippocampus functions to support memory processes. Our structure-function study revealed that, relative to their peers, the CH group had reduced left hippocampal volumes and weaknesses in verbal memory (Rovet, Desrocher, Sheard, Wheeler, & Skocic, 2007; Wheeler & Rovet, 2007; Wheeler et al., 2011). In addition, a recent fMRI study which utilized a visuospatial associative memory novelty detection task demonstrated that adolescents with CH recruited additional hippocampal resources relative to typically developing (TD) peers in order to maintain equivalent accuracy (Wheeler, McAndrews, Sheard, & Rovet, in press). Specifically, the CH group showed greater activation in both left and right hippocampi compared to TD controls when they had to determine if pairs of objects were presented with their original partner or were re-paired. The CH group also had increased activation in the left hippocampus when they had to determine if the object pairs were presented in their original configuration or had switched places. Overall, this study demonstrated that adolescents with CH required additional hippocampal resources to successfully detect associative novelty, activating the left hippocampus to a greater degree than their TD peers across conditions and activating their right hippocampus when their TD peers did not. Additionally, this study showed that the degree to which additional hippocampal resources were recruited was predicted by the severity of hypothyroidism experienced early in life (Wheeler et al., in press).

In summary, research to date has shown that the early period of hypothyroidism experienced by individuals with CH affects memory abilities, hippocampal volumes, and hippocampal functioning during a visuospatial paired associates novelty detection task. However, since our previous structure-function study suggested that verbal memory abilities and the left hippocampus may be more vulnerable to the effects of CH, we also wanted to investigate how the hippocampus functions in adolescents with CH during verbal associative memory processing. Specifically, we modified a verbal paired associates task used by Giovanello and colleagues
(2004) that selectively engaged the hippocampus in adults when processing associations (deciding if words reflected intact versus re-arranged pairings) versus items (deciding whether words had been previously studied irrespective of pairing). We hypothesized that while both adolescents with CH and TD controls would show increased hippocampal activation for correctly recognized associations versus items, hippocampal activity supporting these processes would differ with the CH recruiting more hippocampal resources than controls. In addition, we hypothesized that within the CH group, the severity of TH insufficiency early in life would be predictive of atypical hippocampal functioning during verbal associative memory at adolescence.

4.1 Method

4.1.1 Participants

Participants who ranged in age from 11.0 to 15.5 years were mainly recruited from existing databases of CH and TD individuals. Additional CH participants were recruited through the Division of Endocrinology at the Hospital for Sick Children (SickKids) while additional TD participants were recruited via posters placed within the hospital. Exclusionary criteria included exposure to alcohol or other teratogens during pregnancy, preterm birth, head injury, or a debilitating or chronic medical condition. To prevent MRI contraindications, any participant wearing braces or having other metal implants was also excluded.

The CH group originally consisted of 17 adolescents. However, two cases were eliminated due to poor compliance/data corruption and one for a diagnosis of pseudoparahypothyroidism determined on the basis of her current MRI scans. Thus the final CH sample consisted of 14 participants (8 male) with a mean age of 13.41 ± 0.99 years, one of whom was left handed. All children except two were identified by the Ontario newborn screening program, which uses a thyroid stimulating hormone (TSH) test. The two exceptions were a boy who was diagnosed
clinically at 62 days of age because his sample failed to be delivered to the provincial laboratory by the courier and a boy born abroad. All of the Canadian-born children had their CH diagnosed by staff endocrinologists at SickKids with the majority (9/14) first being treated within the first two weeks of life (mean age = 19.6 days). Three children with borderline TH levels were not treated until their T4 levels declined; two at one month of age and the third was unknown as he was diagnosed abroad. On the basis of technetium scans conducted at the initial diagnostic visit, 8 children had ectopic glands, 2 had athyrosis, 2 had dyshormonogenesis; and two had unknown etiologies because the parents of one refused the scan and the child born abroad was not assessed in this way. Medical chart review revealed that our CH group at diagnosis had a mean thyroid stimulating hormone (TSH) level of 406.8nmol/L, free thyroxine (T4) of 8.5pmol/L and total T4 of 44.2nmol/L, which fall well outside of the normal range for newborns (Rose & Brown, 2006). Their mean starting dose of L-T4 was 10.3 μg/kg/day.

The TD participants included children with a normal newborn screen and no evidence of later thyroid or other medical disorders. Any child with a history of learning disabilities or known neurological or psychiatric disorders was excluded. Of the 17 TD participants who met these criteria, one was eliminated due to a periventricular cyst that was found on scanning and considered significant by the neuroradiologist and two due to a lack of responses during the fMRI session. Thus the total TD control sample consisted of 14 adolescents (8 male) with a mean age of 12.85 ± 1.48 years, two of whom were left-handed.

All procedures were approved by the research ethics board at SickKids and the University of Toronto.
4.1.2 Procedures

As part of a larger study examining the memory and the hippocampus in adolescents with CH, all participants underwent two days of testing. On the first day, they received a battery of clinical tests, which included tests of general intelligence and clinical tests of memory abilities (not reported here). On the second day, two fMRI studies were conducted in separate 1-hour sessions, the first involved a visuospatial memory paradigm (which is the topic of a separate paper) and the second, a verbal paired associates task, which is the focus of this report.

Participants were given a gift certificate to use for lunch and/or a snack break and on completion of the scanning, a CD with a structural picture of their own brain, a certificate of participation in the study, and a letter to use for mandatory high school volunteer hours. Parents were compensated for parking and/or travel expenses. Neuroradiological reports were sent to the children’s physicians with recommendations for follow-up if necessary.

4.1.2.1 Verbal Paired Associates Task

The verbal paired-associates task was based on a paradigm developed by Giovanello et al. (2004) and redesigned to be child friendly and age appropriate. This involved the presentation of task requirements as games with logos to keep the participant engaged and on-task, age-appropriate stimuli, a reduced number of stimuli in each word pairs list, and an increased length of stimulus presentation to allow participants more time to process information and respond. All stimuli were programmed for presentation using E-Prime software.

