Hippocampal subfield volumes and relations to associative memory in children and adolescent survivors of pediatric brain tumors

By

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A thesis submitted in conformity with the requirements for the degree of Master of Arts
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Abstract

Pediatric brain tumor survivors (PBTS) treated with cranial radiation therapy (CRT) experience debilitating cognitive deficits, including impaired learning and memory. These impairments may be linked to volume abnormalities in the hippocampus. Animal studies suggest that radiation induces memory impairments in part, by disrupting neurogenesis in the hippocampal dentate gyrus. However, the effects of CRT on hippocampal subfields and relations to impaired memory in humans remain unknown. We used MRI and segmented hippocampal subfields (CA1, CA2/3, dentate gyrus/CA4, stratum radiatum, lacunosum, moleculare (SR/SL/SM) and subiculum) in PBTS and typically developing children using an automated segmentation tool. Hippocampal subfield volumes in PBTS were assessed in relation to memory performance. We observed that PBTS displayed smaller dentate gyrus/CA4, CA1, and SR/SL/SM volumes compared to typically developing children. Moreover, smaller dentate gyrus/CA4 and SR/SL/SM volumes correlated with lower memory performance. These findings suggest that damage to hippocampal subfields may in part underlie memory deficits in PBTS.
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Chapter 1
Background and Rationale

1 Introduction
1.1 Overview

Cranial radiation therapy (CRT) is a standard of care in the treatment of malignant brain tumors and has largely contributed to increasing survival rates over the past several decades (Merchant, Pollack, & Loeffler, 2010). However, CRT disrupts cognitive and brain development in young children and leads to debilitating deficits in executive functioning, attention, processing speed, quantitative skills and declarative memory (Mulhern, Merchant, Gajjar, Reddick, & Kun, 2004; Lassaletta, Bouffet, Mabbott, & Kulkarni, 2015). As a greater number of children survive from malignant brain tumors, mitigating the cognitive side effects of treatment has become increasingly critical. However, prior to developing neuro-protective or rehabilitative strategies, a better understanding of the underlying neuropathological mechanisms mediating cognitive dysfunction following brain cancer treatment is required.

Declarative memory impairment is a particularly devastating cognitive late effect of brain tumor treatment. Previous research investigating the neuropathology underlying impaired declarative memory has focused on the effects of brain tumor treatments on measures of whole hippocampal volumes. This research has demonstrated that brain tumors and their treatments are linked to long-term reductions in whole hippocampal volumes (Monje et al., 2013; Riggs et al., 2014; Jayakar, King, Morris, & Na, 2015). However, the hippocampus is comprised of distinct subfields that can be differentiated based on their unique cytoarchitecture (Insausti and Amaral, 2004). These subfields include the four cornus ammonus fields (CA1-4), the dentate gyrus, and the subiculum. Interestingly, hippocampal subfields have unique developmental profiles (Abrahám et al., 2010; Jabès & Nelson, 2015), are implicated in different types of hippocampal memory processing (Bakker et al., 2008; Jabès & Nelson, 2015), and exhibit distinct vulnerabilities to central nervous systems illnesses (Gemmell et al., 2012; Padurariu, Ciobica, Mavroudis, Fotiou, & Baloyannis, 2012). Animal models of radiation injury suggest that the dentate gyrus of the hippocampus may be particularly sensitive to radiation exposure (Raber et al., 2004; de Guzman et al., 2015). Irradiated rodents display smaller dentate gyrus volumes than
sham-treated rodents, and this effect is exacerbated by an earlier treatment age (de Guzman et al., 2015). Histological studies in rodents indicate that these changes may reflect disruption to the dentate gyrus microenvironment. For example, radiation is linked to neural precursor cell dysfunction, perturbed neurogenesis, increased apoptosis and neuroinflammation within the dentate gyrus (Tada, Parent, Lowenstein, & Fike, 2000; Mizumatsu et al., 2003). While animal models of radiation injury have examined localized damage in the hippocampal dentate gyrus, there is a lack of understanding of how cancer treatments affect hippocampal subfields in the developing human brain. Moreover, although animal models of radiation injury show that the dentate gyrus is vulnerable, other subfields may also be affected by CRT. For example, neuroinflammation, which is one biological response of the central nervous system to radiation, perturbs synaptic connectivity between the dentate gyrus and CA2 and CA3 (Llorens-Martín, Jurado-Arjona, Avila, & Hernández, 2015).

Elucidating the vulnerability of hippocampal subfields to CRT may inform our current understanding of radiation-induced memory impairment. Human neuroimaging studies (Bakker et al., 2008; Lacy et al., 2011; Yassa, Mattfeld, Stark, & Stark, 2011), as well as animal behavioral (Lee & Kesner, 2004; Jerman, Kesner, & Hunsaker, 2006) and neurophysiological studies (Leutgeb, Leutgeb, Moser, & Moser, 2007) suggest that hippocampal subfields play unique roles in memory processing. The dentate gyrus and CA3 have been implicated in encoding and pattern separation (Leutgeb, Leutgeb, Moser, & Moser, 2007; Deng, Aimone, & Gage, 2010; Yassa & Stark, 2011), a process that enables unique events to be encoded distinctly from one another. The CA3 and CA1 are implicated in retrieval and pattern completion (Hoang & Kesner, 2008; Hunsaker & Kesner, 2013), a process that enables partial cues in our environment to re-activate a complete memory. Mounting evidence also suggests that neurogenesis may contribute to hippocampal function (Deng, Aimone, & Gage, 2010; Ming & Song, 2011). In fact, reduced dentate gyrus neurogenesis in irradiated rodents results in impaired performance on spatial memory tasks (Raber et al., 2004). Given the heterogeneity of the hippocampus, it is critical to examine the impact of central nervous system injury during development at the level of hippocampal subfields. This thesis aims to do so, and is organized into three specific aims:
1. Examine the effects of brain tumor treatments, including CRT, on hippocampal subfield volumes in PBTS
2. Identify medical and demographic factors (age, sex, age at diagnosis, time since diagnosis, hydrocephalus) that predict hippocampal subfield volumes in PBTS
3. Examine the relationship between hippocampal subfield volumes and memory performance in PBTS

1.2 Aims
1.2.1 Aim 1

Radiation exposure perturbs the dentate gyrus microenvironment (Monje, Toda, & Palmer, 2003; Mizumatsu et al., 2003) and leads to smaller dentate gyrus volume in rodents (Hellström, Björk-Eriksson, Blomgren, & Kuhn, 2009; de Guzman et al., 2015). However, the impact of radiation on hippocampal subfields in humans is unknown. In Aim 1, we examined hippocampal subfield volumes in PBTS in relation to typically developing controls (TDC). An automated segmentation tool (MAGeT Brain) was used to segment hippocampal subfields (CA1, CA2/3, dentate gyrus/CA4, stratum lacunosum, stratum radiatum, stratum moleculare (SR/SL/SM) in both groups. Due to the challenge of distinguishing hippocampal subfield boundaries on standard MR images, a single label was derived for hippocampal subfields CA2 and CA3 (“CA2/3”), for the dentate gyrus and CA4 (“dentate gyrus/CA4”), and for the SR, SL and SM layers of the hippocampus (“SR/SL/SM”).

1.2.2 Aim 2

The severity of neurodevelopmental abnormalities following treatment for brain tumors is exacerbated by specific known risk factors, including female sex and young age at diagnosis (Mulhern, Merchant, Gajjar, Reddick, & Kun, 2004). The purpose of Aim 2 is to investigate the neurodevelopmental consequences of these risk factors on hippocampal subfields. In Aim 2, we examine the influence of age, sex, age at diagnosis, time since diagnosis and hydrocephalus treatment on hippocampal subfield volumes in PBTS. To provide a benchmark for the ‘normal’ effects of demographic factors on hippocampal subfield volumes, we also examine the effect of age and sex on subfield volumes in TDC. Findings from Aim 2 provide insight into the risk factors that exacerbate neurodevelopmental abnormalities in hippocampal subfields following
brain tumor treatment. In doing so, predictors of post-treatment neurodevelopmental outcomes in hippocampal subfields are identified.

1.2.3 Aim 3

No study to date has examined the relationship between perturbed hippocampal subfield development and memory function in PBTS. In Aim 3, hippocampal subfield volumes in the left hemisphere that are identified as smaller in PBTS than TDC are examined in relation to verbal associative memory performance. Findings from Aim 3 may provide insight into the neuroimaging correlates of impaired memory in PBTS.

1.3 Declarative Memory

Human memory is not a unitary construct, but is instead comprised of multiple systems. The terms ‘declarative’ and ‘explicit’ memory refers to the process of engaging in conscious recall of events or facts. Declarative memory is differentiated from non-declarative or implicit memory (“knowing how”), including skill based learning, simple conditioning, and priming (Squire & Wixted, 2011). Lesion studies demonstrate that these memory systems can operate independently and rely on separate underlying structures. The hippocampal system and medial temporal lobe structures are particularly critical for declarative memory (Squire, Stark, & Clark, 2004). Damage to the medial temporal lobe can impair declarative memory, but preserve implicit memory (Scoville and Milner, 1957; Graf, Squire, & Mandler, 1984). Conversely, damage to structures that support implicit memory can spare declarative memory (Gabrieli, Fleischman, Keane, Reminger, & Morrell, 1995; Bechara et al., 1995).

