The Use of Functional Magnetic Resonance Imaging in the Assessment of Pediatric Patients Post Concussion

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December 2016

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy in Neuroscience.

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Abstract

Concussion, also known as mild traumatic brain injury, is an entity that is being increasingly recognized as an important public health problem with incidence rates reaching epidemic proportions. It is widely recognized that important long-lasting sequelae can be seen in patients who suffer concussions and adequate diagnosis and follow-up are essential in treating them. This issue is even more important in the pediatric population who, after having suffered a concussion at a young age, have a high chance of suffering other concussions later on. Furthermore, the effect of concussion on the developing brain may have more important implications than what is known so far. Unfortunately, standard structural imaging in these patients it typically normal and no objective clinical tests are currently available for medical professionals to aid with the assessment of patients post-concussion. Clinicians must rely on subjective symptom reporting, which is often fraught with inconsistencies. A number of studies in the literature have demonstrated the utility of functional MRI (fMRI) as an assessment tool in concussion but very few studies have examined its application to children.

Consequently, the current thesis describes three fMRI studies examining the use of specific fMRI paradigms in children. Study 1 was carried out in normal children and adults and demonstrated that the fMRI tasks that were developed in adults could be validly used in children. Study 2 used a working memory task that was previously proven to be useful in adults and demonstrated similar attenuation of fMRI signals in concussed children. However, unlike the concussed adults, the children had a diminished ability to recruit other brain regions and performed significantly worse than controls on the working memory task, a finding not seen in adults. Study 3 examined the use of a novel navigational memory task that was more complex, employing a number of cognitive functions. In this study as well, an attenuation of fMRI signal

was seen in the concussed subjects, with additional peaks in the hippocampus, suggesting that these subjects remained in a "learning phase" of the task.

Taken together, these studies demonstrate that, not only can these tasks be applied in children, they also show tremendous potential as an assessment tool in the context of pediatric concussion.

Résumé

La commotion cérébrale, également connue sous le nom de traumatisme crânien léger, est une entité qui est de plus en plus reconnue comme un problème de santé public important avec des taux d'incidence qui atteignent des proportions épidémiques. Il est généralement reconnu que des séquelles importantes de longue durée peuvent être observées chez les patients qui souffrent de commotions cérébrales. Un diagnostic et un suivi adéquat deviennent donc essentiels pour le traitement. Ce problème est encore plus important parmi la population pédiatrique qui, après avoir subi une commotion cérébrale à un jeune âge, ont de fortes chances de souffrir de commotions cérébrales subséquentes. De plus, une commotion cérébrale sur un cerveau durant son développement pourrait avoir des conséquences plus importantes que ce qui est connu jusqu'à présent. Malheureusement, l'imagerie structurelle standard pour ces patients est typiquement normale et il n'y a pas actuellement de tests cliniques objectifs disponibles aux professionnels de la santé pour aider à l'évaluation des patients suite à une commotion cérébrale. Les cliniciens doivent donc compter sur les rapports subjectifs des symptômes, qui sont souvent incohérents. Un certain nombre d'études dans la littérature a démontré l'utilité de l'IRM

fonctionnelle (IRMf) comme un outil d'évaluation pour les commotions cérébrales, mais très peu d'études ont examiné son application aux enfants.

Par conséquent, la thèse actuelle relate trois études utilisant l'IRMf et examinant l'utilisation des paradigmes d'IRMf spécifiquement conçus pour les enfants. La première étude a été réalisée chez des enfants et des adultes normaux et a démontré que les tâches d'IRMf qui ont été développées chez les adultes pouvaient être utilisées de façon valide chez les enfants. Dans la deuxième étude, une tâche de mémoire de travail dont l'utilité a déjà été établie chez les adultes a été utilisée et cette étude a démontré une atténuation similaire des signaux d'IRMf chez les enfants commotionnés. Cependant, contrairement aux adultes commotionnés, les enfants montraient une diminution de la capacité de recruter d'autres régions du cerveau et leurs performances à la tâche de mémoire de travail étaient significativement amoindries comparées aux contrôles, une constatation qui n'a pas été observée chez les adultes. La troisième étude a examiné l'utilisation d'une nouvelle tâche de mémoire de navigation qui est plus complexe et faisant appel à plusieurs fonctions cognitives. Dans cette étude, une atténuation des signaux de l'IRMf a été observée chez les participants commotionnés, avec des activations supplémentaires dans l'hippocampe, ce qui suggère que ces sujets sont restés dans la "phase d'apprentissage" de la tâche.

Dans l'ensemble, ces études démontrent que ces tâches peuvent être appliquées chez les enfants. De plus, elles montrent aussi un énorme potentiel comme outil d'évaluation dans le contexte des commotions cérébrales pédiatriques.

Acknowledgements

The journey has been quite challenging and it is certainly not something I could have done alone. I am eternally grateful to my supervisor, Dr. Alain Ptito for all his support and advice over the years. His mentorship has been invaluable to me. I would also like to express my sincere gratitude to my co-supervisor Dr. Judith Marcoux who has served as a great role model for me over the years, and committee member Dr. Isabelle Gagnon for providing me only the best of advice and guidance. I would also like to give a special thanks to Dr. Jen-Kai Chen who spent countless hours helping me with testing subjects and data analysis, even coming in evenings and weekends to help me get the work done. I would also like to thank all my co-authors for all their work and advice on the papers included in this thesis. Each of them played key roles in getting this work accomplished. I would also like to thank Dr. Frank Litvack and the Fonds de recherche du Québec – Santé for providing financial support to allow me to complete my research.

I would also like to express a special thanks to my family and friends who have been there for me throughout the good times and bad. All my life, my parents, Inder and Dipak, and my brother, Sanjeet, have been nothing but supportive and I would not have made it anywhere without their help and support. To my beloved wife, Nahil Dajani, words cannot express how grateful I am to you for everything you do to support and encourage me in all aspects of life. You are always there for me and I could not have accomplished any of this without you. And, finally, to my wonderful son, Rohan, thank you for always being such a great little man and cheering me up whenever I am down or frustrated. My accomplishments would never have come to fruition were it not for all the wonderful people who have supported me throughout my life and I never would have made it anywhere without them.

Preface and Contribution of Authors

The current document contains three studies that represent original scholarship. Study 1 (Chapter 3) has been recently submitted for publication. Studies 2 and 3 (Chapters 4 and 5) have been published in the Journal of Neurotrauma in 2014 and 2015, respectively. The elements of the studies that constitute original scholarship and contributions of authors are as follows:

Study 1: This study evaluates the use of two fMRI tasks, a working memory task and a navigational memory task, in adults and adolescents to determine what impact level of development plays in the activation patterns of adolescents versus the effect of performance. This study demonstrated that in the working memory task, there was very little age-related change seen as performance seemed to influence the activation considerably. Conversely, with the navigational memory task, age as well as performance both had strong influences on the strength of activations seen. However, although in both tasks, the overall areas of peak activation did not change with age, the strength of the activation did. As a result, we demonstrated that the two tasks that had been developed initially in adults, could also be used in the pediatric population as similar areas of peak activation were observed. Additionally, the results of this study lend support to the prevailing theory in the literature that functional maturation of the brain occurs progressively based on increasing task complexity.

Contributions of Authors:

Rajeet Singh Saluja: study design, subject recruitment, subject testing, data analysis, manuscript preparation, manuscript revision

Jen-Kai Chen: study design, subject testing, data analysis, manuscript revision

Rosanne Aleong: subject recruitment, manuscript revision

Sonja C. Huntgeburth: adult subject recruitment and testing, manuscript revision

Michael Petrides: study design, supervision, manuscript revision

Alain Ptito: study design, supervision, funding, manuscript revision

Study 2: This paper examined the use of an externally-ordered working memory task in the assessment of children after mTBI. While this task had been proven useful in the past in adult concussed athletes, this was the first time this task was used to test children post mTBI. Like in the adult population, we demonstrated decreased activation in several regions including the dorsolateral prefrontal cortex in the mTBI group relative to the normal controls. However, as opposed to the adults, we did not see the typical additional peaks such as the middle temporal activation seen in adults. Furthermore, the pediatric mTBI subjects performed significantly worse on the fMRI task than their normal counterparts, a finding that was not seen in adults. The findings of this study suggested that children had a diminished ability to recruit additional areas of the brain and, consequently, had decreased ability to compensate for affected areas after mTBI.

Contributions of Authors:

Michelle Keightley (co-first author): funding, study design, manuscript preparation, manuscript revision

Rajeet Singh Saluja (co-first author): study design, subject recruitment, subject testing, data compilation and analysis, manuscript preparation, manuscript revision

Jen-Kai Chen: study design, subject testing, data analysis, manuscript preparation, manuscript revision

Isabelle Gagnon: subject recruitment, manuscript revision

Gabriel Leonard: neuropsychological testing, manuscript revision

Michael Petrides: study design, manuscript revision

Alain Ptito: study design, supervision, funding, manuscript revision

Study 3: This study examined the use of a novel fMRI task, the navigational memory task, in the assessment of pediatric subjects post-concussion. This was the first time such a task was used to assess concussions in any age group. Despite little differences in task performance, we demonstrated significant decreases in task-related activation in the concussed group as compared to the healthy controls. In addition, we demonstrated that there was increased activity in areas such as the hippocampus, suggesting that the concussed subjects remained in a "learning phase" while attempting to complete the task.

Contributions of Authors:

Rajeet Singh Saluja: study design, subject recruitment, subject testing, data compilation and analysis, manuscript preparation, manuscript revision

Jen-Kai Chen: study design, subject testing, data analysis, manuscript revision

Isabelle Gagnon: subject recruitment, manuscript revision

Michelle Keightley: funding, study design, manuscript revision

Alain Ptito: study design, supervision, funding, manuscript revision

Chapter 1.

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability in children and young adults worldwide and is considered a significant public health issue (Beaglehole et al., 2003; Langlois et al., 2006). It is responsible for nearly half of all trauma related deaths and results in many years of productive years lost at great economic costs for both individuals and society (Basso et al., 2006). Mild traumatic brain injury (mTBI), often referred to as concussion, accounts for 80-90% of all TBI (Basso et al., 2006; Bazarian et al., 2005). While these injuries were traditionally thought to only cause temporary fluctuations in the level of consciousness, we now know that patients suffering these injuries can have long-lasting problems such as headaches, cognitive deficits, sleep disturbances, and depressive symptoms (McCrory et al., 2013). With incidence rates exceeding 600 per 100 000/year (Cassidy et al., 2004), mTBI has reached the level of epidemic proportions and is an important issue before the public health community worldwide. Youths are particularly at risk with higher incidence rates (McKinlay et al., 2008; Rutland-Brown et al., 2006) and, with a higher risk of multiple concussions over the

lifetime, they are consequently at higher risk of cumulative effects of these injuries (Gaetz et al., 2000; Guskiewicz et al., 2003; Rabadi and Jordan, 2001). With growing evidence linking multiple mTBIs to important chronic neurological conditions, mTBI has, over the last 20 years, become an essential area of intense study.

Obtaining the proper diagnosis and evaluating when it is safe to return to activities is essential in treating mTBI. Patients who suffer mTBI tend to have slower reaction times (Pare et al., 2009) and postural instability (Geurts et al., 1996; Guskiewicz, 2001), resulting in a higher risk of suffering another injury if allowed to continue high risk activities such as contact sports while still symptomatic (Delaney et al., 2000). This scenario is potentially devastating as it can lead to a rare but clinically significant condition known as second impact syndrome, an entity that carries with it a *mortality* rate of up to 50% (Cantu, 1998b; Cantu and Voy, 1995; McCrory and Berkovic, 1998). Unfortunately, diagnosis and monitoring of recovery have proven difficult for mTBI as the currently used clinical tools often reveal normal results and are unable to detect subtle abnormalities (Mami and Nance, 2008; Ptito et al., 2007). As a result, the mainstay of diagnosis, and subsequent monitoring, has been patient-reported symptomatology. The lack of objective measures to test for concussions has led clinicians to rely primarily on unreliable patient self-reporting of symptoms. Depending on the context, patients have been known to either minimize or exaggerate their symptoms, misleading the clinicians that are monitoring recovery. For example, professional athletes have been known to deny symptoms so that they can continue playing, an activity that is the source of their livelihood. Conversely, many clinicians treating mTBI will report that there are patients who tend to magnify symptoms, particularly in the presence of secondary gain(Chang et al., 2011). Clearly, more objective tests to diagnose concussions and monitor recovery are essential.

There have been a number of studies in the literature that have sought to develop objective testing modalities for the study of mTBI. One method that has shown significant promise thus far is functional MRI (fMRI). Several studies in the literature, including some written by co-authors of the studies presented here, have shown that fMRI combined with working memory tasks has potential as an objective diagnostic tool in concussion (Ptito et al., 2007). However, the vast majority of studies in the literature examining the utility of fMRI in the context of concussion have been restricted to adults. Prior to the studies presented in this thesis, very few studies looked at the use of fMRI for the diagnosis of concussion in the pediatric age group (Keightley et al., 2012). Given that brain maturation and development is not yet complete in children, we cannot assume that the findings in adults who have suffered concussions will hold true for children. As a result, the current body of work examines the potential use of two fMRI tasks as potential diagnostic modalities in the context of pediatric mTBI. The objectives of the following original work will be to: 1) determine what role age plays in the fMRI activation patterns using working memory and navigational memory tasks ; 2) to determine if the peak activation areas in normal children are the same as those seen in normal adults (allowing us to apply these tests to children); 3) determine if, using the previously published working memory tasks, the fMRI findings seen in concussed adult athletes are the same as those seen in concussed children; 4) Explore the use of a novel fMRI task, the navigational memory task, to determine if similar activation attenuation is seen in concussed children.

Chapter 2

Background

2.1. Review of mTBI/Concussion.

2.1.1. Definition of mTBI/Concussion.

While the definitions of the terms mTBI and concussion may seem trivial, it is an area of unresolved controversy in the literature. Part of the disagreement stems from the use of the two terms by different contingents of the expert pool. In general, the word concussion has been the favoured term in the literature among sports medicine specialists while mTBI is the preferred term among other medical specialists including neurosurgeons, neurologists, and rehabilitation specialists (Tator, 2009). Furthermore, the lay-population is much more familiar with the term concussion than that of mTBI. There is, however, often a misinterpretation by the lay-population that concussions are not considered brain injuries (Dematteo et al., 2010), although this is less of an issue with increasing awareness.

While the terms are often used interchangeably, there are subtle differences in the commonly used definitions of the two entities. The most widely used definition of mTBI comes from the American College of Rehabilitation Medicine (ACRM) that defines mTBI as "*A traumatically induced disruption of brain function, as manifested by at least one of the following: any LOC, any loss of memory for events immediately before or after the accident, any alteration in mental state at the time of the accident, and focal neurological deficit(s) that may or may not be transient; but where the severity of the injury does not exceed the following: LOC of approximately 30 min or less, after 30 min an initial GCS score of 13–15, and posttraumatic amnesia (PTA) not greater than 24 h*" (Kay et al., 1993).

A definition similar to that of the ACRM was adopted and promoted by the World Health Organization (WHO) task force on mTBI. They defined mTBI as "*an acute brain injury resulting from mechanical energy to the head from external physical forces. Operational criteria for clinical identification include (1) 1 or more of the following: confusion or disorientation, LOC for 30 min or less, posttraumatic amnesia for less than 24 h, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; (2) Glasgow Coma Scale score of 13–15 after 30 min post-injury or later upon presentation for healthcare. These manifestations of MTBI must not be due to drugs, alcohol, medications, caused by other injuries or treatment for other injuries (e.g., systemic injuries, facial injuries or intubation), caused by other problems (e.g., psychological trauma, language barrier or coexisting medical conditions) or caused by penetrating craniocerebral injury*" (Carroll et al., 2004a). This WHO definition stressed the transient nature of any neurological symptoms and introduced the concept of the absence of confounding factors such as intoxication or other potential problems.

What complicates matters further is that, in the clinical setting, most institutions use the Glasgow Coma Scale (GCS) score (Teasdale and Jennett, 1974) to assess severity of traumatic brain injury. Traditionally, patients presenting with a GCS of 3-8 are considered severe traumatic brain injuries, 9-12 moderate, and 13-15 mild. While this definition of mTBI is similar to others presented above, it does not cover the nuances of what constitutes a traumatic brain injury versus a minor blow to the head that does not result in any symptomatology. Furthermore, GCS-based definition includes all injuries, including large hematomas and contusions, so long as the GCS score is 13-15. There are many who feel that this does not truly reflect the reality of a mTBI and this has led to the relatively newer Mayo Classification. In this classification, there is more of an effort to re-classify any TBIs with any sign of traumatic lesion of the brain as moderate-severe, and reserving the term mild TBI (stratified into possible and probable mTBI) for patients with normal neuroimaging, with the exception of isolated skull fractures which are still considered mTBI (Malec et al., 2007).

With regards to concussion, it is an entity with ancient roots, dating back to the first description in the Corpus Hippocratum, later translated as *commotio cerebri* (Clark and Guskiewicz, 2016). The term concussion has always been a distinct diagnostic entity in the International Classification of Disease used by health care organizations worldwide to classify illnesses and this continues to be the case today (Tator, 2009). Although many definitions have been used over the years, currently the most commonly used definition comes from the 4th International Conference on Concussion in Sport. Their consensus statement posited that "*Concussion is a brain injury and is defined as a complex pathophysiological process affecting the brain, induced by biomechanical forces*" (McCrory et al., 2013). While this definition focuses more on the pathophysiological process rather than the clinical parameters, most agree

that the two terms mTBI and concussion are, in fact, referring to the same entity. As a result, in the current body of work, the terms will be used interchangeably, although for the purposes of the research carried out, the WHO criteria was used to establish clinical admissibility in our studies.

2.1.2. Epidemiology.

Defining the true epidemiology of mTBI has been very difficult over the years. Many earlier studies that utilised hospital records established incidence rates in the range of approximately 100 to 300 per 100 000/year (Hirtz et al., 2007). However, many patients who suffer these injuries never present to a hospital setting. Some are seen in clinics or by primary care physicians, and some patients simply never seek medical advice at all. This has led to estimates of the true incidence exceeding 600 per 100 000/year (Cassidy et al., 2004). To provide some context, the incidence rates of other "common" neurological ailments are as follows: multiple sclerosis 4.2 per 100 000/year; epilepsy 48 per 100 000/year; stroke 183 per 100 000/year (Hirtz et al., 2007). As a result, the incidence rates of mTBI make it one of the most common neurological disorders, reaching epidemic proportions as well as an important public health issue worldwide (Basso et al., 2006).

The statistics become even more troubling when we examine the pediatric population separately. TBI incidence in children aged 0 to 14 years presenting to hospitals in the United States reached a level of approximately 800 per 100 000/year (Rutland-Brown et al., 2006). Furthermore, McKinlay and colleagues performed a cohort study of children in New Zealand followed up until the age of 25 years, looking at the incidence of TBI, treated both in-hospital and out-of-hospital settings. They noted incidence rates ranging from 1100 per 100 000/year

(age 5 to 10 years) to 2360 per 100 000/year (age 15-20 years), with 90% of their subjects suffering mTBIs (McKinlay et al., 2008).

With respect to Canadian data in children, two studies in the literature quoted much lower rates but both studies are felt to be low estimations of the true incidence. Gordon and colleagues reported an incidence rate of approximately 200 per 100 000/year from retrospective data obtained from parents that participated in the Canadian National Health Population Survey (1996-97), with over 28 million people from the overall population. However, they only included in their study "any injuries that were serious enough to limit their normal activities" (Gordon et al., 2006). This restriction, along with the retrospective nature of the data, likely led to an underestimation of the true incidence. Willer and colleagues reported a symptomatic concussion rate of approximately 135 per 100 000/year from incident reports from the Ontario school system involving head injuries in the year 2000. While the "head injury" rate was 3980 per 100 000/year, they estimated that only 135 per 100 000/year had symptoms of concussion (Willer et al., 2004). However, these data only included children for whom incident reports were filed and did not include concussions suffered outside of school settings such as in recreational sports. Indeed this last point can lead to significant underestimation of the incidence rate since, as pointed out by Gordon et al., over 50% of concussions in the pediatric population occur during participation in sports activities.

2.1.3. Biomechanics and Pathophysiology

With the help of animal studies, it has long been established that the primary mechanism by which mTBI occurs is via forces created by linear and rotational accelerations of the head, though not necessarily through direct impact (Denny-Brown and Russell, 1940; Denny-Brown

and Russell, 1941; Holbourn, 1943; Holbourn, 1945). These early studies established that it was very difficult to cause concussions when the head was fixed in place and that concussions were primarily created by abrupt changes in velocity, either by sudden acceleration or deceleration. These changes in velocity result in the creation of inertial forces that induce strain on brain tissue causing injury (Clark and Guskiewicz, 2016). The mechanism by which this occurs is thought to be related to differing densities and rigidities of the various intracranial contents (i.e. grey matter, white matter, CSF, meninges, dura). As a result of these differing properties, some tissues are thought to accelerate faster than others resulting in tissue strain at the interfaces of these different tissues (Viano et al., 1989; Viano and Lovsund, 1999).

On the cellular level, animal models have demonstrated a great deal regarding the chemical and metabolic cascades that occur in concussion. Immediately after injury, it is thought that a massive efflux of potassium occurs resulting in a large, diffuse release of neuronal glutamate (Katayama et al., 1990), likely due to shear and strain forces on the neurons (Clark and Guskiewicz, 2016). This release of glutamate then causes random depolarization of a large number of neurons with a resulting increase in energy demand of the cerebral tissues. However, this increased demand occurs at a time of decreased cerebral blood flow, leading to a large deficit between energy demand and glucose delivery (Barkhoudarian et al., 2011; Barkhoudarian et al., 2016; Giza and Hovda, 2001). Following this period of metabolic stress, a period of hyperglycolysis and oxidative dysfunction with a related glucose hypometabolism occurs that can persist for many days post injury and may be related to prolonged post-concussive symptoms (Barkhoudarian et al., 2016). While these pathophysiological explanations seem plausible and useful, they are mainly based on animal studies and there may be other mechanisms involved in the brains of humans.

2.1.4. Symptomatology and Classification

In the immediate post-injury period (i.e. within the first few minutes), signs and symptoms that have been used to define concussions and mTBI were thought to consist mainly of alterations in level of consciousness and memory dysfunction in the form of post-traumatic amnesia (PTA)(Cantu, 1986). The alterations in level of consciousness can range from mild confusion or disorientation to a brief period of loss of consciousness, typically less than 30 minutes (Carroll et al., 2004a). Any loss of consciousness beyond that would be deemed a higher level of injury such as moderate or severe TBI. With regard to PTA, it is a short-lived memory disorder that is associated with traumatic brain injury and can take the form of either retrograde or anterograde amnesia (Cantu, 2001). Retrograde amnesia typically consists of the partial or total loss of the ability to remember the events immediately prior to the injury (Cartlidge and Shaw, 1981). Anterograde amnesia, however, involves the diminished ability to form new memories after the injury (Cantu, 2001). As with loss of consciousness, if the PTA persists longer than a certain period of time (24 hours in this case), the injury is considered to be of a higher grade than mTBI (Kay et al., 1993; Nakase-Richardson et al., 2011). There are a number of other symptoms that can occur in the acute setting including: headache, light-headedness, vertigo and instability, tinnitus, experience of seeing bright lights or blurred vision, diminished concentration, fatigue/lethargy, and personality changes (Cantu, 2001). However, the previously perceived common presence of loss of consciousness and/or PTA at the time of injury led to their inclusion in some of the definitions of mTBI and, subsequently, the focus of most classification systems involving concussions.