Stimuli were words generated by the MRC Psycholinguistic Database: Machine Usable Dictionary (Wilson, 1987) and were selected for the following parameters: 1 or 2 syllables, four to six letters in length, score of at least the mean or better for concreteness and imageability, Thorndike-Lorge written frequency of AA (appearing at least 100 times per million words
written), and age of acquisition earlier than four years. All words were manually screened by two independent-raters to ensure age-appropriateness of the stimuli and any word deemed to be overly emotional, inappropriate, or difficult was removed. This resulted in a database containing 441 words, 361 of which were randomly selected for inclusion in the task. A subset of words selected for pretraining had the same general parameters, however these words could also contain a minimum of three letters or more than six letters. Stimuli were randomly paired for both pretraining and testing and no words were repeated from the pretraining in the test phase.

Participants received extensive practice prior to scanning. This entailed using sample cards initially to illustrate the concept of each game and then practice with the computer generated games that involved different materials than those used later during scanning. In addition, although the task was to be performed silently in the scanner, the participants also gave verbal responses to the task during the pre-training session to ensure that they were performing the task as instructed. In the scanner, participants studied lists of word pairs by silently forming a sentence containing the two words. The sentences had to be robust, more than a simple interaction or linkage of the words, with the words in the same order as in the original pair. The use of sentences as the encoding task was designed to promote engagement of the hippocampus required for the recollection of associative information during the subsequent recognition trials, rather than relying on non-hippocampal processes required for the recognition of item information (Cohn & Moscovitch, 2007; Mayes et al., 2007; Prior & Bentin, 2003; Quamme et al., 2007). The appropriateness of sentences was ensured during the pre-training phase. All stimuli were presented via MR-compatible goggles.

The retrieval portion of the task consisted of three different types of retrieval trials presented as ‘games’. The first assessed associative memory by requiring the participants to decide if the
words were previously paired together. The second, assessing item memory, required them to determine if the words were presented before regardless of their current pairing. The third was a control task which required participants to respond to the @ sign by indicating on which side of the screen the symbol appeared. An important feature of this design was that the retrieval tasks of interest (involving word pairs) differed only on the type of question to which the subject responded (pairs versus items), not to the particular stimuli that were encoded or the number of words presented. Thus, from one encoded list both memory for item pairing and item identity could be assessed and compared.

The task was presented in four runs, each with an encoding and retrieval phase. During the encoding phase, a list of 14 word pairs was presented with each pair shown for 7 seconds to allow the subject to silently create a sentence. During the retrieval phase, four blocks alternating the pair and item retrieval tasks were presented sequentially, with the game presented first counterbalanced across participants. At the beginning of each retrieval task, the instructions, game logo/cue, and response button reminders were displayed together for 5 seconds. During each retrieval trial lasting 4 seconds, the game logo/cue remained on the screen to remind the participant of the retrieval task type. During each item memory retrieval block, participants were presented with trials consisting of a) word pairs containing two words seen before but not together (Rearranged Items [RI]), b) one word seen before and one not seen before (Old/New Items [ONI]), and c) two new words (New Items [NI]). Participants indicated YES if both items were seen before or NO if either word is new. During each association memory retrieval block, participants were presented a) previously seen pairs (Intact Pair [IP]), b) pairs containing words both seen before but not previously paired (Rearranged Pairs [RP]), and c) pairs containing two new words (New Pair [NP]). Participants indicated YES if the pair was presented together before as a pair or NO the words were not previously paired. The control condition included
throughout both types of retrieval tasks was presented for 2, 2.5, or 3 seconds (one of each length in each block) to introduce jitter. Each retrieval block consisted of 12 trials: nine were experimental and included three of each type of retrieval trial such that three required YES responses and six required NO responses, and three control responses. The trials were pseudo-randomly presented during each block with the constraint that no more than two trials of the same type were presented consecutively and a control trial was always presented last. The assignment of YES and NO responses to the left and right response keys was assigned randomly and counterbalanced across participants.

4.1.3 Data Acquisition and Analysis

Functional data were acquired using echo-planar imaging (EPI) (TR=2sec, 25 slices, 240mm FOV, 64 X 64 matrix, resulting in a voxel size of 3.75 X 3.75 X 5mm) with an 8-channel head coil on the 1.5T Sigma GE SickKids’ research scanner. Slices were acquired in a coronal-oblique orientation perpendicular to the long axis of the hippocampus. In each of the four retrieval runs, 200 functional volumes were acquired with the first 3 being dropped from the analyses to allow for signal equilibrium. E-prime software recorded behavioural data (accuracy and reaction time (RT) for each trial) for each participant. SPSS 17.0 was utilized to analyze all behavioural and TH variables.

All functional imaging data were pre-processed and analyzed using SPM5 (Statistical Parametric Mapping 5; Wellcome Department of Imaging Neuroscience). Pre-processing included realigning and screening for excessive motion. For each run for each participant, movement exceeding ±1mm and/or ±1 degree rotation from baseline was noted. Runs containing large motion spikes had the relevant scans removed and any run containing excessive motion throughout was discarded from the analyses. Further pre-processing included slice timing
correction to the middle slice, coregistering to the participant’s T1-weighted structural image, normalizing using the Montreal Neurological Institute EPI template, resampling at a voxel size of 4 x 4 x 4mm, and smoothing using a Gaussian kernel of 8 mm full-width half maximum. Each stimulus event was modeled by SPM5’s canonical hemodynamic response function beginning at the onset of each stimulus presentation. First-level analyses of individual subjects’ data were processed using a fixed-effects model to assess condition differences and those contrasts were then entered into second-level random-effects analyses to assess group differences. Since the previous study demonstrated greater hippocampal activation for recognizing associations between words compared with recognizing individual items (Giovanello et al., 2004), specific contrasts of interest for the present study were Association>Item (collapsed across trial types) and Old Associations->Old Items. All contrasts included trials collapsed across all runs of the task but only those trials in which the participant responded correctly were retained for analyses.

