SUB-ACUTE HIPPOCAMPAL ATROPHY IN THE FIRST YEAR FOLLOWING MODERATE TO SEVERE TRAUMATIC BRAIN INJURY

by

Danielle D. DeSouza

A thesis submitted in conformity with the requirements for the degree of Master’s of Science

Graduate Department of Rehabilitation Science
University of Toronto

© Copyright by Danielle D. DeSouza
(2009)
SUB-ACUTE HIPPOCAMPAL ATROPHY IN THE FIRST YEAR FOLLOWING MODERATE TO SEVERE TRAUMATIC BRAIN INJURY

Degree of Master of Science, 2009
Danielle D. DeSouza
Graduate Department of Rehabilitation Science
University of Toronto

Abstract

Rationale: Ng et al. (2008) demonstrated that sub-acute hippocampal atrophy occurred between 4.5 and 24 months following moderate-to-severe traumatic brain injury (TBI); it remains to be determined if atrophy occurred before 24 months. Objectives: (1) to determine if sub-acute hippocampal atrophy occurs by the first year of injury; (2) to determine associated clinical and demographic variables. Methods: Ten moderate-to-severe TBI patients underwent MRI at 5 and 12 months post-injury. Glasgow Coma Scale (GCS) and demographic variables were correlated with change. Results: Significant hippocampal volume decreases were observed for right ($P < 0.002$, Cohen’s $d = 0.34$) and left ($P < 0.036$, Cohen’s $d = 0.22$) sides. GCS was significantly correlated with right ($r = -0.663$, $P < 0.037$), but not left percent hippocampal volume change ($r = -0.327$, $P < 0.356$). No significant correlations were observed for demographic variables. Conclusion: Sub-acute hippocampal atrophy occurs between 5 and 12 months post-injury and is associated with injury severity.
Acknowledgements

First, I would like to thank my supervisor Dr. Robin Green for this wonderful learning opportunity and for introducing me to the world of neuroimaging. I would also like to thank my committee members Dr. David Mikulis and Dr. Nancy Lobaugh for their expertise along the way.

Thank you to all of my GDRS friends and lab mates who have been extremely supportive and made the past two years some of the most memorable. In particular, thanks to Ephrem Pano, April Arundine, Diana Frasca, Yuko Koshimori, Alexandra Arnold-Oatley, Sabrina Agnihotri, Melanie Andre, Jake Yoon and Quoc Hao Mach for all of their support (not to mention the statistics and formatting help)!

Thank you to all of the participants from Toronto Rehab. This study would not have been possible without your co-operation.

Last, but not least, thank you to my wonderful family for all of their patience, love and support throughout this process. Mom, Dad, Danita and Tanya this thesis is as much yours as it is mine. Michael, your support and understanding have meant the world to me.
Table of Contents

Abstract .......................................................................................................................... ii
Acknowledgements .......................................................................................................... iii
Table of Contents .............................................................................................................. iv
List of Figures .................................................................................................................. vi
List of Tables ................................................................................................................... vii
List of Abbreviations ....................................................................................................... viii

Chapter 1 General Introduction ...................................................................................... 1
  1.1 Rationale .................................................................................................................. 1

Chapter 2 Literature Review .......................................................................................... 4
  2.1 Introduction ................................................................................................................. 4
      2.1.1 Definition and Classification of TBI ................................................................. 4
      2.1.2 General Behavioural Characteristics of TBI ...................................................... 5
      2.2 Pathophysiological Manifestations of Moderate to Severe TBI ......................... 6
          2.2.1 Overview ........................................................................................................... 6
          2.2.2 Acute Injury ...................................................................................................... 7
              2.2.2.1 Primary Injury .............................................................................................. 7
              2.2.2.2 Secondary Injury .......................................................................................... 9
      2.2.3 Sub-Acute Atrophy & Behavioural Change ....................................................... 14
          2.2.3.1 Animal Studies of Sub-Acute Atrophy .......................................................... 15
          2.2.3.2 Human Studies: Global Sub-Acute Atrophy .................................................. 17
      2.3 Hippocampal Injury Following TBI ...................................................................... 19
          2.3.1 Human Studies: Sub-Acute Hippocampal Atrophy ......................................... 20
          2.3.2 Controlling for Limitations of Previous Human Studies Examining Sub-Acute Atrophy: Ng et al. (2008) and Greenberg et al. (2008) .................................... 21
      2.4 Correlates of Outcome from TBI ......................................................................... 23
          2.4.1 Correlates of Behavioural Outcome ................................................................. 23
          2.4.2 Correlates of Pathophysiological Outcome ...................................................... 25
          2.4.3 Summary of Studies Examining Correlates of TBI Outcome ......................... 27
      2.5 Summary of Literature Review ............................................................................. 27
      2.6 Current Study ......................................................................................................... 28
          2.6.1 Objectives/Hypotheses ..................................................................................... 29

Chapter 3 Methods ......................................................................................................... 31
  3.1 Participants ............................................................................................................... 31
      3.1.1 TBI Participants ................................................................................................. 31
          3.1.1.1 Inclusion/Exclusion Criteria ........................................................................... 31
      3.1.2 Healthy Control Convenience Sample .............................................................. 32
  3.2 Materials ................................................................................................................... 32
      3.2.1 Demographic and Injury Variables .................................................................. 32
      3.2.2 Structural MRI Protocol .................................................................................. 33
List of Figures

Figure 3.1 Tail of Left Hippocampus in Coronal View……………………………37
Figure 3.2 Body of Left Hippocampus ..........................................................37
Figure 3.3 Left Hippocampus in Sagittal View...........................................38
Figure 4.1 Mean Right Hippocampal Volume for the TBI and Control Groups…..45
Figure 4.2 Mean Left Hippocampal Volume for the TBI and Control Groups……46
Figure 4.3 Left and Right Percent Hippocampal Volume Changes for Individual
TBI Participants..........................................................................................48
Figure 4.4 Left and Right Percent Hippocampal Volume Changes for Individual
TBI Participants..........................................................................................49
Figure 4.5 Correlation between Right Percent Hippocampal Volume Change and
GCS Score..................................................................................................50
List of Tables

Table 4.1 Group Data for TBI and Control Participants ................................................. ..45
List of Abbreviations

Aβ
Amyloid Beta

ANOVA
Analysis of Variance

APOE4
Apoplipoprotein E ε4 allele

APP
Beta Amyloid Precursor Protein

ATP
Adenosine Triphosphate

BVC
Brain Volume Change

CA
Cornu Ammonis

Ca²⁺
Calcium ion

CBF
Cerebral Blood Flow

CCI
Controlled Cortical Impact

CDR
Clinical Dementia Rating

CIMT
Constrained Induced Movement Therapy

CSF
Cerebrospinal Fluid

CT
Computerized Tomography

DAI
Diffuse Axonal Injury

FCC
Focal Cortical Contusion

GCS
Glasgow Coma Scale

GOAT
Galveston Orientation and Amnesia Test

GOS
Glasgow Outcome Scale

GOSE
Extended Glasgow Outcome Scale

GM
Gray Matter

ICC
Intraclass Correlation Coefficient

ICP
Intracranial Pressure

K+
Potassium ion

MRI
Magnetic Resonance Imaging

MVA
Motor Vehicle Accident

Na+
Sodium ion

NS
Not Significant

SAH
Subarachnoid Hemorrhage

SD
Standard Deviation

SDH
Subdural Hematoma

TBI
Traumatic Brain Injury

VBP
Volume of Brain Parenchyma

VBR
Ventricle-to-Brain Ratio

WM
White Matter
Chapter 1 General Introduction

1.1 Rationale

With advances made in brain injury prevention and safety promotion awareness (e.g., recommendations by the Canadian Medical Association) (Elford, http://www.phac-aspc.gc.ca) as well as in medical treatment of traumatic brain injury (Hedges et al., 2009; Lane et al., 2000; Rosner, 2003), more and more people are surviving serious brain injuries (Chiu et al. 2007; De Silva et al., 2009; Lane et al., 2000). Indeed, traumatic brain injury (TBI) is currently one of the leading causes of morbidity worldwide (Dikmen et al., 2003; Yattoo and Tabish, 2008). Because TBI commonly affects young adults, survivors can endure decades of disability (Centers for Disease Control and Prevention, 1999) caused by impairments to cognitive, motor and emotional functioning (Draper and Ponsford, 2008; Jorge et al., 2007; Walker and Pickett, 2007). In Canada, approximately 50,000 individuals are affected annually (Ontario Brain Injury Association [OBIA], 2001), with 11.4/100,000 individuals sustaining a severe TBI (Zygun et al., 2005) and associated costs estimated at $3 billion per year (Brain Injury Association of Canada [BIAC], 2008).

Given the enormous impact of TBI, research into the underlying causes of disability is critically needed in order to facilitate the development of treatments that can mitigate its consequences. To this end, the focus of the current research is on gaining a better understanding of a putative impediment to recovery from TBI. There is growing evidence from the animal and human literature for a second wave of damage to the brain following serious TBI, including the hippocampi (Ariza et al., 2006; Bigler et al., 2002; di Paola et al., 2008; Tomaiuolo et al., 2004). Given the importance of the hippocampi to memory
- and therefore to all aspects of day-to-day life - gaining a better understanding of whether or not this sub-acute atrophy occurs and over what time course can help us to design treatments to pre-empt atrophy, and thereby improve recovery from TBI. While whole brain sub-acute atrophy can occur following TBI (MacKenzie et al., 2002; Trivedi et al., 2007), the focus of the current study is on the hippocampi due to its known vulnerability in trauma and its important contributions to memory processes.

A number of studies have demonstrated the acute loss of hippocampal volume after TBI (Bigler et al., 1997; Bouilleret et al., 2009; Immonen et al., 2009), however very few studies have specifically examined whether atrophy can occur **sub-acute**ly; that is, atrophy commencing **after** the acute events of injury (e.g., edema) have resolved. A recent study conducted in our lab (Ng et al., 2008) - and one which obviated methodological limitations of prior studies examining the possibility of sub-acute atrophy - demonstrated significant hippocampal volume loss. Ng et al. (2008) measured sub-acute atrophy with an initial scan taken at 4.5 months post-injury and a follow-up scan taken at 24 months post-injury; however, it is possible that atrophy had occurred earlier than 24 months post-injury. This result is important in and of itself; however, an important further question is whether atrophy occurred earlier than 24 months post-injury. Indeed, preliminary findings from our lab suggest that sub-acute atrophy may occur by the first year of injury.

Also, Ng et al. (2008) did not investigate the correlates of atrophy. Although a cross-sectional study by Bigler et al. (1997) determined injury severity (as measured by the Glasgow Coma Scale) was positively correlated with hippocampal volume 100+ days post-TBI, a comprehensive review of the literature indicated that few longitudinal studies have examined the relationship between clinical and demographic variables and the
presence and/or amount of sub-acute hippocampal atrophy following TBI (Bendlin et al., 2008; Trivedi et al., 2007).

Therefore, the primary objective of the current study was to determine if sub-acute hippocampal atrophy occurs in the first year of injury in a sample of moderately and severely impaired TBI patients. A secondary, exploratory objective was to examine correlates of sub-acute hippocampal atrophy.