Declarative memory is further subdivided into two forms: episodic and semantic memory (Squire, Stark, & Clark, 2004). Episodic memory supports the capacity to recall or ‘re-live’ one’s own past experiences and is accompanied by rich contextual details, such as the time and the place that an event occurred. Semantic memory refers to fact-based knowledge about the world and is not accompanied by contextual details or a feeling of ‘re-living’ a prior event. Phenomenologically, what differentiates semantic and episodic memory is that episodic memory is accompanied by rich contextual details (e.g., recollecting events tied to the context of a beach
vacation), whereas semantic memory is non-contextual (e.g., knowing the name of the beach) (Moscovitch, Cabeza, Winocur, & Nadel, 2016).

An effective declarative memory system depends on specific processes including precise encoding and recollection. Encoding refers to a process in which information that is consciously attended to is transformed into representations that can be accessed in memory (Davachi & Dobbins, 2008). Recollection refers to a conscious “re-experiencing” of a previously encoded event. Recollection can occur spontaneously such as when memories ‘pop’ into mind. However, recall often requires retrieval processes. Retrieval processes depend on interactions between goal-directed control processes in the prefrontal cortex, and declarative memory systems in the medial temporal lobe (Davachi & Dobbins, 2008).

Neuropsychological evaluation of declarative memory is achieved using a variety of tasks that assess memory for different types of material. For example, declarative memory tasks may test memory for verbal, visual, and/or visual-spatial information. Moreover, declarative memory tasks can measure multiple forms of memory processing, including free recall, recognition and familiarity. General index scores are typically derived from performance on multiple subtests. These subtests assess memory for specific types of information (i.e.: verbal, visual information) and examine specific forms of memory processing (i.e.: free recall, recognition, familiarity). As such, general index scores may depend on multiple underlying structures that support declarative memory. On the other hand, subtests that assess specific forms of memory processing (i.e.: recollection for visual items) may be more sensitive to pathology in specific structures. For example, tasks that assess relational memory over short and long delays may be sensitive to hippocampal pathology (Squire & Wixted, 2011, Olsen et al., 2012; Yee, Hannula, Tranel, & Cohen, 2014). In contrast, tasks that assess familiarity for visually presented items may be sensitive to perirhinal cortex damage (Bowles et al., 2007).

1.4 The Role of the Hippocampus in Declarative Memory

Human neuropsychological studies have demonstrated that the hippocampus is necessary for episodic memory formation. The most striking feature of hippocampal lesions is severe anterograde amnesia, or an inability to form context-rich episodic memories (Scoville and
Milner, 1957). Interestingly, patients with developmental amnesia are capable of learning new facts (Vargha-Khadem et al., 1997), although are unable to remember the context in which the facts were learned. Despite an inability to form long-term memories, amnesiacs are capable of remembering information for short time periods (30 seconds). These findings have led to a theory of hippocampal function that states that the hippocampus is specialized for long-term but not short-term memory. However, this account is unsupported by behavioral studies in amnesiacs (Giovanello, Verfaellie, & Keane, 2003; Turriziani, Fadda, Caltagirone, & Carlesimo, 2004; Quamme et al., 2007) and neuroimaging studies (Giovanello, Schnyer, & Verfaellie, 2004), which suggest that the hippocampus may be involved in binding arbitrary aspects of our experience, regardless of the retention interval. These studies suggest that hippocampal involvement may reflect the degree to which relational binding is required. This ‘relational view’ is supported by evidence that hippocampal damage leads to a disproportionate impairment in memory for arbitrary relationships, compared to memory for single, and even unitized items (Giovanello, Verfaellie, & Keane, 2003; Quamme et al., 2007). Therefore, during episodic memory formation, the hippocampus may bind unrelated elements of our experiences (the people present, their actions, and a place) together in memory (Eichenbaum, 2004; Moscovitch, Cabeza, Winocur, & Nadel, 2016).

Importantly, declarative memory processes differ in the extent to which relational binding is required (Mayes, Montaldi, & Migo, 2007). For example, autobiographical memory requires a high degree of relational binding (associating people and sequences of actions to a context), whereas memory for single items requires a low degree of relational memory processing (associating a single item to an invariant context). Hippocampal function may be assessed using tasks that require the formation of relational associations. These include autobiographical memory tasks, or tasks in which arbitrary items must be associated, such as word-word or object-word associations (Moscovitch, Cabeza, Winocur, & Nadel, 2016).

1.5 Hippocampal Anatomy and Circuitry

The hippocampus is located at the end of an information processing hierarchy and receives projections from numerous separate neocortical association areas (Eichenbaum, 2004).
Information perceived in the neocortex is relayed to the perirhinal cortex and parahippocampal cortices, which project to the entorhinal cortex the major input into the hippocampus. The hippocampus itself is comprised of histologically distinct subfields, including CA1-4, dentate gyrus, and subiculum. These subfields are highly interconnected through a system of distinct synaptic circuits (Amaral & Witter, 1989). For example, in the trisynaptic circuit (dentate gyrus – CA3 – CA1) (Figure 1), input from the entorhinal cortex enters the dentate gyrus, and is relayed in a unidirectional manner to the CA3 (via the mossy fibers) and then to the CA1 (via the shaffeur collaterals) (Neves, Cooke, & Bliss, 2008). Inputs from CA1 are then relayed back to the entorhinal cortex, or to the subiculum (Figure 1) (Andersen, P., 2007). Projections from CA1 and subiculum to the entorhinal cortex complete the hippocampal information processing loop.

The hippocampus also contains other synaptic networks that are critical for information processing. For example, CA3 neurons display a high degree of synaptic connectivity with one another via a system of ‘recurrent collateral’ projections. Based on computational models, these recurrent collateral projections may operate as a single network within CA3 (commonly termed the ‘auto-associative’ network) (Wiskott, Rasch, & Kempermann, 2006). In addition, the entorhinal cortex has independent projections to CA3, CA1 and subiculum (Andersen, P., 2007) (Figure 1).

Notably, information processing in the hippocampus depends on a high degree of synaptic connectivity between regions. The SR/SL/SM layers contain synaptic contacts that mediate communication between hippocampal subfields (Amaral & Witter, 1989). For example, the SL and SM regions harbor synaptic contacts that give rise to the perforant pathway that connects the entorhinal cortex layer II to the CA1 (Figure 1). Moreover, the SR region contains synaptic connections that form the recurrent collateral “CA3-CA3” synaptic contacts, the “CA3-CA1” synaptic “shaffeur collateral” connections, as well as projections from the entorhinal cortex layer II to CA3 perforant pathway (Andersen, P., 2007). In neurological populations, hippocampal abnormalities localized to the SR/SL/SM regions may reflect impaired hippocampal synaptic connectivity. In fact, patients with Alzheimer’s Disease exhibit reduced volume in the SR/SL/SM (Kerchner et al., 2013). Critically, volumetric abnormalities in hippocampal neuropil have been associated with poorer hippocampal-dependent memory performance (Kerchner et al., 2012).
1.6 Hippocampal Subfields and Memory

Human neuroimaging studies (Bakker et al., 2008; Lacy et al., 2011; Yassa, Mattfeld, Stark, & Stark, 2011) and neuro-computational models (Wiskott, Rasch, & Kempermann, 2006) suggest that hippocampal subfields provide unique contributions to declarative memory processing. The unique processing mechanisms mediated by subfields are thought to be critical for successful encoding and retrieval of information. These processing mechanisms include pattern separation (which occurs during encoding), associative binding, and pattern completion (which occurs during retrieval) (Rolls, 2013). Neuro-computational models suggest that pattern separation enables distinct, but highly similar memories to be encoded independently from one another (Wiskott, Rasch, & Kempermann, 2006). By encoding highly similar experiences independently, interference between similar memories may be prevented. As a primary input into the hippocampus, it is thought that the dentate gyrus (and perforant path connecting the entorhinal cortex to the dentate gyrus) is particularly suited for facilitating encoding and pattern separation processes (Yassa, Mattfeld, Stark, & Stark, 2011; Hunsaker & Kesner, 2013).
Theories of pattern separation state that neural inputs from the entorhinal cortex are encoded in unique, non-overlapping sets of dentate gyrus neurons (Hunsaker & Kesner, 2013). Accordingly, damage to the dentate gyrus would be expected to lead to imprecise encoding of neural input that ultimately results in interference between memories (Deng et al., 2010; Bakker et al., 2008). Functional neuroimaging studies in humans provide evidence that activity consistent with pattern separation occurs in the dentate gyrus (Bakker et al., 2008). Moreover, structural neuroimaging studies suggest that damage to the perforant path connecting the entorhinal cortex to the dentate gyrus and CA3, and microstructural changes in dentate gyrus/CA3 may predict pattern separation deficits in older adults (Yassa, Mattfeld, Stark, & Stark, 2011).

According to computational models of pattern separation and completion, distinct inputs encoded in the dentate gyrus are relayed to CA3. The abundance of recurrent collateral connections between CA3 neurons allow CA3 to operate as an ‘auto-associative network’ enabling associations to be generated between encoded input (Wiskott, Rasch, & Kempermann, 2006). Partial cues can then activate part of the network in CA3, which may lead to activation of an entire circuit that represents a whole memory. The activation of a complete memory following a partial cue is a process that is termed ‘pattern completion’. CA1 may be particularly important for memory retrieval by way of pattern completion processes. In particular, CA1 is thought to be critical for decoding activity in CA3 associative networks to enable retrieval of a complete memory (Wiskott, Rasch, & Kempermann, 2006).

1.7 Hippocampal Neurogenesis

Neural precursor cells in restricted regions of the brain continue to generate new neurons throughout the lifespan in a process known as adult neurogenesis. This process occurs in both the olfactory bulb (in rodents only) and the subgranular zone of the hippocampal dentate gyrus (in rodents and humans) (Ming & Song, 2011). Proliferating neural precursor cells in the subgranular zone give rise to immature cells that differentiate into dentate granule neurons (Zhao et al., 2006; Deng et al., 2010). After only a few days, these immature neurons form axons that project to CA2 and CA3 (Ming & Song, 2011; Llorens-Martín, Jurado-Arjona, Avila, & Hernández, 2015). The dentate gyrus microenvironment may be particularly permissive to the differentiation and integration of newborn neurons. For example, proliferating neural precursor
cells reside close to hippocampal microvasculature, which releases factors that promote neurogenesis (Zhao et al., 2006).