Since the primary focus of the present study was hippocampal activation, we applied a small volume correction using an ROI mask for the hippocampus from MAsks for Region of INterest Analysis software (MARINA) (Walter et al., 2003) with a threshold of p<0.05 and a minimum of five contiguous voxels. Covariates of no interest entered into the analyses were age and accuracy in order to remove any age effects and the impact of ‘correct guessing’ from the analyses. In order to determine the relationship between early TH levels (T4 and TSH at diagnosis) and hippocampal activation, multiple regressions were conducted in SPM5 for both Association>Item and Old Associations(IP)>Old Items (RI) contrasts where the TH variable was the predictor of interest and age and accuracy were entered as covariates of no interest.
4.2 Results

4.2.1 Demographic and Behavioural Data

Analyses of demographic data revealed CH and TD groups did not differ in age \(t(26)=1.185, p=0.25\), handedness \(\chi^2=0.373, p=0.54\), or sex ratio \(\chi^2=0.144, p=0.71\). Since there were no differences for either accuracy \(t(27)=0.847, p=0.41\) or RT \(t(27)=0.202, p=0.84\) for control trials between associative or item blocks, all control trials were collapsed across blocks for all further analyses. Mean accuracy levels across all trial types were 77 and 70% for TD and CH groups respectively (Figure 3). Groups did not differ in mean accuracy for either associations \(t(26)=1.304, p=0.20\) or items \(t(26)=1.291, p=0.21\) whereas they did differ in overall mean RT \(t(26)= 2.079, p<0.05\). Further analyses revealed that the CH group responded faster than the TD group for all item memory trials \(t(26)=2.137, p<0.05\) but not for associative memory trials \(t(26)=1.604, p=0.12\) or for the control condition \(t(26)=0.813, p=0.42\) (Figure 4). In addition, a significant interaction was observed in RT between group and recognition conditions \(F(1,26)=10.303, p<0.01\) which reflected the significantly slower RTs for item trials than associative trials \(t(13)=-2.590, p=0.02\) in the TD group and the slightly faster RTs for item trials than associative in the CH group, although this failed to reach significance \(t(13)=2.005, p=0.06\). In terms of the relationship between accuracy and RT, these were unrelated in the TD group for associations or items \(r=0.102, p=0.73; r=0.165 p=0.57\) respectively), whereas for the CH group, RT was significantly correlated with for both associations and items \(r=0.657, p<0.01; r=0.618, p=0.01\) respectively) reflecting that with longer RTs, accuracy was improved.

Inclusion of age as a covariate in the behavioural analyses performed did not alter the results and age did not correlate in either the TD or CH group with accuracy \(r=0.490, p=0.08; r=0.043, p=0.88\) respectively) or RT \(r=-0.025, p=0.93; r=-0.305, p=0.29\) respectively).
When correlations between accuracy, RT and CH variables were assessed, results indicated that accuracy for IP (r=-0.574, p=0.03) and RP (r=-0.567, p=0.03) trial types, total associative accuracy (r=-0.599, p=0.02), and overall accuracy (r=-0.572, p=0.03) were predicted by age at diagnosis. This pattern of results indicates that the longer it took for a diagnosis to be made and treatment given (i.e. the longer the postnatal TH insufficiency), the worse the child’s performance, particularly on associative trials. Total associative accuracy (r=-0.700, p=0.04), accuracy for ONI (r=-0.701, p=0.04) and NI (r=-0.700, p=0.04) trial types, total item accuracy (r=-0.706, p=0.03), and overall accuracy (r=-0.718, p=0.03) were all predicted by total T4 at diagnosis reflecting decreased performance when initial TH insufficiency was not as severe. No other significant correlations between any CH variable and behavioural measures were observed.

4.2.2 fMRI Results

To investigate the role of the hippocampus in associative relative to item memory, two planned contrasts investigating hippocampal activation were conducted. The first involved contrasting all associative memory trials (IP+RP+NP) versus all item memory trials (RI+ONI+NI) and the second involved contrasting activation during memory for old associations (IP) versus memory for old items (RI) (see Table 5 and Figure 5). All contrasts had age and accuracy entered as covariates of no interest. Similar to adults, as reported by Giovanello et al. (2004), both the TD and CH groups activated bilateral hippocampi to a greater degree for associative versus item memory in the contrast involving all trial types and the contrast between old associations (IP) and old items (RI). However, the contrasts between the TD and CH groups revealed group differences in hippocampal activation. Specifically, for the contrast including all associative and item trial types, the CH group activated left hippocampus to a greater degree than the TD group whereas for the contrast comparing old associations (IP) and old items (RI), CH activated
bilateral posterior hippocampus to a greater degree than TD. In addition, the reverse contrast revealed TD adolescents activated a more anterior area of right hippocampus to a greater degree than did adolescents with CH.

Multiple regression analysis to investigate whether CH variables predicted hippocampal activation for the associative>item contrast, revealed a positive correlation between TSH at diagnosis and left hippocampal activation [z=1.74, p<0.05]. Also observed were negative correlations between free T4 levels at diagnosis and areas in left anterior and posterior hippocampus [z=1.86, p<0.05; z=1.73, p<0.05]. For the IP>RI contrast, a negative correlation was found between free T4 at diagnosis and left hippocampal activation [z=1.87, p<0.05], but not for TSH at diagnosis. These findings indicate that greater activation of the left hippocampus may be associated with a more severe TH insufficiency at diagnosis.