Many classification systems exist in the literature for concussion (Cantu, 1986; Jordan et al., 1989; Kelly and Rosenberg, 1997; Nelson et al., 1984; Ommaya, 1985; Report of the Sports

Medicine Committee, 1991; Roberts, 1992; Torg, 1991). It is important to keep in mind when discussing these classification systems that they are primarily based on expert opinion. Despite this, three of these classification systems have been used in the clinical setting to characterize concussion severity. These three main classification schemes are summarized in table 1 below:

Grade	Cantu Classification	Colorado Classification	American Academy of Neurology Classification
I	• No LOC • PTA < 30 min	• Confusion; no amnesia • No LOC	• Confusion • No LOC • Symptoms last < 15 min
II	• LOC < 5 min or • PTA > 30 min but < 24 hrs	• Confusion with amnesia • No LOC	• Confusion • No LOC • Symptoms last > 15 min
III	• LOC > 5 min or • PTA >24 hrs	• Any LOC	• Any LOC, brief (seconds) or prolonged (minutes)

Table 1. Summary of major concussion classification schemes. LOC = loss of consciousness; PTA = post traumatic amnesia. (Cantu, 2001).

While these grading systems have previously had widespread use in the clinical setting, there is no proven value to the use of these classification systems. In fact, the American Academy of Neurology updated their practice guideline in 2013 and moved away from classification of injury and, instead, stressed the use of screening tools to establish a diagnosis of concussion and subsequently enacting concussion protocols including return-to-play guidelines (Giza et al., 2013). Furthermore, some more recent studies have demonstrated that loss of consciousness may be present in less than 10% of sports-related concussion cases and PTA in 25-30% of cases (Dikmen et al., 2010; McCrea et al., 2003; Meehan et al., 2010). This has led to less of a focus on simply those two findings in the context of concussion.

Beyond simply the first few hours of injury, patients can suffer a multitude of symptoms to varying degrees for prolonged periods of time. The constellation of symptoms can be broadly divided into four main categories: *physical* including headache, dizziness, nausea/vomiting, balance problems, and fatigue; *cognitive* including altered level of consciousness, PTA and other memory problems, psychomotor slowing ("feeling slowed down"), and difficulty concentrating; *emotional* including irritability, emotional lability, and depressed mood; and *sleep disturbances* including difficulty falling asleep, sleeping more or less than usual, and drowsiness (Clark and Guskiewicz, 2016). While patients can suffer any of these symptoms to varying degrees, the most common symptoms include headache, dizziness, psychomotor slowing and fatigue (Dikmen et al., 2010; Kraus et al., 2005; Lucas et al., 2014). A patient who suffers any of the post-concussion symptoms after a blow to the head or other injury where significant acceleration/deceleration motions of the head are thought to have occurred should be evaluated as a suspected concussion (McCrory et al., 2013).

In the vast majority of cases, post-concussion symptoms typically resolve by about 7-10 days after injury, although children and adolescents tend to take longer to recover than adults (McCrea et al., 2013; McCrea et al., 2003; McCrory et al., 2013). There is, however, a subset of patients in whom symptoms persist longer than the typical time course, sometimes months longer, the so called slow-to-recover patients (Alves et al., 1993; Carroll et al., 2004b; Rutherford et al., 1979). Unfortunately, at present, there is no way of determining which patients will be slow-to-recover, although certain risk factors, such as history of previous traumatic brain injury, do sometimes relate to prolongation of symptoms (McCrory et al., 2013). Despite symptom resolution, however, there is evidence that in some patients, particularly children, some neurological deficits, such as problems with balance, may persist beyond symptom resolution

(Gagnon et al., 1998; Gagnon et al., 2001; Gagnon et al., 2004a; Gagnon et al., 2004b; Gagnon et al., 2005). This calls into question whether the brains of these patients are truly recovered once post-concussive resolve or if they are still in a stage of healing.

2.1.5. Clinical Management

Beginning from the time of injury, the first, and often most important step, in any suspected mTBI is proper initial evaluation. This assessment is not always carried out by a healthcare professional, especially in the context of sports, where athletic therapists often conduct initial evaluation, or coaching staff who have some training in the evaluation of injuries. When a TBI is suspected, thorough evaluation by a medical doctor is warranted. The initial medical evaluation primarily focuses on potentially more serious injuries including intracranial hemorrhages that may require further intervention. This evaluation typically includes a thorough history including exact mechanism of injuries, loss of consciousness, presence of PTA, headache, other symptoms, previous history of head injuries or other major illnesses or bleeding disorders. In addition to a thorough history, a full neurological exam is carried out to establish if any neurological deficits are present. If any suspicion of potentially higher grade of injury is present, further evaluation with computed tomography (CT) scan is typically undertaken.

When more serious injury is ruled out, further evaluation is variable among clinicians. Many medical professionals will simply document detailed histories of the present illness focusing primarily on the symptoms typically associated with concussions. These clinicians subsequently use the self-reporting of symptom presence or resolution to guide further management. Some others may go further with their evaluations employing the use of standardized batteries of tests such as the Sport Concussion Assessment Tool, 3rd edition

(SCAT3), which includes a modified version of the Balance Error Scoring System (BESS) to evaluate patient's balance (Guskiewicz et al., 2013). This is done in an attempt to standardize the testing elements and guide further management.

The recommendations on the treatment of patients with concussion comes largely from the sports medicine realm where considerable emphasis has been placed on the return-to-play protocols. To date, there are no clinically proven methods of active treatment for patients with concussions. Adequate treatment of these patients is critical with potentially serious implications. It has long been thought that returning to play too early can be detrimental in the evolution of concussion symptoms leading to worsening of neurocognitive symptoms and delayed recovery (Majerske et al., 2008). Furthermore, patients who return to play prior to the resolution of symptoms put themselves at higher risk of having a second concussion (Delaney et al., 2000; Guskiewicz et al., 2003; Laurer et al., 2001; Longhi et al., 2005; McCrea et al., 2009; Signoretti et al., 2010), mainly because of postural instability (Geurts et al., 1996; Guskiewicz, 2001) and impaired reaction time (Pare et al., 2009). The consequences of a second concussion before the first has resolved can have devastating consequences in the form of second-impact syndrome, an entity that carries with it a mortality rate of up to 50% and a morbidity rate approaching 100% (Cantu, 1998b).

The current mainstay of treatment is based on a graduated return-to-play and other activities, allowing adequate time and rest for the acute symptoms to resolve (McCrory et al., 2013). It is important to note that, in this context, rest includes both physical rest and *cognitive* rest. This latter point is often misunderstood among patients and a regimen of cognitive rest that includes reducing school/work demands, less "screen time" (e.g. computer, television, and cellular telephone usage), and less exposure to loud music should be employed (Silverberg and

Iverson, 2013). In the past, a regimen of complete rest was advocated until all symptoms of concussion resolved (Ingebrigtsen et al., 2000; Symonds, 1942; The Management of Concussion/mTBI Working Group, 2009). However, a recent review of the available evidence demonstrated that complete rest beyond three days post injury has no proven benefit and may even have detrimental effects (Silverberg and Iverson, 2013). As a result, the current return to play recommendations include a short period of complete rest (less than three days) followed by a gradual increase in activity as tolerated by the patient. If the patient is feeling well, the level of activity is gradually increased but, if symptoms worsen, a "step-back" approach is typically taken whereby the patient returns to the previous level of activity that caused no symptoms, then gradually increased again (McCrory et al., 2013). This continues until the patients return back to full activities in an asymptomatic state.

2.1.6. Potential Long-Term Consequences

Over the last two decades, considerable interest has developed regarding the potential long-term effects of multiple traumatic injuries to the brain. Certainly, a number of studies have shown links between mTBI and various illnesses including chronic post-concussion syndrome (PCS), chronic traumatic encephalopathy (CTE), post traumatic Parkinsonism (Jordan, 2013), and depression (Chen et al., 2008a; Johnston et al., 2004; Jordan, 2013). Chronic PCS is diagnosed when the symptoms of concussion persist beyond the typical recovery period (< 3 months), sometimes lasting months to years. Care must be taken when treating these patients to exclude the potential presence of other comorbid conditions such as post-traumatic stress disorder (PTSD). In these cases, treatment of these other conditions is vital (Mott et al., 2012). The association between concussion and depression has been often seen (Johnston et al., 2004), and the potential link has been further highlighted in the media with the recent suicides of

professional athletes who after pathological evaluation had brains consistent with previous traumatic injuries (Breslow, 2014; Cole, 2015). Indeed, a recent Canadian study demonstrated that patients who had a history of concussion had a long term risk of suicide three times that of the general population (Fralick et al., 2016).

The entity with the most correlation with concussions is CTE. Previously known as dementia pugilistica because it was first linked to dementia seen in former boxers (Roberts, 1969), it is typically seen in patients who have suffered multiple concussive, or possibly subconcussive, traumatic injuries to the brain (Dashnaw et al., 2012; Jordan, 2013). It typically presents as behavioural changes such as mood swings and depression. Cognitive issues develop over time including impaired attention/concentration and memory. Motor symptoms can develop over time including tremor, resulting in a Parkinsonian-like syndrome (Jordan, 2013). Neuroimaging typically only demonstrates non-specific changes such as diffuse cerebral atrophy. On microscopic evaluation of pathological tissue, CTE is considered a tauopathy with the perivascular deposition of phosphorylated tau-protein in the form of neurofibrillary tangles, thorned astrocytes, and neurites (Huber et al., 2016). This condition is gradually degenerative with increasing deposition of neurofibrillary tangles (McKee et al., 2013) and follows the clinical course of a progressive neurodegenerative disorder.

There has been great interest in exploring the potential long term effects of concussion in children, where brain development is still ongoing. The question whether concussion can cause permanent dysfunction by disrupting the proper development of a particular cognitive function is one that has important implications. There exist critical periods in brain development when significant acquisition of cognitive skills occur over time (Paus, 2005). Multiple reports exist suggesting that traumatic brain injuries suffered during an important period of development lead

to permanent impairments in the development of particular tasks (Freund et al., 1994; Levin and Hanten, 2005; Wiseman-Hakes et al., 2000). Furthermore, there has been suggestion in the literature that multiple concussions can have cumulative, long-lasting effects (Gronwall and Wrightson, 1975; Moser and Schatz, 2002). Beauchamp and colleagues reported a number of factors that had a relationship with 5-year outcomes after injury including "injury severity, age at injury, premorbid characteristics, psychosocial factors, access to resources, interventions, rehabilitation programs, and post-injury adjustments" (Beauchamp et al., 2010). However, the potential effects of concussion on normal brain development is not completely clear in the literature, particularly with regard to the long term effects of multiple concussions in the pediatric age group, with some studies showing detrimental effects of multiple concussions and others showing no effect (Yumul and McKinlay, 2016). With the potential of permanent cognitive deficits from impaired brain development and other possible long-term consequences, the realm of pediatric concussion research has become an area of intense interest.

2.2. Structural Neuroimaging in Concussion.

2.2.1. Traditional Structural Neuroimaging.

While structural neuroimaging has typically not shown any lesions in the context of concussion, it still plays an important role in the assessment of these patients to exclude more serious injuries with clinically relevant structural lesions such as hematomas. The typical mainstay of imaging for TBI in the emergency setting is computed tomography (CT) scanning. CT employs the use of ionizing radiation from rotating x-ray tubes and detectors. By measuring the attenuation of the x-ray beams as they travel through the tissue, they are able to create two-

dimensional images of the brain and skull in planes at various distances apart (slices) which can then be analyzed to create three-dimensional images and/or detect structural lesions in or around the brain parenchyma or bony fractures. Large hematomas, contusions and fractures can typically be seen with CT making it a relevant test to exclude large structural lesions that may require intervention or follow-up. Unfortunately, CT has limited resolution for soft tissues and cannot pick up small microstructural injuries (Gentry et al., 1988; Orrison et al., 1994). Furthermore, because of its use of ionizing radiation, the medical community has been hesitant to employ this technique unnecessarily, particularly in children where the developing brain is very sensitive to ionizing radiation. Indeed, a typical CT scan of the brain has an effective dose of 2 mSv and this dosage alone translates to an estimated increased lifetime risk of cancer of 0.016% in males and 0.026% in females (BEIR VII Committee to Assess Health Risks From Exposure to Low Levels of Ionizing Radiation, 2006), not to mention the potential risk to the developing brain (Kempf et al., 2013).

In adults, the Canadian CT Head Rule was created to establish clear guidelines when CT head was warranted (Stiell et al., 2001). It typically includes traumatic brain injuries whose characteristics surpass those in the typical definition of mTBI (e.g. Prolonged loss of consciousness) but it also allows some leeway for scanning individuals who sustained a dangerous mechanism of injury or those with potential bleeding disorders (endogenous or iatrogenic) that could increase the risk for potentially dangerous hematomas (Stiell et al., 2001). However, these criteria specifically exclude children under the age of 16 years. While management of these injuries can vary from one pediatric institution to the next, the preference at the Montreal Children's Hospital and other hospitals in North America, has been to rely more on

prolonged in-hospital observation of children post mTBI rather than CT scanning, unless clearly warranted.

In the current clinical context, structural MRI also plays a role in the evaluation of patients post mTBI. Because of the higher cost and more limited availability of this technology, MRI has been typically reserved for patients with persistent symptoms despite normal CT scans or to further characterize findings seen on positive CT scans (Honce et al., 2016). Unlike CT, MRI employs the use of high energy magnetic fields to align spinning protons in the tissue, then spin them out of equilibrium using radiofrequency pulses and measuring the time taken and amount of energy released when the protons realign with the magnetic fields. Different tissues have different magnetic properties and, by measuring these properties, images can be created with various tissue contrasts reflecting these magnetic properties (Gentry et al., 1988; Orrison et al., 1994).

Given its higher resolution for different tissue properties, MRI is superior to CT scan in detecting parenchymal lesions of the brain including small contusions and diffuse axonal injury (DAI) (Honce et al., 2016; Mittl et al., 1994). While MRI has a higher sensitivity in detecting extraparenchymal hematomas, such as subdural and epidural hematomas than CT scan (Jenkins et al., 1986; Kelly et al., 1988; Lee et al., 2008), the latter is more sensitive in detecting subarachnoid hemorrhages (Gentry et al., 1988; Orrison et al., 1994). In addition to this, there are various sequences that take advantage of specific tissue properties to make them more sensitive to specific pathologies. Fluid attenuated inversion recovery (FLAIR) sequences are very sensitive at detecting edema within the brain tissue making it easier to see contusions that can be missed on CT scan (Honce et al., 2016), and it is more sensitive in detecting small extra-axial hematomas (Morais et al., 2008). Another sequence, T2*-weighted gradient-echo imaging

(GRE) is particularly sensitive to the magnetic field distortions created by paramagnetic materials such as deoxygenated hemoglobin. As a result, this sequence is particularly sensitive in detecting microscopic hemorrhages that are not visible with other modalities (Ashwal et al., 2014; Kuzma and Goodman, 2000).

Despite this higher sensitivity to parenchymal injuries as compared to CT scan, the vast majority of patients with concussion, do not exhibit any of these abnormalities. As a result, traditional structural neuroimaging techniques including MRI and CT, while useful in excluding more serious injury, have limited utility in the diagnosis and management of the majority of patients with concussions.

2.2.2. Newer Structural Neuroimaging Techniques

With the advancement of MRI technologies, newer sequences have emerged that take advantage of certain specific properties of brain tissue to allow further characterization of microstructural changes in the tissue. One such sequence is susceptibility-weighted imaging (SWI), a variation of the GRE sequence discussed above, which is sensitive to hemorrhagic lesions due to the paramagnetic nature of blood breakdown products such as hemosiderin. While GRE has never been found to be useful in the context of concussion, SWI has shown more promise. It is a newer modality that involves further processing of the GRE data in various planes. Using sensitive measurements of the disruption of the magnetic field, a contrast can be generated resulting in the SWI image. In the case of traumatic brain injury, the theory is that micro-hemorrhages, which are typically undetectable by conventional imaging modalities, result in iron deposition in small static pools of blood, causing disruption of the magnetic field, resulting in the contrast mentioned above. Using this contrast method, SWI significantly

increases the sensitivity of the sequence to detect micro-hemorrhages within the brain tissue (Helmer et al., 2014).

The theory behind such a modality is certainly intriguing in the context of concussion and a few studies have shown the potential of SWI in detecting micro-hemorrhages in concussed patients where conventional imaging showed nothing obvious (Beauchamp et al., 2013; Helmer et al., 2014; Spitz et al., 2013). However, a recent paper has suggested that some of these findings may be called into questions as flawed methods in post-processing manipulation of these images may have led to false positive results (Li et al., 2015). Moreover, even if the imaging finding are in fact true, while it could be useful diagnosis, it is still unclear how this information could be applied clinically in aiding return-to-activity decisions.

Another MRI technique that has shown some promise is diffusion tensor imaging (DTI). This modality can be used to examine the integrity of white matter tracts that connect different areas of the brain. By measuring the restricted diffusion of water molecules along axonal tracts, the DTI data can be used to develop three-dimensional images of white matter tracts within the brain. While assessing and quantifying the diffusivity of water along the long axis of the axonal tracts, we can make observations about the integrity of these tracts in concussed patients by comparing them to those of normal controls (Vilanova et al., 2006). From the data collected, a number of parameters can be measured, the main one being the fractional anisotropy (FA) ratio. The FA ratio, which has values that fall between 0 and 1, is a measure of the fractional diffusivity of water along the long axis of the white matter tracts, where 0 corresponds to complete isotropic movement of water molecules (no restriction whatsoever) and 1 corresponds to complete restriction along a single axis. Other measures that can be assessed include the axial diffusivity (AD), which is the diffusivity along the long axis of the fibre bundle; radial diffusivity

(RD), which is the diffusivity perpendicular to the long axis of the primary diffusion; and mean diffusivity (MD), an overall measure of average diffusivity (Vilanova et al., 2006).

From pathological data, we know that shearing forces can exist in the context of severe traumatic brain injury that can result in disconnection of the grey and white matter of the brain (Adams et al., 1989). However, the importance of this in the setting of concussions is not as well understood. As a result, DTI can be an interesting tool to determine if white matter connectivity derangement plays a role in concussion pathology. To date, a number of experimental studies have used DTI in concussion research but the findings are unclear. Some studies have demonstrated decreased FA ratios or increased MD suggestive of microstructural white matter damage (Cubon et al., 2011; Honce et al., 2016; Kraus et al., 2007; Kumar et al., 2009; Lipton et al., 2008; Messe et al., 2011; Niogi et al., 2008; Rutgers et al., 2008a; Rutgers et al., 2008b), while others have revealed increased FA ratios or decreased MD in mTBI, possibly suggestive of axonal swelling (Bazarian et al., 2007; Borich et al., 2013; Henry et al., 2011; Ling et al., 2012; Wilde et al., 2008). Meanwhile, other papers reported mixed or no difference at all between DTI findings of mTBI subjects as compared to controls (Bazarian et al., 2012; Lange et al., 2012; Zhang et al., 2010). As a result, the use of this newer technique in the context of mTBI is still questionable. It is possible that a more targeted approach, focusing on specific areas such as those involved in areas of functional changes, may produce more fruitful results. Studies with this technology are ongoing.

2.3. Functional Neuroimaging

While structural neuroimaging has not shown great utility in the realm of concussion other than to exclude more serious injury, the same cannot be said about functional neuroimaging. A number of important findings have been seen over the years with regard to functional changes after concussion, both relating to symptom severity and recovery (Chen et al., 2007; Chen et al., 2008b). Indeed, the Consensus Statement from the 4th International Conference on Concussion in Sport stated that "Concussion may result in neuropathological changes, but the acute clinical symptoms largely reflect a functional disturbance rather than a structural injury" (McCrory et al., 2013). As a result, a great deal of interest has developed over the years on the functional findings seen in patients after concussion, using different imaging modalities.

2.3.1. Positron Emission Tomography

Positron emission tomography (PET) is an imaging modality that makes use of radiolabelled compounds injected intravenously. A positron is a form of anti-matter that has all the characteristics of an electron but with a positive charge instead of negative. Positrons only exist in our universe for fractions of seconds because immediately after they are emitted, they collide with an electron (present in abundance in all matter in our universe), resulting in the annihilation of the two particles and subsequent energy release in the form of two gamma rays in opposite directions (i.e. 180^{o} apart). Radioisotopes capable of emitting positrons such as fluorine-18 (^{18}F) can be created with the help of a cyclotron then used to synthesize a compound that may be an analogue of another common compound, such as ^{18}F-fluorodeoxyglucose (FDG) which is a form of glucose which replaces one of the hydroxyl groups with ^{18}F. Since FDG is an analogue of glucose, it will localize to tissues with higher glucose uptake and, consequently, higher metabolic demand (Bailey, 2005; Meyer, 1998). As a result, the FDG-can be used as an

important radiolabelled-compound in PET-scanning since it will localize in metabolically active areas of the brain. The PET scanner has gamma ray detectors arranged all around the head and, by analysing the different times at which the gamma rays reach said detectors 180^{o} apart, the FDG compounds can be localized in 3-dimensional space, allowing us to determine which areas of the brain are active by seeing where the FDG is concentrating.

The PET studies to date on concussion have mainly employed the measurement of resting metabolic activity. The first such study was by Humayun et al. in 1989 where they compared, using ^{18}F-FDG PET, 3 concussed subjects to 3 control subjects, within 3 to 12 months of injury. In their study, they found that the concussed subjects had decreased ^{18}F-FDG uptake in the medial temporal, posterior temporal, and posterior frontal regions (Humayun et al., 1989). A later study, however, demonstrated decreased uptake in the anterior temporal and frontal lobes (Ruff et al., 1994). The majority of studies looking at PET findings in the context of concussion demonstrated changes seen in the temporal and frontal lobes that seemed to correlate with worse outcome (Roberts et al., 1995; Umile et al., 2002), though one study demonstrated decreased cerebellar uptake in 12 mTBI soldiers who experienced blast injuries (Peskind et al., 2011). In addition to these resting activity paradigm studies, one study employed a PET study that included both a resting state and spatial working memory task. In their study, contrary to others, their resting state scans demonstrated no difference between the concussed and control groups but, with the spatial working memory task, less regional cerebral blood flow was seen in the prefrontal areas in the concussed subjects in comparison to the control group (Chen et al., 2003). Although some hope existed that this PET technology could be used as an objective diagnostic test for concussions, it was complicated by the finding that alcoholism and migraines produced an altered PET pattern similar to those seen with concussion (Mehr and Gerdes, 2001).