Hippocampal neurogenesis has received considerable attention as recent investigations suggest newborn granule neurons contribute to hippocampal-dependent learning and memory (Zhao, Deng, & Gage, 2008). Ablating neurogenesis in animals results in hippocampal-dependent memory deficits that are consistent with impaired pattern separation (Clelland et al., 2009). Interestingly, increasing neurogenesis in animals enhances discrimination between highly similar stimuli in memory (Yassa and Stark, 2011; Sahay et al., 2011). Neuro-computational models also support the theory that dentate gyrus neurogenesis contributes to memory through enhancing pattern separation. These theories state that a constantly available supply of newborn neurons (generated through neurogenesis) ensures that a vast number of new experiences can be effectively encoded in memory (Wiskott, Rasch, & Kempermann, 2006).

1.8 Neuroanatomical Segmentation of Hippocampal Subfields

Neuroanatomical segmentation of hippocampal subfields is typically performed using high-resolution structural magnetic resonance images (MRI) (Pipitone et al., 2014). Manual tracing is considered the ‘gold standard’ of segmentation methods and involves an expert manually tracing the hippocampal anatomy (Chakravarty et al., 2013). However, manual segmentation is time consuming and therefore impractical with large datasets (Chakravarty et al., 2013). To overcome this issue, automated atlas-based segmentation methods are increasingly being used to segment hippocampal subfields (Pipitone et al., 2014). Atlas-based approaches use a single or multiple manually labelled atlases to segment unlabeled MR images automatically. In multi-atlas based segmentation, manually derived labels from each atlas are propagated to unlabeled images in a dataset. This produces multiple labels for each image in the dataset, that are then fused to produce a single label for a structure on each image (Pipitone et al., 2014). Many existing multi-atlas approaches have been shown to be a reliable alternative to manual segmentation (Pipitone et al., 2014).
1.9 Current Treatment Strategies for Pediatric Brain Tumors

The majority of central nervous system tumors diagnosed within the first decade of life arise in the posterior fossa (PF) (Habrand & De Crevoisier, 2001). Due to a tendency for pediatric tumors to disseminate within the central nervous system, surgical removal of the tumor is often an ineffective measure for eliminating the cancer (Merchant et al., 2010). CRT is considered a highly effective treatment option, as many pediatric tumors are highly radiosensitive. Consequently, CRT has been widely adopted in treatment protocols for malignant pediatric brain tumors and has shown to be highly effective for tumor eradication. Consequently, treatment for pediatric brain tumors typically involves surgical resection, with or without adjuvant chemotherapy, and CRT (Mueller & Chang, 2009). CRT has largely been attributed to increasing survival rates over the past several decades, however the majority of survivors treated with CRT exhibit life-long cognitive deficits.

1.10 Declarative Memory Outcomes in Pediatric Brain Tumor Survivors

All forms of central nervous system treatment, including CRT, chemotherapy and surgery, as well as the injury sustained from the tumor itself may contribute to cognitive decline (Levisohn, Cronin-Golomb, & Schmahmann, 2000; Dietrich, Monje, Wefel, & Meyers, 2008). However, compared to children treated without CRT, children treated with CRT experience more severe cognitive deterioration (Ellenberg et al., 2009; Mulhern, Merchant, Gajjar, Reddick, & Kun, 2004). The severity of cognitive impairment is linked to the total dose and volume of radiation delivered to the central nervous system as well as that delivered to the medial temporal lobe (Abayomi, 2002; Mulhern, Merchant, Gajjar, Reddick, & Kun, 2004; Tallen et al., 2015). Amongst the cognitive deficits exhibited by PBTS, declarative memory impairment is thought to be particularly debilitating. Impaired declarative memory is present in the majority of children treated with CRT (Monje & Fisher, 2011) and is thought to contribute to poor academic achievement (Dennis et al., 1992).
Declarative memory outcomes in PBTS are often assessed using standardized clinical batteries that provide age-normed indices for both verbal and visual information (Dennis et al., 1992; Spiegler, Bouffet, Greenberg, Rutka, & Mabbott, 2004; Nagel et al., 2006; Riggs et al., 2014). One disadvantage of this method is that studies often report general index scores derived from performance on multiple subtests. As noted, general index scores may reflect broad measures of functioning but may be insensitive to specific underlying neuropathology (e.g.: hippocampal damage). However, standardized clinical batteries allow for comparisons to be drawn between clinical samples and the normative population. This method has enabled memory outcomes for PBTS to be evaluated in relation to population averages in both cross-sectional (Riggs et al., 2014; Dennis et al., 1992; Nagel et al., 2006; Kieffer-Renaux et al., 2000), and longitudinal studies (Spiegler, Bouffet, Greenberg, Rutka, & Mabbott, 2004; Mabbott et al., 2011).

PBTS exhibit memory deficits for both verbal and non-verbal information (Nagel et al., 2006; Riggs et al., 2014). Longitudinal evaluations demonstrate the long-term nature of impaired declarative memory in PBTS (Spiegler, Bouffet, Greenberg, Rutka, & Mabbott, 2004; Mabbott et al., 2011). In fact, studies with adult survivors of pediatric brain tumors who were treated as children indicate that these memory deficits persist into adulthood (Ellenberg et al., 2009; Monje et al., 2013).

1.11 Pathogenesis and Radiation-Induced Memory Impairment

The onset of radiation-induced cognitive deterioration can be delayed up to 6 months to a year following the completion of treatment (Mulhern, Merchant, Gajjar, Reddick, & Kun, 2004; Dietrich, Monje, Wefel, & Meyers, 2008). Perplexingly, in some cases impaired memory occurs in the absence of obvious neuropathology (Monje & Fisher, 2011). This suggests that radiation may cause subtle pathological changes that significantly impact cognition (Mulhern & Palmer, 2003).

Brain regions such as the hippocampus that continually undergo neurogenesis throughout the lifespan are particularly sensitive to radiation-induced neurotoxicity (Monje, Mizumatsu, Fike, & Palmer, 2002; Hellström, Björk-Eriksson, Blomgren, & Kuhn, 2009). For example, radiation exposure decreases neurogenesis in the dentate gyrus for up to months in adult rodents.
(Monje, Mizumatsu, Fike, & Palmer, 2002). Human post-mortem histological analysis of hippocampal tissue has revealed significant decreases in neurogenesis in patients treated for brain tumors, even decades following treatment with radiation. In animals, decreases in neurogenesis are linked to long-term performance deficits on hippocampal-dependent memory tasks (Madsen, Kristjansen, Bolwig, & Wörtwein, 2003; Raber et al., 2004). These animal findings have led to the speculation the damage to the dentate gyrus micro-environment may contribute to hippocampal-dependent memory deficits following CRT in humans.

Despite the evidence that radiation induces hippocampal dysfunction in animals, only one study has assessed hippocampal development longitudinally in PBTS. This study showed that PBTS exhibited bilateral hippocampal volume loss that continued for 2-3 years following treatment, after which hippocampi returned to a positive normal growth pattern (Nagel et al., 2004). However, whether increases in volume compensated for the initial volume loss is unclear. Moreover, as evidenced by animal studies, hippocampal subfields may exhibit differential patterns of recovery following CRT.

1.12 Moving Forward

An investigation into the long-term impact of CRT on hippocampal subfield development and relations to memory impairment in PBTS is warranted. Animal studies suggest that radiation impairs the dentate gyrus microenvironment and leads to volume deficits. However, other subfields, including CA2 and CA3 may also be sensitive to disease mechanisms triggered following radiation. As previously noted, synapse formation between neurons in the dentate gyrus and CA2 and CA3 may be disrupted due to neuroinflammation caused by CRT. Thus, it is hypothesized that:

1) PBTS will exhibit smaller CA2/CA3 and dentate gyrus/CA4 volumes than TDC
2) Age will positively correlate with CA2/3 and dentate gyrus/CA4 volumes in TDC, but not in PBTS, reflecting disrupted volumetric development following exposure to brain cancer treatment in PBTS
3) Smaller subfield volumes in PBTS will correlate with lower verbal associative memory performance
2 Methods

2.1 Participants

The sample included 29 PBTS treated with CRT and 30 TDC (Table 1). Participants were recruited between 2009 and 2015 as part of three larger studies at the Hospital for Sick Children. The protocols for these studies were approved by the Research Ethics Board. PBTS were identified through the brain tumor program at the Hospital for Sick Children and recruited by a study coordinator during a clinic visit or through letter mailings. TDC were recruited through hospital advertisements, through the families of PBTS (i.e.: they were siblings of PBTS) and through family and friends of investigators and study team members. Participants were eligible if they were between 5 and 18 years of age, had either English as their native language or had at least two years of schooling in English at the time of participation, did not require sedation for MR imaging, and were free of any metal implants (i.e.: braces, cochlear implants). TDC were eligible if they had no history of traumatic brain injury, neurological disorder, developmental delay, or learning disability. PBTS were eligible if they had been diagnosed with a brain tumor at least one year prior to participation, had been treated with CRT, were not receiving palliative care or treatment for recurrent disease at the time of participation, and had no premorbid history of neurological disorder or learning disability. Each participant or their parent (where applicable) provided written informed assent or consent prior to participation.