Having a better understanding of when sub-acute hippocampal atrophy occurs can aid in the development of interventions aimed at offsetting this secondary wave of atrophy by providing information on when to introduce treatment. Furthermore, determining which clinical and demographic variables are related to the pathophysiological outcome from TBI will increase our understanding of which patients are at a greater risk of sub-acute pathophysiological outcome. This understanding is important for long-term clinical and occupational planning as well as identifying which patients should be targeted for prophylactic treatment. Before discussing the methods of the current study, a review of the relevant literature will be provided.
Chapter 2 Literature Review

This chapter will review the literature that is most germane to the objectives of this thesis. The literature on mechanisms of damage in moderate to severe TBI will be examined with a particular emphasis on the findings of Ng et al. (2008), which the current study was designed to extend. With regard to a secondary exploratory objective, the literature concerning correlates of clinical and pathophysiological outcomes from moderate to severe TBI will be provided.

2.1 Introduction

Given the deleterious clinical and economic burden of TBI and the increasing numbers affected, it is of value to have a thorough understanding of the pathophysiology of TBI in order to design interventions that will optimize outcome for this group.

2.1.1 Definition and Classification of TBI

TBI refers to an externally inflicted trauma to the brain that may result in significant impairments in physical, cognitive, social and affective functioning (NIH Consensus Development Panel, 1999; Salmond and Sahakian, 2005), which can persist into the longer term (Dikmen et al., 2003; Green et al., 2008; Levine et al., 2008).

TBI is typically classified as mild, moderate and severe, both in clinical practice and in the scientific literature. These designations are commonly made using broad clinical measures of injury severity such as the Glasgow Coma Scale (GCS), which measures depth of coma, length of time in post-traumatic amnesia (PTA; i.e., the length of time a
person is disoriented and unable to encode new events following the injury) (Ruijs et al., 1994), and acute care length of stay (ACLOS).

### 2.1.2 General Behavioural Characteristics of TBI

Following moderate and severe brain injuries, a wide range of impairments may be sustained. Patients may often experience sensory deficits affecting auditory, olfactory and visual domains (Haxel et al., 2008; Jury et al., 2001; Kapoor and Ciuffreda, 2002). Motor function is often compromised (Walker and Pickett, 2007), including impairments in motor speech (Wang et al., 2005), fine motor coordination (Neistadt, 1994), spastic paralysis (Zafonte et al., 2004), balance and gait (Walker and Pickett, 2007). Changes in mood and anxiety are common, both in reaction to lost participation in day-to-day life (Hiott and Labbate, 2002; Jorge and Robinson, 2002; Parsons et al., 1995) and due to direct organic changes. Emotion and behaviour regulation deficits, including anger and antisocial behaviour are also common (Tonks et al., 2007).

Arguably the most disruptive consequences of TBI, however, are cognitive deficits. While all domains of cognitive functioning can be affected, executive functioning (Draper and Ponsford, 2008; McDonald et al., 2002; Ponsford et al., 2008), attention and speed of processing (Draper and Ponsford, 2008; Madigan et al., 2000; Vitaz et al., 2003) and memory (Levin et al., 1988; Levine, 1989; Ponsford et al., 2008; Schacter and Crovitz, 1977; Vakil et al., 2005; Vitaz et al., 2003) have been reported, with memory impairment being one of the most frequent complaints of patients and their family members (Arcia and Gualtieri, 1993; Oddy et al., 1985; Vakil, 2005).

Memory impairments are due in part to damage to the hippocampi and to white matter projections into and out of this structure. The formation of new long-term
memories, a process critically dependent on the hippocampi (Nadel and Moscovitch, 1997; Scoville and Milner, 1957; Squire, 1992; Winocur, 1985), as well as structures in the diencephalon (McKee and Squire, 1992; Squire, 1987) and the frontal lobes (Fletcher and Henson, 2001; Lee et al., 2000), is typically impaired after moderate and severe TBI. It has been well established that individuals with TBI are impaired on verbal learning, recall and recognition tasks (Ariza et al., 2006; Baddeley et al., 1987; Ferri-Campos et al., 2008; Millis and Ricker, 1994; Vanderploeg et al., 2001; Wiegner and Donders, 1999; Zec et al. 2001) as well as visuo-spatial learning, recall and recognition (Ariza et al., 2006; Brooks, 1976; Brooker and George, 1984; Lehnung et al., 2003; Shum et al., 2000).

The physiological mechanisms of injury to the hippocampi and other regions of the brain are complex. Injury can occur both acutely and sub-acutely, and within these periods, a variety of mechanisms may operate to produce damage that is both focal and diffuse.

2.2 Pathophysiological Manifestations of Moderate to Severe TBI

2.2.1 Overview

During TBI, a number of mechanical forces act on the brain to cause irreversible damage. Although injuries whereby an individual remains static may result in TBI, the most common mechanism of damage is from acceleration-deceleration injuries. This type of injury occurs when inertial forces from the trauma cause the brain to move within the skull. The result is typically a combination of focal damage where the brain strikes the inner surface of the skull (for example in the form of cortical contusions or cerebral haemorrhaging) and diffuse damage throughout the brain (Banich, 2004; Besenski et al.,
2002; Lezak et al., 2004). Damage may occur not only at the moment of impact, as is the case with primary injury, but also minutes to months post-injury as pathophysiological changes initiated by the primary injury take place (Lezak et al., 2004; Morganti-Kossman et al., 2007).

Focal injuries include cortical contusions, infarcts, hematomas and tissue compression secondary to raised intracranial pressure, with midline shift and herniation secondary to edema and haemorrhaging occurring in very severe cases (Kan et al., 2006; Valadka et al., 2000). Diffuse damage occurs to vulnerable axons as they twist and shear during the trauma (de la Plata et al., 2007; Smith et al., 2003) and as small blood vessels supplying axons are disrupted via petechial hemorrhaging (Povlishock and Katz, 2005), causing mechanical and biochemical injury. This widespread white matter damage is called diffuse axonal injury (DAI), and it largely contributes to the morbidity and mortality seen in moderate and severe TBI (Adams et al., 1991). Furthermore, DAI can also contribute to gray matter damage to areas remote from the initial impact site as slower, chronic mechanisms acting on the white matter may lead to eventual cell body death (Anderson et al., 1996). Eventually, these cellular mechanisms occurring post-injury lead to gross atrophy, whereby large brain areas can deteriorate even after the acute phase of injury.

2.2.2 Acute Injury

2.2.2.1 Primary Injury

Traumatic damage to the brain occurring at the moment of impact has been widely studied. Biomechanically, TBI can result from a violent blow to the head, directly deforming and damaging brain tissue or it can result from acceleration-deceleration
incidents (e.g., a motor vehicle accident [MVA]) resulting in DAI and petechial white matter haemorrhaging (Povlishock and Katz, 2005). TBI can also cause contusions to the brain as the brain collides with the inner surface of the skull (Besenski et al., 2002). In this scenario, various forces act on the brain to cause damage without the skull being penetrated. Commonly, the forces involved in this type of injury (e.g., linear, rotational or angular) produce hemorrhaging in the protective meningeal layers of the brain or pronounced focal contusions in a coup and counter-coup pattern (Besenski, 2002).

During TBI, not all areas of the brain are equally susceptible to damage; the frontal and temporal lobes are reported to be the most vulnerable on impact (Bigler, 2007; Lundy-Ekman, 2007; Pierallini et al., 2000). Although this is in part due to the action of the linear forces involved (Besenski et al., 2002), it is also the result of the brain’s orientation within the skull. Compartments in the skull known as the anterior and middle cranial fossa surround the surface of the frontal and temporal lobes, respectively (Bigler, 2007). Consequently, bony surface protuberances cause damage, particularly in these areas, when a sudden force causes the brain to move and forcefully make contact with these protrusions.

Gray matter damage caused during primary injury usually results in subsequent tissue loss due to irreversible neuronal damage and cell death. Cell death can occur by way of three mechanisms that are not mutually exclusive: apoptosis, necrosis and autophagy, with necrosis appearing to be the dominant means in primary injury (Kovesdi et al., 2007). Necrosis is a passive process whereby mechanical deformation of the neuron results in membrane damage, primarily to the cell soma, and also activation of an inflammatory response leading to secondary injury (Kovesdi et al., 2007). At the moment of impact, a cell may undergo immediate swelling and organelle breakdown, but if the
cell is damaged beyond a certain point, the swelling causes it to rupture, prompting the secondary inflammatory response that is characteristic of necrosis (Goodlett and Horn, 2001).

In addition to the focal cortical damage that occurs in primary injury, DAI can also occur in very severe circumstances when axons immediately rupture on impact (Maxwell et al., 1993; Povlishock and Katz, 2005). More commonly, DAI is a delayed response that occurs days to months following the TBI.

2.2.2.2 Secondary Injury

In the minutes to days post-injury, a number of additional events may take place to cause increased neuropathology. It is important to note that some events may also occur months post-injury. Although these events are also considered secondary, they occur following the acute phase of injury. These sub-acute mechanisms will be described in another section.

As previously discussed, necrosis is initiated during primary injury. In contrast, the inflammatory response that causes the recruitment of neighbouring cells to also undergo cell death, contributing to atrophy beyond the initial lesion site, is a secondary process (Goodlett and Horn, 2001). The release of intracellular components after cell membrane damage causes the inflammatory response associated with necrosis. Unlike apoptosis, whereby cell signals are sent to phagocytes (of the immune system) to engulf the dying cell, necrotic cells lack a signal (Sahuquillo et al., 2001). This makes it more difficult for the immune system cells to locate and remove cells that have died by necrosis. Furthermore, when cells die by necrosis they can release harmful enzymes usually stored
within the lysosome. Once released, these enzymes can trigger a chain reaction of further cell death to neighbouring cells.

Also, TBI causes damage to surviving cells via secondary complex cellular mechanisms, such as glutamate excitotoxicity, triggered by the initial event (Fujimoto et al., 2004). Additionally, several secondary neurological effects such as edema, hypoxia, herniation and hemorrhaging may occur acutely and contribute to the morbidity of this population (Bigler et al., 1992; Pierallini et al., 2000; Povlishock and Katz, 2005).

Acutely, several disruptions to ionic balance may take place following injury. On impact, neurotransmitter release, mitochondrial dysfunction, and subsequent membrane depolarization of the injured cell may occur (Buki et al., 2000; Katayama et al., 1990; Marmarou, 2007). Although the primary mechanical deformation initiates these events, many of these neuronal changes are delayed and classified as secondary. These changes are delayed because it is the combination of slower molecular processes (e.g., increased axolemma permeability, increased intracellular calcium, cytoskeletal degradation) acting together over time that can cause further damage. An example of when several molecular processes co-occur is during glutamate excitotoxicity, a process involving the extracellular accumulation of glutamate, the most abundant excitatory neurotransmitter in the human nervous system. This accumulation results in the over-stimulation of glutamate receptors, prolonged depolarization, and ionic imbalances including increases in intracellular calcium, which subsequently leads to further cell dysfunction. Although elevated levels of glutamate post-TBI have been reported in both animal and human studies (Matsushita et al., 2000; Yamamoto et al., 1999), the use of glutamate antagonists (i.e., NMDA receptor antagonists) as a treatment to offset these chemical cascades has yielded mixed results (Beauchamp et al., 2008). As a result, recent research has focused
on metabolic consequences of injury and examined impaired glucose metabolism as a potential cause of impairment.

In a study by Bergsneider et al. (2000), decreased cerebral glucose utilization (as determined using $^{18}$F fluorodeoxyglucose positron emission tomography) was regionally obtained in 88% of their sample of mild to severe TBI patients (n=42). Also, when global reduction was examined, the prevalence was higher in severe TBI patients (86%) versus their mild to moderately impaired counterparts (67%).