2.3.2. Functional MRI

Functional MRI is a technique that has shown interesting potential in the evaluation of concussion. Briefly, the principle of fMRI is based on the differences in the magnetic properties of oxygenated versus deoxygenated hemoglobin. Deoxyhemoglobin is *paramagnetic* in that it has magnetic susceptibility effects on the tissue, a property that is detected with T2*-weighted imaging as a decreased signal intensity. Oxyhemoglobin, on the other hand, is *diamagnetic*, meaning that it has very little effect on the T2*-weighted images produced. With this ability to differentiate oxygenated from deoxygenated hemoglobin, fMRI is able to evaluate the difference between the venous oxyhemoglobin and deoxyhemoglobin, a technique called blood oxygen level-dependent (BOLD) contrast. When a portion of the cortex becomes activated, local cerebral blood flow to that area increases in response to the activation related increased demand. Since the oxygen-extraction ratio changes very little, the increased blood flow results in a relative increase in the concentration of oxyhemoglobin compared to deoxyhemoglobin. As these relative changes in concentration can be measured by the fMRI, areas that are activated will result in increased MRI signal using the T2*-weighted imaging (Huettel et al., 2014).

There are numerous studies in the literature looking at the use of fMRI in concussion but the vast majority of these studies were studies on adults. FMRI was first used in the context of mTBI by McAllister et al. in two studies from 1999 and 2001. They used a working memory task that was an auditory version of a task known as n-back. Here, the subjects are tested for varying working memory loads ranging from 0-back to 2-back using a series of stimuli, and adding a 3-back condition in subsequent studies. In the 0-back paradigm, the subject simply has to answer whether the stimulus presented was the same as the one presented immediately before. In the 1-back condition, the subject needed to determine if the stimulus was identical to the one

presented before the last stimulus. During the 2-back condition, the subjects had to determine if the stimulus was the same as the one presented two stimuli back. As a result, this test assesses varying working memory loads (McAllister et al., 1999; McAllister et al., 2001a). In the two studies, McAllister and colleagues demonstrated that while the healthy control subjects demonstrated progressively increasing brain activation with increasing working memory load, the mTBI subjects had an altered activation pattern with the increasing load despite the fact that their performance was comparable to the normal controls in the task. In their mTBI cohort, they had a diminished brain activation compared to the control subjects in the low working memory load condition. However, with moderate load (2-back>1-back contrast), the mTBI subjects had more extensive brain activation, particularly in the right superior parietal and dorsolateral prefrontal cortex (DLPFC). With the highest load condition (3-back>2-back contrast), the mTBI group did not show any further increases in brain activation whereas the normal control subjects continued to have increased activation with the higher load. The authors felt that these findings likely represented altered resource allocation in the mTBI subjects and that this led to altered brain activation patterns (McAllister et al., 1999; McAllister et al., 2001a). In these studies, however, the authors did not try to examine the potential relationship between the altered activation patterns and symptomatology.

Soon after these initial studies, Chen and colleagues carried out three studies to further examine the use of fMRI in the context of concussion (Chen et al., 2007; Chen et al., 2004; Chen et al., 2008b). In their studies, they employed an externally ordered working memory task that did not have varying working memory loads like the n-back. In their task paradigm developed by Dr. Michael Petrides, the subjects were shown four items followed by a fifth item at a set interval. The subjects then had to answer whether this fifth item was a repeat of one of the first

four or whether the fifth item was a novel one (Petrides, 2005; Petrides, 2013; Petrides et al., 2001). While this is also a working memory task, it is considered a discrete working memory task whereas the n-back test is a continuous working memory task that requires constant surveillance of stimuli and, therefore, requiring a higher cognitive demand (Bryer et al., 2013). In their studies, Chen et al. demonstrated that, despite the fact that they performed just as well on the task as the normal controls, the concussed subjects had diminished task-specific percent BOLD signal increases seen in the typical areas of activation, particularly the DLPFC. Furthermore, they noted that the concussed athletes in their group had additional activation peaks seen in areas not typically activated with the task used (Chen et al., 2004). This finding was later supported in another study years later (Pardini et al., 2010). Chen and colleagues later demonstrated that these percent BOLD signal alterations correlated well with levels of symptomatology as measured by the post-concussion symptom (PCS) score – revised, with increased PCS score correlating with diminished activation (Chen et al., 2007). Furthermore, with the aid of serial testing, Chen et al. were able to demonstrate that, as symptoms resolved, the activation patterns became similar to those seen with normal controls while subjects who remained symptomatic continued to have altered activation patterns (Chen et al., 2008b). Taken together, these studies demonstrated that fMRI, in addition to providing interesting insight into the pathophysiology behind concussions, they also showed that fMRI could have some potential as a diagnostic tool in the assessment of concussion and monitor recovery.

In addition to these studies, a number of other groups have investigated the use of fMRI in concussion using various tasks that mostly related to frontal lobe/executive functioning, particularly with working memory related tasks. The majority of the literature looking at working memory has utilized the n-back task in various forms with relatively comparable results

(Dettwiler et al., 2014; Lovell et al., 2007; McAllister et al., 2006; McAllister et al., 1999; McAllister et al., 2001a; Pardini et al., 2010; Smits et al., 2009; Stulemeijer et al., 2010). In general, using the n-back task as the working memory fMRI paradigm, the studies have shown that concussed subjects had increased activation with the low and moderate-load conditions, while at the highest load, they tended not to show as much increase as the normal controls. As can be seen, this is the stark opposite of the findings seen in studies using the externally-ordered working memory task where hypoactivation was seen in the concussed individuals(Chen et al., 2007; Chen et al., 2004; Chen et al., 2008b; Gosselin et al., 2011). The difference likely resides in the task construct. While both the n-back task and the Petrides task are meant to study working memory, the Petrides task can be seen as a discrete working memory task which has tended toward relative hypoactivation while the n-back task is considered a continuous working memory task which, particularly at higher loads, is considered to have a higher cognitive demand (Bryer et al., 2013). Furthermore, other studies have shown that, despite its common use as a working memory task, the n-back task itself has very weak correlations with other working memory tasks such as the working memory span task (Kane et al., 2007).

Other fMRI tasks that have been studied in concussion include auditory orienting tasks (Mayer et al., 2009), route learning (Slobounov et al., 2010), word listening (McAllister et al., 2006), counting Stroop which assesses attention (Smits et al., 2009), auditory oddball (Witt et al., 2010), and a combination of finger sequencing, serial calculation, and digit span (Jantzen et al., 2004). The differences in activation between concussion and healthy controls in these studies varied with some demonstrating concussion-related relative hypoactivation in regions of interest (Mayer et al., 2009; McAllister et al., 2006; Witt et al., 2010) and others showing hyperactivation (Jantzen et al., 2004; Slobounov et al., 2010; Smits et al., 2009). In the vast

majority of studies in the literature, the performance measured on the tasks was no different between concussed subjects and healthy controls (McDonald et al., 2012).

In addition to these task related fMRI studies, a few groups have examined the use of resting-state fMRI (rs-fMRI) in the context of concussion. In the resting-state condition, no task is performed by the subjects and it is thought to reflect the intrinsic functional activity and connectivity of the brain. The theory behind the use of rs-fMRI in concussion is based on the concept that concussion likely results in a disturbance of the normal structural connectivity through axonal disruption resulting in asynchrony of the neural signals on the fMRI (Yuh et al., 2014). Research in this area is still early in its evolution and findings to date have mostly been with small numbers of mixed severities and variable times after injury. The various studies, however, have shown some interesting data. Research to date has shown disruption of functional connectivity in thalamocortical pathways (Tang et al., 2011), between the frontal lobe and the default mode network (DMN) (Mayer et al., 2011), within the DMN (Johnson et al., 2012; Sours et al., 2013; Zhang et al., 2012; Zhou et al., 2012), and widespread multiple network disruption (Stevens et al., 2012). Studies using this technique are still in their early stages and clinical utility of this type of scan is still to be established.

2.3.2.1. Correlation with Event-Related Potentials

The earlier findings of Chen and colleagues were further confirmed and correlated with event-related potentials (ERP) by Gosselin et al. where they used the same Petrides working memory task in both fMRI and ERP paradigms (Gosselin et al., 2011). In their paper, they studied 14 mTBI subjects with persistent symptoms beyond 2 months post-injury (mean 5.7±2.9 months). As in the previous studies by Chen et al. their data demonstrated that subjects with

persistent symptoms had hypoactivation in various regions including the mid-DLPFC, findings that correlated well with symptomatology. Furthermore, they measured ERPs using the same working memory task and noted decreased amplitudes in the N350 component in the frontal region. They concluded in their study that in patients with prolonged symptoms post-injury, there was evidence of continued cerebral dysfunction and, therefore, their symptoms could not simply be discounted as psychological or malingering (Gosselin et al., 2011). Gosselin and colleagues followed up this study with an additional paper examining the use of ERP alone in 44 patients with mTBI. In this paper, they noted that similar ERP changes were seen in this group as well (Gosselin et al., 2012).

2.3.2.2. Functional MRI in Children

While a number of fMRI studies have been carried out in concussed adults, very few studies have looked at the use of fMRI in children after concussion. Prior to the research contained in the following chapters, only three other papers in the literature addressed fMRI in children after concussion (Keightley et al., 2012). The three papers each used different tasks and had somewhat differing results. Yang et al. utilized an auditory orienting task in a group of 14 mTBI adolescents and 14 matched healthy controls. In their study, they demonstrated significantly decreased task-related activation in various brain regions in mTBI adolescents compared to control subjects (Yang et al., 2012). Another study by Krivitsky et al. examined a group of 13 mTBI subjects and matched controls using a Tasks of Executive Control (TEC) paradigm which is a combination of the n-back task and the go/no-go task (testing inhibitory control) developed by their group. In their study, the activation patterns did not differ between the two groups on the working memory portion of the TEC but they did demonstrate increased activation with the mTBI group in the inhibitory control portion in the posterior cerebellum

(Krivitzky et al., 2011). In the third report by Talavage et al., the main thesis of the paper revolved around the finding that subjects with multiple subconcussive hits could develop similar activation patterns on fMRI to those with diagnosed concussions. However, in their paper, they reported fMRI findings of 4 patients with clinically diagnosed concussions using an n-back task and demonstrated hyperactivation with the lower load memory tasks (Talavage et al., 2014). These findings are somewhat contradictory to those presented in the Krivitzky paper that did not find any differences between mTBI patients and normal controls using the n-back portion of their task. None of these studies related fMRI findings to symptomatology. Clearly, there is a paucity of literature examining the use of fMRI in children after concussion.

2.4. Summary

Concussion is recognized as an increasingly important issue with rates reaching epidemic proportions. The adequate assessment of patients after concussion is an essential issue with important potential long term consequences. This is particularly true in children who, as they are young at the time of injury, have a high likelihood of repeated concussions over time. Furthermore, the impact of concussion on the developing brain may have tremendously important consequences. Currently, the mainstay of assessment has been the reliance on notoriously unreliable subjective symptoms with a complete lack of truly objective measures to assess and follow patients with concussion. Of all the technologically advanced diagnostic tools at our disposal, the method that has shown the most promise in the assessment of concussion to date has been fMRI. However, there has been a relative dearth of literature examining its use in the pediatric age group. While some tend to see children as "miniature adults", increasingly the

majority of the medical community knows this assertion to be false, particularly with regards to the brain. Throughout the childhood years, the brain undergoes tremendous structural and functional development (Paus, 2005). As a result, we cannot simply assume that the fMRI findings that we see in adults will hold true in children.

The studies contained in the following chapters have, therefore, sought to tackle this issue by first examining whether the fMRI findings seen in normal adults are the same as those seen in normal children, establishing whether our fMRI paradigms can be applied to children and what role age plays in the fMRI activation patterns. Thereafter, we applied the same working memory task that had been proven in the adult population to be useful in the assessment of concussions to determine if similar attenuations of activation were seen in children. We then examined the use of a novel navigational memory task that employs multiple cognitive functions to determine if it had similar utility in the assessment of children after concussion.

Chapter 3

Functional MRI Testing in Adults Versus Children

3.1. Preface.

While the overarching goal in these studies was to examine the use of specific fMRI in the assessment of concussions in children, before these tests could be applied to children, we needed to determine if the tests themselves could be validly applied to children in general. In order to answer that question, we sought to determine if the fMRI findings seen in healthy children were similar to those seen in healthy adults. Although this task seems relatively simple, as will be discussed further later in this chapter, it is more complicated than it seems. In order to test the effect of age on the functional activation, it is essential to control for performance as this can be a very important confounding factor. As a result, what follows is a study that seeks to determine if age plays a role in the functional activation patterns seen in adults versus children, while controlling for performance, using an externally-ordered working memory task and a navigational memory task.

Working Memory and Navigational Memory Tasks: Effects of Age and Performance on Functional MRI Activation

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Submitted for publication.

3.2. Abstract

Several neuroimaging studies have examined functional activation in various cognitive and motor tasks in relation to the development of the brain. Variation in results across studies has led to controversy with respect to the contribution of developmental changes versus the effect of differential levels of performance on the functional activation patterns observed. Nevertheless, the prevailing theoretical idea remains that there is progressive functional maturation of the brain with simple and basic functions developing first and more complex ones developing later over time. The current investigation tests this idea by examining a group of 64 subjects, aged 10 to 55, using two tasks designed to measure fundamental processes. The first task measures the monitoring of information in working memory free of strategic planning and the second, a navigational memory task, requires retrieval from long term memory of location to navigate towards a target, planning of the shortest route from the current location, and online tracking along the route of any errors in navigation that would require a reformulation of the planned shortest route. The aim of comparing performance on these two tasks was to determine if, when controlling for the effect of different levels of performance, age-related changes could be observed in the peak activation patterns associated with these tasks. In the verbal working memory task, only two peaks of activation showed significant correlations with age and only one in the visual working memory task, although peak activations in several areas were shown to correlate with performance. By contrast, in the navigational memory task which requires several cognitive processes, six areas demonstrated significant correlations with age and seven areas correlated with performance. These results suggest that, after age 10, there are few age-related changes in the functional activation patterns related to working memory and none to the lateral prefrontal areas known to be critical for the monitoring of information in working memory.

However, in a more complex task, such as the navigational task, which requires the coordination of several cognitive processes, there were significant age-related effects. These findings support the model of progressive functional development dependent on task complexity.

3.3. Introduction

The developmental maturation of the human brain has long been an issue of great interest to neuroscience. Numerous structural neuroimaging studies have demonstrated major developmental changes occurring with age and most of these studies focused on the childhood and adolescent stages of life. During adolescence, in particular, white matter density has been shown to rise with increasing age (Giedd et al., 1999). Grey matter, however, has been shown to increase with age up to the ages of 10-12 years, after which grey matter volume is said to decrease, especially in the frontal and parietal regions (Giedd et al., 1999; Sowell et al., 2001). Other studies have suggested progressive grey matter changes that correspond with cognitive maturation (Casey et al., 2005). These grey matter changes appear to begin around puberty in the sensorimotor region and then in the association cortical areas of the frontal and parietal lobes. The dorsolateral prefrontal cortex (DLPFC) and posterior aspect of the superior temporal gyrus seem to be the last regions to be affected by these maturational changes (Gogtay et al., 2004; Sowell et al., 2004). With all of these structural changes occurring, it is of importance to understand what happens in terms of the functional organization of the brain with progressive age.

Over the last two decades, interest in the functional development of the brain has exploded. The advent and advancement of functional magnetic resonance imaging (fMRI)

techniques has permitted non-invasive acquisition of functional activation data related to the performance of various cognitive tasks. Many studies have shown changes in the strength of functional activation as a function of age (Casey et al., 2005; Luna et al., 2010; Paus, 2005). In general, most of these studies suggest that functional maturation of the brain begins with sensory and motor functions followed by association areas in a progressive manner (Casey et al., 2005). However, many of the earlier studies did not take into account the effect of levels of performance on functional activation (Paus, 2005). This begs the question of whether activation changes were indeed related to age per se, or confounded by performance.

The effect of age on functional brain activation patterns during the performance of a verbal working memory task has been investigated in a number of studies that showed higher percent BOLD signal changes in the prefrontal cortex as a function of age (Casey et al., 1995; O'Hare et al., 2008; Thomason et al., 2009; Vogan et al., 2016). These studies, however, did not adequately take into account the effects of the individuals' performance measures on functional activation patterns. While the other studies did not address the effect of performance, Thomason and colleagues (2009), using a variable load working memory task, controlled for performance by comparing adults with the highest load condition (six items presented) to children with the lowest load condition (two items presented). This was done because performance on these conditions was comparable and revealed similar patterns of functional activations. A study by Kwon and colleagues used a visuospatial working memory task and showed that increased activation in the prefrontal cortex persisted after taking into account the effect of performance (Kwon et al., 2002). However, a large study by Satterthwaite and colleagues investigated 951 young participants aged 8-12 years old using an n-back working memory task and found a stronger association between activation changes and performance than with age (Satterthwaite et

al., 2013). This study further highlights the importance of taking into account performance when conducting developmental studies.

Other studies examining the developmental effects on contextual memory and episodic retrieval have demonstrated important age-related changes in the medial temporal lobe structures (Demaster and Ghetti, 2013; Ghetti et al., 2010). Navigation tasks have proven reliable in eliciting functional activation patterns in the medial temporal region during memory encoding and retrieval (Iaria et al., 2007; Spiers and Maguire, 2007). However, only a few studies have examined the effect of brain development on functional activation changes using spatial navigation memory tasks. A study of only eight adolescents and eight adults found that age correlated well with functional activation changes particularly in the temporoparietal region and the cerebellum (Pine et al., 2002). A more recent study examined memory of landmarks as a component of spatial navigation in subjects aged 8-18 years (van Ekert et al., 2015). This study focused on the medial temporal structures and the prefrontal cortex; the authors noted that, while activity in these regions remained relatively stable across age, the parahippocampal gyrus and anterior cingulate cortex showed activations that increased with age. However, these findings did not take into account the potential effects of performance on the functional activation patterns.

The prevailing hypothesis in the literature to date is that functional maturation of the brain proceeds progressively throughout childhood and adolescence into young adulthood, beginning with relatively basic cognitive functions, which increase in their complexity. However, to the best of our knowledge, to date, there have been no studies that have examined the differential effects of age and performance on functional brain activation using tasks that assess distinct cognitive processes *within the same population*. The present study addressed this

issue by examining the functional activation peaks related to performance during two distinct cognitive tasks in the same group to determine whether age-related changes are more prominent in one task relative to the other. We chose a working memory task as is thought to employ more simple cognitive processes, and a navigation memory task, thought to represent more complex cognition.

3.4. Materials and Methods

3.4.1. Subject Recruitment.

Research ethics approval for this study was obtained from the Montreal Neurological Institute at McGill University. In total, 64 subjects (31 female) were recruited to participate in the study. This sample included 49 adolescents (ages ranging from 10 to 17 years; mean=14.52 years, standard deviation=2.05) and 15 adult subjects (ages ranging from 18 to 55 years; mean=31.17 years, standard deviation=12.73, median=28 years,). In addition to direct referrals, the majority of the pediatric participants were recruited from a database of subjects that had taken part in a previous study conducted by one of the co-authors (R.A.). The recruited subjects either had themselves participated in the previous study or were the younger siblings of previous participants. Adult subjects were recruited via self-referrals. All subjects were screened prior to participation in the study to rule out any underlying neurological and/or other medical conditions, and to ensure that there were no contraindications to MRI examinations. All testing was carried out over the course of one single, half-day and all subjects were compensated for their time.

3.4.2. Functional MRI Scanning.

Two cognitive tasks were performed by the subjects during the fMRI scanning sessions: a task assessing monitoring of information in working memory and a navigational memory task. The working memory task was an adapted version of the externally ordered working memory task designed by Petrides to evaluate the specific contributions of the lateral prefrontal cortex to the monitoring of information in working memory (Petrides, 2005; Petrides, 2013). This task was selected for two reasons: a) it has been shown in a series of human and nonhuman primate lesion studies that task performance depends critically on specific areas of the prefrontal cortex and, therefore, this is a validated task of prefrontal function and b) the cognitive process that is being measured has been analyzed in great detail. Two versions of this task were used in the present study: a verbal and a non-verbal version (see Figure 1a). In both versions of this task, subjects were first familiarized with five stimulus items, abstract drawings for the nonverbal version and pseudo-words for the verbal version of the task. On each trial of the task, four of the five stimulus items were randomly selected and presented in arbitrary sequence. Subjects were required to keep track of the four items presented on each particular trial. After a one-second delay, a test item was presented which was either one of the four stimuli already presented or the item that had not been presented on that particular trial. Subject had to decide, by pressing an appropriate button, whether or not the test item had been amongst those that had just been presented (right button for yes, left button for no). The next trial was then delivered in the same manner. The responses for each trial were recorded for all subjects and the accuracy (i.e. percentage of correct responses) was calculated and used as the primary performance measure.

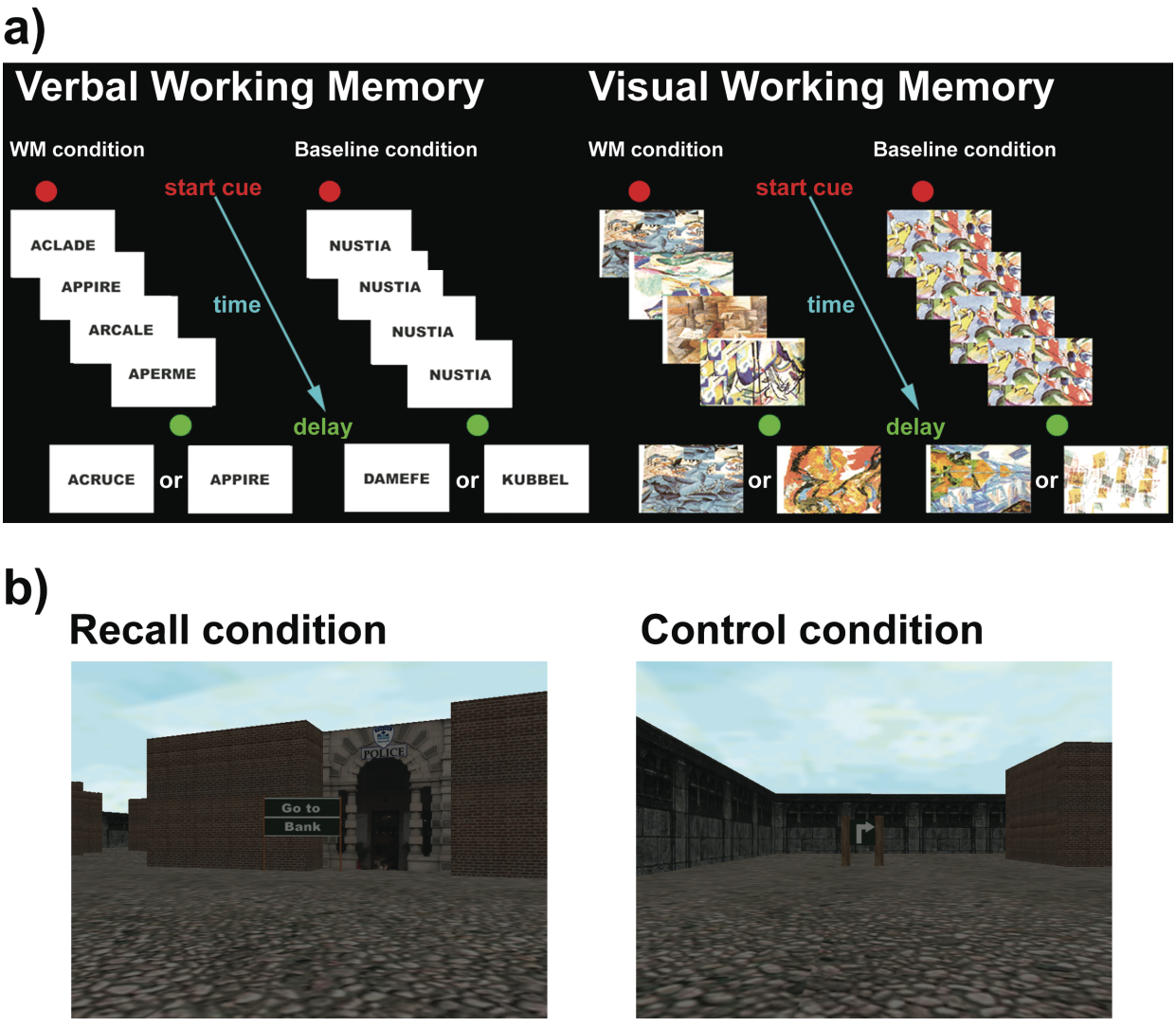

Figure 1. a) Schematic illustration of the verbal and visual working memory tasks and their respective control conditions. b) Examples of the navigational memory recall task and control condition.