4.3 Discussion

4.3.1 Memory Performance

Since associative memory abilities have been previously related to hippocampal integrity (Eichenbaum et al., 2007; Holdstock et al., 2005; Mayes et al., 2004; Mayes et al., 2007) and the hippocampus has been shown to be affected in CH (Wheeler et al., 2011; Wheeler et al., in press), it was anticipated that individuals with CH would exhibit significantly worse memory than their TD peers on associative memory trials in the present study. However, there were no significant differences observed between CH and TD groups in memory accuracy for any associative or item trial type. The lack of significant differences may reflect the current alterations of the original task that were implemented in order to make the task easier (i.e. shorter lists at encoding) as well as the lack of power in the analyses of behavioural data since the focus of the present study was hippocampal activation rather than behaviour. There were, however,
significant differences in RT with the CH group responding significantly faster than the TD group for all item trial types but not for associative or control trials. An interaction between RT for associations versus items and group reflected the tendency for the TD group to be slower for item than associative trials and the CH group to be slightly faster for items than associations, although the latter finding just failed to reach significance (p=0.06). In addition, a speed/accuracy trade-off was noted for the CH group but not for the TD group.

Despite no group difference in accuracy, we did see relationships between severity and length of hypothyroidism early in life and behavioural performance on the task within the CH group. Total T4 at diagnosis predicted accuracy for some item trial types (ONI, NI, total item) as well as total associative accuracy such that greater TH was associated with worse performance. This relationship was in the opposite direction to what would be anticipated however these correlations were based on a small N and total T4 is not as good of a predictor of outcome as free T4 or TSH levels. Thus these results may not adequately reflect the true relationship between degree of hypothyroidism and performance. However, as anticipated age at diagnosis predicted accuracy for some associative trial types (IP, RP, total associative) as well as overall accuracy such that a longer delay to diagnosis resulted in poorer memory performance. These results signify that the longer the hypothyroid period lasted early in life the worse the child’s performance on an associative memory task later in life and therefore support previous findings in the literature demonstrating that children, adolescents and adults experience memory problems relating to the period of hypothyroidism experienced early in life (Oerbeck et al., 2005; Rovet, 1999; Rovet, 2002). Also, results are consistent with an extensive literature on rodent models of CH which has demonstrated permanent alterations to hippocampal structure and function as a result of hypothyroidism during the perinatal period and that this does not recover once euthyroidism is achieved. These effects were seen to reflect a number of structural and
functional abnormalities in the hippocampus including fewer mature glial cells in area CA1, impaired migration of cells (Martínez-Galán et al., 1997), reduced cell acquisition (Rami, Patel et al., 1986), morphological alterations in the dentate gyrus, altered arborization of dendritic fields in Ammon’s horn (Rami, Rabie et al., 1986), and impaired of long-term potentiation and altered population spikes in the dentate gyrus (Gilbert & Sui, 2006) and area CA1 (Gilbert, 2004). Alterations to the hippocampus such as these may underlie the observed differences in neural indices within the CH group in the present study.

4.3.2 Hippocampal Functioning

Similar to the original study of healthy adults (Giovanello et al., 2004), increased hippocampal activation was observed for verbal associative versus item memory for TD adolescents as well as adolescents with CH. However, similar to our previous study of visuospatial associative memory in CH (Wheeler et al. in press), the present study demonstrated that hippocampal activity related to associative memory in adolescents with CH differed from their TD peers. TD adolescents had hippocampal activation similar to that previously reported in adults with bilateral anterior hippocampi more active for remembering verbal associations versus items (Giovanello et al., 2004). Although adolescents with CH also had bilateral hippocampal activation, their recruitment of specific hippocampal resources differed from controls. The contrast comparing all associative trials with all item trials revealed the CH group had increased activation of the left hippocampus relative to controls. As well, the contrast between old associations (IP) and old items (RI) revealed greater activation in bilateral posterior hippocampus in the adolescents with CH relative to controls. However, the reverse contrast (i.e. TD>CH) revealed an area of right anterior hippocampus was more active for the TD controls than the adolescents with CH. These results therefore signify that to successfully remember verbal associations, adolescents with CH
recruit different hippocampal resources than their peers. Notably, the group differences were present despite the lack of a significant group difference in task performance and even after statistically controlling for accuracy and age in the analyses.

Interestingly for both sets of contrasts, the degree of increased activation reflected the severity of TH levels early in life. In the contrast comparing all association trials with all item trials, for which the CH group had increased activation in left hippocampus relative to controls, degree of left hippocampal activation was predicted by more severe hypothyroidism at diagnosis early in life as indicated by decreased levels of TH and elevated levels of TSH, which are produced when TH is insufficient. Therefore, both a high TSH and a low T4 were associated with greater activity within the left hippocampus. Similarly, in the contrast comparing old associations and old items for which the adolescents with CH had bilateral areas of greater hippocampal activation relative to controls, greater activation of the left hippocampus was predicted by decreased levels of TH. Overall, these results signify that within the CH group, the degree to which additional left hippocampal resources are recruited when successfully remembering verbal associative information is predicted by the severity of TH insufficiency early in life.

The present study is concordant with previous studies that have suggested the left hippocampus is more vulnerable to the effects of CH. Previous studies in our lab have shown reduced verbal memory abilities in the presence of reduced left hippocampal volumes (Rovet et al., 2007; Wheeler & Rovet, 2007; Wheeler et al., 2011). In addition, our previous fMRI study of visuospatial associative memory showed that adolescents with CH activated their left hippocampus to a greater degree than their TD peers in both object pairs and spatial location conditions (Wheeler et al., in press). Therefore, the present results demonstrating that adolescents with CH recruit left hippocampus to a greater degree than their TD peers and the
correlation between increased left hippocampal activation with severity of hypothyroidism early in life support the hypothesis that the left hippocampus is more affected by a lack of TH during early life than the right. This differential sensitivity may be reflected in studies of individuals with CH that have found more difficulties with verbal than visual memory (Oerbeck et al., 2005; Wheeler et al., 2011) since the left hippocampus is typically associated with memory for verbal material (Frisk & Milner, 1990; Milner, 1968a) and the right for visuospatial (Milner, 1968b; Smith & Milner, 1981).