Ionic imbalances can also cause abnormal water accumulation in the intercellular spaces between cells or within the brain cells themselves resulting in edema (Yi and Hazell, 2006). Acute, secondary edema is one of the leading causes of death following trauma to the brain. It results from an accumulation of fluid in the brain parenchyma and it is highly associated with elevations in intracranial pressure (ICP) (Marmarou, 2007). Together, these changes are frequent causes of mortality in this population. Two types of edema may take place following brain injury: vasogenic and cytotoxic (Klatzo, 1967). Although vasogenic edema has been highly implicated as the source of rapid, acute increases in ICP following TBI, recent literature has determined that cytotoxic edema or fluid accumulating within cells can co-exist with the former type, and is actually the predominant type of edema when ionic imbalances are sustained over time, usually as the result of impaired ATP-dependent Na$^+$/K$^+$ pumps (Ito et al., 1996; Marmarou, 2007; Sweeney et al., 1995).

Previous research has suggested that the hippocampus is particularly vulnerable to these excitotoxic mechanisms (Tate and Bigler, 2000). In a study by Sun and Faden (1995), glutamate release was prohibited using sodium channel blockers in an animal model of TBI. The prevention of glutamate release reduced the amount of hippocampal
neuronal loss in the CA1 and CA3 pyramidal cell fields from 59% to 22% in the ipsilateral side. The CA1 area of the hippocampus appears to be particularly vulnerable to the effects of TBI. Bramlett et al. (1999) demonstrated that following a secondary hypoxic insult in a rodent model of TBI, there was a significant increase in the frequency of damaged neurons in the CA1 areas of the hippocampus.

Another common process following TBI is secondary axonal damage. In contrast to the theory that the twisting and shearing of axons caused by TBI are the immediate cause of swollen axon balls or disruption of the injured axon segment (Adams et al., 1982; Gennarelli et al., 1982; Povlishock et al., 1983), more recent findings (e.g., Li et al., 1999; Pettus et al., 1994; Wolf et al., 1999) have determined that focal changes at the axon membrane, primarily following acceleration-deceleration TBI, results in progressive secondary changes that hinder axonal transport causing swelling of the axon and eventual detachment from its downstream segment over time (Park et al., 2008; Maxwell et al., 1993; Povlishock et al., 1983; Smith and Meaney, 2000). Additional studies have also provided evidence for impaired axonal transport, whereby organelle delivery via anterograde transport is hindered as a result of permeability changes in the axon membrane caused by the mechanical deformation of the brain at the moment of impact (Buki et al., 1999; Smith and Meaney, 2000). These primary changes in permeability, caused by the formation of pores in the axolemma, result in an increased secondary influx of extracellular calcium ions (Ca$^{2+}$) within the cell. This atypical increase produces local degradation of the cytoskeleton (Buki et al., 1999; Smith and Meaney, 2000) organelle accumulation, axonal swelling and eventual detachment (Buki and Povlishock, 2006).

Adding to this, there is evidence for the rapid accumulation of proteins following brain injury. In particular, β-amyloid precursor protein (APP) and amyloid-β (Aβ)
peptides, typically known for their role in Alzheimer’s disease histopathology, have been examined (Smith et al., 2003). Although several epidemiological studies have reported a link between TBI and the development of AD (Heyman et al., 1984; Rasmusson et al., 1995; Schofield et al., 1997), until recently it was not understood how Aβ peptides (derived from APP) aggregate to form plaques rapidly following brain injury. Much of the evidence points to damaged axons as the main source of Aβ plaque formation following TBI. APP is typically found in large quantities in axons, as it is carried via fast axonal transport mechanisms. Thus, when white matter areas are damaged, this protein has been shown to accumulate in damaged axons (Sheriff et al., 1994), and also, APP and Aβ can co-accumulate in the terminal swellings of disconnected axons following brain trauma, as shown in a swine model of TBI (Smith et al., 1999). Moreover, identifying APP via immunocytochemical methods has been shown to be a useful tool for identifying DAI in humans (Sherriff et al., 1994).

With all of these secondary pathological changes occurring on a cellular and molecular level, including excitotoxicity and white matter damage, it is not surprising that sub-acute atrophy is a consequence of moderate to severe TBI. Although sub-acute atrophy can occur to the brain globally, some structures, in particular the hippocampus, are particularly vulnerable to trauma. In the next section, studies examining sub-acute atrophy post-TBI will be discussed. These studies include those that focused on global brain changes and those that examined changes to the hippocampi, specifically.
2.2.3 Sub-Acute Atrophy & Behavioural Change

In addition to the mechanisms of injury described above, there is now growing evidence from our lab and others that atrophy takes place during the sub-acute stage of recovery, after the acute neurological events of injury (e.g., edema, hematoma, microglial proliferation, encephalomalacia, clearing of traumatized cells) have resolved (Di Giovanni et al., 2005; Ng et al., 2008; Greenberg et al., 2008; Trachtenberg, 1983). For the current purposes, sub-acute atrophy refers to progression of volume loss and tissue damage that cannot be attributed to the resolution of acute injury (e.g., volume loss in a region of the brain that is actually caused by resolution of edema or hematoma). No particular mechanisms of atrophy are presumed. In the current study, the period after which any observed atrophy is considered sub-acute is from approximately 4.5 months onwards, when edema is presumed to have resolved. This is different from early atrophy which occurs at the time of injury or shortly thereafter.

Following early recovery from TBI, studies have demonstrated that the brain may not be stable, as presumed. Some studies have provided evidence that following early recovery (i.e., within the first year of injury), some individuals may show subsequent cognitive decline (Himanen et al., 2005; Millis et al., 2001; Till et al., 2008). For example, Millis et al. (2001) examined neuropsychological performance at 1 and 5 years post-injury in a sample of mildly to severely impaired TBI patients. The results indicated that some individuals improved or remained the same; however, approximately 15% actually declined in performance over time.

Additionally, a study conducted in our lab (Till et al., 2008) examined the neuropsychological performance of moderate to severe TBI patients from a baseline of 12-months post-injury to a follow-up conducted on average 2 years later (SD= 0.99). It
was determined that statistically significant cognitive decline was apparent on at least two neuropsychological measures in approximately 27% of the group. Although results were variable, decline was most commonly observed for verbal fluency and delayed recall (tests that rely on memory, executive functioning and psychomotor speed) (Spreen and Strauss, 1998; van Beilen et al., 2004). Sub-acute atrophy is one explanation for the cognitive declines observed in these patients.

In fact, our laboratory has found preliminary evidence suggesting that memory recovery is affected by sub-acute atrophy. In a study examining the relationship between hippocampal atrophy and memory change over time, our lab determined that memory recovery from 4.5 to 12 months post TBI was negatively correlated with hippocampal atrophy over this same time period: therefore, the greater the atrophy, the less the memory recovery from 4.5 to 12 months post-injury. No significant correlations were obtained for memory recovery and hippocampal atrophy from 4.5 to 24 months post-injury. Thus, these findings indicate that sub-acute hippocampal atrophy may indeed occur by 12 months post-injury.

2.2.3.1 Animal Studies of Sub-Acute Atrophy

Animal studies in TBI research have been critical, as they have allowed for the controlled and reproducible examination of TBI. Specifically, the animal literature has provided evidence for sub-acute atrophy that occurs following the acute phase of moderate to severe injury (Colicos et al., 1996; Immonen et al., 2009; Rodriguez-Paez et al., 2005; Smith et al., 1997). These studies have examined both gray matter and white matter regions of the brain and have outlined potential mechanisms underlying these sub-acute changes. It is important to examine sub-acute white matter pathology even when
focusing on sub-cortical gray matter structures. Over time, the secondary loss of axons, in addition to the primary cortical damage, can lead to further widespread damage to cortical and sub-cortical gray matter structures, as important input and output connections may be lost (Loftus et al., 2000).

In a study by Rodriguez-Paez et al. (2005), it was determined that chronic axonal changes were present following TBI in a population of male rats that underwent moderate parasagittal fluid-percussion brain injury. In this study, animals were sacrificed and examined at one of seven time points: 3 days, 15 days, 1 month, 3 months, 6 months, 9 months or 12 months post-TBI. Compared to sham animals, there were significant decreases in the estimated number of myelinated axons in the fimbriae (portion of the fornix), external capsules and white matter tracts of the thalami in the TBI animals. Although not statistically significant, the number of myelinated axons continued to decrease over time in the fimbriae (from 3 to 12 months) and external capsules (from 15 days to 12 months). In contrast, myelinated axons in the thalami significantly decreased over time, indicating that damage to sub-acute white matter occurred in addition to initial acute damage. This study was one of the first to quantitatively describe chronic white matter axonal damage post-TBI.

Similarly, Smith et al. (1997) examined rats following severe parasagittal fluid percussion injury at nine different time points: 1 hour (h), 2h, 48h, 1 week, 2 weeks, 1 month, 2 months, 6 months and 1 year post-TBI. These authors determined that substantial and progressive tissue loss occurred in several structures including the cerebral cortex and hippocampus of these animals. Most notably, at all time points between 48h and 1- year post-injury, including from 6- months to 1- year, significant decreases in the pyramidal cell layer of the ipsilateral hippocampi were observed. These
authors suggested that a progressive, degenerative process might have been initiated by the initial trauma.

2.2.3.2 Human Studies: Global Sub-Acute Atrophy

Using MR-based quantitative tools for analyses, several studies have reported sub-acute decreases in brain parenchyma following TBI and associated increases in cerebrospinal fluid (CSF) volume, indicative of atrophy. A portion of these studies have been cross-sectional in design and have either collected control volumes for comparison (Ariza et al., 2006; Bigler et al., 2002; Blatter et al., 1997; Jorge et al., 2007; Levine et al., 2006; Levine et al., 2008; Tomaiuolo et al., 2003; Yount et al., 2002) or have used normative databases to compare TBI findings, such as the one compiled by Blatter and colleagues (1995). Collectively, these studies have found that increased injury severity, as measured by GCS or PTA, is related to greater atrophy following trauma as demonstrated by decreases in total brain volumes, hippocampal volumes, posterior cingulate gyrus volumes, increases in ventricle-to-brain ratio (VBR), and increases in CSF volumes. However, the cross-sectional design poses a limitation in determining individual differences within this heterogeneous population. Also, the patients in these studies were assessed at varying lengths of time post-injury. For example, Ariza et al. (2006) examined patients that acquired MRI scans from 189-603 days post-injury. Variability in the length of time between the injury and the MRI make it difficult to determine the time course of these sub-acute changes.

A small number of human studies have employed longitudinal designs to examine sub-acute atrophy post-TBI (Bendlin et al., 2008; Greenberg et al, 2008; Mackenzie et al., 2002; Ng et al., 2008; Trivedi et al., 2007). The advantage of this strategy is that within-
subject factors are the same at all time points of analysis. Therefore any longitudinal
decreases in volumes of interest are due to increases in sub-acute atrophy, and not merely
differences between the groups (i.e., the TBI and control samples). As individual
decreases in volume can be tracked over time, longitudinal imaging is a particularly
valuable tool for the assessment of progressive neurological disorders (Whitwell, 2008).