In the working memory task, subjects are simply required to mentally tag the stimuli from the target set that are presented on each trial and, therefore, the task measures the monitoring (epoptic) process within working memory (Petrides, 2005; Petrides, 2013). Several studies using this task have shown: a) that prefrontal lesions which impair performance on this task importantly do not impair pure maintenance of the exact same information in working memory. This demonstrates that the essential cognitive process affected by dorsolateral prefrontal lesions is the mental tagging (monitoring) of the information and not its maintenance

in working memory (see Petrides 2013 for a review). An important advantage of this particular task, apart from its validation in lesion studies, is that it measures the monitoring (mental tracking) of occurrence compared to non-occurrence of a few stimuli and thus performance is not confounded by issues of strategic approaches, mental planning, and the overcoming of prepotent responses, problems present in many other working memory tasks (see Petrides, 2013).

A control condition was used to provide a baseline for the experimental task condition. Trials of the control condition were interspersed with the trials of the experimental condition at set intervals during the fMRI testing session. In the control condition, the same type of stimuli were used as in the experimental condition (i.e. abstract drawings and pseudo-words) but the stimuli employed in the control condition were novel stimuli. Here, one novel item was displayed four times in a row, after which a delay of one second occurred before a fifth item was displayed (i.e. one of two other items previously associated with a particular button press). The same two items were used throughout the control task trials. As in the experimental condition, subjects had 1.5 seconds to provide their response by pressing the appropriate button before the administration of the next trial. Thus, the control condition was comparable to the experimental one, except that in the control condition, subjects did not have to keep track of the occurrence of the particular stimuli from the specific target set during the presentation phase and, during the test trials, the decision which button to press was not based on the mental tagging (monitoring) process, but rather when subjects viewed the control stimuli, they were required to respond based on pre-instructed knowledge of which test stimulus required a left or right button response.

The navigational memory task is a modified version of a task devised and validated at our institution that employed a virtual three-dimensional environment (Iaria et al., 2007). In this task, subjects are required to navigate freely within a virtual environment (i.e. a virtual town), in

a video game-like manner with the aid of a four-button keypad. Prior to the scanning session, subjects are given the opportunity to explore the environment freely and thus learn the locations of six landmarks in this virtual town (e.g. police station, bank, etc). The free exploration allowed the subjects to form a mental representation of the environment (i.e. a cognitive map of the town) necessary to navigate from any one of the embedded landmark locations in the town to any other. Once subjects were comfortable with the virtual environment, they practiced a few route-finding trials during which they started at one location (e.g. the police station) and were instructed by a sign to move to another landmark location (e.g. go to bank) using the most direct route (Figure 1b). The starting point and destination were different across each trial during the fMRI scanning session, such that efficient completion of the task could only be achieved by using the previously learned cognitive map of the virtual environment. During the fMRI scanning session, subjects were given 15 minutes to complete as many trials as possible, up to a maximum of 24 trials. Their performance was measured by recording the number of trials completed within the allotted time and the total time taken to complete all trials (excluding incomplete and control trials), and the average time taken on the completed trials was subsequently calculated. The same virtual environment was utilized for the baseline control trials, but this time subjects were simply asked to follow signs with arrows on them to reach particular destinations in the environment (Figure 1b). Thus, these control trials provided a baseline condition for the experimental navigation task, since the procedure in the control task had the same requirements as the experimental task, except that no cognitive retrieval of the shortest route to a specific destination was required in the control condition since navigation was guided by signs with arrows. Thus, as in the working memory task, the control trials replicated the structure of the experimental task and provided a control for the motor, perceptual, general decision making, and other aspects of performance

required in the experimental task, without tapping into the essential processes (i.e the retrieval of the shortest route from a pre-established cognitive map of the virtual town). The control trials were interspersed between the cognitive navigation trials within the 15-minute scanning period.

3.4.3. Image Acquisition.

All structural and functional MRI scanning was performed on a 3-Tesla Siemens Magnetom Trio A Tim System with a 32-channel head coil. Each session began with the acquisition of high-resolution T1-weighted 3D anatomical images using a 3D Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence (TR = 23ms, TE = 2.98ms, Slice Thickness = 1mm, Image Matrix = 256 x 256, Flip Angle = 30 degrees, FOV = 256mm, interleaved excitation). This was followed by fMRI acquisitions with T2* weighted gradient echo (GE) echo-planar images (EPI) for the acquisition of the blood oxygenation level dependent (BOLD) signal. For the different tasks employed in the present study, the following parameters were used: *working memory task* - TR = 3000ms, TE = 30ms, flip angle: 90°, slice thickness = 4mm, in-plane resolution = 2.34 x 2.34 mm, FOV = 300 mm, 128 x128 image matrix, number of slices: 37, interleaved excitation; *navigational memory task* - TR = 4500ms, TE = 30ms, flip angle: 90°, slice thickness = 4mm, in-plane resolution = 4 x 4 mm, FOV = 256 mm, 64 x64 image matrix, number of slices: 38, descending excitation. For the working memory tasks, four scans were obtained (two each for the verbal and visual versions). Each of the scans lasted six minutes with activation and baseline control conditions alternating every eight trials (60 seconds). For the navigation task, the scanning time lasted 15 minutes, regardless of the number of trials completed by the participants. Prior to the scanning sessions, all subjects were introduced to the tasks outside the scanner by completing at least 48 practice trials of the

working memory tasks and by having the opportunity to familiarize themselves with the virtual environment and practice navigating within the virtual town.

3.4.4. Imaging Analysis.

The fMRI data analysis has been described in our previous publications (Keightley et al., 2014; Saluja et al., 2015). Briefly, all images were corrected for motion artifacts using the 3-D prospective acquisition correction technique implemented by Siemens for real-time motion correction of BOLD data (Thesen et al., 2000). These motion-corrected images were then spatially smoothed with a 6-mm full-width at half-maximum Gaussian filter to increase the signal-to-noise ratio and these data were subsequently analysed statistically using fmristat (Worsley et al., 2002b). Significant BOLD changes between the control condition and the experimental task condition were determined at each voxel, based on a linear model with correlated errors. To obtain the average group t-maps, all individual MRI data were first normalized to the Montreal Neurological Institute (MNI) stereotaxic space constructed from the average stereotaxic MRI of 305 normal subjects (Evans et al., 1993) and were then combined using a mixed effects linear model. The resulting t-statistic images were thresholded using the minimum given by a Bonferroni correction and random field theory to correct for multiple comparisons (Worsley et al., 1996). The threshold for significance was established at $t = 4.10$ for the activation peaks, or $t = 3.10$ for activation clusters greater than 222 mm^3, corresponding to a $p<0.05$, based on the number of resolution elements in the acquisition volume (2,880 resels). Once significant areas of activation were identified, individual percent BOLD signal change values were extracted for further analyses.

3.4.5. Statistical Analyses.

Further analyses were performed with IBM Statistical Package for the Social Sciences (SPSS) version 23.0 for Mac. Regression analyses and Pearson correlations were performed to examine the relationships between age and performance for each of the tasks. Partial correlations, a statistical method used to describe the relationship between two variables while subtracting out the potential effects of a third control variable, were performed to compare the peak activation values obtained from fMRI to age when performance was set as a control variable. Further, partial correlations were performed to compare peak activation values to performance while using age as a control variable.

3.5. Results

3.5.1. Age-Performance Correlations.

Correlations between age and accuracy of responses (percent correct responses) on the verbal working memory (ρ=0.06, p=0.635) and the abstract visual working memory tasks were not significant (ρ=-0.09, p=0.48), showing that age did not have an influence on the proportion of correct responses made. The correlation between age and reaction time (a secondary measure of performance) reached statistical significance for the abstract visual working memory task (ρ=0.261, p=0.038), but was not significant for the verbal working memory task (ρ=0.124, p=0.328). For the navigational memory task, age was significantly correlated with the average trial completion time (ρ=0.300, p=0.017), indicating that the older subjects required more time to complete the trials (i.e. inferior performance). Visual examination of the graphed data

demonstrated a non-linear relationship between performance and age (Figure 2) with an improvement of performance up to the age of about 30 years followed by a subsequent decline in performance with increasing age. Quadratic regression analysis revealed a statistically significant result for the non-linear correlation between performance and age for the navigational memory task (R^2=0.341, F=15.492, p<0.001). This analysis was repeated for the working memory performance correlations but did not demonstrate any other significant correlations.

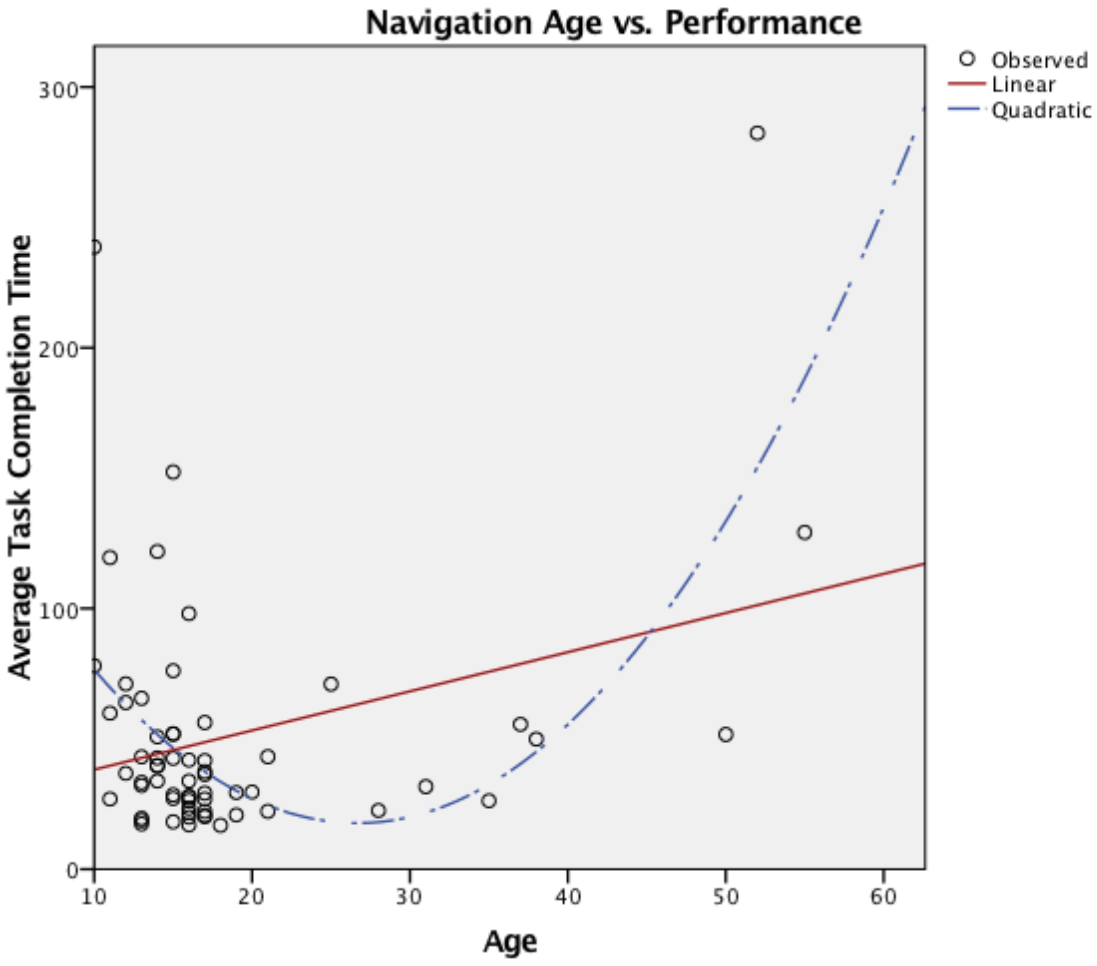

Figure 2. Age-performance regression using linear and quadratic analyses. The x-axis represents the age of the subjects. The y-axis denotes the average task completion time and, as a result, represents the inverse of performance.

3.5.2. Working Memory Task-Related Peak Activations.

Average t-maps were computed by comparing the experimental and control conditions of each task across participants, and the areas of functional activation, represented as BOLD signal changes, were identified for both, verbal and visual, working memory tasks. As can be seen in Table 1, the t-statistic maps obtained from the verbal working memory task showed significant functional activation patterns, bilaterally, in the frontopolar and lateral prefrontal cortex, the anterior insular and cingulate cortex, caudate nucleus, thalamus, superior parietal lobule and occipital lobe, cerebellar hemispheres and the vermis of the cerebellum, and unilaterally in the right orbitofrontal area. Analysis of the visual working memory task revealed activation patterns in similar regions as seen in the verbal working memory task, with the addition of a few extra activation clusters (Table 1 and Figure 3). To ensure that the activation patterns were comparable across age groups, the t-maps of the youngest 15 subjects and those of the eldest 15 subjects were directly compared. No differences were found in this direct group comparison between the areas of activation observed across the two groups (see Figure 4).

3.5.3. Navigational Memory Task-Related Peak Activations.

As for the working memory tasks, an average t-map was created for the navigational memory task using the entire subject pool. This t-map demonstrated peak activations, bilaterally, in the frontopolar and lateral prefrontal cortex, anterior cingulate cortex, premotor area, thalamus, posterior parahippocampal gyrus, retrosplenial area, superior parietal lobule, lingual gyrus and cerebellar hemisphere regions (Table 1 and Figure 3).

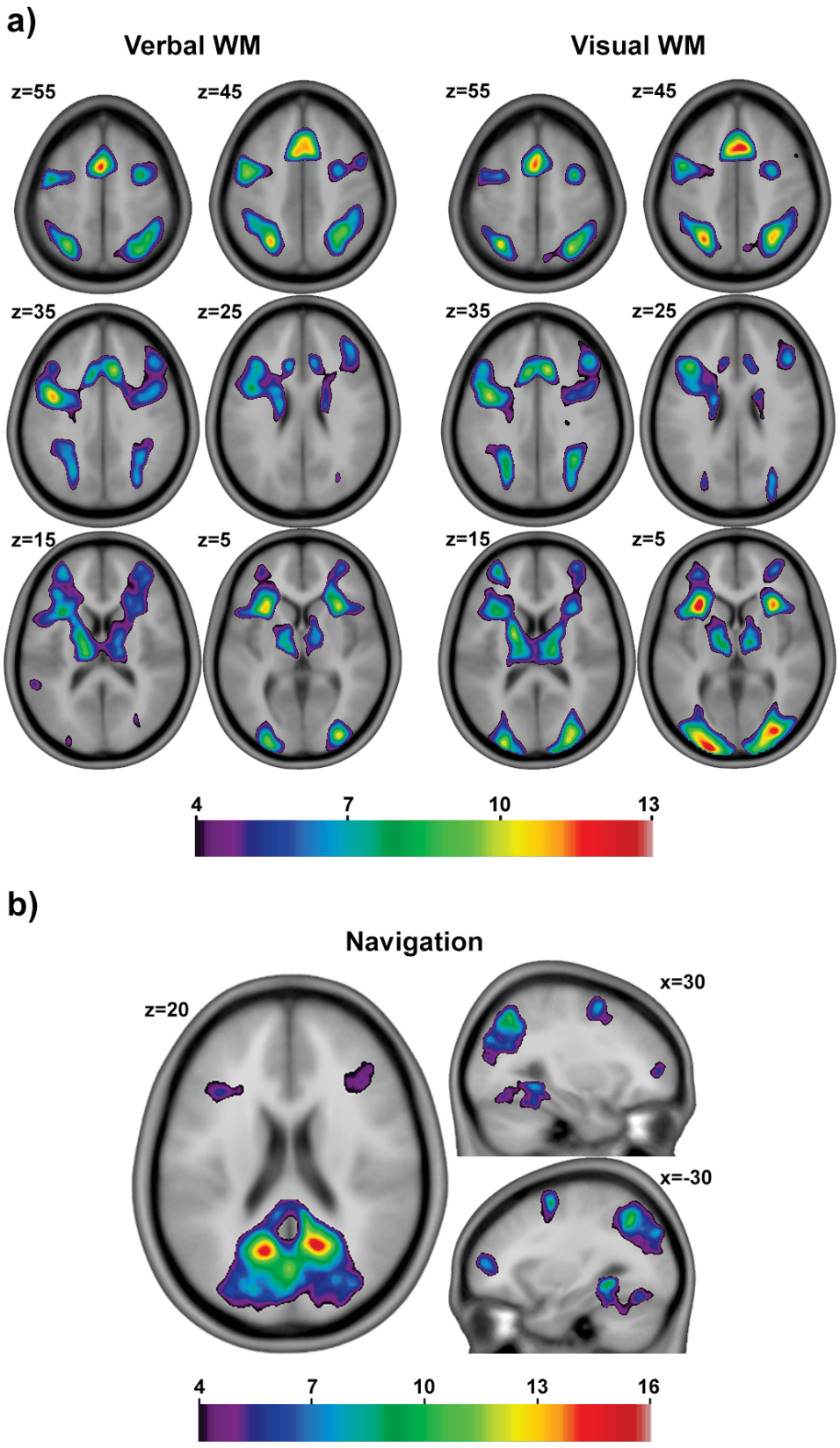

Figure 3. Average t-maps for each of the task paradigms.

Verbal Working Memory Task				
Region	**x**	**y**	**z**	**t**
Right Frontopolar	30	50	10	6.93
Left Frontopolar	-32	48	12	7.33
Right Orbitofrontal	24	50	-10	6.31
Right DLPFC	44	26	30	7.33
Left DLPFC	-40	24	26	8.05
Right Anterior Insula	32	24	-2	11.92
Left Anterior Insula	-30	18	4	11.10
Right Cingulate	12	20	36	9.07
Left Cingulate	-8	14	42	9.98
Right Caudate	10	2	8	6.41
Left Caudate	-12	2	8	6.01
Right Thalamus	14	-12	8	7.03
Left Thalamus	-14	-12	10	9.78
Right Superior Parietal Lobule	30	-62	50	9.88
Left Superior Parietal Lobule	-28	-62	48	11.51
Right Occipital	32	-92	-2	12.74
Left Occipital	-26	-96	-2	11.31
Right Cerebellar Hemisphere	30	-62	-24	10.19
Left Cerebellar Hemisphere	-34	-58	-30	8.56
Right Vermis	8	-72	-24	9.07
Left Vermis	-6	-72	-24	8.56
Visual Working Memory Task				
Region	**x**	**y**	**z**	**t**
Right Frontopolar	32	50	6	6.60
Left Frontopolar	-32	50	14	8.45
Right Orbitofrontal	26	48	-8	6.36
Left Orbitofrontal	-24	50	-12	5.74
Right DLPFC	44	28	30	8.20
Left DLPFC	-40	22	26	9.81
Right Caudate	12	2	4	5.86
Left Caudate	-12	2	2	5.99
Right Thalamus	12	-16	10	7.59
Left Thalamus	-12	-16	10	8.82
Right Anterior Insula	32	22	0	13.63
Left Anterior Insula	-32	22	2	12.64
Right Cingulate	8	18	40	10.91
Left Cingulate	-6	16	42	10.67
Right Premotor	32	-2	52	9.31
Left Premotor	-40	-2	36	10.05
Right Superior Parietal Lobule	30	-62	50	11.16
Left Superior Parietal Lobule	-28	-62	46	11.90
Right Occipital	36	-80	-8	13.38

Left Occipital	-38	-74	-8	13.50
Right Cerebellar Hemisphere	36	-58	-28	10.67
Left Cerebellar Hemisphere	-36	-64	-26	9.07
Right Vermis	6	-76	-22	9.19
Left Vermis	-4	-76	-22	10.42
Navigational Memory Task				
Region	**x**	**y**	**z**	**t**
Right Frontopolar	28	52	4	7.10
Left Frontopolar	-30	52	8	8.07
Right DLPFC	42	28	30	8.07
Left DLPFC	-42	24	30	7.42
Right Anterior Cingulate	8	26	38	9.91
Left Anterior Cingulate	-4	28	36	9.69
Right Premotor	30	4	58	8.61
Left Premotor	-30	2	58	9.26
Right Thalamus	16	-24	14	6.56
Left Thalamus	-10	-24	12	6.45
Right Posterior Parahippocampal Gyrus	24	-42	-10	8.94
Left Posterior Parahippocampal Gyrus	-26	-42	-8	11.20
Right Retrosplenial	14	-58	18	14.55
Left Retrosplenial	-14	-60	12	15.52
Right Superior Parietal Lobule	30	-66	50	10.56
Left Superior Parietal Lobule	-30	-64	45	10.45
Right Lingual Gyrus	10	-76	-10	11.20
Left Lingual Gyrus	-8	-76	-10	11.53
Right Cerebellar Hemisphere	30	-60	-28	7.53
Left Cerebellar Hemisphere	-36	-60	-30	6.13

Table 1. Areas of significant activation for each task paradigm, across all subjects.

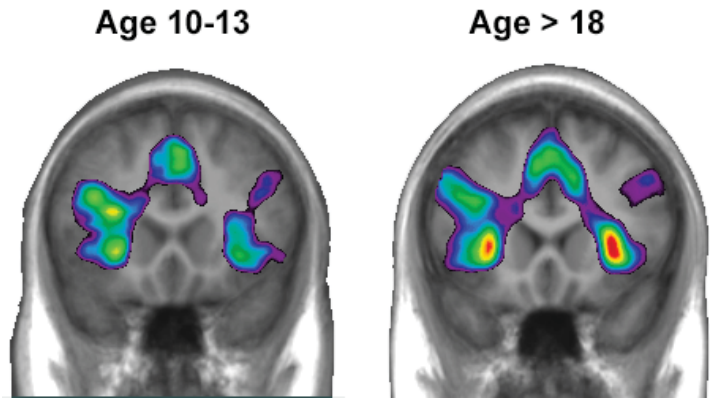

Figure 4. T-maps of the 15 youngest and the 15 oldest subjects demonstrating comparable areas of peak activation in the visual working memory task.