2.2 Patient Medical Variables

Table 1 details patient medical variables. The PBTS were treated for medulloblastoma (n=26), pineoblastoma (n=2) and recurrent ependymoma (n=1). The medulloblastoma survivors were treated with CRT (plus a boost to either the tumor bed (TB) or posterior fossa (PF)), surgery and adjuvant chemotherapy. This was the case for all medulloblastoma survivors, except for one who was treated without chemotherapy. Although ependymomas are typically effectively treated without CRT, the ependymoma survivor in our sample was treated with CRT (plus a boost to the PF) and surgery due to tumor recurrence. This patient participated in the study following completion of treatment for recurrent tumor. The two pineoblastoma survivors in the sample
were treated with CRT plus a boost to the TB, (located in the third ventricle in one patient and the pineal region in the other), adjuvant chemotherapy, and a surgical resection or a biopsy.

**Table 1. Demographic and medical information.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PBTS $n=29$</th>
<th>TDC $n=30^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male: Female (No.))</td>
<td>17:12</td>
<td>17:13</td>
</tr>
<tr>
<td>Age at assessment (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>12.95 (3.75)</td>
<td>12.92 (3.55)</td>
</tr>
<tr>
<td>Range</td>
<td>7.5 – 18.99</td>
<td>5.15 –18.93</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Right</td>
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<td>27</td>
</tr>
<tr>
<td>Left</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
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<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
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<td></td>
</tr>
<tr>
<td>Range</td>
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<tr>
<td>Time since diagnosis to assessment (years)</td>
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<td></td>
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<tr>
<td>Mean (SD)</td>
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<tr>
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<td></td>
</tr>
<tr>
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<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Pineoblastoma</td>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
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<td></td>
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</tr>
<tr>
<td>Pineal region</td>
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<tr>
<td>Surgical Outcome/Extent of resection</td>
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<td></td>
</tr>
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<td>Greater than 95% of the tumor resected</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Between 50-95% of the tumor resected</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
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<td></td>
</tr>
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<td>Hydrocephalus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No hydrocephalus</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>
Hydrocephalus requiring treatment 16

Radiation dose and type

Head/Spine + PF boost (No.) 18
  Range head/spine (Gy.) 23.4 – 36
  Range PF (Gy.) 18 – 55.8

Head/spine + tumor bed (No.) 11
  Range head/spine (Gy.) 18 – 36
  Range TB (Gy.) 18 – 54.8

Chemotherapy (No.) 27

* The TDC did not differ significantly from the PBTS group in terms of sex ($\chi^2(1) = 0; p=1$), age at the time of assessment ($t(56.6)=-0.03, p = 0.98$) or handedness ($\chi^2(1) = 0.19; p=0.67$)

**Chemotherapy agents included Cisplatin, Cyclophosphamide, Vincristine, Lomustine, Etoposide, Amifostine

2.3 Procedure

Each study visit included a 3T structural MRI scan. A subset of the PBTS (n=11) also completed memory testing with the Children’s Memory Scale (CMS) or Wechsler’s Memory Scale (WMS III) during the same study visit. Demographic and medical variables for this subset of participants can be found in Table 3. Participants were compensated for their participation with a gift card or a toy valued at approximately $15. Reimbursement for lunch and transportation was also provided.

2.4 MRI Acquisition and Post-Processing

T1-weighted MR imaging data were acquired at the Hospital for Sick Children using a Siemens 3T whole body scanner with a 12 channel head coil. The scanning protocol included a 3D Magnetization prepared rapid acquisition gradient echo imaging (3D MPRAGE) sequence. The scan parameters used to acquire the T1-weighted images included TR = 2300ms, TE = 3.91ms, inversion time = 900ms, voxel size = 1mm isotropic, number of excitations = 1, pixel bandwidth = 240, acquisition matrix = 256x224, 160 contiguous axial slices, FOV= 256x224mm, Flip angle 9°.

All T1-weighted dicom files were converted to Analyze 7.5 file format. FSL’s Brain Extraction Tool (BET) was used to generate a preliminary outline on each MR image that
separated the brain from non-brain tissue (i.e.: the skull and extra-cerebrospinal fluid) (Smith, 2002; Jenkinson & Smith, 2005). This outline was manually corrected, slice by slice, in the axial plane and was used to obtain estimates of intracranial volume (ICV) for each subject, which was controlled for in all subsequent statistical analyses.

For hippocampal subfield segmentations, T1-weighted MR data of brains within skulls were converted to MINC file format, such that they were compatible with the MAGeT Brain segmentation tool (Chakravarty et al., 2013).

2.5 Hippocampal Subfield Segmentation

Hippocampal subfield segmentations were performed using Multiple Automatically Generated Templates for different brains (MAGeT Brain), a multi-atlas based automated segmentation algorithm (Pipitone et al., 2014; Chakravarty et al., 2013). Whole hippocampal segmentations produced by MAGeT Brain on 1.5T images correspond more accurately to manual segmentations than other automated methods, such as FreeSurfer and FSL FIRST (Pipitone et al., 2014). Furthermore, hippocampal subfield segmentations on 3T images produced by MAGeT Brain show an acceptable degree of overlap with manually derived segmentations (Pipitone et al., 2014).

Figure 2 illustrates each step in the MAGeT Brain algorithm. The MAGeT Brain algorithm generates labels in three primary steps. First, manually defined atlas labels\(^1\) of hippocampal subfields are propagated to a subset of unlabeled images in the dataset. These images become ‘template’ images that comprise a ‘template library’\(^2\). This first step requires that each image chosen as a template is aligned to each of the atlas images through non-linear registration. A non-linear transformation is estimated via each atlas-to-template non-linear registration, and is applied to each atlas label. This generates numerous labels for each subfield on the template images (Figure 2a). Specifically, the number of template labels generated for each subfield is equal to the number of input atlases. In the second step, the newly defined

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\(^1\) Atlas labels refer to previously defined manual segmentations on high resolution MR images

\(^2\) Template library refers to a subset of MR images from the dataset being analyzed. The template images are segmented automatically using the atlas labels
template labels are propagated to the entire set of images being analyzed in the same fashion as in step one (Figure 2b). Specifically, each template image is registered to the entire dataset of images. Next, a non-linear transformation estimated and applied to each template label. This generates numerous labels for each subfield. The newly-generated labels are then fused to produce a single label for each subfield (Figure 2c).

Figure 2. Depiction of the steps involved in segmenting hippocampal subfields using MAGeT Brain.

For the purposes of this study, five T1-weighted atlases were used for hippocampal subfield segmentation (Winterburn et al., 2013). These atlases are 3T MR images that have an isotropic voxel dimension of 0.3 mm and were acquired from healthy adult volunteers between 29 and 57 years of age (2 males, 3 females). The atlases include manually defined labels for CA1, CA2 and CA3 (CA2/3), dentate gyrus and CA4 (dentate gyrus/CA4), stratum radiatum, stratum lacunosum, stratum moleculare (SR/SL/SM) and the subiculum. The atlas
Segmentations were derived manually based on contrast differences on the MR images. However, in the case that contrast differences were not apparent, subfield boundaries were approximated based on hippocampal subfield boundaries described in Duvernoy, 2005 and Mai et al., 2008.

For the present analysis, the five atlases were aligned to twenty-one template images via non-linear registration (Chakravarty et al., 2013). A non-linear transformation was then applied to each atlas label, which generated five labels for each subfield on each template image. Next, each template image was registered to each unlabeled subject image in the dataset (n=59) through non-linear registration. A non-linear transformation was then applied to each template label. This procedure yielded 105 separate labels for each subfield (5 atlas labels x 21 template labels) on each of the 59 subject images. The label fusion step described previously was then used to generate a single label for each subfield on each image. The fusion method involves a voxel-by-voxel voting procedure, in which the most frequently occurring label (i.e.: the mode label) at each voxel covering the hippocampus contributes towards the final label for each subfield.

As far as we know, MAGeT Brain has never been used to quantify hippocampal subfields in a cohort of patients with extensive gross brain abnormalities. Thus, we sought to examine the quality of the final labels produced using two unique template libraries to optimize the quality of the final labels. The majority of PBTS in our sample exhibit cerebellar damage from tumor growth and surgical resection, as well as ventricular enlargement resulting from hydrocephalus (a common neurological complication of brain tumors). We aimed to determine whether including PBTS scans in the template library compromised the quality of the final labels. MAGeT Brain was therefore run once using a template library comprised of TDC only, and a second time using templates comprised of both PBTS and TDC. Aside from the images included in the template library, all other parameters (i.e.: number of atlases and templates, registration and fusion methods) remained identical between each run.

Figure 3 depicts an example of the MAGeT Brain segmentation for a representative subject. Each of the final labels underwent a visual quality inspection performed by an expert rater. This inspection included rating each label on accuracy (i.e.: whether the label covered the hippocampus, but not extra-hippocampal areas) and coverage (i.e.: whether the label adequately
covered the hippocampal anatomy). Visual inspection revealed that the labels generated using TDC scans as templates were of higher quality than those generated using both PBTS and TDC scans. Thus, subsequent analyses were conducted on labels produced using a template library comprised of TDC. None of the final labels in this set had to be excluded due to poor accuracy or coverage.

**Figure 3. MAGeT Brain hippocampal subfield segmentation for a representative subject.**

Prior to analysis, raw volumes were adjusted for individual differences in ICV (Free, et al., 1995). Regression coefficients were calculated based on the relationship between measures of ICV and hippocampal subfield volumes. This regression coefficient was used to adjust each subfield volume for ICV (see formula for adjusting raw hippocampal subfield volumes below). This step is crucial as smaller raw subfield volumes in PBTS may simply reflect smaller overall brain volumes. Group differences in subfield volumes that have been adjusted for ICV may reflect a vulnerability of hippocampal subfields to brain tumor treatments.  