Of the studies that employed longitudinal designs (Bendlin et al., 2008; Greenberg et
al., 2008; Mackenzie et al., 2002; Ng et al., 2008; Trivedi et al., 2007), differences in the
times of MR data acquisition have been observed. MacKenzie and colleagues (2002)
retrospectively assessed total volume of brain parenchyma (VBP) from MR images in
mild-moderate TBI patients at two time-points: within the first three months of injury and
again within three months of the initial scan. Although this study did not find significant
decreases in VBP as compared to controls, the longitudinal analysis did show that the rate
of atrophy was greater in the TBI group.

Trivedi and colleagues (2007) used MR-derived analyses to demonstrate decreases in
percent brain volume change (%BVC) in mild to severe TBI patients, as compared to
controls within approximately the first year of injury. This study supports the possibility
that TBI can lead to progressive neurological changes as soon as one year post-injury.

These longitudinal studies provide stronger evidence that sub-acute atrophy occurs.
However, one particular limitation was evident in all but the studies of Ng et al (2008)
and Greenberg et al (2008) from our lab. A sub-set of the initial MRI scans were taken
during the acute phase of injury. For example, in the MacKenzie et al. (2002) study,
initial scans were acquired as early as seven days post-TBI (range: 7-430 days).
Similarly, in Trivedi et al. (2007), some of the initial MRI scans were taken as early as 39
days following injury (range: 39-109 days). Therefore, because some individuals
acquired their first scans very early post-injury, any observed volume loss may reflect the resolution of edema or hematoma. In other words, the brain may have looked smaller over time because the edema or blood products that accrued acutely had now been eliminated. As the authors themselves noted, this could have accounted for some or all of the sub-acute atrophy observed. As previously mentioned, two types of edema may occur following TBI: vasogenic and cytotoxic. The latter of these two commonly occurs when ionic imbalances persist over time (Ito et al., 1996; Marmarou, 2007). The hippocampus, and in particular the pyramidal neurons of this structure, have been shown to be particularly vulnerable to ionic imbalance as a result of excitotoxic mechanisms (Figiel et al., 1997). The edema that results could be a potential partial confound of these studies (Mackenzie et al., 2002; Trivedi et al., 2007) if scans were taken too early.

2.3 Hippocampal Injury Following TBI

In addition to these studies examining global brain atrophy post-TBI, some studies have specifically examined hippocampal volume decrease following trauma. Although the hippocampi sit deep in the temporal lobes, several studies have demonstrated that this structure is particularly vulnerable to sub-acute atrophy following TBI (Bigler et al., 1996; Bigler et al., 1997; Bigler et al., 2002; Tate and Bigler, 2000). This is in part due to excitotoxic injury and other mechanisms that act on the many white matter fibres that project to, from and within this structure. These findings are not surprising considering memory impairments are of the most common and persistent cognitive sequelae
following TBI (Auerbach, 1986). Thus, hippocampal atrophy and associated memory impairments have been a particularly common research focus in the literature.

2.3.1 Human Studies: Sub-Acute Hippocampal Atrophy

In a cross-sectional study mentioned above, Tate and Bigler (2000) used MRI scans to examine the hippocampi and fornices (major efferent fibres from hippocampi) of 86 TBI patients of all severities, as measured by the GCS. MRI scans were acquired at least 2- months post-injury and were compared to 46 control participants. It was determined that sub-acute atrophy of both the fornix and hippocampi occurred following TBI, with the degree of atrophy being related to the severity of the injury.

Additionally, Bigler et al. (1996) examined two TBI groups: an early group comprised of patients who had MRI scans up to and including 90 days post-injury, and a late group that included patients who acquired scans more than 90 days post-injury. Both groups consisted of individuals with GCS scores between 3 and 15; however, the authors noted that they were generally in the moderate to severe range of injury severity. Compared to a sample of control participants (n=72), it was determined that hippocampal volume for the late TBI group was significantly smaller than those of controls, but for the left hippocampus only. The early TBI group did not have significantly smaller hippocampi for either side.

In another study by Bigler et al. (1997), hippocampal volumes were significantly smaller in individuals with TBI as compared to normal controls. Like the previous study, TBI participants with GCS scores ranging from 3 to 15 were divided into two groups: those that had MRI scans up to and including 100 days post-injury (early group, n=45) and those that had MRI scans acquired more than 100 days post-injury (late group, n=55).
The results indicated that only the late group had significantly smaller hippocampi as compared to controls and hippocampal volume was positively correlated with GCS. Unlike the previous study by this group (Bigler et al., 1996), both left and right hippocampal volumes were significantly smaller than those of normal controls. Furthermore, the authors themselves noted that the decreases in hippocampal size stabilized, “some time after 100 days following injury,” and thus atrophy was taking place sub-acuteley.

Although these studies provide evidence for sub-acute hippocampal volume loss following TBI, the main limitation of these studies is that they were cross-sectional in design.

2.3.2 Controlling for Limitations of Previous Human Studies Examining Sub-Acute Atrophy: Ng et al. (2008) and Greenberg et al. (2008)

In order to obviate some of the limitations of previously conducted studies, Ng et al. (2008) conducted a longitudinal study to quantify global hippocampal volume changes, and their first MRI acquisition was taken after acute neurological events were presumed to have resolved based on expert opinion. In this repeated-measures design, MRI scans of TBI patients (n=14) were taken at two sub-acute time-points: 4.5 months (SD= 0.5) and 24 months (SD= 4.4) post-injury. In the first part of this study, three expert neuroradiologists reviewed all 14 pairs of scans and were informed that they belonged to TBI patients and were taken at two sub-acute time-points. The early and late scans were randomized into rows and the expert raters were asked to determine if deterioration, no observable change or improvement occurred. The raters unanimously agreed that eight of
the 14 pairs of scans were indicative of progression of atrophy; for two of the scans, there was agreement that progression of atrophy occurred by two of the three raters.

In the second part of the study, hippocampal and CSF volumes were obtained via MRI volumetric analyses. These quantitative analyses revealed significant increases in CSF volume and decreases in left and right hippocampal volume. Moreover, when the annual percent volume change was compared to published normative data, there was far greater volume loss; this provided evidence that volume loss was not attributable to age-related decline. Most importantly, as initial scans were acquired at approximately 4.5 months post-injury, it was very unlikely that the acute effects of the trauma contributed to the volume decreases. Therefore, this study provided strong evidence for bona-fide sub-acute hippocampal atrophy from approximately 4.5 months to 24 months following brain injury.

Corroborating these sub-acute findings, another study from our lab (Greenberg et al. 2008) observed sub-acute decreases in white matter integrity, as measured by MRI-diffusion tensor imaging (DTI). Similar to the Ng et al. (2008) study, sub-acute changes were observed from approximately 4.5 months (SD= 0.4) to 29 months (SD= 4.0) post-injury in a sample of moderately to severely impaired TBI patients. The areas that were significantly compromised were the deep frontal and temporal white matter fibres of both hemispheres. Thus, not only has evidence been provided for sub-acute hippocampal atrophy, but our lab has also provided evidence for sub-acute decreases in white matter integrity in regions of interest within the frontal and temporal lobes.

Although our lab has found evidence for significant sub-acute hippocampal atrophy and progressive damage to some white matter post-injury, the time course for these sub-acute changes remains to be determined. In other words, sub-acute atrophy was observed
between 4.5 and 24 months post-injury, but it is possible that these changes could have occurred earlier. As mentioned earlier, our lab has found preliminary cognitive evidence suggesting that this might be the case.

2.4 Correlates of Outcome from TBI

Although sub-acute hippocampal atrophy may occur for some TBI patients by the first year of injury, the degree of atrophy may not be the same for everyone. The outcome from TBI can be quite variable from patient to patient. Therefore, if it is determined some individuals with moderate to severe TBI demonstrate sub-acute hippocampal volume loss by the first year of injury, it is important to have an understanding of which factors moderate this pathophysiological outcome. In order to determine which variables may be of most relevance, we can look to literature that has examined correlates of pathophysiological outcome. We can also examine studies concerning behavioural outcomes, as these are logically dependent upon what is taking place at a neurophysiological level.

2.4.1 Correlates of Behavioural Outcome

The clinical outcome from TBI can vary markedly between patients and a large number of studies have examined variables that put patients at risk for poor behavioural outcome. With regard to pre-morbid factors, the results of studies have been mixed, but older age, pre-injury unemployment, lower education, and pre-injury substance abuse, have been frequently been found to be related to poor functional and cognitive outcomes.
(Demetriades et al., 2004; Green et al., 2008; LeBlanc et al., 2006; Wilde et al., 2004; Ponsford et al. 2008) including ongoing disability, for example, in a systematic review by Willemse-van Son et al. (2007).

Injury severity indices (e.g., GCS and PTA) have also been correlated with outcome from TBI. Perhaps the most commonly used index is the GCS (Teasdale and Jennett, 1974). GCS has been shown to be useful in the clinical management and prognosis of TBI (Saatman et al., 2008; Sherer et al., 2007). The GCS is composed of three elements: eyes, verbal and motor. A total score out of 15 is given, with scores ≤8 classified as severe, scores between 9 and 12 moderate, and scores ≥13 mild. Several studies have determined that a relationship exists between GCS and functional outcome from TBI (e.g., Dikmen et al., 1995; Park et al., 2009; Ponsford et al., 2008; Utomo et al., 2009), but a study by Zafonte et al. (1996) determined that GCS as a single measure has limited value as a predictor of functional outcome, a finding that was also corroborated in a review by Zasler et al. (2007).

Another measure of severity is post-traumatic amnesia (PTA), which captures the length of time a person is disoriented and unable to encode new events following the injury (Ruijs et al., 1994). PTA is collected daily in the acute period following TBI, whereas initial GCS is only measured once (Wilde et al., 2006). Length of time in PTA is often measured using the Galveston Orientation and Amnesia Test (GOAT) (Levin et al., 1979). The GOAT, a test administered repeatedly following closed head trauma, has been shown to predict long-term recovery (Levin et al., 1979); however, because it is labour-intensive to measure PTA, it is often not collected. (I removed the paragraph describing
2.4.2 Correlates of Pathophysiological Outcome

Few studies have directly examined risk factors for pathophysiological outcome in the brains of traumatically injured individuals (Bigler et al., 1997; Bigler et al., 2006; Ding et al., 2008; Levine et al., 2008; Wilde et al., 2006). Of those that have, one study conducted by Levine et al. (2008) determined that TBI severity, as measured by the GCS at discharge from the trauma unit, was negatively correlated with parenchymal volume at one year post-injury; thus, the more severe the TBI, the more sub-acute brain atrophy. The authors noted that the frontal and temporal regions showed the most robust correlations with this severity measure. Also, patients with moderate and severe TBI could be differentiated from patients with mild TBI, and mild TBI participants could be differentiated from an age and education matched control group.

A cross-sectional study by Bigler et al. (1997) also used GCS to examine the relationship between injury severity and hippocampal volume following TBI. It was determined that injury severity was positively correlated with hippocampal volume 100+ days post-TBI. This correlation was not evident for a group of TBI patients that acquired MRI scans earlier than 100 days following injury. This suggests that sub-acute decreases in hippocampal volume occurred and were related to initial injury severity.

Bendlin et al. (2008) also utilized GCS as a measure of severity to determine its relationship with five different brain measures at approximately 1-year post-injury (range= 252-380 days). Analyses were conducted using MR-DTI and included fractional
anisotropy of white matter, mean diffusivity of white matter, mean diffusivity of gray matter, white matter volume and gray matter volume. Significant correlations were obtained between GCS scores collected within 24 hours of injury and all five pathophysiological measures. Of most relevance to the current study, a significant negative correlation was obtained between GCS and mean diffusivity the fornix, the major efferent tract from the hippocampus. Thus, lower GCS scores within 24 hours of injury were associated with greater mean diffusivity of the fornix (indicative of pathology) at approximately 1-year post-injury.