3.5.4. Age and Performance-Related Changes in Activation for the Working Memory Task.

Percent BOLD signal change values were extracted for each of the subjects in the areas of peak activation listed above. Partial correlations with BOLD activation signal were carried out for the variable age, while controlling for performance (i.e. accuracy). Partial correlations were also calculated for performance, using accuracy as the performance measure, and peak activation BOLD signal while controlling for age. The results demonstrated that, for the verbal working memory task, age correlated with BOLD activation in the left occipital lobe and the vermis regions (Table 2). For the visual working memory task, there was a significant correlation with age in the left superior parietal region. When correlating functional activation with the variable performance (i.e. accuracy), significant correlations bilaterally in the caudate nucleus, thalamus, and superior parietal regions were observed for the verbal working memory task, and correlations approached significance in the left cingulate cortex and the vermis regions. For the visual working memory task, significant correlations between percent BOLD signal change and performance were noted bilaterally in the anterior insular cortex, the cerebellar and vermis regions, along with the left thalamus, cingulate cortex, superior parietal region, and the right premotor area. The areas that approached significance included the right DLPFC, caudate nucleus, superior parietal and the left occipital areas.

Verbal Working Memory		
Age Controlling for Performance		
Region	**ρ**	**p-value**
Left Occipital	-0.267	*0.034*
Left Vermis	-0.277	*0.028*
Performance Controlling for Age		
Region	**ρ**	**p-value**
Right Caudate	0.276	*0.029*

Left Caudate	0.319	*0.011*
Right Thalamus	0.252	*0.047*
Left Thalamus	0.297	*0.018*
Right Superior Parietal Lobule	0.249	*0.05*
Left Superior Parietal Lobule	0.275	*0.029*
Left Cingulate	0.239	0.059
Left Vermis	0.244	0.054
Visual Working Memory		
Age Controlling for Performance		
Region	**ρ**	**p-value**
Left Superior Parietal Lobule	0.274	*0.03*
Performance Controlling for Age		
Region	**ρ**	**p-value**
Left Thalamus	0.263	*0.037*
Right Anterior Insula	0.292	*0.02*
Left Anterior Insula	0.272	*0.031*
Left Cingulate	0.254	*0.045*
Right Premotor	0.274	*0.03*
Left Superior Parietal Lobule	0.366	*0.003*
Right Cerebellar Hemisphere	0.366	*0.003*
Left Cerebellar Hemisphere	0.380	*0.002*
Right Vermis	0.314	*0.012*
Left Vermis	0.318	*0.011*
Right DLPFC	0.244	0.054
Right Caudate	0.237	0.061
Right Superior Parietal Lobule	0.238	0.060
Left Occipital	0.243	0.055
Navigational Memory		
Age Controlling for Performance		
Region	**ρ**	**p-value**
Left Frontopolar	0.471	*<0.001*
Left DLPFC	0.417	*0.001*
Right Premotor	0.270	*0.034*
Right Posterior Parahippocampal Gyrus	0.279	*0.028*
Right Superior Parietal Lobule	0.320	*0.011*
Left Lingual Gyrus	0.261	*0.041*
Left Anterior Cingulate	0.223	0.082
Right Thalamus	0.222	0.083

Performance Controlling for Age		
Region	ρ	**p-value**
Right DLPFC	0.296	*0.02*
Left Anterior Cingulate	-0.330	*0.009*
Right Thalamus	0.249	*0.05*
Left Thalamus	0.472	*<0.001*
Left Posterior Parahippocampal Gyrus	-0.352	*0.005*
Right Retrosplenial Area	-0.273	*0.032*
Left Cerebellar Hemisphere	-0.312	*0.014*
Left Frontopolar	-0.234	0.067

Table 2. Areas of significant (or approaching significance) correlations for age and performance, each controlling for the other.

3.5.5. Age and Performance-Related Changes in Activation in the Navigational Memory Task.

Partial correlation analyses were calculated between age and percent BOLD signal change in the areas of peak activity while controlling for performance (i.e. average time taken), and between performance and percent BOLD signal change while controlling for age. Significant correlations were obtained between age and percent BOLD signal change in the left frontopolar cortex, DLPFC, lingual gyrus, right premotor area, posterior parahippocampal gyrus, and superior parietal lobules. Correlations between age and percent BOLD signal change approached significance in the left anterior cingulate cortex and the right thalamus (Table 2). Performance (i.e. the average time taken on completed trials) correlated significantly with percent BOLD signal in the thalamus bilaterally, in the left anterior cingulate cortex, posterior parahippocampal cortex, and cerebellar regions, as well as in the right DLPFC, and retrosplenial regions. Furthermore, correlations between the average time taken to complete the trials and percent BOLD signal change approached significance in the left frontopolar region. Given that performance was measured as the average time taken to complete the trials, negative correlations

represent improved task performance (i.e. less time taken to reach a target landmark). All fMRI data for the navigational memory and working memory tasks were plotted and visually examined to ensure the absence of curvilinear relationships.

3.6. Discussion

3.6.1. Performance and Age Correlation.

The results of the present study showed that age was not a factor influencing significantly the participants' accuracy of performance (response accuracy) when performing the working memory task. Somewhat unexpected was the finding that performance measures (i.e. average completion time) obtained from the navigation task showed a significant worsening with age. Based on this result, the data were further examined in detail and a curvilinear relationship between age and performance was identified. Such a curvilinear relationship is in agreement with improving task performance as the developing brain matures and decreasing task performance as age increases.

3.6.2. Task-Related Peak Activation.

The areas of peak activation for the abstract visual and verbal working memory and navigational memory tasks are shown in Table 1. The identified areas correspond well to the findings of our previously published studies which employed the same tasks in adults and children (Chen et al., 2004; Iaria et al., 2007; Keightley et al., 2014; Petrides, 2005; Petrides, 2013; Saluja et al., 2015). The areas that typically show increased activation changes in the working memory task include the bilateral DLPFC, caudate nuclei, and thalami, which were

strongly activated in the present study. However, the bilateral premotor activations that were previously observed in our pediatric concussion study (Keightley et al., 2014) were not present in the verbal working memory task, but were present in the visual working memory task of the present study. The significance of this finding is unclear but given the fact that the right premotor area showed a significant performance correlation with the visual working memory task, the lack of activation in these regions in the verbal working memory task as seen in this study may represent a performance bias compared to the previous study (Keightley et al., 2014).

In the navigation task, the regions of activation when contrasting the experimental with the control condition included the posterior parahippocampal gyri, retrosplenial areas, and parietal cortical areas. These findings are in line with those of previous studies (Iaria et al., 2007; Saluja et al., 2015; Spiers and Maguire, 2007). The present study showed additional activation peaks in the frontopolar region, bilaterally, which had not been reported in those previous studies. It has been suggested that the frontopolar region may play a role in the re-formulation of a route when a detour is required to reach a target (Spiers and Gilbert, 2015; Xu et al., 2010). As a result, the frontopolar region may play an important role in navigation by allowing for a re-direction to the target location in the event of an obstacle.

3.6.3. Age and Performance-Related Changes in Peak Activation.

Partial correlations were performed to allow us to separate the effects of age and performance on task-related activation patterns. As seen in Table 2, in the working memory tasks, few areas correlated with age (i.e. the left occipital region and vermis showed correlations between age and the verbal working memory task, and the left superior parietal lobule demonstrated a correlation between age and the visual working memory task). However, a

number of areas showed significant correlations with performance (accuracy). While earlier fMRI studies observed much more robust correlations between age and percent BOLD signal change, the current findings are in line with the study by Satterthwaite and colleagues which demonstrated, in a large sample, a highly significant effect of performance, rather than age, on fMRI activation patterns (Satterthwaite et al., 2013). As pointed out earlier, many of the previous studies that had shown a significant effect of age on functional activation patterns had not controlled for the effect of performance, and those that did were studies with a rather small sample size. It is important to note that the present study included only subjects above the age of 10 years old, based on previous experience that children above the age of 10 are able to perform the tasks employed here more reliably within the MRI scanner, compared to their younger peers. It is possible that a stronger age effect might have been apparent had this study included younger subjects. Nevertheless, the present findings support the view that, in individuals above 10 years of age, there is relatively little effect of age on the areas of activation needed to perform successfully the working memory tasks. Rather, there is a significant effect of performance on the areas of peak activation when engaged in working memory tasks.

With the navigational memory task, however, significant correlations of peak activation values with both age and performance were demonstrated in a number of distinct areas. It is of interest to note that the regions that showed correlations with age were all different from the ones that correlated with performance, although the significance of this observation remains unclear. In contrast to the working memory task, significant correlations between age and activation peaks, as well as between performance and activation peaks were observed for the navigational memory task, which suggests that age continues to play an important role in the levels of activation during navigation. This is in agreement with findings in the literature, suggesting that,

in contrast to working memory, there is still a significant effect of brain development on navigation skills (Pine et al., 2002).

Although the particular working memory tasks used in this study may be considered, by no means, 'simple' tasks, they are nevertheless relatively pure measures of mental tracking (monitoring) of information within working memory. Subjects are simply required to monitor the occurrence or non-occurrence of a few stimuli from a target set. This task was explicitly designed not to involve strategy, planning or the overcoming of prepotent responses common to many complex tasks (Petrides, 2013). In contrast, one may argue that the navigation memory task requires more complex cognitive processes in order to be performed successfully. The experimental condition of the navigation task demands that participants recall from a pre-established cognitive map in long-term memory a destination point in relation to the starting position, and that they plan the most direct route in order to navigate to that destination landmark. In the case of a navigation error along the route, subjects who are monitoring their surroundings must re-formulate the planned route in order to reach the target landmark. Thus, in addition to retrieval of the target location from long term memory, planning the route and tracking the success/failure of the decisions while travelling along the route is a necessary. If we accept that the navigation task is a cognitively more complex task compared to the working memory task, then the findings of the present study support the view that the functional development of the brain follows a task-dependent progression of maturation based on task complexity.

3.7. Conclusion

Taken together, the findings of the working memory and navigational memory tasks demonstrate an effect of age on navigational ability that is much less apparent with the relatively pure process of monitoring information in working memory. As both of these tasks were performed by the same subject group, the results provide evidence that supports the theoretical idea of a progressive functional maturation of the brain based on increasing complexity of the cognitive task.

3.8. Acknowledgements

This study was supported by grants from the Canadian Institutes of Health Research. R.S.S. was a recipient of the Frank Litvack Fellowship for Clinician Scientists and an award from the Fonds de recherche du Québec - Santé for resident physician scientists. J-K.C. was a recipient of a postdoctoral award from Fonds de recherche du Québec - Santé.

Chapter 4

Working Memory fMRI in Children Post Concussion

4.1. Preface.

In the previous chapter, we demonstrated that, with the working memory task, the overall areas of peak activation were the same in both the healthy children and adults, and largely in agreement with previously published literature. Furthermore, we showed that age had very little effect on the intensity of activation in the majority of the regions typically activated in the working memory task. As a result, we could now validly assess the use of the working memory task in concussed children. The working memory task that was used in the previous studies by Chen and colleagues (Chen et al., 2007; Chen et al., 2004; Chen et al., 2008b; Gosselin et al., 2011) were used again in the context of childhood mTBI because, in the previous studies, they demonstrated robust and reproducible attenuations of activation in concussed adult athletes. In those studies, despite the fact that the subjects performed just as well as the control subjects on the working memory task, their activation intensity in the areas of interest was severely diminished. In the current study, we sought to determine if these findings hold true for children as well.

An fMRI Study of Working Memory in Youth Following Sports-related Concussion: Is it Still Working?

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Keightley M.L., Saluja R.S., Chen J.K., Gagnon I., Leonard G., Petrides M., Ptito A. "An fMRI Study of Working Memory in Youth Following Sports-related Concussion: Is it Still Working?" Journal of Neurotrauma. March 1, 2014; 31(5):437-51. DOI: 10.1089/neu.2013.3052

4.2. Abstract

In children, the importance of detecting deficits after mild traumatic brain injury (mTBI) or concussion has grown with the increasing popularity of leisure physical activities and contact sports. While most post-concussive symptoms (PCS) are similar for children and adults, the breadth of consequences to children remains largely unknown. To investigate the effect of mTBI on brain function, we compared working memory performance and related brain activity using blood-oxygen-level-dependent (BOLD) functional MRI in 15 concussed youths and 15 healthy age-matched control subjects. Neuropsychological tests, self-perceived post-concussive symptoms, levels of anxiety and depression were also assessed. Our results showed that, behaviorally, concussed youths had significantly worse performances on the working memory tasks, as well as on the Rey figure delayed recall and verbal fluency. Functional MRI results revealed that, compared to healthy children, concussed youths had significantly reduced task-related activity in bilateral dorsolateral prefrontal cortex, left premotor cortex, supplementary motor area and left superior parietal lobule during performance of the verbal and nonverbal working memory tasks. Additionally, concussed youths also showed less activation than healthy controls in the dorsal anterior cingulate cortex, left thalamus and left caudate nucleus during the nonverbal task. Regression analysis indicated that BOLD signal changes in bilateral dorsolateral prefrontal cortex were significantly correlated with performance such that greater activities in these regions relative to the control condition were associated with greater accuracy. Our findings confirmed functional alterations in brain activity following concussion in youths, a result similar to that observed in adults. However, significant differences were noted. In particular, the observation of reduced working memory accuracy suggests that youths may be

unable to engage compensatory strategies to maintain cognitive performance following mTBI. This has significant implications for safe return to daily activities, including competitive sport.

4.3. Introduction

One of the most commonly reported injuries in children who participate in sports is concussion or mild traumatic brain injury (mTBI). For the purposes of this paper these two terms will be used interchangeably. Children and youth involved in organized sports are nearly six times more likely to suffer a severe concussion than those involved in other leisure physical activities (Browne and Lam, 2006). While the most common cognitive sequelae of mTBI appear similar for children and adults, the recovery profile and breadth of consequences in children remain largely unknown (McCrory et al., 2004). This dearth of literature is compounded by the recent scrutiny youth participation in competitive contact sports (such as hockey and football) has received.

Concussion is defined as "a complex pathophysiological process affecting the brain, induced by traumatic biomechanical forces" that may or may not involve loss of consciousness (McCrory et al., 2009). Concussion is a concern in contact sports, such as hockey and football because, once cleared to play after the resolution of symptoms, the players return to an environment in which a brain injury has a reasonable likelihood of recurring. The variability of the developmental impact of concussion in youths appears due to the complex interplay between age at time of injury, its severity and the mechanism of injury (Goldstrohm and Arffa, 2005).

Children's brain injuries were traditionally thought to be offset by physiological and adaptive factors such as the young brain's plasticity or "reserve" which served to increase impact tolerance and recovery after insult (Browne and Lam, 2006; Kirkwood et al., 2006; Satz, 1993). A growing amount of literature however, strongly indicates that the immature brain is in fact more vulnerable, not more "plastic", to diffuse injury (Kirkwood et al., 2006; McCrory et al., 2004). Specific underlying neural mechanisms make the developing brain more vulnerable to neuronal injury possibly because pathophysiological differences exist. These include the mechanical and compositional properties of the brain such as brain water content, level of myelination, skull geometry, suture elasticity and neck strength which may result in a markedly diminished shear resistance of the immature brain tissue (Bauer and Fritz, 2004). Consequently, similar mechanical load as that applied to the mature brain could in fact induce more intense brain tissue displacements. A concussion sustained prior to or during critical developmental periods may permanently alter and impair the development of a particular function or skill such as working memory (the ability to temporarily store and manipulate information for the purpose of carrying out a complex cognitive task) (Freund et al., 1994; Wiseman-Hakes et al., 2000).

While it has been suggested that individuals with mTBI typically recover within 1-2 weeks (Lovell et al., 2003; McCrea et al., 2003), symptoms and related performance deficits persisting for several weeks to months post-injury have been reported in a small, but significant number of youths (Gagnon et al., 2009). Most recently, Baillargeon *et al*. reported that adolescents are more susceptible to short-term neuropsychological and neurophysiological deficits following concussion than younger children and adults (Baillargeon et al., 2012).

Relatively recent advances in magnetic resonance imaging (MRI) technology have enhanced its sensitivity to detect traumatic abnormalities among concussed athletes, including

the characterization of diffuse axonal injury defined as foci of abnormal signal intensity (Doezema et al., 1991; Gale et al., 2005; Hughes et al., 2004; Mittl et al., 1994). While MRI can furnish knowledge of structural anatomical abnormalities, functional techniques such as fMRI reveal the pathophysiological and functional sequelae of injury.

While working memory performance in adults has been found to be comparable to healthy controls following concussion, notable differences in brain activation patterns accompanying task performance have been observed and include both increased and decreased activations relative to control subjects across frontal, parietal and temporal areas, with increased activity found in regions not typically employed during working memory (i.e. temporal cortices) (Chen et al., 2007; Chen et al., 2004; Jantzen et al., 2004; McAllister et al., 1999; McAllister et al., 2001a; Pardini et al., 2010). Together, these results suggest that recruitment of other brain regions in adults may represent cerebral compensatory mechanisms to maintain cognitive performance.

Despite the availability of research findings in adult athletes, research utilizing neuroimaging approaches such as fMRI to investigate the neural impact of concussion have largely neglected the pediatric population (Keightley et al., 2012). This represents a critical oversight as findings from adult data cannot be applied to this population. Developmentally, brain changes in healthy children and youth on working memory tasks involve increased localization in regions associated with increased age such as the dorsolateral prefrontal and parietal cortices (Cantu, 1998a; Crone et al., 2006; Nagel et al., 2005).

A recent scoping review identified only five studies investigating the neural impact of pediatric concussion (19 years of age or younger) as measured with structural and functional

MRI techniques (Keightley et al., 2012). Only two of these studies were focused specifically on sports-related concussion and presented fMRI data (Maugans et al., 2011; Talavage et al., 2014).

Specifically, Yang *et al*. found hypoactivation during an auditory orienting task in mTBI youth relative to healthy controls, but this difference did not correlate with task performance and there were no statistically significant differences between the groups (Yang et al., 2012). However, because moderate effect sizes and trends for group differences in performance were found, the authors argued that the lack of group differences may be due to the small sample size. Talavage *et al*. also recorded functional changes in brain activity associated with a working memory n-back task from pre-season to in-season assessment in football players with clinically diagnosed concussion, as well as in a subset of players who sustained a high number of high magnitude impacts, but did not present with clinically identified concussion (Talavage et al., 2014). Specifically, fMRI activation patterns shifted from greater activity during the more complex task to greater activity during the less complex task, suggesting hyperactivation associated with the simpler task condition. However, the authors did not report whether there were performance differences between the groups on the tasks. Hyperactivation in the frontal and parietal lobes on a working memory task has also been found to predict the length of time to recovery in concussed high school athletes, with the mean age of participants being approximately 18 years (range of 13-24 years) (Lovell et al., 2007). Taken together, the paucity of literature in adolescent athletes indicates that more research is needed to better understand the clinical and neural implications of concussion in youth.

Thus the purpose of the present study was to examine and compare working memory performance and related brain activity using fMRI in concussed youths and healthy age-matched control subjects. We employed the same working memory task previously described by Chen *et*

al. in concussed adult athletes (Chen et al., 2007; Chen et al., 2004). As decreased working memory performance has been observed in children and youths following mTBI (Levin et al., 2004; Moran and Gillon, 2004), we hypothesized that unlike studies involving adult participants, concussed youths would demonstrate observable differences in working memory performance compared to age-matched healthy controls. Furthermore, we hypothesized that deficits in performance would be linked to reduced change in activation from baseline to task performance. This reduced change in activation is hypothesized to occur primarily in the dorsolateral prefrontal cortex and brain areas previously shown to be preferentially involved during working memory in both children and adults (Chen et al., 2007; Chen et al., 2004; Ciesielski et al., 2006; Lovell et al., 2007; McAllister et al., 2001b; Pardini et al., 2010).

4.4. Methods

4.4.1. Participants

Participants included 15 concussed youths (8 females, 7 males, mean age = 14.47 ± 2.29 years) who had previously sustained a concussion ranging from 9 – 90 days at the time of testing, as well as 15 age-matched control subjects (7 females, 8 males, mean age = 14 ±2.3 years). Inclusion criteria for the mTBI groups were: 1) a diagnosis of mTBI, as per the WHO task force definition (Cassidy et al., 2004), by a physician at the Montreal Children's Hospital where the children were recruited. Operational criteria for clinical identification included: (i) confusion or disorientation, loss of consciousness for 30 minutes or less, posttraumatic amnesia for less than 24 hours, and/or transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; (ii) Glasgow Coma Score of 13-15 after 30 minutes post-injury or

later upon presentation for health care; 2) No history of mTBI at least in the previous year; and 3) a functional knowledge of French or English. Exclusion criteria included: 1) a pre-morbid diagnosis of learning disabilities, ADHD and/or behaviour problems. Because many children present with features of ADHD without having been formally diagnosed, parents filled out the Conners' Rating Scale (Waschbusch and Willoughby, 2007) while their child underwent testing. This scale provides additional information for screening for attention, learning and behavioural problems. Based on cut-off scores provided in the literature, we excluded those children who displayed 1) severe clinical criteria for ADHD (90^{th} percentile on all scales); 2) severe pain, vestibular, neurological (other than mTBI) or musculoskeletal problems (other than upper extremity injuries); 3) violation of standard criteria regarding eligibility for the MRI scan. All participants were right-handed and screened to ensure there was no history of psychological or neurological illness apart from the current concussion. Of the 15 concussed subjects, 3 had a history of a previous concussion more than one year prior. All the control subjects were given the same screening questionnaire as the concussed-subjects to ensure that they had no major medical issues, no history of any pre-natal or perinatal insults, learning disabilities, history of ADHD, neurological/psychiatric conditions, history of any oncologic conditions, nor any history of recurrent ear infections. In addition the control subjects also reported an absence of any previous concussions.

Data regarding the age, gender, presence or absence of loss of consciousness (LOC), time since injury and degree of post-concussion symptoms present at the time of testing are presented in Table 1. Structural scans for the mTBI subjects were reviewed by a neuroradiologist who was blind to their injury status. All were reported as normal. Post-concussion symptoms were assessed using an adapted version of the Post-Concussion Scale – Revised (Lovell and Collins,

1998). The scale consists of 21 symptoms (i.e. headache, nausea, dizziness etc.) assessed by the subject on a scale from zero to six, six being a severe problem. Total post-concussion symptom scores reflect the following severities: 0 - 5 = within normal limits; 6 - 21 = low post-concussion symptoms; 22 - 84 = moderate post-concussion symptoms and 85 - 132 = high post-concussion symptoms (Chen et al., 2007; Chen et al., 2004). Each participant provided informed written consent as approved by the Ethics Committee at McGill University, Montreal Neurological Institute.