Additionally, the pattern of hippocampal activation observed in the present study may have implications for understanding memory functioning in CH. Since different regions of the hippocampus are recruited by the CH and TD groups in order to successfully retrieve verbal associations, it is possible that verbal associative memory is processed differently by adolescents with CH than their TD peers. There is some evidence in the literature to suggest a functional dissociation along the long axis of the hippocampus with respect to flexible retrieval of associations versus the reinstatement of studied episodes. A study by Preston, Shrager, Dudukovic, & Gabrieli (2004) showed hippocampal activity was greater for ‘mediated’ associations (i.e. two faces, each originally studied in a pair with the same house at encoding, but were not presented together) rather than studied face pairs (i.e. faces shown together at encoding). This study further demonstrated that recognition of items in the mediated association condition was linked with anterior hippocampal activation and recognition of studied face pairs was associated with a more posterior region (Preston et al., 2004). Additionally, as a follow-up study to their original verbal paired associates study, Giovanello, Schnyer, & Verfaellie (2009) showed that anterior hippocampus is associated with flexible retrieval of verbal associative information (i.e. recognizing words as belonging to a pair when presented in the reverse order) whereas posterior regions are more associated with reinstatement of studied episodes (i.e.
recognizing words as belonging to a pair when presented in the studied order). In addition, accuracy on the verbal-paired associates task was correlated with anterior, but not posterior, hippocampal activation (Giovanello et al., 2009). Thus, studies investigating hippocampal activation during recognition have shown more posterior activations for reinstatement of studied episodes and more anterior activation for flexible retrieval of associative information. These data may have implications for the dissociation along the long axis of the hippocampus observed in the present study which showed that adolescents with CH activated posterior hippocampal regions to a greater degree than TD adolescents who instead activated anterior hippocampus. These results may suggest that although CH and TD groups had equivalent accuracy, the way in which the associations were retrieved differed such that the CH group retrieved bound associations whereas the TD group retrieved the associative information in a more flexible manner.

Thus, the lack of group differences in memory accuracy reported in this study may also reflect the fact that flexible retrieval of associations was not required to perform the task. It is possible that a task requiring flexible retrieval would have resulted in poorer performance in the CH group compared to their TD peers, since hippocampal activation associated with associative retrieval in the CH group was in hippocampal regions that do not seem to support flexible retrieval of associations (Giovanello et al., 2009; Preston et al., 2004). However, exactly how the hippocampus functions under such circumstances in CH remains speculative. In addition, since the present study was not designed to investigate activity within the entire episodic memory network, it is not known if altered neural processing in other brain areas in the CH group are contributing to the present findings. Furthermore, it is not known if hippocampal activity for the present task is stable or changes with development within either the TD or CH
group. Finally, TH levels at time of assessment and scanning, which could have affected cognitive functioning (Rovet, 2002; Song et al., 2001), were not determined.

In conclusion, although both adolescents with CH and their TD peers had increased hippocampal activation for verbal associations versus items, how the hippocampus was engaged differed between the groups. The lack of TH that the adolescents with CH experienced during the perinatal period resulted in different spatial recruitment of hippocampal resources for successful recognition of verbal associations. In addition, severity of TH insufficiency at the time of diagnosis predicted additional recruitment of left hippocampal resources. Together, these results suggest that the period of hypothyroidism adolescents with CH experienced early in life has lasting effects on hippocampal functioning, particularly on the left, and demonstrates how a structure altered early in development functions subsequently.
Table 5: Hippocampal activation as a function of group and contrasts (SVC, p<0.05) with age and accuracy as covariates of no interest

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[TD=typically developing; CH=congenital hypothyroidism]
Figure 3: Mean accuracy and standard error by trial type for typically developing (TD) and congenital hypothyroidism (CH) groups

[IP=Intact Pairs; RP=Rearranged Pairs; NP=New Pairs; RI=Rearranged Items; ONI=One Old and One New Item; NI=New Items; C=Control task]
Figure 4: Mean reaction time and standard error by trial type for typically developing (TD) and congenital hypothyroidism (CH) groups

Figure Legend: IP=Intact Pairs; RP=Rearranged Pairs; NP=New Pairs; RI=Rearranged Items; ONI=One Old and One New Item; NI=New Items; C=Control task; * =p<0.05
Figure 5: SPM results for the congenital hypothyroid (CH)>typically developing (TD) contrast. 
A: TD>CH for Intact Pairs>Rearranged Items Contrast; B: CH>TD for Intact Pairs>Rearranged Items Contrast
Chapter 5
General Discussion

5.1 Summary of Results

5.1.1 Are there specific memory weaknesses in CH?

Since previous research has shown CH is associated with slightly lower scores on some learning and memory measures (Oerbeck et al., 2005; Rovet & Daneman, 2003; Rovet, 1999; Rovet, 2005), it was anticipated that memory weaknesses would be observed in the present studies. However, there were no areas of weaker performance in any memory domain tested in the CH group relative to their TD peers. These disparate results may reflect the specific cohort of adolescents included in the present study. It is possible that since participants recruited to the study were followed from birth and/or throughout childhood by endocrinologists at Sickkids, they may have received better quality of care than a cohort obtained via other means. Nevertheless, the relationships between greater severity and longer duration of hypothyroidism with poorer memory abilities reported previously in the literature (Rovet, 2005; Song et al., 2001) were also observed in the present study. These relationships support the notion that treatment factors impact outcome and thus may have resulted in the lack of group differences observed in the present research. Additionally, since the participants were recruited primarily for the fMRI studies, the clinical analyses may be underpowered as a result of the sample size. Indeed, a larger analysis performed by Wheeler et al (2011) of memory abilities associated with CH that included the subjects who participated in the present studies revealed weaker memory in adolescents with CH versus their TD peers (Wheeler et al., 2011).

In conclusion, results of the present clinical neuropsychological study of memory indicate that the present cohort of adolescents with CH do not show significant memory deficits relative to
their TD peers. However, since memory abilities are predicted by the severity and duration of
the period of hypothyroidism early in life in the CH group, the importance of prompt diagnosis
and adequate treatment is highlighted.