Wilde, Bigler, Pedroza and Ryser (2006) examined the relationship between PTA and ventricle-to-brain ratio (VBR), a common measure of global cerebral atrophy, at least 90 days post-injury (range: 92-2,748 days). The authors noted their rationale for using PTA to predict long-term atrophy was that PTA is collected repeatedly in the acute period following TBI, whereas GCS is typically measured once. The results of this study indicated that a strong relationship was evident between PTA and the probability of abnormal VBR, with the longer durations of PTA being associated with increased probability of abnormal VBR. These studies provide evidence that certain variables, including measures of injury severity such as GCS scores and duration of PTA, are related to the extent of sub-acute pathophysiological outcome in TBI patients.

2.4.3 Summary of Studies Examining Correlates of TBI Outcome

In summary, many studies have examined predictors of functional and cognitive outcomes using demographic variables and measures of injury severity. While there exists some evidence that demographic variables such as age and years of education are
related to functional and cognitive outcome (Demetriades et al., 2004; Green et al., 2008; LeBlanc et al., 2006; Williamse-van Son et al., 2007; Zafonte et al., 1997), other studies did not find these relationships (e.g., Bush et al., 2003; Girard et al., 1996; Novack et al., 2001). Understanding the relationship between demographic variables and measures of injury severity on cognitive and functional outcome from TBI is important because these variables may also be related to pathophysiological outcome. It is presumed that pathological brain changes resulting from the trauma underlie impairments in function and cognition. If this is the case, then these variables may also be related to the pathophysiological changes in structures known to be involved in commonly impaired cognitive and functional domains. It remains to be determined if the demographic variables of age and years of education are correlates of the degree of sub-acute hippocampal volume change following moderate to severe TBI.

Additionally, a few studies have provided evidence that injury severity, as measured by GCS and PTA, is significantly correlated with sub-acute pathophysiological outcome from TBI (Bendlin et al., 2008; Bigler et al., 1997; Wilde et al., 2006). Although these studies have observed a relationship between these measures and sub-acute measures of atrophy, they did not specifically examine the relationship between injury severity and sub-acute hippocampal volume change.

2.5 Summary of Literature Review

The literature has revealed that sub-acute brain atrophy, and in particular, hippocampal atrophy occurs following TBI (Bigler et al., 1996; Bigler et al., 1997; Ng et al., 2008; Tate and Bigler, 2000). Several studies have used structural MRI volumetry as
a tool to capture volumetric changes in the hippocampi (Bigler et al., 1997). However, of the studies that examined hippocampal volume post-TBI and that were longitudinal in design, only one (Ng et al., 2008) assessed hippocampal volume at two sub-acute time points where observed volume loss could be confidently attributed to atrophy rather than resolution of acute neurological effects, such as edema. This study determined that sub-acute hippocampal volume decreases occurred approximately between 4.5 months and 24 months post-TBI in moderately to severely impaired patients. However, it remains to be determined if these sub-acute changes occurred earlier. Recent cognitive evidence from our lab suggests that this may be the case, and that sub-acute hippocampal atrophy may be occurring by the first year of injury.

Also, few studies have examined correlates for sub-acute brain atrophy (Bendlin et al., 2008; Bigler et al., 1997; Wilde et al., 2006). For example, Levine et al. (2008) determined that GCS scores at discharge were related to amount of parenchymal volume loss observed in TBI patients approximately one year post-injury as compared to age and sex-matched controls, and Wilde et al. (2006) found a relationship between PTA and abnormal VBR. However, it is unclear if these factors will also be related to sub-acute hippocampal atrophy following moderate to severe TBI.

2.6 Current Study

Although Ng et al. (2008) determined that sub-acute hippocampal atrophy occurred following TBI, to date, there has yet to be a longitudinal study in humans that has examined sub-acute hippocampal atrophy in the first year of injury. Thus, the aim of the current study was to quantify hippocampal volume at two sub-acute time points in the
first year of injury, using structural MR-derived measures of volumetry, in a sample of moderately to severely impaired TBI patients.

Furthermore, previous research has determined that some demographic variables are related to functional and cognitive outcome, while clinical measures of severity, such as GCS scores and PTA scores have been related to cognitive, functional and pathophysiological outcome from TBI. Nonetheless, the relationship between injury severity, demographic variables and sub-acute hippocampal atrophy following moderate to severe TBI has yet to be examined. Therefore, a secondary, exploratory aim of the current study was to determine if any of these variables correlate with the amount of sub-acute hippocampal atrophy observed.

2.6.1 Objectives/Hypotheses

Objective 1: To determine if sub-acute hippocampal atrophy occurs by the first year in moderate and severe TBI patients. This objective was addressed using MR-derived hippocampal volumetric measurements to quantify left and right hippocampal volumes at 5 and 12 months post-injury. Volume change in patients was examined longitudinally and was also compared to a convenience control sample tested in the current study and to normative data from the literature.

Hypothesis 1: Based on the previous neuroimaging findings that sub-acute hippocampal atrophy occurred between 4.5 and 24 months post-injury (Ng et al., 2008), and the preliminary cognitive findings from our lab indicating that hippocampal atrophy may be underlying poor memory recovery from 4.5 to 12 months post moderate to severe TBI, it
was hypothesized that significant sub-acute hippocampal volume loss would be observed from 5 to 12 months post-injury.

**Objective 2 (Exploratory):** *To determine the impact of injury severity, years of education and age on sub-acute hippocampal atrophy.* This was achieved by correlating GCS, age and years of education with percent hippocampal volume change from 5 to 12 months post-injury for each hippocampus. This secondary objective was exploratory and its main purpose was to generate hypotheses for future research. As previous research has yet to specifically examine the relationship between these variables and sub-acute hippocampal volume atrophy, a specific hypothesis was not tested. The main purpose of this objective was to generate hypotheses for future research.
Chapter 3 Methods

3.1 Participants

3.1.1 TBI Participants

Ten participants with TBI were recruited from a larger, ongoing prospective study examining cognitive and motor recovery following moderate-severe TBI. The Research Ethics Board at the Toronto Rehabilitation Institute (TRI) approved this study. All patient participants were recruited from the in-patient program at TRI.

3.1.1.1 Inclusion/Exclusion Criteria

Inclusion criteria for the larger recovery study are as follows: 1) acute care diagnosis of TBI, 2) PTA of one hour or more and/or GCS score of 12 or less determined either at Emergency or at the scene of the trauma and/or positive CT or MRI findings in acute care, 3) mechanism of injury associated with trauma, 4) between 18 to 65 years of age, 5) able to follow simple commands in English, and 6) able to provide informed consent to participate in the study or availability of a legal decision maker to provide informed consent.

Exclusion criteria for the larger study are as follows: 1) diagnosis of a disease primarily or frequently affecting the central nervous system (CNS) (e.g., Alzheimer’s disease, multiple sclerosis), 2) previous history of psychotic disorder, 3) TBI acquired secondary to another brain injury, and 4) subsequent brain injury occurring over the course of the study.

In addition to the above criteria, to be included in the current study patients had to have acquired their first MRI already, and at the time of recruitment, they needed to be
within one year of injury. Thirteen patients qualified for the current study. Three were lost to follow-up. Therefore, a retention rate of 77% was achieved, which is very high compared to previous longitudinal studies of TBI where retention rates were under 40% (e.g., Dikmen et al., 2003; Himanen et al., 2006).

### 3.1.2 Healthy Control Convenience Sample

Five healthy control participants were recruited to participate in the current study. This convenience sample acted as a comparison group to provide evidence that any decreases in hippocampal volume from 4.5 to 12 months post-injury observed in patients could not be explained by normal, age-related decreases in hippocampal volume. These individuals were either employees of TRI or were family members of employees. Control participants were eligible for the current study if they: 1) were between the ages of 18-65 years of age, 2) had no previous history of TBI or any other disease primarily affecting the CNS.

### 3.2 Materials

#### 3.2.1 Demographic and Injury Variables

All demographic and clinical variables were obtained retrospectively from medical records. Demographic variables acquired were: age and years of education. Injury severity was measured by the GCS.
3.2.2 Structural MRI Protocol

All patient MR scans were acquired on a GE Signa-Echospeed 1.5 Tesla scanner, located at Toronto General Hospital, part of the University Health Network (UHN), using a standard quadrature head coil. The high-resolution 1mm isotropic T1 weighted, three-dimensional radio-frequency spoiled-gradient recalled-echo (SPGR) images were acquired in the axial plane (TR = 11.74 ms, TE = 5.14 ms, and flip angle = 20°, 160 slices). The scans of two of the five control participants were acquired on the same platform as above. Three of the five were acquired on a 3.0 Tesla scanner at the Toronto Western Hospital, also part of UHN, using a GE Signa-EXCITE 3.0 Tesla scanner (TR = 25 ms, TE = 5ms, and flip angle = 45°, 166 slices, 8-channel headcoil). Higher resolution of the 3.0 Tesla scanner would allow for increased detection of any decline in the controls; therefore this difference between the patients was conservative, biasing the hypothesis against rather than in favour. All participants underwent MRI scans of the entire head.

3.3 Image Processing and Analysis for Hippocampal Volumes

3.3.1 Pre-Processing Steps

MRI scans were obtained in Digital Imaging and Communications in Medicine (DICOM) format, the most common way to receive scans from hospitals, and transformed to Medical Imaging Network Common Data Form (MINC). MINC, developed by Neelin (1993) of the McConnell Brain Imaging Centre of the Montreal Neurological Institute at McGill University, allows for the storage and manipulation of medical images that can be used across a variety of computer operating systems.
Once subject scans were converted to MINC format, they were anonymized and processed.

### 3.3.2 Processing Steps

Processing involved the submission of the MINC files to the TBI pipeline, an automated file processing command that converts a T1-weighted image from its native state to a state that is ready for analysis. A number of steps are involved in this process. First, there was a non-uniformity correction that corrected for any inhomogeneities of the images that occurred as a result of a non-uniform signal (Sled et al., 1998). Next, the corrected image was registered to a Montreal Neurological Institute (MNI)-Talairach space using a linear stereotaxic transformation (Collins et al., 1994) and resampling onto a 1mm voxel grid using a linear interpolation kernel (Mazziotta et al., 1995). The traditional Talairach atlas (Talairach and Tournoux, 1988) is a coordinate system used to describe the location of brain structures regardless of individual differences in size and shape. The MNI group created a new, more representative brain atlas derived from the traditional Talairach model. The new template was created by averaging 305 MRI scans of normal, right-handed individuals. It is this average 305-brain template that was used to register each image to the same space for comparison.

After the images were registered to the MNI-Talairach space, each voxel comprising the image slices was classified into one of three tissue types: gray matter (GM), white matter (WM) or cerebrospinal fluid (CSF). As T1-weighted images were acquired in the current study, GM appeared darker than WM, and CSF was the darkest of the three tissue types.
Next, the tissue-classified image was used to create a 3-dimensional (3D) reconstruction of the cortical surface. The skull and scalp surrounding the cortical surface were removed using this 3D surface reconstruction map as a mask.