Subject ID	Group	Gender	Age (Years)	Time Since Injury (days)	Mechanism of Injury	PCS	Number of Previous Concussions
TC079	mTBI	F	10	41	Hit wall in gym class	2	0
TC051	mTBI	M	11	61	Kicked in head playing soccer	14	0
TC033	mTBI	M	12	90	Fell playing soccer	17	0
TC078	mTBI	F	13	12	Fell playing ringette	62	0
TC050	mTBI	M	13	34	Hit head while swimming	32	0
TC068	mTBI	F	14	49	Hit head playing football	39	0
TC056	mTBI	F	14	62	Hit head against wall while swimming	47	0
TC007	mTBI	F	15	16	Hit in head by ball in soccer	45	1
TC074	mTBI	F	15	20	Fell playing ringette	12	0
TC053	mTBI	M	15	19	Struck on head by a knee while on trampoline	35	0
TC069	mTBI	F	17	72	Hit in head by ball playing soccer	29	0
TC001	mTBI	M	17	26	Fell while skiing	39	0
TC076	mTBI	M	17	9	Hit head playing football	54	1
TC077	mTBI	F	17	44	Hit head playing soccer	33	0
TC067	mTBI	M	17	62	Hit head playing football	0	1
TC009	Control	F	10	N/A		2	0
TC003	Control	M	11			10	0
TC022	Control	M	12			3	0
TC045	Control	F	13			33	0
TC021	Control	M	13			15	0
TC004	Control	F	14			20	0
TC055	Control	M	14			9	0
TC011	Control	F	15			13	0
TC010	Control	F	15			7	0
TC049	Control	M	15			6	0

TC041	Control	F	17		6	0
TC040	Control	M	17		5	0
TC036	Control	M	17		21	0
TC029	Control	F	17		32	0
TC037	Control	M	17		21	0

Table 1. Demographic, medical and injury information for all participants. Note: PCS=Post-concussion symptoms (as assessed on the day of scanning using the Post-concussion Symptom Scale Revised).

4.4.2. Neuropsychological Testing

All participants completed a comprehensive neuropsychological battery, comprised of tests that demonstrate the greatest sensitivity to the effects of mTBI. Tests administered included the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999), Rey Complex Figure (Meyers and Meyers, 1995), Verbal Fluency (Miller, 1984), Rey Auditory Verbal Learning Test (Lezak et al., 2004), Stroop Color and Word Test (Golden, 1978), Symbol Digit Modality Test (Smith, 1968), Children's Color Trails Test (Williams et al., 1995), the Place and Remove tasks of the Grooved Pegboard Test (Bryden and Roy, 2005). We also assessed behaviour and emotional functioning by obtaining parent reports on the Conners' Rating Scales (Waschbusch and Willoughby, 2007) and the Child Behavior Checklist (CBCL) (Achenbach and Edelbrook, 1983). The Conners' Rating Scales-Revised (CRS-R) evaluate problem behaviours by obtaining reports from a child's parents and teachers across a number of domains (i.e. oppositional behaviour, inattention, hyperactivity etc.). The CBCL is a series of parent/teacher/self-report questionnaires designed to assess competencies, adaptive functioning and problems in child behaviour that map onto the major mood and behavioural disorders listed in the Diagnostic and Statistical Manual of Mental Disorders (4^{th} edition). Finally participants' self-perceived levels of anxiety and depression were assessed via the Beck Youth Inventories (Beck et al., 2005). The testing was performed by a neuropsychologist blinded to the injury history of all subjects and was completed in a standardized order. All testing was completed over a period of 1.5-2 hours in a single session, either before or after the MRI testing, depending on scheduling constraints.

4.4.3. fMRI Experimental Task

The experimental task used during the fMRI session was an adapted version of the externally-ordered working memory task devised by Petrides (Petrides et al., 2001) and validated in studies of patients with lateral frontal lesions, monkeys with dorsolateral prefrontal cortex lesions and functional neuroimaging work with PET and fMRI (Petrides et al., 1993; Stern et al., 2000). This task has also been used with adult concussed athletes where less activity in the dorsolateral prefrontal cortex was found during working memory performance compared to baseline task performance (Chen et al., 2007; Chen et al., 2004). We replicated the methodology (imaging protocol and working memory tasks) in order to compare findings from adults to concussed youth. There are two versions of the task: verbal and nonverbal (see Figure 1). In each, subjects were familiarized with a set of 5 items that were to be used throughout the test (5 abstract drawings or 5 pseudo words). On each trial, four of the five items were presented successively in random order at the center of a computer screen. The subject had to monitor their occurrence and identify the one item from the set that had not been presented. The four items presented were randomly selected from the five items. After the presentation of the fourth item, a 1-s delay occurred. Immediately after this delay, a test item was presented and the subject had to indicate within 1.5 seconds, by pressing a mouse button (yes = right button, no = left button), whether this test item was one of the four items presented prior to the delay or whether it was the item from the set of five that had not been presented.

A control condition was introduced to "subtract out" any activation related to the motor and perceptual components of the working memory task. In the control condition, the format and type of stimulus presentation, mode of response, and timing of events were identical to those in the experimental working memory task. The stimuli used in the control condition were similar,

but not previously used for the experimental task. During stimulus presentation in each trial, a single item (abstract design or pseudo-word for the nonverbal and verbal conditions, respectively) was presented four times in succession at the centre of the screen, followed by a delay of 1 second. After the delay, one of two items associated with either a left or a right mouse button press was presented at the center of the screen and the subject had 1.5 seconds to respond. The subjects learned prior to scanning which one of the two items (2 abstract designs or 2 pseudo-words) was associated with a left mouse button press and which one with a right button press. Thus, in the control condition, the subject was making an identical response as in the experimental task (i.e. press left or right), but these motor responses were based on particular conditional associations learned before scanning rather than a decision based on the monitoring of information in working memory (i.e. whether a particular item from the expected set of five had or had not been presented during the trial). Thus, the crucial difference between the experimental and control conditions was that the working memory task required constant monitoring of which items from a familiar set of stimuli had or had not been presented (i.e. an executive function depending on the dorsolateral prefrontal cortex), whereas in the control task the decision was made on the basis of learned associations. The experimental task was administered by two trained examiners. A graphical illustration of the task can be found in Figure 1.

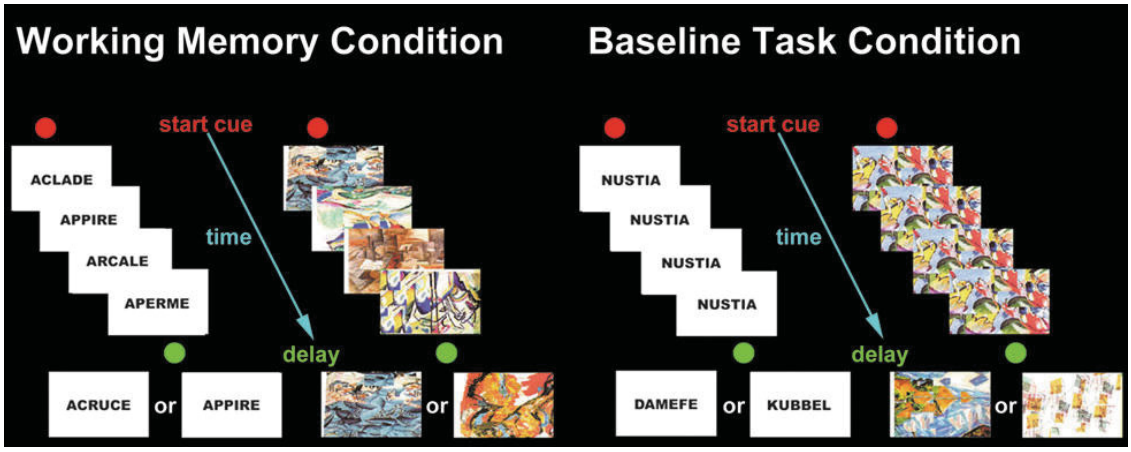

Figure 1. Schematic diagram of the externally ordered working memory task.

4.4.4. Image Acquisition

The fMRI scanning was carried out using a 3 Tesla Siemens Magnetom Trio A Tim System with a 32 channel head coil. Each fMRI session started with the acquisition of high-resolution T1-weighted 3D anatomical images using 3D Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence (TR = 23ms, TE = 2.98ms, Slice Thickness = 1mm, Image Matrix = 256 x 256, Flip Angle = 30 degrees, FOV = 256mm, interleaved excitation), followed by acquisitions of T2* weighted gradient echo (GE) echo-planar images (EPI) for blood oxygenation level dependent (BOLD) fMRI (TR = 3000ms, TE = 51ms, flip angle: 90°, in-plane resolution = 2.34 x 2.34 mm, FOV = 300, 128 x128 image matrix, slice thickness = 4mm, number of slices: 37, interleaved excitation).

Four functional scans (two for the verbal working memory condition and its control and two for the nonverbal working memory condition and its control) were acquired in a single session. Each functional scan lasted 6 minutes, with activation and baseline conditions alternating every 8 trials (60 seconds). Before entering the scanner, all participants were introduced to the tasks and given at least 48 practice trials (i.e. two runs) outside the scanner prior to entering the magnet

4.4.5. Statistical Analyses

All behaviour data analyses were conducted using the IBM Statistical Package for the Social Sciences (SPSS) version 19.0 for Windows.

4.4.6. Post-Concussion Symptom Scale - Revised

A total score for participants' endorsement of post-concussion symptoms was generated by summing the ratings for each of the 21 symptoms. Graphing revealed that the data was positively skewed, so a non-parametric test (Mann Whitney U) was performed to compare degree of perceived symptoms for mTBI versus control subjects. Spearman correlations were carried out to determine if performance on the fMRI experimental task was significantly correlated with participants' degree of endorsement of post-concussion symptoms.

4.4.7. Neuropsychological Testing and Parental Questionnaires

The statistical analyses performed on the neuropsychological testing data included descriptive statistics calculated by group. For all tests, student t-tests were performed to determine if any group differences existed using an α of 0.01 given the multiple comparisons performed. For tests consisting of multiple component measures (i.e. RAVLT), multivariate analysis of variance (MANOVA) was performed using the group (concussed versus control) as the independent variable and each of the sub-measures (i.e. immediate memory recall, delayed memory recall etc.) as the dependent variables.

4.4.8. fMRI Experimental Task Data

Descriptive statistics for the sample were calculated for the total sample and by group. A multivariate analysis of variance (MANOVA) was calculated where group (concussed versus control) was the between subjects variable and working memory performance measures (verbal control condition accuracy, verbal control condition reaction time (RT), verbal working memory condition accuracy, verbal working memory condition RT, nonverbal control condition accuracy, nonverbal control condition RT, nonverbal working memory condition accuracy and nonverbal working memory condition RT) were the within subjects variables.

4.4.9. Functional Neuroimaging Data

Whole-brain voxel-wise statistical analysis of the motion corrected fMRI time series was performed with fMRIstat (Worsley et al., 2002a). The fMRI data were first converted to percentage of whole volume. Significant BOLD changes between experimental (i.e., working memory task) and baseline (i.e., control task) conditions were determined at each voxel, based on a linear model with correlated errors ($Y = X\beta + e$). A design matrix containing the explanatory variables (X), and their respective onset time and duration was first convolved with a hemodynamic response function modeled as a difference of two gamma functions, and corrected for slice-timing to coincide with the acquisition of each slice (Friston et al., 1998). Temporal and spatial drifts, and other estimated errors (e) were modeled and removed. The linear model was then fit with the fMRI time series (Y), solving parameter estimates (β) in the least squares sense, yielding estimates of effects, standard errors, and t-statistics for each run.

Data from each individual run was then normalized to the Montreal Neurological Institute template (MNI305) using an in-house algorithm (Collins et al., 1994), and combined together

using a fixed effects analysis for the following comparisons: 1) verbal working memory minus verbal control condition, and 2) nonverbal working memory minus nonverbal control condition. Within group average across participants was achieved by using a mixed effects linear model with fixed effects standard deviations taken from the previous analysis. A random effects analysis was performed by first estimating the ratio of the random effects variance to the fixed effects variance, then regularizing this ratio by spatial smoothing with a Gaussian filter. The variance of the effect was then estimated by the smoothed ratio multiplied by the fixed effects variance. The amount of smoothing was chosen to achieve 100 effective degrees of freedom. The resulting T statistic images were thresholded using the minimum given by a Bonferroni correction and random field theory to correct for multiple comparisons, taking into account the non-isotropic spatial correlation of the errors (Worsley, 2005). Threshold for significance was established at $t = 4.10$ for the activation peaks, or $t = 3.10$ for activation clusters greater than 222 mm^3, based on the number of resolution elements in the acquisition volume (2,880 resels).

Finally, to address differences in brain activity between mTBI and healthy controls, a group subtraction analysis was carried out on the data for the group average analysis, using a fixed effects model. In Control – mTBI subtraction, positive t statistics show brain regions that have greater increase in activity during working memory against control task for control group relative to mTBI group, and vice versa for the mTBI – Control subtraction. The % BOLD signal change relative to the control task was also extracted at voxels of interest (VOIs) obtained from this analysis, which were identify as 6-mm radius gray matter volume centred at the voxel with the highest t-value from the group subtraction analysis.

To identify brain regions where performance modulated BOLD signal changes, whole-brain, voxel-wise linear regressions were carried out using performance (accuracy) as the

covariate in separate analyses for each working memory condition against the its respective control task condition. This analysis was completed in order to look for systematic variation in BOLD signal change as a function of performance and determine if there is a link between performance and BOLD signal change in those brain areas where the concussed group may differ from the control group.

4.5. Results

4.5.1. Post-Concussion Symptom Scale – Revised

A Mann Whitney U Test for Independent Samples revealed that the total score for the Post-Concussion Scale – Revised (PCS-R) was higher for the mTBI compared to the control group ($p = 0.011$). Furthermore, perceived severity of post-concussion symptoms was not significantly correlated with any of the fMRI experimental task performance measures.

4.5.2. Neuropsychological Testing and Parental Questionnaires

Using student t-tests, of all the variables examined using an α of 0.01, only the Rey figure delayed recall ($p=0.0083$) and verbal fluency ($p=0.0078$) showed a statistically significant difference between the concussed subjects and controls. In both cases, as expected, the controls performed better than the concussed subjects. Using MANOVA, none of the tests demonstrated statistically significant differences between the two groups. The results are summarized in Table 2.

Test		Mean		Standard deviation		T-test	MANOVA
		Control	mTBI	Control	mTBI	p-value	p-value
WASI	IQ score	115.3	110.5	13.7	9.2	0.23	
Rey Figure	Copy	30.7	30.4	3.6	4.0	0.74	0.81
	Copy Time	301.7	325.6	88.8	139.7	0.47	
	Immediate Recall	22.2	18.5	5.6	6.1	0.078	0.095
	Immediate Recall Time	203.7	194.9	82.1	60.8	0.68	
	Delayed Recall	21.0	17.2	3.9	4.8	*0.0084**	0.072
	Delayed Recall Time	160.1	144.3	57.0	62.5	0.52	
	Recognition	21.4	20.8	1.4	2.8	0.51	0.46
Verbal Fluency	Animals	24.2	21.2	7.1	6.6	0.09	0.24
	Food/Drink	23.9	21.9	8.4	5.4	0.35	0.45
	S words	15.7	11.1	7.1	3.6	*0.0086**	0.031
	F words	13.7	11.4	6.1	4.2	0.19	0.25
	Total	77.6	65.6	24.0	16.3	*0.0078**	0.12
RAVLT	I	7.4	6.3	1.8	1.3	0.033	0.23
	II	9.9	9.8	2.1	2.1	0.75	0.96
	III	12.6	11.2	1.5	2.2	0.06	0.079
	IV	13.3	13.0	1.3	1.8	0.66	0.40
	V	13.5	12.9	1.4	1.7	0.39	0.21
	B	6.9	5.9	2.0	1.4	0.15	0.76
	Immediate	12.3	11.5	2.0	2.2	0.20	0.20
	Delayed	12.3	11.0	1.9	2.3	0.09	0.084
	Word Recognition	14.2	13.7	1.1	1.7	0.47	0.57
Beck Youth	BSCI	40.5	39.4	7.8	7.1	0.71	
	BAI	14.5	16.7	8.4	9.9	0.54	
	BDI	8.5	11.2	5.7	8.8	0.39	
	BANI	11.8	12.5	6.4	9.7	0.84	
	BDBI	6.5	6.3	4.2	4.4	0.94	
Stroop	Word Score	92.5	89.1	32.0	21.4	0.70	
	Color Score	70.6	63.6	16.0	17.5	0.25	
	Color-Word Score	45.1	37.5	10.4	13.3	0.13	
	Interference Score	-19.1	-22.9	16.1	13.8	0.55	
SDMT		61.3	52.2	13.8	12.8	0.094	
Color Trails	CT-1 Time	14.0	20.8	4.2	13.4	0.084	
	Standard Score	103.3	100.8	15.2	16.8	0.72	0.64
	CT-2 Time	29.3	37.5	6.3	13.8	0.099	
	Standard Score	101.9	94.0	8.9	17.1	0.20	0.30
PASAT	2.8	18.9	16.7	1.2	3.5	0.12	0.088
	2.4	16.6	14.7	1.3	3.9	0.12	0.18
	2.0	16.9	15.0	2.5	4.7	0.33	0.25
	1.6	11.8	11.2	2.5	2.8	0.54	0.58
	1.2	13.7	12.9	2.6	2.8	0.47	0.32
Pegboard (s)	Right Mean	61.0	60.2	8.5	10.6	0.82	
	Left Mean	64.9	68.1	9.9	8.5	0.36	
Pegboard Removal	Right Mean	20.5	23.0	3.0	9.4	0.30	
	Left Mean	20.5	20.6	2.7	4.2	0.93	

(s)							
CPRS (T-score)	Oppositional	51.6	48.2	10.8	17.2	0.47	
	Cognitive Problems/ Inattention	46.4	51.9	4.7	12.8	0.11	
	Hyperactivity	47.6	51.0	5.1	6.7	0.12	
	ADHD Index	48.1	49.6	6.0	9.3	0.39	
CBCL (T-score)	Activities	47.7	49.8	5.9	12.4	0.55	
	Social	48.6	54.3	8.3	8.7	0.032	
	School	53.1	50.6	3.7	7.6	0.34	
	Total Competence	49.5	52.8	6.7	13.3	0.34	
	Anxious/ Depressed	53.3	54.5	5.5	6.7	0.65	
	Withdrawn/ Depressed	54.3	55.1	5.8	7.5	0.78	
	Somatic Complaints	55.5	61.8	4.2	8.6	0.030	
	Social Problems	53.1	54.9	4.7	5.9	0.26	
	Thought Problems	52.9	55.9	4.3	6.6	0.23	
	Attention Problems	52.7	53.8	3.5	4.9	0.48	
	Rule-breaking Behavior	52.2	52.4	3.9	3.6	0.91	
	Aggressive Behavior	53.3	52.4	5.4	3.3	0.66	
	Internalizing Problems	50.2	55.2	9.2	9.9	0.25	
	Externalizing Problems	48.4	48.1	8.6	7.0	0.93	
	Total Problems	47.6	51.2	9.6	8.0	0.32	
CBCL DSM-IV	Affective Problems	54.7	55.3	7.3	7.1	0.82	
	Anxiety Problems	53.8	53.7	6.4	5.5	0.97	
	Somatic Problems	55.6	60.9	5.0	7.7	0.034	
	ADH Problems	52.9	53.2	5.1	3.9	0.85	
	Oppositional Defiant Problems	54.7	52.6	4.9	2.6	0.19	
	Conduct Problems	51.9	52.2	3.9	3.7	0.79	

Table 2. Summary of neuropsychological testing and parental questionnaires.

4.5.3. fMRI Experimental Task Data

Table 3 depicts the means, standard deviations and 95% confidence intervals for the mTBI and control groups on the working memory and associated control tasks, respectively. MANOVA results revealed that the mTBI group performed significantly worse than the control

group across all accuracy conditions of the task including verbal control [F(1,28) = 11.92, p = 0.002], verbal working memory [F(1,28) = 10.79, p = 0.003], nonverbal control [F(1,28) = 5.44, p = 0.027] and nonverbal working memory [F(1,28) = 17.58, p < 0.001]. A subsequent MANOVA performed on the RT data did not reveal any significant group differences in speed of responding across the four conditions (p > 0.05).

	Mean		Standard Deviation		95% Confidence Interval	
	mTBI	Control	mTBI	Control	mTBI	Control
Task Condition						
Verbal Control Acc	86.07	95.87	9.84	4.88	81.96-90.18	91.76-99.98
Verbal Control RT	1019.07	933.40	124.04	121.35	954.17-1083.96	868.51-998.30
Verbal WM Acc	61.07	71.73	6.65	10.67	56.36-65.77	67.03-76.44
Verbal WM RT	1233.60	1146.47	84.24	153.06	1168.26-1298.94	1081.13-1211.81
Nonverbal Control Acc	88.27	94.67	9.85	3.99	84.29-92.24	90.69-98.64
Nonverbal Control RT	967.73	912.33	120.65	97.16	909.80-1025.67	854.40-970.27
Nonverbal WM Acc	61.53	74.93	7.41	9.91	56.91-66.16	70.31-79.56
Nonverbal WM RT	1181.20	1117.33	91.77	130.78	1121.45-1240.95	1057.59-1177.08

Table 3. fMRI experimental task data: Accuracy and reaction time for mTBI and control subjects.

4.5.4. Functional Neuroimaging Data

4.5.4.1. Working Memory > Control Conditions

Independent sample t-tests compared the mean percentage of BOLD signal change for the verbal and nonverbal working memory conditions to their respective control conditions. Significant peak activations and three dimensional t-maps depicting these activations can be found in Table 4 and Figure 2. Brain regions demonstrating greater bilateral activation for the verbal working memory versus control conditions included the dorsolateral prefrontal cortex

(Brodmann Area (BA) 9/46, 46), premotor cortex (BA 6), superior parietal lobes (BA 7), inferior occipital cortices (BA 18, 19), insula, caudate nucleus and thalamus. In addition, the supplementary motor area, left superior temporal gyrus and dorsal anterior cingulate cortex (BA 32) also showed greater percent BOLD signal change for the verbal working memory versus control condition. The nonverbal working memory condition demonstrated greater percent BOLD signal change in all of the above areas as well as some additional regions. These were the bilateral fusiform gyrus (BA 37) and primary visual cortices (BA 17).