**ICV correction formula:**  
Raw volume – (beta value * (Individuals’ ICV - Grand Mean ICV of the sample = Adjusted volume))

Once each subfield was adjusted for ICV, bilateral hippocampal volumes were calculated. This was accomplished by summing the volume of a given subfield in the left

---

3 MAGeT Brain also provides segmentations for amygdalae, which were not analyzed for the purposes of this study
hemisphere with the corresponding subfield volume in the right hemisphere (i.e.: left CA1 + right CA1 = bilateral CA1).

2.6 Memory Assessment

A trained research coordinator administered the CMS or WMS to a subset of PBTS (n=11). PBTS younger than 16 years of age completed the CMS (n= 5), whereas PBTS 16 years and older (n = 6) completed the WMS-III. Both standardized tools are well validated and have high internal consistency and reliability (Vaupel, 2001; Lo, Humphreys, Byrne, & Pachana, 2012). Although the CMS and WMS are comprised of numerous subtests that measure different aspects of declarative memory performance (visual, visuospatial and verbal memory), in the present study, only performance on the verbal paired associates subtest of the CMS and WMS were examined. This task was chosen because it places a high demand on associative memory processing, which is thought to depend on hippocampal function (Olsen, 2012). Thus, this task is considered more of a ‘pure’ hippocampal task in relation to tasks assessing memory for single items.

During the verbal associate word pair task of the CMS, the examiner verbally presents a list of 14 word pairs to the participant. The examiner then provides the first word of each word pair and the participant is asked to verbally provide the second word from memory. This is repeated twice. A verbal paired associate raw ‘learning score’ is calculated based on the total number of correctly recalled word pairs over the course of three trials. Similarly, during the corresponding task on the WMS, the examiner verbally presents a list of 8 word pairs to the participant, followed by the first word of each pair. The participant is asked to provide the second word pair from memory. This is repeated three times. A verbal paired associate raw ‘verbal paired I’ score is calculated based on the total number of correctly recalled word pairs over the course of four trials. Age-normed standard scores are derived from raw scores on each task.
2.7 Statistical Analyses

All analyses were conducted using R statistical software. P-values equal to or less than 0.05 were considered statistically significant. Separate analyses were conducted for each aim and are described below.

2.7.1 Do PBTS have Smaller Dentate Gyrus/CA4 and CA2/3 Volumes than TDC?

The entire sample of 59 participants (PBTS = 29, TDC = 30) were included in this analysis. To ensure that each group was demographically similar, chi square analyses were conducted to assess group differences in sex and handedness. Additionally, T-tests were conducted to determine whether there were significant age differences between groups.

A repeated measures analyses of variance was used to investigate group differences in hippocampal subfield volumes, with group (PBTS, TDC) as the between subject factor, and hemisphere (right, left) and subfield (CA1, CA2/3, dentate gyrus/CA4, SR/SL/SM, subiculum) as within-subject factors. As part of the omnibus analysis, planned tests of simple effects were used to examine the a priori hypothesis that PBTS have smaller dentate gyrus/CA4 and CA2/3 than TDC.

2.7.2 What are the Demographic and Medical Predictors of Hippocampal Subfield Volumes in PBTS and TDC?

The relationship between subfield volumes and demographic variables were assessed for each group separately. T-tests were used to examine sex differences in hippocampal subfield volumes. Pearson’s r correlations were used to examine relationships between age and subfield volumes.

The effects of medical variables (age at diagnosis, time since diagnosis, and hydrocephalus) on subfield volumes were examined in PBTS using either Pearson’s r correlation or T-tests. Specifically, Pearson’s r correlations were used to assess the effects of age at
diagnosis and time since diagnosis on subfield volumes. T-tests were used to examine the effects of hydrocephalus treatment on subfield volumes. All contrasts were adjusted for the family-wise error rate using the false discovery rate correction. Contrasts surviving 5% false discovery rate correction were considered statistically significant.

An exploratory analysis was conducted to directly examine the consequences of an earlier vs. later diagnosis age on volumes in relation to TDC. In this analysis, PBTS were stratified by a diagnosis age of before and after 6 and compared to TDC. This analysis provides information beyond simply correlating volumes with diagnosis age in PBTS, as volumes in PBTS diagnosed at < 6 and > 6 are examined in relation to ‘normal’ volumes in TDC. Five one-way ANOVAs were conducted with group as the independent variable (PBTS diagnosed at less than 6 (n=14), PBTS diagnosed at greater than 6 (n=15), and TDC (n=30)) and bilateral subfield volumes as the dependent variables. In the case that omnibus effects were observed, planned post-hoc T-tests were conducted to identify statistically significant group differences in subfield volumes.

2.7.3 Do Smaller Left Hippocampal Subfields in PBTS Correlate with Impaired Memory?

Only PBTS who completed memory testing (n=11) were included in this analysis. Volumes of subfields in the left hemisphere that were identified as smaller in PBTS (based on previous analyses) were correlated with memory performance using Pearson’s r correlations. Standard scores derived from the WMS and CMS verbal associative memory task were the measure of memory performance. Standard rather than raw scores were assessed because the WMS and CMS verbal memory tasks differ with regards to the number of word pairs and trials, and the types of words pairs presented to the participant. Standard scores were derived from the verbal paired ‘learning’ raw CMS score and the ‘verbal paired I’ raw WMS. All contrasts were adjusted for the family-wise error rate using false discovery rate correction and contrasts surviving 5% false discovery rate correction were considered statistically significant.
3 Results

3.1 Group Differences in Hippocampal Subfield Volumes

The chi square analyses indicated that groups did not differ in terms of sex ($\chi^2(1) = 0; p=1$) or handedness ($\chi^2(1) = 0.19; p=0.67$). Additionally, age at assessment was not different between groups ($t(56.6) = -0.03, p = 0.98$).

The repeated measures ANOVA revealed a significant main effect of group ($F(1, 57) = 19.07; p < 0.001$), subfield ($F(4, 228) = 2431, p < 0.001$) and hemisphere ($F(1, 57) = 9.7, p < 0.002$). There was a significant subfield × group interaction ($F(4, 228) = 3.94; p < 0.004$), but no significant group × subfield × hemisphere interaction ($F(4, 228) = .9; p = .47$). Planned analyses of simple effects were conducted on the significant subfield × group interaction. This analysis revealed significantly smaller bilateral dentate gyrus/CA4 ($p < 0.01$), CA1 ($p < 0.01$), and SR/SL/SM volumes ($p < 0.001$) in PBTS than TDC (Figure 4a, b, c). There were no statistically significant group differences in subiculum volumes ($p=0.68$) or CA2/3 ($p=0.59$), although the results were in the expected direction, such that PBTS displayed smaller volumes in these regions than TDC (Figure 4d, e).

Figure 4. Bilateral hippocampal subfield volumes as a function of group.
3.2 Demographic and Medical Predictors of Hippocampal Subfield Volumes

3.2.1 Demographic Predictors of Subfield Volumes

Results from analyses involving demographic variables are summarized in Table 2. In TDC, there were no relationships between age at assessment and subfield volumes. In PBTS, age at assessment was significantly correlated with SR/SL/SM (r=0.52, p = < 0.01, q = 0.03) and subiculum volumes (r = 0.48, p = < 0.01, q = 0.05). No sex differences in subfields volumes were identified in either PBTS or TDC.

3.2.2 Medical Predictors of Subfield Volumes

Results from analyses involving medical variables in PBTS are summarized in Table 2. Diagnosis age in PBTS was positively correlated with CA1 (r = 0.53, p = < 0.01, q = 0.03), CA2/3 (r = 0.58, p = < 0.001, q = < 0.01) and SR/SL/SM volumes (r = 0.68, p = < 0.001, q = <0.01), such that a younger diagnosis age predicted smaller volumes (Figure 5a, b, c). Neither subiculum (r = -0.234, p = 0.22, q = 0.5) nor dentate gyrus/CA4 volumes (r = 0.43, p = 0.02, q = 0.09) were correlated with diagnosis age. Time since diagnosis to assessment was positively correlated only with subiculum volume (r = 0.66, p = < 0.001, q= < 0.01), such that a greater time since diagnosis to assessment predicted a larger volume. There were no relationships identified between subfield volumes and hydrocephalus treatment.
The analysis comparing PBTS diagnosed at less than and greater than six to TDC revealed an omnibus effect of group for CA1 (F(1, 57) = 18.75, p = < 0.001), dentate gyrus/CA4 (F(1, 57) = 20.87, p = < 0.001), CA2/3 (F(1, 57) = 22.51, p = < 0.001) and SR/SL/SM volumes (F(1, 57) = 50.78, p = < 0.001) (Figure 6). In contrast, there was no significant effect of group on subiculum volumes (F(1, 57) = 2.74, p=0.10). Post-hoc analysis revealed that only PBTS diagnosed at less than six had smaller volumes in dentate gyrus/CA4, CA1, and CA2/3 (p = <0.001, p= < 0.01, and p < 0.001, respectively). In these regions, PBTS diagnosed at greater than six had volumes that were similar to TDC. In contrast, SR/SL/SM volumes were smaller than TDC irrespective of diagnosis age, such that both PBTS diagnosed at less than six, and greater than six had smaller volumes than TDC (p= < 0.001 and p = 0.05, respectively).
Figure 6. Hippocampal subfield volumes in PBTS diagnosis before and after 6, and TDC.

Table 2. Summary of results from statistical tests involving demographic and medical variables.