### 3.3.3 Quality Control

After the T1-weighted scans were processed via the TBI pipeline, a series of quality control steps were taken to visually inspect the results of each stage of processing. This included (1) giving one of five quality ratings ranging from excellent (e.g., perfect alignment of lobes; no over/under-scaling; proper rotation; proper translation) to terrible (e.g., complete failure of alignment; brain is distorted, stretched or rotated in abnormal ways) (adapted from the Centre for Addiction and Mental Health instructional laboratory document, “Visual Quality Control Guide” (Richards, 2005), (2) flagging problematic scans, and (3) determining if dura mater and scalp were present. Quality control steps were required since errors could have been made during the processing stage that rendered the scans unusable without manual alteration of the problematic areas. For example, poor or terrible ratings on registration could cause structures to appear much larger than they actually were, making volumetric interpretations of these structures inaccurate. Therefore, if any of the scans received poor or terrible ratings and could not be manually corrected, they were not included in the current study. One scan was excluded for this reason.
3.3.4 Manual Segmentation of the Hippocampi

Once quality control was completed and the scans were judged to be useable, the program DISPLAY was used to manually segment the hippocampi of each subject. DISPLAY, an interactive software program developed at the Brain Imaging Centre at the Montreal Neurological Institute, allowed for the simultaneous viewing of scan slices in three orientations: coronal, sagittal, and horizontal. The majority of the manual segmentations were completed in coronal view with the exception of the most anterior portion or head of the hippocampus; this section was completed predominantly in sagittal view. Hippocampi labels were created by manually tracing and filling in the voxels that comprised the structure of interest. As each voxel comprised a space of 1mm$^3$, DISPLAY automatically calculated the volume of the structure being segmented by summing the number of voxels that were labelled.

The protocol used to determine the boundaries of the hippocampus for segmentation was developed by Preussner et al. (2000). Segmentation of each hippocampus began at the most posterior aspect of the hippocampus, where the tail bulges slightly into the trigone of the lateral ventricle. The most medial border of the trigone of the lateral ventricle was used as the medial border for the tail of the hippocampus. The superior border of the tail was marked by the superior row of GM voxels (see Figure 3.1), while one or two rows of voxels were left unlabelled at the lateral border to ensure that the tail of the caudate nucleus was excluded. As segmentation moved anteriorly through the brain, the tail of the hippocampus descended and changed into the body. When the body of the hippocampus was viewed in coronal section, the fornix, the major efferent pathway from the hippocampus could be viewed, but was not included. A portion of WM called the fimbria was included. The fimbria is the part of the fornix that is embedded in the GM
of the hippocampus. To ensure that only the fimbria and not fornix was included, only voxels that were surrounded by GM, and thus “embedded” were incorporated into the hippocampal volume.

Figure 3.1 Tail of Left Hippocampus in Coronal View

Tail of left hippocampus circled in coronal view. Rectangle identifies most superior row of GM voxels.

Additionally, the inferior and lateral borders for labelling the hippocampal body remained the same as for the tail. However, the medial border was created by extending the WM below the hippocampus superior-medially at a 45 degree angle to ensure that entorhinal cortex, located below the hippocampus, was not included (Figure 3.2). Also, a “safety” row of voxels was left unlabelled laterally and superiorly to ensure that the choroid plexus of the inferior horn of the lateral ventricle was not included as hippocampus.
Lastly, in the coronal view, the body of the hippocampus transitioned into the head of
with the appearance of the gyrus intralimbicus (posterior border of entorhinal cortex),
which appeared as a medial bulge. At this point, the sagittal view was also used to
identify hippocampal borders (Figure 3.3). Similar boundaries were used for this section
as were used for labelling the body, however, due to the subtle transition of the
hippocampal head to the amygdala, the sagittal view helped with identifying a thin WM
line called the alveus. The alveus separated the head of the hippocampus from the
amygdala and was included in the volume. It should be noted that for the TBI
participants, these borders were not always evident due to atrophy. In these cases, borders
that were visible were used to guide the labelling of the unclear border. For example, if
the alveus surrounding the head of the hippocampus was not visible, then the most
inferior aspect of the lateral ventricle was used to determine approximately where the
head of the hippocampus ended.
3.4 Inter- and Intra- Rater Reliability Assessment

As the hippocampus is a highly variable structure in both normal and TBI populations, a series of reliability correlations were conducted on practice TBI scans prior to working on patient scans from the current study. Reliability refers to the degree to which multiple assessments of a subject agree or are reproducible (Bartko, 1991). The intraclass correlation coefficient (ICC) can be used to assess both inter- and intra-rater reliability. Also, it has been shown to be a useful tool for evaluating continuous data (Bartko, 1966) such as hippocampal volumes. The aim of the ICC is to determine the amount of measurement error due to subjective interpretation or human error (Shrout and Fleiss, 1979). A high ICC for inter-rater reliability suggests that two or more raters, in this case those conducting the hippocampal labelling, achieved similar results (i.e., little variability) with regard to hippocampal volume acquired. On the other hand, a low ICC on inter-rater reliability suggests that the raters achieved different results due to human error, which could affect statistical analyses and findings. (Shrout and Fleiss, 1979).
Similarly, the ICC can be used to assess the amount of error produced by an individual rater between rounds (intra-rater reliability). A high ICC on intra-rater reliability suggests that the rater is consistent in reproducing similar hippocampal volumes between rounds, whereas low intra-rater reliability suggests that individual rater results between rounds were not highly reproducible.

For the current study, a minimum of five rounds of rater reliability were conducted at least two weeks apart. Inter-rater reliability was conducted against an experienced quantitative MRI analyst trained on the Preussner (2000) method. This individual also trained the rater of the current study. The results of these analyses indicated that for left side, an “almost perfect” agreement was reached ($\kappa = .871$), while a “substantial” agreement was reached for the right side ($\kappa = .801$) (Landis and Koch, 1977). With regard to intra-rater reliability, “almost perfect” agreements were reached for both the left ($\kappa = .934$) and right ($\kappa = .898$) sides (Landis and Koch, 1977).

### 3.5 Design and Procedure

#### 3.5.1 Design

The study employed a longitudinal design with a within subjects factor (time: 5 vs. 12 months post-injury) and a between subjects factor (group: patients vs. controls). The dependent variables were hippocampal volume (objective 1), and age, years of education and GCS score (objective 2).
3.5.2 Procedures

TBI patients who gave their informed consent were recruited to the larger study on TBI recovery. As part of that study, they underwent neuropsychological and motor testing while in-patients. All testing took place at the Toronto Rehabilitation Institute. After discharge, they underwent two more neuropsychological and motor assessments and two MRI scans. Structural MRI scans (as well as behavioural testing) took place at two sub-acute time-points: 5-months post injury (mean= 5.23 months, SD= 0.53, range= 4.7-6.2 months) and 12-months post-injury (mean= 12.4 months, SD= 1.5, range= 11-15.2 months). MRI scans were completed at the Toronto General Hospital.

Individuals in the control group who provided their informed consent also underwent two MRI scans at the Toronto General Hospital. There were varying lengths of time in between the two scans (mean= 24.6 ± 7 months, SD= 7.4, range= 14-32 months).

3.6 Data Analysis

3.6.1 Objective 1: Detection of Sub-Acute Hippocampal Atrophy by the First Year of Moderate and Severe TBI

In order to test the hypothesis that the TBI group would demonstrate greater hippocampal volume loss over time than the control group, a repeated measures 2 (group) x 2 (time) analysis of variance (ANOVA) was conducted for each hippocampus followed by planned comparisons (time 1 vs. time 2 for each group) with effect sizes. Given the small sample size, effect size permits a determination of the strength of these differences or the degree to which the null hypothesis was believed to be false (Cohen, 1988) while minimizing the impact of sample size.
3.6.2 Objective 2 (Exploratory): Examination of Correlates of Sub-Acute Hippocampal Atrophy

To examine the relationship between demographic and injury severity variables with sub-acute hippocampal volume change, bivariate, zero-order Pearson correlations were conducted for left and right percent hippocampal volume change with (1) injury severity, as measured by GCS scores, (2) age and (3) years of education.

Percent change scores for the left and right hippocampi were separately calculated using the following formula: \[
\frac{(\text{hippocampus volume at time 1} - \text{hippocampus volume at time 2})}{\text{average hippocampus volume at times 1 and 2}} \times 100,
\]
as recommended by colleagues at the Brain Stimulation and Neuroimaging laboratory based at the Alfred Psychiatric Research Centre (a Department of Monash University). Therefore, decreases in hippocampal volume over time were captured by positive values, while negative values represented increases in volume over time.

Estimated annual percent hippocampal volume change scores derived from a published normative database (Bigler et al., 1997) were also utilized in order to compare TBI patients to age-matched controls. The method used to derive these change scores has previously been described by Ng et al. (2008). Briefly, Bigler et al. (1997) collected normative hippocampal volumes of individuals between the ages of 16 and 65 years of age. Average hippocampal volumes were grouped for each of the five decades spanning this age range. Change scores were calculated by taking the mean hippocampal volume of the age band that followed the age band of interest and subtracting the mean hippocampal volume of the age band preceding the age band of interest. These scores were then divided by 20 (as the mean span across these age bands was 20) and then annual percentages were calculated from this value.
All statistical analyses, including those for the ICC, were conducted using the Statistical Package for the Social Sciences, version 16.0 (SPSS 16.0) for Windows.
Chapter 4 Results

4.1 Demographic and Injury Characteristics

Table 1 presents the injury and demographic variables for the TBI and the control groups. The convenience control group had more years of education, a difference which was not statistically significant, but for which Cohen’s d was large ($P < 0.152$, Cohen’s $d = 0.83$). Significant differences between groups were observed for age ($P < .007$, Cohen’s $d = 1.76$), with the TBI group being older, and for interval between scan times, which was shorter in the TBI group ($P < .000$, Cohen’s $d = 3.22$). While the older age of the TBI sample biased the findings in favour of the hypothesis (i.e., because they were older, they were more likely to show atrophy from 5 to 12 months post-injury), the shorter time between scans in the TBI group biased the results against the hypothesis of the study.

Consistent with the literature, the TBI group consisted of more males (60%) than females (40%). This contrasted with the control group, which was composed of more females (60%) than males (40%). These differences were not statistically significant ($\chi^2 (1, n= 15) = 0.536$, N.S.), although the result should be interpreted conservatively because of acceptance of the null and the small sample size.