5.1.2 Does the hippocampus function differently during associative memory in CH?

In both the visuospatial and verbal paired associates fMRI tasks, differences were observed in
the recruitment of hippocampal resources between the CH and TD groups. In both tasks, the CH
group recruited additional hippocampal resources as compared with their TD peers. In addition
for the verbal task, the CH group failed to recruit an anterior portion of the hippocampus to the
same extent as the TD group.

For the visuospatial paired associates task, the CH group showed greater activation in both left
and right hippocampi for the Objects condition and increased activation in the left hippocampus
for the Place condition. These findings indicate that when adolescents with CH process
visuospatial associations, they need to recruit additional neural resources and accomplish this by
activating the left hippocampus to a greater degree than their TD peers across conditions and by
also increasing the activation within their right hippocampus when their TD peers do not.

For the verbal paired associates task, increased left hippocampal activation was observed for the
contrast comparing all associative trials with all item trials in the adolescents with CH relative to
TD controls. The contrast between old associations (IP) and old items (RI) revealed greater
activation in bilateral posterior hippocampus in the adolescents with CH relative to TD controls.
However, the reverse contrast revealed that there was also an area of right anterior hippocampus
that was more active for the TD controls than for the adolescents with CH. These results indicate
that adolescents with CH recruit additional and different hippocampal resources than their peers
to successfully remember verbal associations. In addition, the pattern of hippocampal activation suggests that adolescents with CH may retrieve verbal associations in less flexible manner than TD adolescents. Notably in both studies, the group differences were present despite the lack of a significant group difference in task performance and even after statistically controlling for accuracy and age in analyses.

In conclusion, the results of the two paired associates tasks indicate that CH results in altered hippocampal activity relative to TD peers despite no differences in overall accuracy for both visuospatial and verbal associations. Interestingly across all conditions in both studies, areas in the left hippocampus were recruited to a greater degree by the adolescents with CH. These results therefore suggest that although the hippocampus, particularly the left, may be altered by a lack of TH early in life, compensatory mechanisms appear to be utilized in order to maintain performance.

5.1.1 Does the severity of CH predict hippocampal functioning?

The severity of TH deficiency at diagnosis predicted the degree of increased activation within the hippocampus in the CH group for both paired associates tasks. For the visuospatial paired associates task, increased TSH at diagnosis, which is produced when TH is insufficient, was associated with greater bilateral hippocampal activation in both Object and Place conditions while decreased TH levels at diagnosis were related to the extent to which the left hippocampus was active during the Object condition. For the verbal task contrast comparing all association trials with all item trials, both increased levels of TSH and decreased levels of TH were associated with greater activity within the left hippocampus. Similarly, for the contrast comparing old associations and old items, greater activation of the left hippocampus was predicted by decreased levels of TH. Interestingly, the activity in the left hippocampus was
consistently related to severity of hypothyroidism at diagnosis across all conditions. These results therefore signify that the degree of hypothyroidism experienced by each individual early in life influences hippocampal recruitment, particularly on the left, during associative memory during adolescence.

In conclusion, the results from both tasks indicate that the degree to which the additional and/or different hippocampal resources were engaged during associative memory reflected the severity of the hypothyroidism experienced early in life at diagnosis. These results are congruent with the findings from the clinical neuropsychological memory study (Chapter 2), which found that memory abilities are predicted by the severity and duration of the period of hypothyroidism in the CH group. Thus, results from both the clinical and fMRI studies emphasize the importance of newborn screening, prompt diagnosis, and adequate treatment to ensure intact memory abilities and typical hippocampal functioning.

5.2 Implications

5.2.1 Animal Literature and Biological Mechanisms

Extensive research on rodent models of TH insufficiency has demonstrated that hypothyroidism early in life results in impaired learning and memory in adulthood, and reflects the length and severity of the hypothyroid period experienced (Akaike et al., 1991; Eayrs, 1959; Gilbert & Sui, 2006; Opazo et al., 2008). In addition, animal research has provided insight into the biological mechanisms that may underlie the behavioural changes in the rodents. Two areas in the hippocampus that have been extensively studied and are known to be affected by a lack of TH in rodent models of CH are the DG and area CA1. Reduced numbers of cells within the DG (Rami, Rabie et al., 1986) and area CA1 (Martínez-Galán et al., 1997) as a result of impaired migration of cells to these regions has been observed (Martínez-Galán et al., 1997; Rami, Rabie et al.,
1986). Also, morphological alterations within the granule cells of the DG and the pyramidal cells of Ammon’s horn result in reduced arborization of dendritic fields (Rami, Patel et al., 1986). In addition, there are functional consequences of hypothyroidism to the developing hippocampus. In both the DG and area CA1 under baseline and LTP conditions, hippocampal neurons are less responsive to input and not as likely to fire from the same level of input as a control animal. In addition, once the input is sufficient to cause the cell to fire, the output is larger (Gilbert, 2004; Gilbert & Sui, 2006). Thus, animal models have shown that activity within the hippocampus is permanently altered by a transient period of hypothyroidism early in life and hippocampal neurons require more input to fire and increased output when the structure is engaged.

In conclusion, the animal literature suggests that structural and functional changes within the hippocampus as a result of hypothyroidism early in life can result in an overall increase in hippocampal activity. These results are congruent with the findings from the present fMRI studies that found areas of increased hippocampal activation in CH relative to the TD group during associative memory tasks. This contention is also supported by the findings from both fMRI memory tasks that demonstrate the severity of hypothyroidism early in life was predictive of how much additional hippocampal resources were needed to be recruited in order to successfully remember associations at adolescence.

5.2.2 Hippocampal Functioning

The developmental literature suggests that early hippocampal insult can have an impact on subsequent functioning. However, the nature and cause of these changes remain unclear. Given the relatively few studies conducted to date and a lack of consistent control over extraneous variables across studies, it is not clear whether the critical factor(s) influencing the results in
these studies are related to differences in the populations studied (i.e. age at test or insult, cause or severity of the hippocampal damage, or memory abilities) or task-related variables (i.e. the nature of the task or stimuli utilized, or performance accuracy). However, the present studies may aide in elucidating the impact of some of these factors to results obtained in fMRI studies of hippocampal functioning.