<table>
<thead>
<tr>
<th>Variable of interest</th>
<th>Group</th>
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<th>t-value</th>
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<th>q value</th>
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</table>
3.3 Relationship between Left Hippocampal Subfield Volumes and Verbal Associative Memory in PBTS

We observed a significant positive relationship between memory performance and left dentate gyrus/CA4 volumes ($r=0.70$, $p = 0.02$) and left SR/SL/SM volumes ($r = 0.59$, $p = 0.05$) (Figure 7a, b), such that greater volumes predicted higher verbal associative memory scores. Further, left CA1 volumes were marginally associated with memory performance ($r = 0.58$, $p = 0.06$).
Discussion

The primary contribution of this thesis is that we examined hippocampal subfield volumes and the relations between smaller hippocampal subfield volumes and verbal associative memory in PBTS treated with radiation. The novel findings from this thesis are that: 1) PBTS treated with radiation exhibited volume abnormalities in specific subfields of the hippocampus. Namely, the dentate gyrus/CA4, a known region of on-going adult neurogenesis, CA1, and SR/SL/SM were smaller in PBTS than TDC. This finding may reflect treatment-induced volume loss, perturbed neuroanatomical development, or a combination of both. 2) Second, among the medical and demographic variables examined, a younger age at diagnosis most clearly predicted smaller hippocampal subfield volumes. In particular, the group of PBTS diagnosed at less than six had the smallest hippocampal subfield volumes. This finding is consistent with evidence from studies with humans (Mulhern, Merchant, Gajjar, Reddick, & Kun, 2004) and irradiated animals (de Guzman et al., 2014) that suggest that the early developing brain is particularly sensitive to the effects of radiation. 3) Third, we observed that smaller left dentate gyrus/CA4 and left SR/SL/SM volumes predicted lower verbal associative memory performance in PBTS. This finding suggests that regional hippocampal volumes may be important predictors of impaired declarative memory in PBTS.
The following sections of the discussion highlight group differences in hippocampal subfield volumes in a regionally specific manner. Our findings are discussed in the context of the known vulnerabilities of hippocampal subfields to disease processes initiated in the central nervous system following radiation. Furthermore, medical and demographic predictors of subfield volumes in PBTS and TDC are highlighted. Last, we explore the relationship between smaller hippocampal subfield volumes and lower verbal associative memory performance in PBTS.

4.1 Dentate gyrus/CA4 Volumes are Smaller in PBTS than TDC

PBTS treated with radiation exhibited smaller bilateral dentate gyrus/CA4 volumes than TDC (mean difference = 95 µm³). This finding is consistent with animal models of radiation injury that show irradiated rodents have smaller dentate gyrus volumes than sham treated rodents (de Guzman et al., 2015). Moreover, these findings align with histological evidence from both post-mortem human (Monje et al., 2007) and animal studies (Mizumatsu et al., 2003) that clearly demonstrate radiation perturbs the dentate gyrus microenvironment.

Determining the pathogenesis of treatment-induced volume abnormalities in the dentate gyrus is beyond the scope of this thesis. However, it is possible that smaller dentate gyrus/CA4 volumes manifest due to pathological mechanisms that are observed in irradiated rodent dentate gyrus. For example, the dentate gyrus in irradiated rodents exhibit decreases in neurogenesis, increases in apoptosis and inflammation, and an altered dynamic between neural precursor cells and microvasculature (Monje, 2008; Palmer, 2003; Monje, Mizumatsu, Fike, & Palmer, 2002). Although examining cellular mechanisms is not currently feasible in humans, in vivo, one study has examined the dentate gyrus in post-mortem tissue samples from brain tumor patients (Monje et al., 2007). This study observed that tissue from brain tumor patients treated with radiation exhibited decreases in neurogenesis and increases in neuroinflammation (Monje et al., 2007). This study suggests that similar changes may be present in PBTS.

Interestingly, the relationship between apoptosis, neurogenesis and volume has previously been examined in animals. Histological animal studies suggest that apoptosis and reduced neurogenesis contribute to decreases in overall cell numbers in the dentate gyrus (van
Interestingly, reduced dentate gyrus neuron numbers is also associated with smaller dentate gyrus volume in animals (van der Beek et al., 2004). Moreover, factors that upregulate or down-regulate neurogenesis such as exercise (van Praag, Kempermann, & Gage, 1999) and stress (Lucassen et al., 2001), respectively, result in corresponding changes in hippocampal volume in humans (Erickson et al., 2011; Zannas et al., 2013; Brown et al., 2015). In order to determine how altered cellular mechanisms in the dentate gyrus contribute to reduced volume, a combined histological and volumetric approach in irradiated animals is required.

4.2 Predictors of Dentate Gyrus/CA4 Volumes in PBTS and TDC

4.2.1 Demographic Predictors of Dentate Gyrus/CA4 Volumes

In TDC, there was no relationship between dentate gyrus/CA4 volumes and age, although this relationship was in the expected positive direction. Our results are inconsistent with evidence suggesting that the dentate gyrus/CA4 increases in volume until early adolescence in healthy individuals (Krogsrud et al., 2014; Lee, Ekstrom, & Ghetti, 2014). Our relatively small sample size may have prevented the detection of subtle effects, which may account for the different results. In PBTS, age was unrelated to dentate gyrus/CA4 volumes. Similarly to TDC, the relationship was in the expected positive direction. It is tempting to speculate that these findings indicate an absence of age-related changes in the dentate gyrus/CA4 in both groups. However, the fact that both relationships were positive, had medium effect sizes, and were markedly similar in strength ($r = 0.30$ and $r = 0.33$, respectively) may suggest otherwise. Longitudinal studies that include a larger sample size will be required to clarify the pattern of volumetric growth associated with dentate gyrus/CA4 development in both PBTS and TDC.

There were no sex differences in the volume of dentate gyrus/CA4 or any other subfield in either TDC or PBTS. The absence of sex differences in TDC are inconsistent with previous research suggesting that healthy developing males have larger hippocampal subfield volumes than females (Krogsrud et al., 2014; Tamnes et al., 2014). However, sex differences in the size of the dentate gyrus during development are observed inconsistently (Daugherty, Bender, Raz, & Ofen, 2016). Discrepant findings in the literature may reflect differences in how and whether
studies normalized raw subfield volumes for intracranial volumes prior to analysis. Indeed, it is possible that males have larger raw dentate gyrus volumes due to larger overall brain volumes than females (Nopoulos, Flaum, O’Leary, & Andreasen, 2000). Therefore, procedures that adjust raw subfield volumes for intracranial volumes may eliminate sex differences. Notably, supplementary analysis of raw hippocampal subfield volumes corroborated our initial findings that sex differences were absent in both groups for all subfields. Together, these finding suggest that sex differences are either too subtle to be detected, or absent in both PBTS and TDC.

4.2.2 Medical Predictors of Dentate Gyrus/CA4 Volumes

There was no significant relationship between dentate gyrus/CA4 volumes and diagnosis age when PBTS were considered as a group. However, stratifying PBTS by a diagnosis age of six revealed that the group of PBTS diagnosed at less than six years of age had smaller dentate gyrus/CA4 volumes than TDC. In contrast, PBTS diagnosed after six years of age had similar dentate gyrus/CA4 volumes to TDC. These findings may reflect that the dentate gyrus/CA4 is particularly sensitive to radiation early in development. Alternatively, it is possible that CRT has the propensity to perturb volumetric growth regardless of age, but that younger children have smaller dentate gyrus/CA4 volumes at the time of treatment. In either case, our results indicate that a young age at diagnosis is a key predictor of perturbed dentate gyrus/CA4 volume development. These findings are consistent with evidence from irradiated rodents suggesting that radiation exposure earlier in life produces more severe dentate gyrus volume abnormalities (de Guzman et al., 2015). Notably, these findings may have implications for our other analyses. It is possible that age-related changes in dentate gyrus/CA4 volumes differ in PBTS diagnosed at greater than and less than six. To tease apart the age-specific consequences of treatment on dentate gyrus/CA4 development, future studies with larger sample sizes should stratify PBTS by diagnosis age.

There was no significant relationship between time since diagnosis and dentate gyrus/CA4 volumes. These findings may suggest an absence of volume change associated with greater time since diagnosis. It is possible that this finding indicates an absence of compensatory growth following treatment-related injury. Longitudinal studies will be required to directly assess the effects of time since diagnosis on volumes.
Hydrocephalus was not a significant predictor of dentate gyrus/CA4 volume, or any other hippocampal subfield volume in PBTS. This finding is encouraging, given that CSF accumulation in the ventricular system may lead to brain trauma, including enlarged ventricles, increased intracranial pressure, blood vessel compression and neuronal damage (Erickson et al., 2001; Del Bigio, 1993). Injury caused by hydrocephalus alters blood flow, which can result in a hypoxic ischemia and a neuro-inflammatory response (Del Bigio, 1993). These central nervous system responses to injury have previously been shown to negatively affect the hippocampus (Abayomi, 2002; Barrientos, Kitt, Watkins, & Maier, 2015). It is important to note that this analysis required that PBTS be subdivided, resulting in a relatively small sample size per group. This may have limited our ability to detect subtle but significant effects. Moreover, the inherent variability within our sample may have also prevented detection of significant effects. Notably, the PBTS in our sample incurred multiple neurological insults because of tumor growth, recurrence, surgical resections, and radiotherapy and chemotherapy.