Table 1 also presents the hippocampal volumes for the groups for scan 1. There were no statistically significant differences in hippocampal volume for the two groups, either for the left ($P > 0.1$ Cohen’s $d = 0.86$) or the right ($P > 0.1$; Cohen’s $d = 0.61$) sides. However, as expected, the hippocampal volumes of the TBI patients were smaller than those of the controls at scan 1.
Table 4.1 Group Data for TBI and Control Participants

<table>
<thead>
<tr>
<th></th>
<th>TBI Group (n=10)</th>
<th>Control Group (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>50.2 ± 10.5</td>
<td>32.4 ± 9.7**</td>
</tr>
<tr>
<td>Months between Scans (mean ± SD)</td>
<td>7.3 ± 1.8</td>
<td>24.6 ± 7.4**</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/4</td>
<td>2/3</td>
</tr>
<tr>
<td>Years of Education (mean years ± SD)</td>
<td>17.1 ± 2.1</td>
<td>19 ± 2.4</td>
</tr>
<tr>
<td>GCS (mean ± SD)</td>
<td>5.8 ± 4.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Injury type (fall/MVA)</td>
<td>6/3§</td>
<td>N/A</td>
</tr>
<tr>
<td>Left Mean Hippocampus Volume, Time 1 (mm3)</td>
<td>2535.4 ± 394.4</td>
<td>2854 ± 346.6</td>
</tr>
<tr>
<td>Right Mean Hippocampus Volume, Time 1 (mm3)</td>
<td>2640.3 ± 415.2</td>
<td>2854.8 ± 267.2</td>
</tr>
</tbody>
</table>

Note: *indicates significance at the .05 level; **indicates significance at the .01 level
§ Data unavailable for one participant

4.2 Objective 1: Detection of Sub-Acute Atrophy in the First Year of Injury

4.2.1 Hypothesis 1: TBI patients will show significant loss of volume across time that is not explained by age-related decline

Figure 4.1 presents the data for the right hippocampal volumes of patients and convenience controls. A repeated measures ANOVA revealed a significant group x time interaction ($F_{1,13}= 7.87; P< .05$). There was a trend for a main effect of time ($F_{1,13}= 3.96$, $P=.068$), and no significant main effect of group, ($F_{1,13}= 1.80$, N.S.).
Planned comparisons revealed that the TBI group demonstrated a significant decrease in hippocampal volume from baseline to 1-year post-injury ($P < 0.005$, Cohen’s $d = 0.34$). The control group demonstrated no significant decline or trend towards a decline; indeed, the absolute mean volume was slightly higher at 1 year post-injury. Taken together, these findings provide support for Hypothesis 1.

The purpose of the control group was to ensure that any decreases in hippocampal volume in the TBI group could not be attributed to age-related volume loss. However, the control group was significantly younger than the TBI group and therefore less vulnerable to age-related decline. This might have exaggerated relative differences in volume change between the groups. Therefore, published age-stratified normative data (from Bigler et al., 1997) were additional used as a second basis for comparison. Here, percent annual
change scores for published healthy individuals matched for age bands (in decades) to each of the patients were computed as in Ng et al. (2008). Annual percent change for the right hippocampus was -0.16% (SD= 0.12), which was approximately 25 times less than that of the TBI group, which was -4.09% (SD= 3.3), although these numbers should be interpreted with caution given the large standard deviations.

Figure 4.2 presents the results of the repeated measures ANOVA for the left hippocampal volume. A trend was observed for the group x time interaction ($F_{1,13} = 3.72$, $P= 0.076$), while the main effects of group and time were not significant ($F_{1,13} = 3.11$, N.S.) and ($F_{1,13} = 0.508$, N.S.), respectively.

Figure 4.2 Mean Left Hippocampal Volume for the TBI and Control Groups.

The TBI group demonstrated a significant hippocampal volume decrease over time for the left side. This was not observed for the control group. Note: * indicates significance at $P< .05$ level.

Although the interaction for the left hippocampus ANOVA showed only a trend towards significance, planned comparisons were nonetheless undertaken because of
the associated directional hypothesis. Mean hippocampal volume for the TBI group was significantly smaller at one-year post-injury compared to baseline ($P< 0.05$, Cohen’s $d= 0.22$). For the control group, there was again no significant difference or trend, and the absolute volume showed a slight increase from baseline to follow-up. Therefore, in support of Hypothesis 1, the left hippocampus demonstrated sub-acute atrophy by the first year of injury and this decrease in volume was not observed for the control group.

Again, published normative data from Bigler et al. (1997) were used to compute annual percent hippocampal volume changes for the left side. The TBI group demonstrated sub-acute annual percent hippocampal atrophy that was over 7 times that of age-matched controls: $-2.59$ (SD= 4.4) vs. $-0.34\%$ (SD= 0.15).

Although significance testing revealed significance, analytic stability is limited in a small sample size. Moreover, Figures 4.1 and 4.2 reveal substantial variability in volume change over time. Therefore, to visually inspect the reliability of these findings, percent change is plotted for each patient individually for both hippocampi in Figure 4.3. As can be seen from the figure, 9/10 patients showed some degree of volume loss in both hippocampi. One patient showed volume loss on only one side, and one patient showed increased volume for both hippocampi.

It is of interest to note that subject 7, who demonstrated the largest overall hippocampal volume loss bilaterally (left hippocampal change = 11.93%, right hippocampal change= 7.58%) and subject 2, who demonstrated the most favourable outcome over time cannot be distinguished demographically from one another. Both were female, older and with equal years of education. Only injury severity and injury
mechanism differed: patient 7 had sustained a more severe injury, and an MVA as opposed to a fall.

Figure 4.4 presents percent change for each control participant individually for each hippocampus. None of the participants showed absolute volume loss bilaterally. Only two demonstrated a unilateral decrease.

Figure 4.3 Left and Right Percent Hippocampal Volume Change for Individual TBI Participants.

Note: Volume decreases are represented by negative bars and increases are represented by positive bars for this figure. Participants 1, 3, 5, 7, 8, 9 and 10 demonstrated bilateral hippocampal volume decreases. Participants 4 and 6 demonstrated unilateral hippocampal volume decrease and participant 2 was the only one to demonstrate no hippocampal volume decrease.
Figure 4.4 Left and right percent hippocampal volume changes for individual control participants

Note: Volume decreases are represented by negative bars and increases are represented by positive bars for this figure. Only participants 4 and 5 demonstrated unilateral hippocampal volume decrease; none demonstrated bilateral volume decreases.

4.2.2 Objective 2 (Exploratory): Preliminary Examination of Correlates of Sub-Acute Hippocampal Atrophy

Figure 4.5 presents the findings of the bivariate correlation between GCS and percent volume change for the right hippocampus. A significant negative correlation was observed for GCS scores and right percent hippocampal change (Pearson $r = -0.663$, $P < 0.05$). Thus, greater injury severity, as indexed by lower GCS scores, was related to larger amounts of right sub-acute hippocampal volume decrease. This relationship was not significant for the left side, however (Pearson $r = -0.327$, N.S.).
A significant negative correlation was obtained for GCS and right percent hippocampal volume change (Pearson $r = -0.663$, $P < 0.05$).

With regard to demographic variables, there were no significant correlations. Age did not significantly correlate with either the left ($r = 0.065$, N.S.) or right ($r = 0.023$, N.S.) percent hippocampal volume change scores nor did years of education for either the left ($r = 0.436$, N.S.) or right ($r = -0.17$, N.S.).
Chapter 5 Discussion

5.1 Summary of key findings

The current study was designed to replicate and extend those of Ng et al. (2008) who had found sub-acute hippocampal atrophy in moderate to severe TBI patients from 4.5 months to 24 months post-injury. No conclusions from that study could be drawn as to whether the atrophy occurred earlier than 24 months, and preliminary cognitive findings from our laboratory had suggested that the atrophy might be occurring earlier, as greater atrophy from 4.5 months to 24 months post-injury correlated with poorer memory recovery from 5 to 12 months post-TBI in an overlapping sample of patients.

Thus, the primary objective of the current study was to determine whether a group of patients with moderate and severe TBI would demonstrate significant sub-acute hippocampal atrophy from 5 to 12 months post-injury. The approach was to examine atrophy within the TBI group and to compare the magnitude of atrophy to a convenience control sample. A secondary, exploratory objective was to examine the correlates of atrophy, examining injury severity (as measured by the GCS) and two demographic variables (age and years of education).

Consistent with the hypothesis, TBI patients demonstrated significant left and right sub-acute hippocampal volume loss within the first year of injury. These changes were not observed in the control convenience sample. The findings were robust, with 7 of 10 patients showing bilateral change in absolute volume in the direction of atrophy. In contrast, none of the controls showed bilateral absolute change in that direction. Less than half showed even unilateral change for the worse.
Severity of injury, as measured by GCS, was related to the amount of sub-acute hippocampal volume change observed for the right, but not the left hippocampus. There were no significant associations between amount of hippocampal volume loss and either age or years of education.

5.1.1 Sub-Acute Atrophy – Relationship to Prior Findings; Mechanisms

The current findings of sub-acute hippocampal atrophy corroborate findings in the animal literature (Smith et al., 1997) and human literature (Ng et al., 2008). Recent research has provided insight as to why these sub-acute decreases in hippocampal volume may be occurring during a time when the brain is presumed to be stable (Blatter et al., 1997; Christodoulou et al., 2001; Farne et al., 2004). Although the hippocampus is a deep gray matter structure, it has many white matter projections that act to bring information to and from this memory structure. Several studies have demonstrated that delayed white matter pathology can occur following TBI (Buki and Povlishock, 2006; Greenberg et al., 2008; Pettus et al., 1994; Rodriguez-Paez et al., 2005; Smith et al., 2003). Thus, it is possible that some of this delayed white matter damage could underlie sub-acute gray matter atrophy, particularly in the hippocampus. For example, destruction of axonal input to the hippocampus from functionally related but distant areas of the brain may result in decreased metabolism and regional cerebral blood flow to this structure (Kwakkel et al., 2004). In the early 20th century, von Monakow developed this secondary brain lesion effect theory, termed diaschisis (Finger et al., 2004); and today it is known as a potential underlying factor contributing to tissue loss in areas with suppressed metabolism, blood flow, and neural activity caused by the deafferentation of axons from distant structures. Contrary to the original notion that diaschisis is usually reversible in early stages post-
injury (Finger et al., 2004; Kwakkel et al., 2004), recent case study evidence has suggested that this may not always be the case, and individuals may present with irreversible diaschisis and associated degeneration depending on the nature of the primary injury (Baheti et al., 2009).

Similar to diaschisis, hippocampal atrophy can cause white matter destruction to efferent fibres resulting in anterograde degeneration to the structures to which it projects (e.g., the mammillary bodies) (Pierpaoli et al., 2001). This process is called Wallerian degeneration (e.g., Stoll and Muller, 1999; Bigler, 2007). In animal models of TBI, destruction to efferent hippocampal pathways has been observed (Hall et al., 2008) as well as afferent loss. In the latter case, decreased neural activity and accompanying atrophy of the structure being projected to may lead to reciprocal atrophy or dysfunction of the hippocampus. Over time, these processes occurring at the cellular level can lead to gross morphological changes. One might speculate that these sub-acute white matter changes may be underlying some of the sub-acute hippocampal atrophy observed following moderate and severe TBI.

Sub-acute hippocampal loss in the first year of injury was particularly evident for the right hippocampus. It has been well demonstrated that there is functional specialization of the hippocampus (Lye et al., 2004; Sass et al., 1990). Specifically, the left hippocampus has been well associated with verbal memory (Sass et al., 1990; Sass et al., 1992), while the right hippocampus has been associated with visual-spatial memory (Gleissner et al., 1998). However, Lye et al. (2004) also demonstrated that there was no evidence of a clear laterality effect for visual reproduction, a non-verbal memory task. These authors also noted that individuals may have been using verbal encoding to help them remember the items on this task. Therefore, it is possible that individuals have a tendency to rely on left
hippocampal function (due to verbal strategy use) more so than the right hippocampus. Also, in a recent study, Leshikar et al. (2009) used functional MRI on individuals while they performed an encoding task that required them to associate items that were either semantically related (easy) or unrelated (difficult). It was determined that as task difficulty increased, there was greater left hippocampal activation. Thus, as cognitive demand increased, the left hippocampus was used more. This is important considering TBI patients commonly have early memory impairments as a result of the initial acute atrophy (Borgaro and Prigatano, 2002; Ferri-Campos et al., 2008). As a result they have to use more mental effort (Kohl et al., 2009; Zasler et al., 2007) and one may postulate that they are more actively engaging the left hippocampus as a result. Work by Verghese et al. (2003) has shown that increased participation in cognitive activities is actually protective against memory decline. This theory has colloquially been termed, “use it or lose it.” Therefore, if TBI patients are using greater effort to remember things, this could cause greater activation of the left hippocampus and thus serve as a protective mechanism of left sub-acute hippocampal atrophy. Contrastingly, the right hippocampus could get used less and thus be more susceptible to sub-acute atrophy.