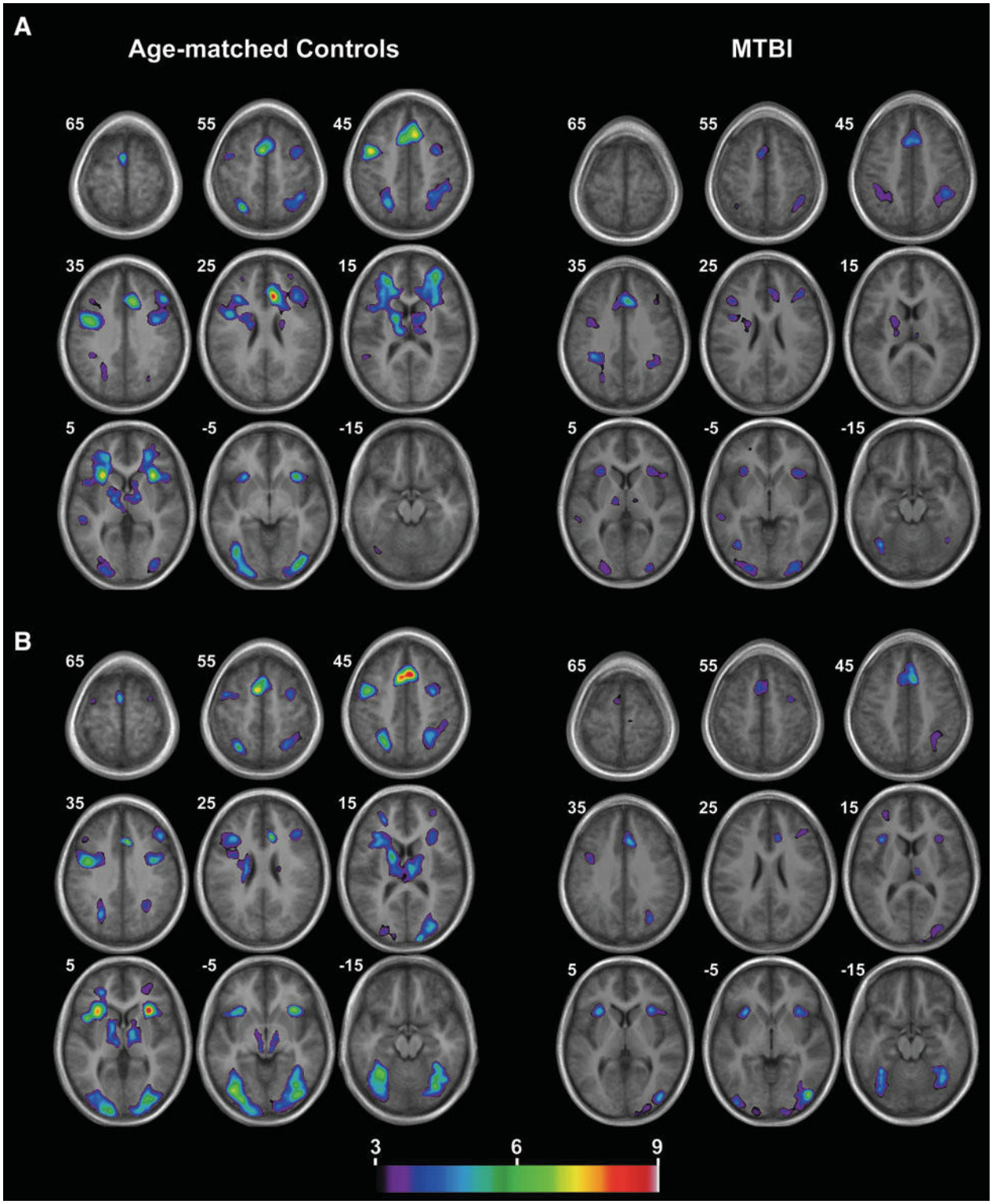

Figure 2. BOLD activation patterns for (A) verbal and (B) nonverbal working memory condition against their respective control condition. The numbers correspond to the z coordinate where the images were sampled.

Verbal Working Memory Condition Activation Peaks

		Control				mTBI			
Region	**BA**	**x**	**y**	**z**	**t**	**x**	**y**	**z**	**t**
Left dorsolateral prefrontal cortex	9/46	-26	40	12	6.55	-	-	-	-
Left dorsolateral prefrontal cortex	9	-32	22	24	5.73	-42	24	26	4.11
Right dorsolateral prefrontal cortex	9/46	32	48	16	6.18	-	-	-	-
Right dorsolateral prefrontal cortex	9	48	26	34	5.82	40	30	28	4.23
Left rostral insula		-30	20	2	7.59	-30	22	2	4.32
Right rostral insula		30	22	0	7.50	34	22	-2	4.13
Dorsal anterior cingulate cortex	32	10	26	26	8.90	4	26	36	5.76
Supplementary motor area		-4	4	58	7.34	8	14	48	5.29
Left premotor	6	-44	-2	44	7.62	-44	4	28	4.60
Right premotor	6	34	-2	50	5.21	38	2	30	3.73*
Left caudate nucleus		-16	-4	16	6.20	-20	-8	20	3.97*
Right caudate nucleus		16	-2	14	4.61	18	2	20	3.39*
Left thalamus		-12	-14	10	6.21	-12	-12	6	4.11
Right thalamus		8	-8	10	4.52	14	-12	8	3.68*
Left superior temporal gyrus	22	-54	-32	4	4.13	-58	-32	-2	3.90*
Left superior parietal lobule	7	-28	-64	54	6.58	-36	-40	34	4.98
Right superior parietal lobule	7	42	-46	48	5.15	40	-46	40	4.96
Left inferior occipital gyrus	19	-40	-68	-4	6.16	-38	-66	-10	4.87
Left inferior occipital gyrus	18	-24	-96	-6	5.51	-26	-92	-6	4.61
Right inferior occipital gyrus	18	34	-86	-2	6.49	26	-94	2	4.78

Nonverbal Working Memory Condition Activation Peaks

		Control				mTBI			
Region	**BA**	**x**	**y**	**z**	**t**	**x**	**y**	**z**	**t**
Left dorsolateral prefrontal cortex	9/46	-26	42	12	5.12	-30	50	12	3.18*
Left dorsolateral prefrontal cortex	9	-34	22	24	5.54	-	-	-	-
Right dorsolateral prefrontal cortex	9	46	30	32	5.29	42	34	22	3.82*
Left rostral insula		-30	22	4	8.04	-30	20	4	5.15
Right rostral insula		30	22	2	9.69	32	22	4	4.88
Dorsal anterior cingulate cortex	32	6	20	42	9.60	6	20	42	5.64
Supplementary motor area		-6	4	56	7.54	8	14	48	6.02
Left premotor	6	-50	-2	42	6.79	-42	4	32	4.14
Right premotor	6	34	-2	50	5.66	34	-4	52	4.43
Left caudate nucleus		-18	-2	18	5.84	-	-	-	-
Left thalamus		-8	-18	12	4.72	-	-	-	-
Right thalamus		6	-14	14	5.54	8	-20	14	4.51
Left superior parietal lobule	7	-28	-62	50	6.86	-	-	-	-
Right superior parietal lobule	7	28	-62	52	4.93	32	-70	34	4.55
Left fuiform gyrus	37	-34	-54	-14	6.74	-36	-50	-18	5.96
Right fusiform gyrus	37	36	-50	-18	6.11	30	-50	-18	4.91
Left inferior occipital gyrus	19	-40	-76	-8	7.51	-38	-74	-14	4.66
Left inferior occipital gyrus	18	-30	-86	-6	6.86	-42	-90	0	4.77
Right inferior occipital gyrus	19	32	-72	-14	6.43	36	-76	-10	4.19
Right inferior occipital gyrus	18	40	-80	-6	6.52	44	-78	-2	6.79
Left primary Visual Cortex	17	-16	-100	2	6.22	-18	-100	-4	3.35*
Right primary Visual Cortex	17	16	-100	12	5.68	14	-100	0	4.07

*Table 4. fMRI t-map activation peaks unique to the verbal and nonverbal working memory task conditions across all subjects. Note: t threshold = 4.10, p <0.05 corrected; * non-significant trend.*

4.5.4.2. Control > mTBI Participants

Independent sample t-tests comparing the mean percentage of BOLD signal change during the verbal working memory condition revealed significantly greater activation for the control versus the mTBI participants in the left (BA 9/46 : [t (2, 28) = -5.59, $p < 0.001$]) and right dorsolateral prefrontal cortex (two activation peaks BA 9/46 : [t (2, 28) = -5.10, $p < 0.001$] and BA 9: [t (2, 28) = -3.92, $p = 0.001$]), left premotor cortex (BA: 6 [t (2, 28) = -3.34, $p = 0.002$], supplementary motor area [t (2, 28) = -3.24, $p = 0.004$] and left superior parietal lobule (BA 7: [t (2, 28) = -5.37, $p < 0.001$]) (see Table 5 and Figure 3A). Of these regions, only the left dorsolateral prefrontal cortex (BA 9/46) was found to be significantly correlated with performance such that greater activity in this region relative to the verbal control condition was significantly associated with greater accuracy for both the mTBI ($r = 0.548$, $p = 0.04$) and control subjects ($r = 0.699$, $p = 0.04$) (Figure 4).

Verbal Working Memory					
Region	**BA**	**x**	**y**	**z**	**t**
Left dorsolateral prefrontal cortex	9/46	-42	40	12	3.00
Right dorsolateral prefrontal cortex	9/46	32	42	16	3.45
Right dorsolateral prefrontal cortex	9	48	26	28	3.28
Left premotor cortex	6	-46	-2	44	4.76
Supplementary motor area		-2	4	56	3.60
Left superior parietal lobule	7	-22	-66	50	3.74
Nonverbal WM					
Region	**BA**	**x**	**y**	**z**	**t**
Left dorsolateral prefrontal cortex	9	-32	22	24	3.00
Right dorsolateral prefrontal cortex	9	42	26	28	2.70
Dorsal anterior cingulate cortex	32	4	18	42	3.56
Left premotor cortex		-48	-4	44	4.37
Supplementary motor area		-6	4	54	3.92
Left superior parietal lobule	7	-22	-68	50	3.74
Left caudate nucleus		-18	-2	20	3.07
Left Thalamus		-4	-16	6	2.93

Table 5. fMRI t-map activation peaks for the Control > mTBI participants.

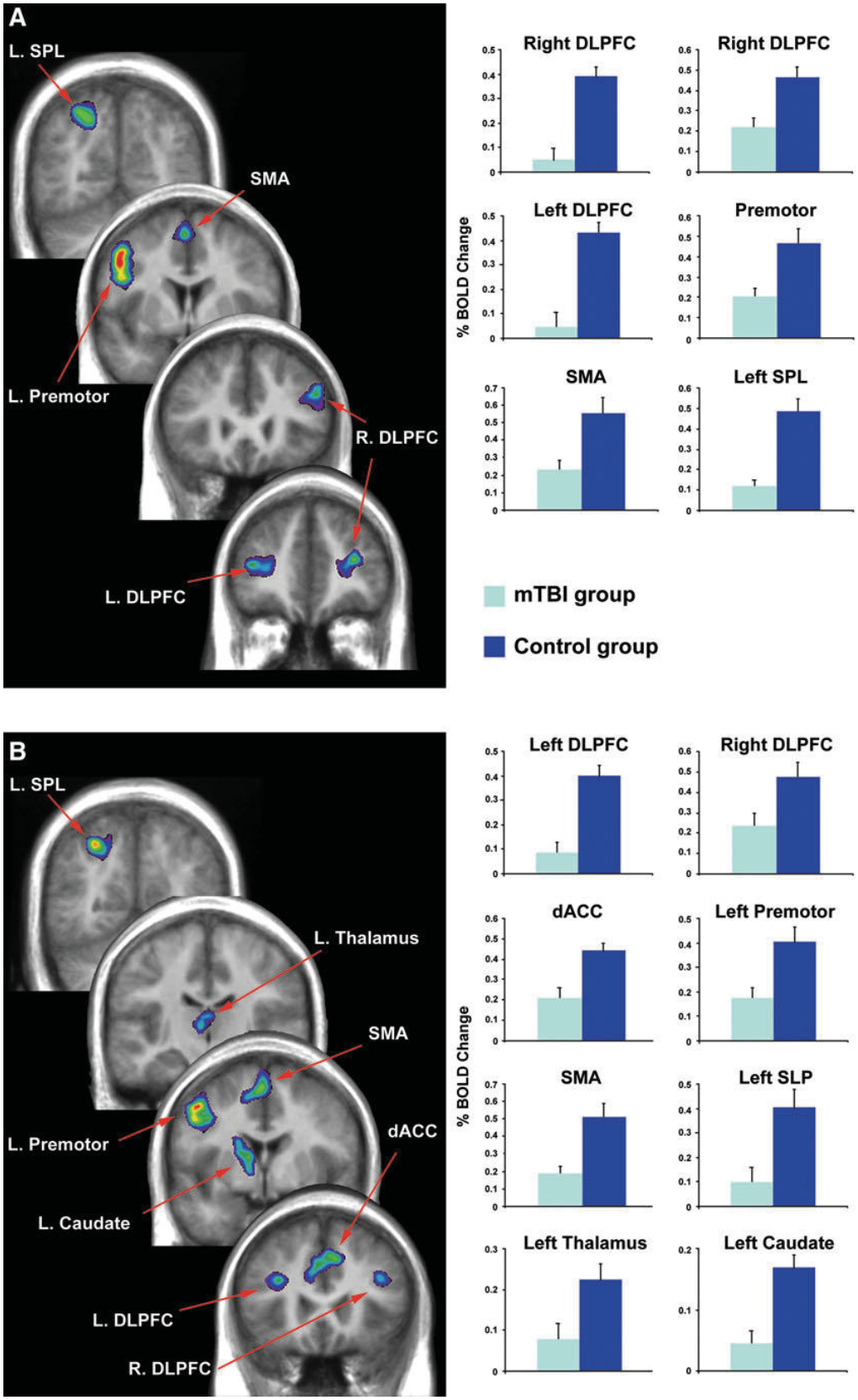

Figure 3. Results from between group subtraction showing significantly greater activation for the control versus the mTBI participants during the (A) verbal and (B) nonverbal working memory condition.

Independent sample t-tests comparing the mean percentage BOLD signal change revealed significantly greater activation for the control group versus the mTBI group in the left (BA 9: [t (2, 28) = -5.95, $p < 0.001$] and right (BA 9: [t (2, 28) = -2.59, $p = 0.015$] dorsolateral prefrontal cortex as well as the dorsal anterior cingulate cortex (BA 32: [t (2, 28) = -3.56, $p = 0.001$] , left premotor cortex (BA 6: [t (2, 28) = -3.16, $p = 0.004$], supplementary motor area [t (2, 28) = -3.44, $p = 0.003$] , left superior parietal lobe (BA 7: [t (2, 28) = -3.23, $p < 0.003$] , left thalamus [t (2, 28) = -2.54, $p = 0.017$] and left caudate nucleus [t (2, 28) = -4.88, $p < 0.001$] during the nonverbal working memory condition (see Table 5 and Figure 3B). Of these regions, the left ($r = 0.808$, $p < 0.001$) and right ($r = 0.895$, $p < 0.001$) dorsolateral prefrontal cortices were found to be significantly correlated with performance for the mTBI subjects only (Figure 4).

The reverse analysis (mTBI > Control) did not reveal any significant regions of greater percent BOLD signal change for the mTBI participants on either the verbal or nonverbal working memory tasks.

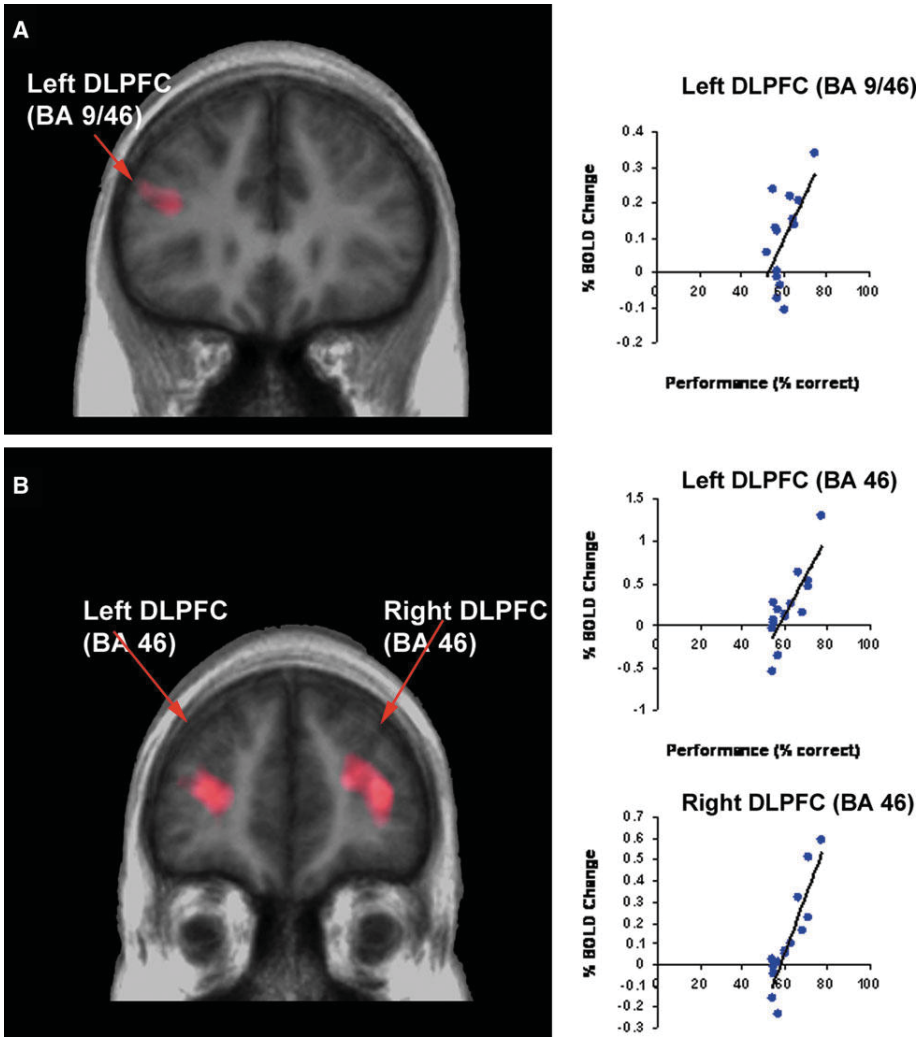

Figure 4. Whole brain regression analysis showing brain areas of the concussed group where BOLD signal changes were modulated by performance accuracy for (A) verbal and (B) nonverbal working memory.

4.6. Discussion

The purpose of this study was to examine and compare working memory performance and related brain activity using fMRI in concussed youths and healthy age-matched control subjects. As hypothesized, the behavioural data revealed significant group differences in performance, where the concussed subjects demonstrated significantly poorer accuracy on both the verbal and visual versions of the task, with no difference in reaction time. However,

concussed subjects also demonstrated significantly poorer performance on the baseline condition of each task. This finding is different from previous studies examining working memory performance in concussed versus control adult subjects, where no difference in group performance had been found (Chen et al., 2007; Chen et al., 2004; Pardini et al., 2010). However, these findings are similar to other studies that document decreased performance on working memory tasks following more severe TBI in children (Levin et al., 2004; Moran and Gillon, 2004) and they suggest that attentional and/or more general associative learning deficits specific to the baseline task may be present as well, given the poorer performance on the baseline task. Neuropsychological findings that demonstrated statistically significant differences in performance between the mTBI and control groups (RAVLT Delayed Memory Recall and Verbal Fluency) also suggest the presence of more general learning challenges in this group. These findings support previous studies suggesting the adolescent group may be more vulnerable to the neuropsychological effects of sports-concussion than younger children or adults (Baillargeon et al., 2012). The PCS scores were also significantly different between the concussed and control groups, indicating that some members of the concussed group were still clinically symptomatic. Therefore it is unknown if these group differences in cognitive performance would persist, or resolve with symptom resolution.

This study is unique and the first of its kind to link behavioural deficits in working memory performance following concussion in youth with functional alterations in brain activity. In one of the few previous investigations involving youth athletes (Talavage et al., 2014), fMRI activation patterns in concussed athletes shifted from greater activity during the more complex task to greater activity during the less complex task, suggesting hyperactivation associated with the simpler task condition (Yang et al., 2012). However the authors did not report whether there

were performance differences between the groups on the tasks. Similarly, while Yang *et al*. found hypoactivation during an auditory orienting task in mTBI youths relative to healthy controls (Yang et al., 2012), this difference did not correlate with task performance and there were no statistically significant differences between the groups in this respect.

Overall, in the present study, similar regions were associated with BOLD signal change for both the verbal and nonverbal abstract design conditions across concussed and age-matched control subjects. Regions demonstrating BOLD signal change included bilateral prefrontal, premotor, parietal and occipital cortices as well as the dorsal anterior cingulate cortex. A direct comparison of percent BOLD change from the baseline task to the verbal working memory task revealed significantly greater change for the controls compared to the concussed subjects in the left premotor cortex. The same analysis with the nonverbal abstract design condition also revealed greater percent BOLD change in the left premotor cortex for age-matched control subjects, as well as greater change in the left dorsolateral prefrontal and dorsal anterior cingulate cortices. The reverse analysis did not reveal any regions where concussed subjects showed greater percent BOLD signal change from baseline to working memory task condition compared to age-matched control subjects.

Given its demonstrated importance for working memory and the observed difference in performance for concussed versus control subjects, we regressed percent change in BOLD signal in this region on working memory performance and found a significant relationship between the two in the left and right hemispheres for the nonverbal visual abstract design condition, and a trend towards significance for the verbal condition. Interestingly, performance was not correlated with post-concussion symptom severity, suggesting that performance may be directly related to the lack of BOLD signal change in this region and not solely due to the presence of

somatic and cognitive post-concussion symptoms such as headache, fatigue etc. This is an important finding given that return to play decisions are made based solely on the absence of post-concussion symptoms.

Taken together, these results suggest that unlike in adults, working memory performance in youth is significantly affected by concussion. Moreover, unlike the adult population where differences in performance are not observed between mTBI subjects and healthy controls (Chen et al., 2007; Chen et al., 2004; McAllister et al., 1999; Pardini et al., 2010), there is a significant relationship between activity in this region and performance in youth, suggesting poorer compensatory resources following mTBI in the immature brain.

These results cannot be solely explained by differences in task methodology as we deliberately employed the same experimental paradigm used by Chen *et al*. in order to compare our findings to adult subjects (Chen et al., 2007; Chen et al., 2004). In addition to lack of BOLD signal change found in the dorsolateral prefrontal cortex, Chen *et al*. also reported regions of increased BOLD signal change in the temporal lobes of concussed adults while performing the nonverbal abstract design working memory task (Chen et al., 2007; Chen et al., 2004). The reason for this pattern of findings is unclear. Regions of hyperactivation have been suggested in many areas of neuroimaging investigation to reflect compensatory strategies in information processing. In the current study we did not find any regions of hyperactivation during either the verbal or nonverbal visual abstract design condition, supporting the idea that youths may be less likely to engage compensatory mechanisms to maintain cognitive performance following a mild neural insult.

Further research is clearly needed to better understand the relationship observed between changes in working memory performance and cortical activity. The lack of ability to engage compensatory mechanisms is one possible hypothesis. Specific underlying neural mechanisms may be related to pathophysiological differences in the immature brain, which make it more vulnerable to neuronal injury. These pathophysiological differences include the mechanical and compositional properties of the brain which result in a markedly diminished shear resistance of the immature brain tissue (Bauer and Fritz, 2004). As such, the immature brain tissue appears to be more susceptible to mechanical alterations, because similar mechanical loads induce a more intense brain tissue displacement. Although our concussed athletes were not young children (the mean age was 14 years for both groups), the frontal lobes demonstrate protracted structural development compared to other regions in the brain and thus continue to develop throughout adolescence and into adulthood (Romine and Reynolds, 2004). This protracted development has been shown to be related to the ability to perform demanding memory tasks (Romine and Reynolds, 2004). More specifically, Crone *et al*. focused on development of non-spatial working memory and revealed that while children made more errors than adolescents and adults, they engaged highly overlapping brain regions during task performance (Crone et al., 2006). A significant positive correlation was found between accuracy and activation in the dorsolateral prefrontal cortex. This region has demonstrated particular involvement for the manipulation of items in working memory in adolescents and adults (Wager and Smith, 2003) but not in 8-12 year olds (Crone et al., 2006).