4.3 CA1 volumes are smaller in PBTS than TDC

We observed that CA1 volumes were reduced in PBTS compared to TDC (mean difference = 85 µm³). In contrast to the dentate gyrus that has been extensively investigated in the context of radiation injury (Monje et al., 2007; Raber et al., 2004; Dietrich, Monje, Wefel, & Meyers, 2008), little is known about the vulnerability of the CA1 to radiation. One study showed that irradiation induces apoptosis in CA1 in animals (Sun et al., 2013). However, the majority of studies investigating the vulnerability of CA1 to central nervous system injury have focused on its marked sensitivity to hypoxic ischemic injury (Nitatori et al., 1995; Amenta, Strocchi, & Sabbatini, 1996). Indeed, the CA1 subfield is known to be selectively vulnerable in conditions involving vascular compromise and hypoxic ischemic injury, including stroke and AD (Kril, Patel, Harding, & Halliday, 2002; Gemmell et al., 2012; Padurariu, Ciobica, Mavroudis, Fotiou, & Baloyannis, 2012). This worth mentioning because it has been speculated that radiation induces vascular pathology that leads to hypoxic ischemic injury in the hippocampus (Tofilon & Fike, 2000; Abayomi, 2002). This speculation raises the possibility that smaller CA1 volumes manifest due to radiation-induced vascular compromise and hypoxic ischemic injury. To
elucidate whether CA1 volumes correlate with vascular health in PBTS will require a coupling of MRI data measuring hippocampal structure and blood flow in the hippocampal microvasculature.

4.4 Predictors of CA1 Volumes in PBTS and TDC

4.4.1 Demographic Predictors of CA1 Volumes

Age was not associated with CA1 volumes in TDC. This finding is inconsistent with previous evidence that CA1 volumes increase throughout childhood until adolescence (Krogsrud et al., 2014). Another study found age-related increases only in the right CA1 (Lee, Ekstrom, & Ghetti, 2014). As noted previously, the small sample size may have prevented the detection of significant effects. In PBTS, age was not associated with CA1 volumes. Given age did not predict CA1 volumes in TDC, the absence of a relationship between age and volume in PBTS is not indicative of pathological development. Future studies should aim to determine whether smaller CA1 volumes in PBTS reflect volume loss or perturbed growth. Moreover, it will be worthwhile to assess neuroanatomical outcomes prior to, and shortly after treatment to clarify the immediate consequences of CRT on volumes.

4.4.2 Medical Predictors of CA1 Volumes

Among the medical variables examined, only diagnosis age correlated with CA1 volumes in PBTS. The direction of the relationship was positive, such that children diagnosed at later ages had larger volumes. Stratifying PBTS by a diagnosis age of six revealed that as a group, only PBTS diagnosed before the age of six had smaller CA1 volumes than TDC. These findings may reflect maturational differences in the size of CA1 at the time of treatment or a greater sensitivity of the early developing CA1 to treatment. Histological analyses in animal models of radiation may contribute to our understanding of CA1’s vulnerability by investigating age-specific responses to radiation.

4.5 SR/SL/SM Volumes are Smaller in PBTS than TDC
We observed that SR/SL/SM volumes were reduced in PBTS compared to TDC (mean difference = 135 µm³). The SR/SL/SM regions contain hippocampal neuropil, including the dendrites of the CA field pyramidal neurons. These regions are the site of numerous synaptic connections that comprise the hippocampal micro-circuitry that is critical for hippocampal information processing (Amaral & Witter, 1989). It is possible that reduced SR/SL/SM volumes manifest due to several cellular mechanisms, such as loss of myelinated fibers, reduced number and size of afferent and efferent projections, and altered dendritic complexity and spine density. Indeed, the adverse consequences of radiation on myelin (Tian, Shi, Yang, Chen, & Bao, 2008) and white matter volume (Nieman et al., 2015) in humans and animals are well documented. Moreover, histological analyses in irradiated rodents suggest that radiation alters dendritic morphology, complexity, and spine density in hippocampal neuropil (Chakraborti, Allen, Allen, Rosi, & Fike, 2012; Parihar & Limoli, 2013). It is possible that these pathological changes to dendrites observed in irradiated rodents contribute to volume reductions in the SR/SL/SM regions.

Interestingly, animal models of AD also demonstrate abnormalities in dendritic architecture in the hippocampus (Shao, Mirra, Sait, Sacktor, & Sigurdsson, 2011). Studies in AD patients have also revealed reduced SR/SL/SM volumes (Kerchner et al., 2012 and 2013). Together both evidence from human and animal studies support the speculation that morphologic changes to dendrites may manifest in smaller SR/SL/SM volumes. It will be critical for future studies to assess the contribution of radiation-induced cellular changes to the reduced neuropil volume in irradiated animals.

4.6 Predictors of SR/SL/SMVolumes in PBTS and TDC
4.6.1 Demographic Predictors of SR/SL/SMVolumes

Age was unrelated to SR/SL/SM volumes in TDC. These findings suggest that age-related volume changes are absent in TDC, or too subtle to detect in our relatively small sample. Previous evidence from healthy individuals suggests that myelination and synapse formation in hippocampal neuropil continues until after puberty (Ábrahám et al., 2010). Notably, volume measures may be insensitive to developmental changes occurring at the cellular level. Indeed,
changes in measures of white matter microstructure are not always commensurate to changes in measures of white matter volume (Tamnes et al., 2010).

There was a positive correlation between age and SR/SL/SM volume in PBTS. These findings are encouraging, particularly if age-related changes manifest due to developmental processes taking place, such as increased dendritic complexity and spine density, synaptogenesis and myelination. As previously noted, sex was unrelated to the volume of SR/SL/SM in both PBTS and TDC.

4.6.2 Medical Predictors of SR/SL/SM Volumes

Diagnosis age was positively associated with SR/SL/SM volumes, such that an older diagnosis age predicted larger volumes. However, both PBTS diagnosed before and after the age of six had smaller volumes than TDC. This finding may reflect the particular sensitivity of white matter (Mabbott et al., 2005; Palmer et al., 2012) and dendritic architecture (Chakraborti, Allen, Allen, Rosi, & Fike, 2012; Parihar & Limoli, 2013) to radiation exposure. Time since diagnosis was positively associated with SR/SL/SM volumes, such that a greater time since diagnosis corresponded to larger volumes. Longitudinal studies in PBTS will be required to confirm whether these observations reflect volumetric growth following treatment.

4.7 No Significant Difference in CA2/3 Volumes in PBTS and TDC

Although PBTS had smaller CA2/3 volumes than TDC (mean difference = 47 µm³), this difference was not significant. Our results failed to confirm our hypothesis that PBTS would have smaller CA2/3 volumes than TDC. Notably, the volume of CA2 and CA3 were combined into a single region, and therefore it is unclear how treatment affected the volume of these subfields individually. Interestingly, previous evidence suggests that cells in CA2 are relatively resistant to death following central nervous system insults, including in hypoxic ischemia and epilepsy (Sadowski et al., 1999; Dudek, Alexander, & Farris, 2016). It will be important for future studies to use segmentation protocols that distinguish between CA2 and CA3 to elucidate
their unique vulnerabilities to radiation. Given PBTS did have smaller CA2/3 volumes that TDC, it is possible that our relatively small sample size prevented a subtle but significant effect from being identified.

4.8 Predictors of CA2/3 Volumes in PBTS and TDC

4.8.1 Demographic Predictors of CA2/3 Volumes

CA2/3 volumes were unrelated to age and sex in TDC and PBTS. However, the correlation between age at CA2/3 volume in PBTS was medium (r = 0.37). This raises the possibility that a statistically significant effect of age on CA2/3 volumes in PBTS may have been observed given a larger sample size.

4.8.2 Medical Predictors of CA2/3 Volumes

Among the demographic and medical characteristics examined, only diagnosis age predicted CA2/3 volumes, such that a younger diagnosis age predicted smaller volumes. Stratifying PBTS by a diagnosis age of before and after six revealed that as a group, only PBTS diagnosed at less than six had significantly smaller CA2/3 volumes than TDC. This raises the possibility that group differences would have emerged had a greater proportion of the PBTS in our sample been diagnosed before the age of six. Future studies could stratify PBTS by diagnosis age to characterize the consequences of an early diagnosis age on neuroanatomical outcomes.

4.9 No Significant Differences in Subiculum Volumes in PBTS and TDC

PBTS had smaller subiculum volumes than TDC (mean difference = 44 µm³), however, this difference was insignificant. This finding is unsurprising given the absence of studies reporting subiculum volume abnormalities following CRT. Moreover, this finding is consistent with research demonstrating that gray matter structures are more resilient to radiotherapy (Tofilon & Fike, 2000).
4.10 Predictors of Subiculum Volumes in PBTS and TDC

4.10.1 Demographic Predictors of Subiculum Volume

Age was marginally positively associated with subiculum volumes in TDC, such that older TDC had larger volumes. Previous studies in typically developing individuals report that age-related increases in subiculum volume occur over the course of childhood until early adolescence (Krogsrud et al., 2014). However, evidence suggests an absence of age-related volume changes (Lee, Ekstrom, & Ghetti, 2014). It is possible that discrepant findings reflect the use of different segmentation protocols with different definitions of subiculum boundaries.

In PBTS, age was positively associated with subiculum volume. The strength of the relationship between age and subiculum volumes in PBTS was similar to the corresponding relationship in TDC. To determine whether this reflects a similar pattern of age-related volume change will require longitudinal studies that directly examine volume changes overtime.

4.10.2 Medical Predictors of Subiculum Volumes

There was a significant relationship between subiculum volume and time since diagnosis in PBTS. In contrast to findings in the other hippocampal subfields, diagnosis age was unrelated to subiculum volume in PBTS. In fact, both PBTS diagnosed before and after the age of six displayed subiculum volumes that were similar to those in TDC. This finding further supports the idea that the subiculum is relatively resistant to radiation injury, irrespective of age at the time of treatment.