The increased recruitment of hippocampal resources for adolescents with CH to successfully perform the paired associates tasks used in the present studies is consistent with some findings described for adult patients with hippocampal damage. There is evidence to suggest that the structural integrity of the hippocampus or the amount of disease burden to the structure may impact hippocampal activation. For example, prior to progressing to Alzheimer’s Disease adults with MCI, showed increased hippocampal activation relative to controls and were able to maintain performance (Dickerson et al., 2005) while adults with greater hippocampal damage and clinical decline as the disease progressed had less hippocampal activation relative to controls (O’Brien et al., 2010). These results suggest that the hippocampus may compensate for a relatively small amount of damage by increasing recruitment of resources. However, as the damage progresses the hippocampus is no longer able to access or coordinate additional resources thus performance declines. In short, hippocampal activity may be predicted by an inverted ‘U’ based on the amount of hippocampal damage with relatively limited damage resulting in increased activation and more severe damage leading to decreased activation. In the present studies, the adolescents with CH performed at a similar level to controls throughout all tests administered but had increased recruitment of hippocampal resources similar to the adults with MCI. Perhaps patients with CH who were not adequately treated or other patient populations of children/adolescents with greater memory difficulties or disease burden would be more similar to the adults with AD and demonstrate reduced hippocampal activity relative to
controls. Additional support for this contention is provided by one study of adolescents born preterm which showed that the degree of increased right hippocampal activation relative to controls was predicted by larger hippocampal volume, suggesting that the severity of hippocampal loss (or disease burden) was predictive of the degree of decreased activation (Gimenez et al., 2005). It is possible that similar correlations would be observed in the CH group in the present studies between volume and activation. Although volumetric data was not included in the present studies, the correlations between severity of CH and increased hippocampal activation suggest that severity of disease also impacts the increased recruitment of hippocampal resources.

There is also evidence showing that accuracy on the memory tasks performed may have an impact on fMRI results. However, studies to date that involve child and adolescent patient populations have not consistently controlled for this factor. In the present research, analyses of the verbal paired associates task revealed that although only correct trials were analyzed, inclusion of age and accuracy as covariates of no interest influenced the results. Although not reported in Chapter 4, analyses performed that did not include the covariates (age and accuracy) indicated no areas of increased hippocampal activation in the CH relative to the TD group whereas inclusion of the covariates revealed areas of increased activation within the CH group. This suggests that accuracy and how it is accounted for statistically may be relevant factors in examining fMRI results and whether or not they are included may influence the findings across studies. Interestingly, the inclusion of covariates for the visuospatial paired associates task did not dramatically influence the results as increased hippocampal activation was observed in the CH relative to the TD group with and without the inclusion of covariates. It is possible that the inclusion of covariates had a larger impact on the verbal than visuospatial task since accuracy on the verbal task was lower for both groups and, as a result, more of the ‘correct’ responses
retained for the verbal task analyses may have been the result of correct guessing. Therefore, the inclusion of accuracy as a covariate may have been beneficial as it was able to account for responses that did not reflect associative memory processing.

In conclusion, while it is clear from the literature and the present studies that early damage to the hippocampus alters its subsequent functioning, many questions remain as to what factors predict relative increases or decreases in hippocampal activity. Results from the present study indicate that task accuracy is one important factor to be considered in fMRI analyses and that once the impact of this factor is accounted for, it is clear that the hippocampus in adolescents with CH requires additional neural resources to support performance of both visuospatial and verbal associative memory tasks. These findings suggest that compensatory mechanisms can be utilized to maintain performance in a group of adolescents with CH who were promptly and adequately treated.

5.2.3 Clinical Relevance

Results from all three studies emphasize the importance of treatment factors in the outcome of patients diagnosed with CH. Early CH variables were shown to have an impact on clinical memory performance with lower TH levels early in life and longer duration of hypothyroidism predicting worse scores. Similarly, the degree to which the recruitment of additional hippocampal resources was required to maintain performance during visuospatial and verbal associative memory tasks was predicted by lower TH levels at diagnosis.

Despite the favourable outcome obtained by the adolescents with CH in the present study with respect to memory abilities, results from the fMRI studies suggest that the compensatory mechanisms engaged by the hippocampus may not be optimal. For example, in the verbal paired associates task the pattern of recruitment of hippocampal resources may provide some insight
into the memory abilities of adolescents with CH. A study by Giovanello et al. (2009) demonstrated that the anterior hippocampus is more associated with flexible retrieval of verbal associative information whereas posterior regions are more associated with reinstatement of studied episodes. In addition, accuracy on the verbal-paired associates task was correlated with anterior, but not posterior, hippocampal activation (Giovanello et al., 2009). Thus data from the present verbal paired associates task showing that adolescents with CH rely on more posterior hippocampal resources may indicate that they have less access to flexible retrieval of information. It is therefore possible that when challenged with memory tasks that require flexible retrieval of associations, their performance may suffer. In addition, since recruitment of these resources was also related to the severity of the hypothyroid period experienced by each individual, these results suggest more severe hypothyroidism may bias memory networks towards less flexible recruitment of hippocampal resources.

These results may be linked to the apparent altered developmental trajectory of the hippocampus previously demonstrated in our lab (Wheeler et al., 2011). If the hippocampus fails to thrive during late childhood and early adolescence, the memory abilities that typically emerge during this period that rely on the continued development of the hippocampus may be affected. Since flexible use of associative memory is not adult-like until the age of 10 years and begins developing later than simple relational memory (Townsend et al., 2010), it is possible that the results from the verbal-paired associates demonstrating that adolescents with CH use hippocampal regions more associated with reinstatement of studied associations rather than flexible retrieval of the information indicates a developmental delay or reflects the failure of the hippocampus to thrive during adolescence. Future research utilizing tasks requiring flexible retrieval of associations throughout development would be required to determine if CH results in
altered processing of flexible associations and if altered hippocampal processing is observed throughout the lifespan.