While the current study was able to reveal that sub-acute atrophy occurs by 12 months post-injury, the findings cannot tell us whether there is continued sub-acute atrophy, beyond the first year. Thus, it is unclear whether the observed sub-acute atrophy is best characterized as a single, second wave of atrophy or more insidiously, as an ongoing, neurodegenerative process. In order to determine this, a within subjects, longitudinal study examining hippocampal volume at three time-points would be an optimal design.
As a coarse comparison made for the purpose of generating hypotheses for future research, percent volume loss in the current study was compared to percent volume loss from 4.5 to 24 months post-injury in the Ng et al (2008) study. In order to control for demographic variance across patients, demographically-matched groups were created. For the left hippocampus, 10 patients (6 male, 4 female) with a mean age of 50.2 years (SD= 10.5) and a mean GCS of 5.8 (SD= 4.0) showed a 2.6% (SD= 4.4) volume decrease from 5 to 12 months post-injury. The comparison group from the Ng et al study (2008), (n= 11; 8 male, 3 female) with a mean age of 40.5 years (SD= 10.7) and a mean GCS of 8.5 (SD= 4.5), showed a 6.3% (SD= 4.5) volume decrease. For the right hippocampus, 10 patients (6 male; 4 female; mean age = 50.2 years, SD= 10.5) showed a 4.1% (SD= 3.3) volume decrease from 5 to 12 months post-injury while the comparison group from the Ng et al study (2008), (n = 11; 8 male, 3 female; mean age of 40.5 years, SD= 10.7) showed a 5.5% volume decrease. These descriptive statistics are compatible with the notion of continued atrophy for the left, but not the right hippocampus. Thus for the left side, findings are compatible with progressive neurodegeneration and for the right side, the findings are more compatible with a second wave of atrophy that completes by approximately the first year post-injury. It is not parsimonious to propose two different mechanisms of change for the two hippocampi, though, and the next phase of the current study will include a third MRI to empirically assess this important question using a repeated measures design.

5.1.2 Correlates of Sub-Acute Atrophy

In general, the literature has found that age, initial GCS score, duration of PTA and neuroimaging findings are correlated with functional and behavioural outcome from TBI
(Green et al., 2008; Levine et al., 2008; Wilde et al., 2006; Zasler, Katz and Zafonte, 2007). In the current study, GCS was significantly correlated with percent hippocampal volume change for the right side, only: lower GCS scores (greater severity) were related to greater right sub-acute hippocampal atrophy. This correlation was not significant for left percent hippocampal volume change, however. These findings are partially in accordance with the cross-sectional study by Levine et al. (2008) where GCS scores at discharge from the trauma unit were positively related to parenchymal volume at one-year post-injury. However, Levine et al did not examine sub-acute atrophy, they looked at one point only.

In contrast to the studies that have determined that demographic characteristics such as age and years of education are related to outcome from TBI (Green et al., 2008; Williamse-van Son et al., 2007), the current study did not find a relationship between these variables and sub-acute hippocampal atrophy in the first year of injury. One potential explanation for this observation is that hippocampi are highly vulnerable to sub-acute atrophy regardless of age or years of education. Thus, the demographic and injury variables examined may be more related to acute rather than sub-acute hippocampal atrophy. The literature has demonstrated hippocampal vulnerability and associated memory impairment under numerous conditions other than TBI including normal aging, hypoxia, prolonged corticosterone administration, transient global ischemia, affective disorders, Alzheimer’s disease and post-traumatic stress disorder (Gozal et al., 2001; Hoschl and Hajek, 2001; Kadar et al., 1998; Ouyang et al., 2007). In fact, animal research has determined that certain areas of the hippocampus, namely the CA1 and CA3 pyramidal cell layers, are linked to cognitive dysfunction and degeneration (Kadar et al., 1998; Kotapka et al., 1991; Royo et al., 2007; Witgen et al., 2005). Other research has
determined that delayed pro-apoptotic genes did not increase in expression at 3, 6 or 12 months post-TBI indicating that apoptotic mechanisms are likely not underlying these sub-acute changes to the hippocampus (Shimamura et al., 2005). Thus, secondary mechanisms involving extra glutamate and calcium involvement may be underlying these sub-acute changes, and are perhaps working in combination with other processes post-TBI, such as decreased neurogenesis in the dentate gyrus and decreased levels of neuroprotective genes (Shimamura et al., 2005; Yoshimura et al., 2003).

Another possible explanation for the lack of findings on significance testing is simply the lack of power in the small sample size of the current study.

5.2 Implications

The findings from the current study are clinically important. Although one recent study (Ng et al., 2008) determined that sub-acute hippocampal atrophy occurred from 4.5 months to 24- months post-moderate and severe TBI in a within-subjects design, the time-course for these changes had not been determined. The current study replicated the findings of Ng et al. (2008) by determining that sub-acute hippocampal atrophy occurs following moderate and severe TBI and moreover, it provided evidence that atrophy occurs by the first year of injury.

These results are highly important because many studies have provided evidence that behavioural and functional recovery is occurring until at least 6 to 12 months post-injury (Choi et al., 1994; Dikmen et al., 1990). Such behavioural improvements may be masking this underlying atrophy, which may in turn be mitigating recovery. Having a better
understanding of whether and when sub-acute hippocampal atrophy occurs is important for identifying windows of opportunity for treatment and rehabilitation purposes.

For example, hippocampal atrophy that occurs during a period when recovery is occurring elsewhere in the brain may be offset by more intensive rehabilitation, which continues throughout the first year of injury. This contrasts the focus of rehabilitation in the 1990’s, which was aimed towards efficiency, reduced costs and shorter rehabilitation periods (High, Sander and Struchen, 2005). Intensive rehabilitation may work to restore connections in the brain that were damaged from the TBI. For example, there is evidence for the effectiveness of constrained induced movement therapy (CIMT) for reduced upper limb function following stroke (French et al., 2007; Hakkennes and Keating, 2005). CIMT involves the restraint of the intact limb over an extended period of time, in combination with several repetitions of task-specific training trials using the affected limb. French et al. (2007) described that this intervention may work because the forced use of the affected limb forms the basis of motor learning; sensorimotor coupling contributes to the recovery or formation of neuronal pathways. Similarly, intensive rehabilitation may work to reform or create neural pathways that were damaged by the trauma or to prevent further deterioration of damaged pathways. For example, some strategies may be to include verbal elaboration training or using repetitive memory drills (Mateer et al., 1996).

Also, because sub-acute hippocampal atrophy may not be a universal phenomenon or it may be greater in some moderate or severe TBI patients over others, it is important to have an understanding of which factors put some individuals at a greater risk of sub-acute hippocampal atrophy over others. In the current study, only GCS was related to sub-acute hippocampal atrophy in the first year of injury. Thus, when injury severity, as measured
by the GCS is known, this may provide the patient, their families or caregivers and rehabilitation therapists an idea of what to expect over the course of the first year of injury.

5.3 Limitations

There were several limitations in the current study that could have affected the results. The first limitation was that there was a small sample size of 10 TBI patients and 5 control participants. Although the repeated measures design of this study helps to reduce within subject error, the sample size nonetheless limits both the stability of effects and the type of analyses that could be conducted.

Also, the control group was a convenience sample and the two groups were not matched for age and length of time between scans. Although the control group had a longer length of time between scans, which biased the results against our hypothesis, they were significantly younger than the TBI group. Therefore, the TBI group may have been more vulnerable to age-related decline, thereby providing an alternative explanation for the findings. Thus, the control group being significantly younger than the TBI group biased the results in favour of our hypothesis.

Furthermore, all TBI patients were recruited from a single, specialized rehabilitation hospital located in an urban area. As a result, the TBI sample in the current study may not be representative of all individuals with moderate and severe TBI. The generalizability of the current study may be limited and the results may not be applicable to others, particularly those that do not have access to urban rehabilitation facilities.
There were some additional limitations that were innate to the materials used in the study. Clinical data surrounding injury severity and pre-morbid characteristics were acquired via a retrospective review of each patient’s medical record. In some cases, patients had more than one GCS score reported (e.g., one at the scene of injury and one at Emergency). When this was the case, the lowest reported GCS score was utilized in the current study. GCS scores were selected over PTA scores to examine the relationship between injury severity and sub-acute hippocampal volume decrease because all patient data were available for this measure unlike PTA.

5.4 Future Direction for Research

The current study has provided preliminary findings that suggest sub-acute hippocampal atrophy occurs in the first year of injury. Future studies should corroborate these findings on a larger scale by incorporating multiple sites and a larger sample size so that findings can be generalized to all moderate and severe TBI patients. Also, results should be compared to a tightly matched control group composed of healthy individuals.

Future research should also address other measures of severity including PTA and ACLOS and their relationship with sub-acute hippocampal atrophy. These measures may be more sensitive in predicting the degree of sub-acute hippocampal atrophy from TBI.

The mechanisms underlying sub-acute hippocampal atrophy should also be a focus of future research. For example, Colicos et al. (1996) noted that neuronal loss following TBI can be broadly divided into three categories: (1) initial cell death from the physical trauma, (2) necrotic cell death from excitotoxic mechanisms and (3) delayed cell death.
Having a better understanding of this delayed cell death may provide insight into the outcome from TBI and may be the focus of future intervention.

Additionally, because TBI typically affects several other cognitive, behavioural and emotional domains, future research should focus on the development of rehabilitative methods that may offset further cognitive decline in these patients, while still being tailored enough to fit individual patient needs. Future work in understanding secondary mechanisms underlying delayed cell death may be useful for the development of pharmacological intervention that can be used in combination with rehabilitation methods.

Lastly, a future follow-up study assessing the patients in the current study at a third time point (approximately 24 months post-injury) should be conducted. This study would allow for hippocampal volume changes from 5 to 12 months to be directly compared to hippocampal volume changes from 12 to 24 months, while controlling for within subject variability. This design would help provide further insight into the time course for sub-acute hippocampal atrophy post moderate to severe TBI.

5.5 Conclusions

In summary the results of the current study suggest that individuals with moderate and severe TBI demonstrate sub-acute hippocampal atrophy in the first year of injury, which is particularly evident for the right hippocampus. An additional finding is that severity of injury, as measured by GCS, may be useful for predicting the degree of sub-acute hippocampal atrophy an individual may experience.
As TBI is one of the leading causes of morbidity and mortality around the world, future research needs to address the time course of sub-acute hippocampal atrophy following moderate and severe injury in order to gain a better understanding of the mechanisms underlying this outcome. A better understanding can aid in the development of precise, mechanism-based interventions aimed at optimizing the recovery of moderate to severe TBI patients. Furthermore, determining which clinical and demographic variables moderate sub-acute atrophy following TBI will increase our understanding of which patients are at a greater risk of poor pathophysiological outcome. This understanding is important for the patient, his/her family and/or caregivers and clinicians to know for the purposes of long-term therapeutic, family and occupational planning.
References


Squire, L.R. (1987). Memory and Brain. USA: Oxford University Press US.