4.11 Smaller Subfield Volumes Predict Lower Verbal Associative Memory Performance in PBTS

An exploratory aim of this thesis was to examine the relationship between smaller hippocampal subfield volumes and lower verbal associative memory in PBTS. It is well established that PBTS treated with CRT suffer from debilitating learning and memory impairments. However, the precise etiology of these impairments is poorly understood. Only one previous study has linked CRT to whole hippocampal volume abnormalities (Riggs et al., 2014). Based on animal models indicating that radiation may differentially affect hippocampal
subfields, we aimed to assess the contribution of subfield-specific compromise to impaired memory in PBTS.

Our results indicate that smaller volumes in the left dentate gyrus/CA4 and left SR/SL/SM were associated with lower verbal associative memory in PBTS. Additionally, smaller left CA1 volumes were marginally associated with lower memory performance. In contrast to subfield volumes in the left hemisphere, subfield volumes in the right hemisphere were unrelated to performance. This finding is unsurprising in light of evidence that verbal memory processing is supported by medial temporal lobe structures in the left hemisphere (Coleshill et al., 2004).

Interestingly, smaller hippocampal volumes in healthy populations are not consistently associated with poorer memory performance (Van Petten, 2004). However, in clinical populations with known hippocampal pathology, smaller hippocampal volumes may represent pathology that interferes with hippocampal memory processing. Our findings are consistent with evidence that hippocampal abnormalities in clinical populations are related to associative memory deficits (Giovanello, Verfaellie, & Keane, 2003; Yassa & Stark., 2011). Moreover, our results support the associative account of hippocampal function by providing evidence that smaller hippocampal subfield volumes in PBTS correlate with lower associative memory, even when retrieval occurs after a short-delay.

Previous research assessing memory outcomes in PBTS have used clinical batteries that assess broad declarative memory abilities. We chose a task that is thought to be sensitive to hippocampal function (Squire & Wixted, 2011). This verbal associative memory task required PBTS to encode a list of word pairs, and subsequently use a partial verbal cue (the first word of a word pair) to retrieve the corresponding appropriate word-pair from memory. Prior research suggests that successful encoding and retrieval depend on several processing mechanisms in the hippocampus. It is possible that smaller subfield volumes in PBTS reflect damage that perturbs these hippocampal processing mechanisms, leading to impaired associative memory performance. For instance, behavioral animal (Gilbert, Kesner, & Lee, 2001) and human neuroimaging studies (Bakker et al., 2008), as well as neurocomputational models (Wiskott, Rasch, & Kempermann, 2006) suggest that pattern separation occurs in the dentate gyrus. These
studies stress that pattern separation enables highly similar but distinct memories to be encoded independently from one another (Wiskott, Rasch, & Kempermann, 2006). Pattern separation is thought to facilitate memory precision by preventing interference between highly similar memories during encoding (Wiskott, Rasch, & Kempermann, 2006; Moscovitch, Cabeza, Winocur, & Nadel, 2016).

Extrapolating from human and animal studies and neuro-computation models, smaller dentate gyrus volumes in PBTS may reflect damage that impairs hippocampal function at the level of encoding. Encoding deficits mediated by perturbed pattern separation processes may impair the precise encoding of word pair associations. Moreover, animal models of radiation injury show that neurogenesis is perturbed in irradiated animals, and that this may mediate performance deficits on hippocampal-dependent memory tasks (Raber et al., 2004; Clelland et al., 2009). Based on these findings from animal models of radiation injury, it is possible that smaller dentate gyrus volumes in PBTS in part reflects perturbed neurogenesis that contributes to impaired memory performance.

In addition to the left dentate gyrus/CA4, the left SR/SL/SM was also significantly associated with task performance. The SR/SL/SM layers of the hippocampus are comprised of neuropil and contain an abundance of synaptic contacts that are critical for hippocampal information processing. For example, the SR/SL/SM contain synaptic contacts that give rise to the trisynaptic circuit, the CA3-CA3 recurrent collaterals and perforant pathways (Andersen, P., 2007). These hippocampal microcircuits play a key role in integrating and regulating information flow throughout the hippocampus (Amaral & Witter, 1989). For example, the SR contains CA3-CA3 recurrent collaterals synaptic connections that have been previously shown to be critical for associative memory formation (Leutgeb, Leutgeb, Moser, & Moser, 2007; Kesner, 2007). These recurrent collateral “auto-associative” connections are thought to facilitate associative memory formation. Based on this ‘auto-associative account’, a partial cue may lead to activation of an entire associative network in CA3 that gives rise to a complete memory (Gilbert & Kesner, 2003). It is possible that smaller SR/SL/SM volumes in PBTS reflect damage that disrupts hippocampal synaptic connectivity and processing. Disrupted hippocampal synaptic connectivity and processing would be expected to impair hippocampal-dependent memory performance, including associative memory. Our results for the relationship between SR/SL/SM volumes and
memory performance are consistent with evidence showing smaller volumes in hippocampal neuropil (the SR region) in AD patients correlate with memory deficits (Kerchner et al., 2012).

We also observed a marginal association between smaller CA1 volumes and lower verbal associative memory in PBTS. In light of our small sample size, it is possible that this association would have reached statistical significance given a larger sample size. Neuro-computational models (Wiskott, Rasch, & Kempermann, 2006) and behavioral (Gilbert, Kesner, & Lee, 2001) studies in animals suggest that the CA1 subfield is involved in retrieval processes and temporal pattern separation. Smaller volumes in the CA1 may therefore reflect damage that impairs retrieval processes on our task. This damage would be expected to manifest as poorer retrieval of word pairs. Given the marginally significant findings for this relationship, it will be important for future studies to clarify the relationship between CA1 compromise and impaired forms of memory in PBTS.

5 Limitations

These findings must be considered in light of several limitations. First, several of the regions examined in this study were comprised of more than a single hippocampal subfield. The segmentation approach that we used generated a single label for CA2 and CA3 (CA2/3), for the dentate gyrus and CA4 (dentate gyrus/CA4), and for the SR, SL, and SM (SR/SL/SM). As a result, we were unable to derive separate volume measures for these regions. This may have implications for our results, as it is possible that brain cancer treatments affect these regions differently. Second, due to a cross-sectional design, we were unable to directly examine how subfield volumes change across development. Employing longitudinal designs to the study of hippocampal subfield structure will be required to directly assess how subfield volumes change over the course of development in PBTS and TDC. Third, the sample size in the present study was relatively small, particularly for the analysis assessing memory-subfield volume relationships. A larger sample size may have allowed for the detection of unidentified relationships. For example, it is possible that both the subiculum and CA2/3 would have been identified as significantly smaller in PBTS compared to TDC if a larger sample of participants had been included. This is supported by the observation that our findings for subiculum and CA2/3 volumes in PBTS were in the expected direction, such that PBTS had smaller volumes
than TDC. Thus, our findings may simply reflect the subfields that are most vulnerable to treatment. Fourth, our memory analysis included scores from both the WMS and CMS. Although the verbal associative memory tasks are similar on the CMS and WMS, the numbers and types of words differ between these two tasks because they are designed for two different age groups of children. While it is unlikely that this affected our results, a single memory task that is standardized across participants would have been ideal. Last, although all of the PBTS in our sample received CRT, they were heterogeneous in terms of the treatments they received (i.e.: extent of surgical resection, hydrocephalus treatment, chemotherapy protocol), as well as the types of brain tumors with which they were diagnosed. Therefore, it is not clear how each separate treatment may have contributed to reduced hippocampal subfield volumes in PBTS. Critically, it is established that radiation particularly impairs hippocampal structure and function in animals, however, certain chemotherapy agents are also associated with disrupted hippocampal neurogenesis and function (Nokia, Anderson, & Shors, 2012; Christie et al., 2012). It will be important for future studies to assess the individual and synergistic effects of cancer treatments by stratifying patients based on the types of treatments they have received.

6 Conclusion

We provide novel evidence suggesting that hippocampal subfields in the developing brain are differentially vulnerable to brain cancer treatments. In particular, dentate gyrus/CA4, CA1, and SR/SL/SM volumes were smaller in PBTS compared to TDC. Furthermore, PBTS diagnosed at younger ages were at a greater risk for smaller hippocampal subfield volumes compared to TDC. We also showed that in PBTS, left dentate gyrus/CA4, left CA1, and left SR/SL/SM were correlated with verbal associative memory performance. Findings from this thesis may contribute to a greater understanding of the sensitivity of the hippocampal subfields to radiation exposure. Our findings also indicate targeted interventions designed to promote repair in specific hippocampal subfields. For example, therapeutic interventions that reduce neuroinflammation and increase neurogenesis may be beneficial for reversing radiation damage in the dentate gyrus. Interestingly, animal models of radiation injury show that physical exercise may restore neurogenesis in the irradiated dentate gyrus in mice (Naylor et al., 2008). Moreover, a recent study found that a 9-week physical exercise training program increased hippocampal volume in PBTS treated with radiation (Riggs et al., 2016). The findings from the present thesis
suggest that it will be important to consider subfield-specific compromise in the context of designing therapeutic interventions aimed to stimulate hippocampal repair following CRT.
## Supplementary Tables

### Table 3. Demographic and medical information for patients who completed memory testing.

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<tr>
<th>Parameter</th>
<th>PBTS $n=11$</th>
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</thead>
<tbody>
<tr>
<td><strong>Sex (Male: Female (No.))</strong></td>
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<tr>
<td><strong>Age at assessment (years)</strong></td>
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<tr>
<td>Mean (SD)</td>
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<td><strong>Handedness</strong></td>
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<tr>
<td>Left</td>
<td>2</td>
</tr>
<tr>
<td><strong>Age at diagnosis (years)</strong></td>
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<td><strong>Time since diagnosis to assessment (years)</strong></td>
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<tr>
<td>Fourth ventricle</td>
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<tr>
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<tr>
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8 Bibliography