In addition, the results from the present fMRI studies are concordant with previous studies that have suggested the left hippocampus is more vulnerable to the effects of CH. Some studies of individuals with CH that have found more difficulties with verbal than visual memory (Oerbeck et al., 2005; Wheeler et al., 2011) thus suggesting that the left hippocampus is more affected than the right given that the left hippocampus is typically associated with memory for verbal material (Frisk & Milner, 1990; Milner, 1968a) and the right for visuospatial (Milner, 1968b; Smith & Milner, 1981). In addition, previous research in our lab showed reduced left hippocampal volumes in the presence of reduced verbal memory abilities (Rovet et al., 2007; Wheeler & Rovet, 2007; Wheeler et al., 2011). In the present research, both the visuospatial and verbal paired associates tasks showed that adolescents with CH activated areas in their left hippocampus to a greater degree than their TD peers across all conditions. In addition, in both studies there were correlations between increased left hippocampal activation with severity of hypothyroidism early in life. Therefore these results support the hypothesis that the left hippocampus is more affected by a lack of TH during early life than the right.

Previous research addressing early hippocampal development may provide insight into the lateralized effects noted in CH. Evidence from rodent research has shown hemispheric differences in gene expression in the hippocampus during early development with a right to left shift of the expression of genes responsible for synaptic formation between postnatal day 6 and 9 (Moskal, Kroes, Otto, Rahimi, & Claiborne, 2006). In addition, a study of humans born preterm between 23 and 36 weeks gestation demonstrated that the right hippocampus is further along its developmental trajectory than the left (Bajic, Ewald, & Raininko, 2010). Together this evidence
may provide an explanation for the differential affect of CH to the left and right hippocampus. Since the hypothyroidism experienced by individuals with CH occurs during late gestation and early life, it is possible that the right hippocampus is more fully formed and thus is not as affected as the left by the lack of TH during the hypothyroid period.

In conclusion, although the results from the present studies do not support previous findings of memory weakness reported in the literature in other studies of CH, the results do support the hypothesis that altered hippocampal functioning, particularly on the left, is present within the population. The results suggest that if patients with CH exhibit memory problems, a history of more severe or poorly treated hypothyroidism is likely and hippocampal function will differ to a greater extent from controls than less severe cases. Since the hippocampus is implicated in CH, patients who experience memory problems may benefit from interventions aimed at implementing more non-hippocampally based memory strategies in order to improve function. For example, instead of relying on memory processes that are more hippocampal dependent such as free recall or associative memory, patients could learn to rely more on familiarity or cued recall of information through the use of more memory aides. In addition, mnemonic strategies utilizing visual imagery may improve memory in individuals with CH as this may serve to engage the more intact right hippocampus to a greater degree.

5.3 Limitations and Future Directions

Although the present studies provided evidence that early TH levels are important for memory abilities and hippocampal functioning at adolescence, there were some limitations to the study design and thus the implications of the findings. First, although the recruitment of altered hippocampal resources seen in both fMRI tasks may have supported accurate memory, it is possible that under more challenging circumstances, an increased need for resources would occur
and the system may no longer be able to adapt or compensate to maintain equivalent performance to controls. It is unclear how the hippocampus would function under such challenges and further research would be required. Another consideration is the sample recruited for the present research, which may not be representative of the general population of patients with CH given that the current cases were treated and followed by staff endocrinologists at Sickkids rather than family doctors in the community. It is possible that other groups of adolescents with CH may have poorer memory scores and different hippocampal activation that reflect differences in management of their condition. Similarly, TH levels at time of assessment and scanning, which could have affected cognitive functioning (Rovet, 2002; Song et al., 2001), were not determined. Other limitations concern the specific CH variables included in the current studies, which had some missing medical chart data for some measures (given the retrospective nature of these analyses) and the lack of precise measurement of when normalization of TH levels was achieved for each participant. Thus, the exact length and severity of each hypothyroid period could not be fully characterized.

Another consideration with respect to the present studies is that it is unclear how applicable the results from the clinical and fMRI tests are to how patients with CH function in more natural situations in their daily lives. Future research could investigate everyday memory abilities with more naturalistic tests or observations in which fewer supports and/or cues would be provided. This would allow a more comprehensive understanding not only of the hippocampus and memory functioning, but also how these skills are integrated into everyday scenarios and how the brain meets the complex challenges of daily life.

In addition, future studies could take a broader approach to the present research questions by a) including analyses of the whole brain and episodic memory network rather than focusing on the
hippocampus, b) exploring a multivariate approach such as a Partial Least Squares approach, and c) investigating the functional connectivity of the hippocampus and the various nodes of the episodic memory network. These analyses may provide greater insight to the changes caused by CH and how the brain compensates.

5.4 Conclusions
Although memory difficulties previously associated with CH were not found in the present studies, hippocampal functioning differed in adolescents with CH versus their TD peers. Within the CH group, accurate memory during the visuospatial and verbal associative memory tasks was supported by additional and different neural resources than the TD group. The different pattern of activation observed between groups on the verbal task suggests that the groups may be retrieving associations differently, with the CH group retrieving associations in a less flexible manner. In addition, memory performance and the degree to which individuals with CH had altered hippocampal functioning relative to controls was predicted by initial severity of CH. Interestingly, altered functioning was more consistently observed in the left hippocampus and the degree of early hypothyroidism more consistently predicted left hippocampal activity, thus supporting the contention that the left hippocampus may be more vulnerable than the right to the effects of CH. Overall, results suggest two key findings: a) early disease and treatment variables are important for predicting later memory abilities and hippocampal functioning in CH, and b) even if performance accuracy is maintained within a patient population, brain activity supporting performance may still be altered and reflect compensatory mechanisms. In conclusion, early disruption of the development of the hippocampus results in altered functioning later in life and engenders different neural strategies to support associative memory.
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