4.6.1. Clinical Implications

The findings from this study regarding poorer cognitive performance associated with differential patterns of cortical activation following youth concussion supports the 4rd

International Consensus Statement which advises greater caution in the clinical management of youths following sports-related concussion (McCrory et al., 2013). This statement, which represents the clinical "Gold Standard" for concussion management, highlights the need to consider the developmental impact of concussive injuries such as diffuse cerebral swelling, and to increase rest and recovery time following injury, accordingly. The statement additionally highlights the need for cognitive rest that includes school and recreational activities such as video games and text messaging. The results from the current study provide the first known empirical data to support this recommendation for concussed youth and further research is clearly indicated in order to better understand the nature of the working memory performance deficits and associated alterations in cortical activity. The impact of concussion on short-term cognitive function has serious implications for the risk of re-injury and Second Impact Syndrome (where the brain swells rapidly and catastrophically if a second injury is incurred prior to complete neuronal recovery from an earlier injury) if youth athletes return to sport participation too early. Overall there is a dire need for systematic evaluation and re-integration following concussion in youth. The results of the regression analysis validate the idea that fMRI activity in well-studied regions of interest (such as the dorsolateral prefrontal cortex) hold significant potential as clinical markers of recovery in youth as well as adults.

4.6.2. Methodological Considerations

The employment of a subtraction technique in the current study does not allow us to determine whether the lack of change in BOLD signal is due to decreased activity for both baseline and working memory task or hyperactivation for baseline task in mTBI group. This is important to consider as some studies have shown hyperactivation following concussion in youth athletes (Lovell et al., 2007; Talavage et al., 2014). Finally, in the current study, concussed

participants were still symptomatic (mean PCS score = 31, SD=18) so not representative of uncomplicated concussion where youth may recover quickly (i.e. 7-10 days following injury).

4.7. Conclusions

Functional alterations in brain activity during a working memory task show some similarity between youth and adults, including a lack of BOLD signal change in the dorsolateral prefrontal cortex. However there are also notable and significant differences. In particular, the observation of reduced working memory accuracy relative to healthy controls is of great concern and warrants further investigation. Combined with a lack of increased BOLD signal typically seen in temporal and parietal regions (Chen et al., 2007; Chen et al., 2004; Lovell et al., 2007), it suggests that youths may be unable to engage compensatory strategies to maintain cognitive performance following neuronal injury. This has significant implications for safe return to daily activities, including competitive sport.

4.8. Acknowledgements

The authors would like to thank CIHR (Grant number 484706, Primary Investigator: M. Keightley, Co-Investigators: A. Ptito, K. Johnston and N. Lobaugh) for their financial support of this study as well as the members of the Trauma Program of the Montreal Children's Hospital, McGill University Health Center for their collaboration.

Chapter 5

Navigational Memory fMRI in Children Post Concussion

5.1. Preface.

In chapter 3, we demonstrated that for the navigational memory task, the overall regions of peak activation were identical between normal adults and children and similar to previously published studies (Iaria et al., 2007; Spiers and Maguire, 2007). Contrary to the working memory task, however, the intensity of activation within those peak areas did show significant correlations with age in a number of areas. Despite this observation, however, since the overall activation pattern was the same, it was felt that as long as the current studies employed the use of age-matched controls, the paradigm could still be applied to children.

In the previous chapter, we saw that the concussed children had similar attenuation of their fMRI signals as seen in adults. However, unlike the adult studies, the children performed significantly worse than the adults on the task itself and they lacked the additional peaks seen in

concussed adults, suggesting that children lacked the ability to compensate for the damaged areas through the recruitment of additional brain regions. We, therefore, used another fMRI paradigm in the navigational memory task, to determine if similar findings could be seen with this task. This new task employed a number of cognitive elements, as described in chapter 3. This made it particularly intriguing to determine if a more complex task would have similar attenuation of fMRI signals and if performance would be different between the concussed subjects and the normal controls. The use of this task in concussion had never been before published prior to the current report.

Navigational memory fMRI: A test for concussion in children

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Saluja R.S., Chen J.K., Gagnon I., Keightley M.L., Ptito A. "Navigational memory fMRI: A test for concussion in children." Journal of Neurotrauma. May15, 2015; 32(10):712-22. DOI: 10.1089/neu.2014.3470

5.2. Abstract

Concussions are high incidence injuries with potentially devastating consequences. Youths are at risk because of a higher threat of repeat injury and cumulative effects of concussions exist, making accurate diagnosis and follow-up essential. This study examines a navigational memory fMRI task to determine whether activation differences exist between concussed children and uninjured controls.

Fifty adolescents were recruited, 35 controls and 15 concussed. All subjects underwent structural and functional MRI testing using our navigational memory task, and a battery of neuropsychological testing. The activation patterns of the 15 concussed subjects were compared to those of 15 age and sex-matched controls. Subtraction and regression analyses were performed using the matched controls along with scatter-plots using means and 95% quantiles of the 35 controls.

While no differences were seen with neuropsychological testing or task performance, concussed subjects had significantly diminished activation in the retrosplenial, thalamic, and parahippocampal areas bilaterally, along with the right dorsolateral prefrontal cortex and left precuneus. Interestingly, they had increased activation in the left hippocampus and right middle temporal gyrus. Regression analysis demonstrated negative correlations between activation and post concussive symptoms in the left premotor cortex, superior and inferior parietal lobules, and parahippocampal gyrus.

Concussed subjects show both diminished and increased activation in specific cerebral regions, differentiating them from controls. This is one of the first studies to look at such

a task using fMRI and its applicability in testing for concussion in children. These findings support navigational memory fMRI as a potential objective test for concussions.

5.3. Introduction

Concussion, often used synonymously with mild traumatic brain injury, is a complex post-traumatic pathological process affecting the brain. Concussion is considered a major public health problem with staggering human and economic costs to society (Coronado et al., 2013). Its incidence is reported to be higher than 600 per 100 000/year (Cassidy et al., 2004). Compared to the incidence of other "common" neurological conditions such as multiple sclerosis (2-3.6 per 100 000/year) (Alonso and Hernan, 2008), Parkinson's disease (17 per 100 000/year) (Twelves et al., 2003), and epilepsy (50 per 100 000/year) (Sander, 2003), the incidence *in the general population* affected by concussion is of epidemic proportion.

Although it was once considered as simply a temporary fluctuation in the level of consciousness, we now know that a significant proportion of patients can suffer from persistent symptoms including physical complaints, emotional disturbances, sleep changes, and cognitive alterations after concussion (post concussion syndrome) (McCrory et al., 2013). With the potential link with other serious neurological illnesses such as depression (Chen et al., 2008a) and chronic traumatic encephalopathy(Jordan, 2013), proper treatment of young concussed patients becomes critical. Furthermore, the recovery profile and breadth of consequences in children and youth remains largely unknown (Keightley et al., 2012).

In younger individuals exposed to concussion, the potential for cumulative effects is real (Gaetz et al., 2000; Guskiewicz et al., 2003; Rabadi and Jordan, 2001), and assessment tools that can reliably detect injury, measure its severity and document recovery are critical for patient management. Unfortunately, diagnosis, treatment, and prognosis have proven difficult because the current tools at our disposal are not sensitive enough to detect subtle abnormalities and usually reveal normal results(Mami and Nance, 2008; Ptito et al., 2007). In efforts to find a suitable tool for use in the management of concussion, much work has been done, particularly from our group, using functional MRI (fMRI) to assess patients post concussion. We previously demonstrated the utility of fMRI and working memory tasks in diagnosing and documenting recovery in concussed adult athletes (Chen et al., 2007; Chen et al., 2008b), while such studies are scarce with pediatric patients (Keightley et al., 2012; Keightley et al., 2014; Talavage et al., 2014).

In addition to working memory difficulties, it has become evident, in recent years, that spatial memory (Chuah et al., 2004) (positions of objects in space) and route learning (referred to as "spatial memory navigation" by Slobounov et al.) may also be impaired in adult concussed subjects (Slobounov et al., 2010). There are, however, no studies in the literature examining spatial navigational memory in concussed children. We therefore sought to examine in concussed children the fMRI activation patterns associated with a virtual reality spatial navigation task. Our goal was to determine if spatial navigation becomes altered in concussed children and explore whether such a test could become a complementary diagnostic tool for concussion.

5.4. Materials and Methods

After obtaining institutional ethics review board approval for our study, preteens and teens aged 10-17 years of age were recruited and divided into two groups: normal uninjured controls and concussed subjects. This age range was selected as subjects 10 and older are able to reliably complete neuropsychological tasks (Waber et al., 2007) during the MRI.

5.4.1. Concussed subjects.

Fifteen concussed subjects aged 10 to 17, within three months of injury, regardless of symptom severity, were recruited from the Concussion Clinic at The Montreal Children's Hospital, McGill University Health Centre as well as from direct local referrals. Both symptomatic and asymptomatic concussed subjects were included in this study in order to capture the full spectrum of recovery. This allowed us to carry out regression analyses with respect to symptom severity and cerebral activation patterns.

Prior to referral, all subjects were evaluated in a clinical setting by medical personnel who performed general physical and neurological examinations. All the subjects had normal neurological status. The concussed subjects all had concussions diagnosed by a physician as per the WHO task force definition of mild traumatic brain injury (Cassidy et al., 2004). Criteria for diagnosis included: 1) confusion or disorientation, loss of consciousness for 30 minutes or less, posttraumatic amnesia for less than 24 hours, and/or transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery (although no subjects in this study had said lesions); 2) Glasgow Coma Score of 13-15 after 30 minutes post-injury or later upon presentation for health care. The criteria for diagnosis in each case were verified by one of the authors (R.S.S.), a neurosurgeon. All subjects were screened prior to testing to rule out any

significant past medical history including attention deficit hyperactivity disorder or other neurological symptoms, any other confounding conditions, or any contraindications to MRI including claustrophobia or major dental work that could interfere with the clarity of the MRI images. Three of the subjects had a previous history of concussion more than one year prior to the one suffered in this current study. In all three cases, the symptoms of the previous concussion had completely resolved.

5.4.2. Normal controls.

A group of 35 normal control subjects ranging in age from 10 to 17 years were recruited from a database of normal controls and their siblings previously used in fMRI studies at the Montreal Neurological Institute by Dr Tomas Paus. All subjects were screened prior to testing with the same questionnaire used for the concussed subjects and excluded if they had any previous history of concussions. From this group, 15 age and sex-matched controls were selected to compare to these 15 concussed subjects. These matched control subjects were used for all between-group comparisons (concussed vs. controls) in this study. For the scatter-plot analysis, all 35 control subjects were used to create a control group mean and 95% quintile.

Post-concussion symptoms were assessed using an adapted version of the Post-traumatic Symptom Scale - Revised (PCS) (Lovell and Collins, 1998) on the day of testing. The scale consisted of 21 symptoms (eg. headache, nausea, dizziness etc.) scored by the subject on a scale from zero to six, six being a severe problem. Total PCS scores were then established by adding up the scores for each symptom.

All testing (neuropsychological testing and MRI) was performed over the course of a single half-day for each subject. All subjects were financially compensated for their time and

presented with a copy of their structural scans including a three-dimensional rendering of the images.

5.4.3. Neuropsychological Testing.

All subjects underwent a battery of standardized neuropsychological tests used in previous studies (Keightley et al., 2014), administered by an experienced, blinded neuropsychologist (G. L.). Additionally, the Beck Youth Inventories (Beck et al., 2005) were used to assess self-perceived levels of anxiety and depression in all participants.

5.4.4. Functional MRI Task.

The navigational fMRI task was adapted from a test that is based on navigation and orientation within a virtual environment developed at our institution by Iaria and colleagues (Iaria et al., 2007). This is the first time this task has been used in a pediatric group. The task consists of a virtual town in which the subject moves about using a four-button keypad. This virtual town consists of several landmarks including a police station, bank, post office, church, clinic, and store. Prior to the functional imaging, the subjects were given the opportunity to explore the town and familiarize themselves with the different locations within the virtual environment. This procedure allowed the subjects to create their own mental representation of the town i.e. a cognitive map. Following this encoding phase, the subjects practiced several way-finding trials in which they started from one location and were instructed to navigate to another location within the town using the most efficient route (figure 1). In each trial, the starting location and the target location were different, such that the only way that the task could be efficiently solved was by using the previously created cognitive map.

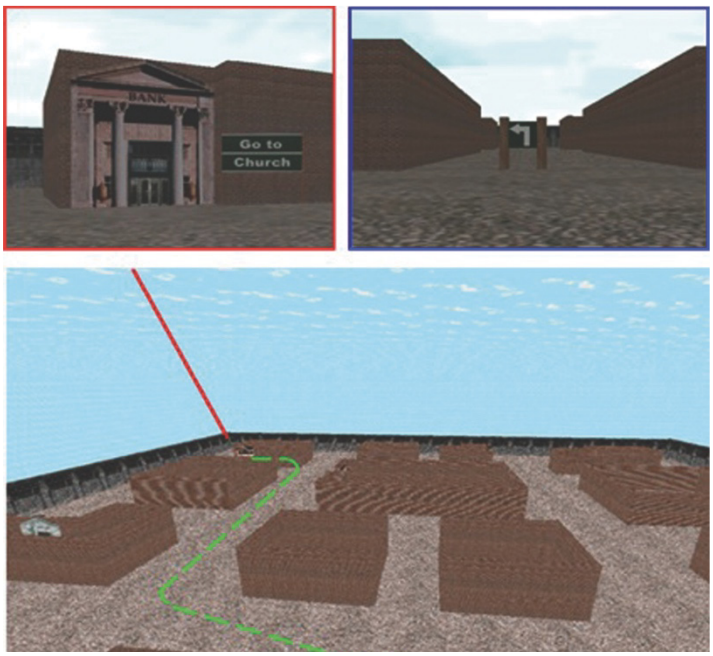

Figure 1. The navigational memory task involved starting at a location and being instructed to go to another location using the most efficient route. The baseline task involved navigating through the environment by simply following the signs with arrows (top right panel).

Once the subjects were comfortable performing the task in the laboratory, they were taken to the MRI where structural and functional scans were performed. The functional scan lasted 15 minutes and the subjects had to complete as many trials as possible up to a maximum of 24. Performance was measured by recording the number of trials that could be completed in the time allotted and the total time needed to complete the trials (excluding baseline tasks and any incomplete trials). Interspersed amongst the trials was a baseline task whereby the subjects simply followed signs with arrows around the town. This baseline allowed us to capture brain activity unique to spatial navigation requiring use of a previously established cognitive map by subtracting the baseline from the navigation task condition. This occurred every sixth navigation trial.

5.4.5. Image Acquistion.

All imaging was carried out using a three-Tesla Siemens Magnetom Trio A Tim System with a 32-channel head coil. Each session started with acquisition of high-resolution T1-weighted 3D anatomical images using 3D Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence (TR = 23ms, TE = 2.98ms, Slice Thickness = 1mm, Image Matrix = 256 x 256, Flip Angle = 30 degrees, FOV = 256mm, interleaved excitation). This was followed by acquisitions of T2* weighted gradient echo (GE) echo-planar images (EPI) for blood oxygenation level dependent (BOLD) fMRI (TR = 4500ms, TE = 30ms, flip angle: 90°, slice thickness = 4mm, in-plane resolution = 4 x 4 mm, FOV = 256 mm, 64 x64 image matrix, number of slices: 38, interleaved excitation). An experienced, blinded neuroradiologist reviewed all structural images and found no significant abnormalities.

5.4.6. Image Analysis.

Images acquired during the fMRI scans were corrected for motion artifacts using 3-D prospective acquisition correction technique implemented by Siemens for real-time motion correction of BOLD data (Thesen et al., 2000). Motion-corrected images were spatially smoothed with a 6-mm full-width at half-maximum Gaussian filter to increase the signal-to-noise ratio of the data. The motion-corrected data were analysed statistically by using fmristat (Worsley et al., 2002b). Significant BOLD changes from control condition to retrieval condition were determined at each voxel, based on a linear model with correlated errors. To obtain the average group t-maps, all individual MRI data were first normalized to the Montreal Neurological Institute (MNI) stereotaxic space constructed from average stereotaxic MRI of 305 normal subjects (Evans et al., 1993) and were then combined using a mixed effects linear model.

The resulting t statistic images were thresholded using the minimum given by a Bonferroni correction and random field theory to correct for multiple comparisons (Worsley et al., 1996). Threshold for significance was established at $t = 4.10$ for the activation peaks, or $t = 3.10$ for activation clusters greater than 222 mm^3, corresponding to a $p<0.05$, based on the number of resolution elements in the acquisition volume (2,880 resels). Finally, between-group comparisons (concussed subjects vs. controls) were carried out in a third-level analysis using fixed effects linear model.

To identify brain regions where symptomatology modulated BOLD signal changes, whole-brain, voxel-wise linear regressions were carried out using PCS and task performance as the covariates in separate analyses for navigation condition against the control task condition. This was carried out using the data obtained from the concussed subjects only. These results were mapped to the average T1 images of the concussed subjects in standard space to identify regions of interest (ROI) with statistically significant positive and negative regressions. Once the ROIs were identified, individual %BOLD signal change values were extracted to perform Pearson correlations to assess the levels of significance.

In order to examine the results of the testing on an individual level, a scatter-plot analysis was performed whereby the %BOLD signal change values for all 35 control subjects was obtained to establish a mean and 95% quantiles for the ROIs obtained from the between group comparisons. The %BOLD signal changes values for each of the 15 concussed subjects was then plotted alongside the results obtained from the 35 control subjects to illustrate how the values obtained from each of the individual concussed subjects compared to the standard group.

As noted above, BOLD signal changes reported in this paper are in comparison to the baseline task. Although we identify this as activation, it should be noted that there is a theoretical possibility that group differences seen reflect differences in BOLD signals of the baseline task itself as opposed to the task condition. Increased and decreased BOLD signals reported in this paper, therefore reflect difference with respect to the subject's own baseline task.

5.4.7. Statistical Analyses.

Neuropsychological, demographic, and behavioural data analyses and descriptive statistics were carried out using the IBM Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows. For all elements of the neuropsychological battery, student t-tests were performed to determine if any group differences existed using an α of 0.01 given the multiple comparisons performed. For tests consisting of multiple component measures (e.g. RAVLT), multivariate analysis of variance (MANOVA) was performed using the group (concussed versus control) as the independent variable and each of the sub-measures (i.e. immediate memory recall, delayed memory recall etc.) as the dependent variables.

5.5. Results

5.5.1. Demographic Data and PCS.

Demographic data and group PCS scores are shown in table 1. As the subjects were age and sex-matched, no differences were seen with respect to demographics. A significant difference was seen with respect to PCS scores between the concussed and control groups ($p<0.001$). As expected, with increasing time since injury, there was a tendency to have

decreased PCS score, though this correlation did not achieve statistical significance (r=-0.469, p=0.078).

		Concussed Group	Control Group	p-value
Average age in years (SD)		15 (2.2)	15 (2.1)	
Sex	M	7	7	
	F	8	8	
Right-Handed		15	15	
Mean PCS score (SD)		32.5 (18.3)	12.0 (8.7)	*<0.001*
Mechanism of Concussion				
			Hit on head playing soccer	5
			Hit on head playing football	3
			Hit head while swimming	2
			Fell playing ringuette	2
			Fell while skiing	1
			Ran into wall in gym class	1
			Hit on head on trampoline	1
Time from injury to testing			Range in days	9 - 72
			Mean (SD)	39.3 (21.5)

Table 1. Summary of demographic data, PCS scores, mechanism of concussion, and time from injury to testing.

5.5.2. Behavioural data.

There were no significant differences in neuropsychological test performance between the concussed and control subjects or parental questionnaires (table 2). A correlation analysis of time since injury versus performance on neuropsychological tests did not reveal any statistically significant results.

Neuropsychological Testing							
Test		Mean		Standard deviation		T-test	MANOVA
		Control	Concussed	Control	Concussed	p-value	p-value
Wechsler Abbreviated Scale of Intelligence (WASI)		114.5	110.5	6.1	8.7	0.19	
Rey Complex Figure	Copy	31.8	30.7	3.0	4.2	0.47	0.25
	Copy Time	285.0	298.6	40.2	79.8	0.59	
	Immediate Recall	24.0	19.3	5.1	5.6	0.04	
	Immediate Recall Time	193.6	202.2	73.2	57.3	0.75	
	Delayed Recall	22.7	18.0	2.5	6.6	0.03	
	Delayed Recall Time	159.6	147.8	55.6	64.9	0.65	
	Recognition	21.3	20.8	1.3	3.0	0.61	
Verbal Fluency	Animals	25.3	22.6	6.5	5.6	0.29	0.10
	Food/Drink	24.9	22.8	6.8	5.2	0.41	
	S words	16.5	11.2	6.8	3.0	0.02	

	F words	13.6	11.7	6.1	4.1	0.36	
	Total	80.3	68.4	22.4	14.2	0.13	
Rey Auditory Verbal Learning test (RAVLT)	I	7.2	6.3	2.1	1.3	0.18	0.13
	II	10.7	10.0	1.8	1.9	0.36	
	III	12.8	11.2	1.2	2.4	0.05	
	IV	13.5	13.2	1.2	1.5	0.57	
	V	13.7	13.1	1.3	1.8	0.31	
	B	7.4	5.9	1.7	1.5	0.03	
	Immediate	12.4	11.6	1.6	2.3	0.33	
	Delayed	12.8	11.3	2.0	2.3	0.10	
	Word Recognition	14.8	13.9	0.5	1.8	0.21	
Stroop Color and Word Test	Word Score	97.5	89.8	15.4	22.4	0.37	
	Color Score	71.2	65.0	10.0	18.4	0.35	
	Color-Word Score	45.5	38.8	9.8	13.8	0.21	
	Interference Score	-16.0	-22.7	16.8	14.4	0.32	
Symbol Digits Modality Test		65.0	53.5	12.7	13.2	0.056	
Children's Color Trails Test	CT-1 Time	13.6	21.3	4.5	14.2	0.12	0.26
	Standard Score	103.9	101.0	15.6	17.2	0.70	
	CT-2 Time	29.9	38.2	5.3	15.8	0.13	
	Standard Score	100.0	92.1	8.8	17.7	0.21	
Paced Auditory Serial Addition Test (PASAT)	2.8	18.7	17.4	1.6	3.2	0.22	0.85
	2.4	16.3	15.6	2.1	3.1	0.55	
	2.0	16.6	16.5	2.7	2.2	0.92	
	1.6	12.6	12.1	1.9	1.2	0.53	
	1.2	14.5	13.9	3.1	1.6	0.64	
Grooved Pegboard	Right Mean (seconds)	57.3	59.3	4.7	10.8	0.57	
	Left Mean (seconds)	63.0	65.9	7.0	6.5	0.30	
G. Pegboard Removal	Right Mean (seconds)	19.0	22.4	2.4	10.0	0.26	
	Left Mean (seconds)	19.6	19.5	1.5	3.4	0.91	
Beck Youth Inventories							
	Self-Concept	37.9	39.2	7.2	7.7	0.68	
	Anxiety	16.7	15.7	8.7	10.1	0.80	
	Depression	10.8	11.8	6.6	9.3	0.78	
	Anger	13.4	12.7	7.5	10.1	0.86	
	Disruptive Behavior	7.4	6.7	4.6	4.6	0.72	
Parental Questionnaires							
Conners Parent Rating Scale (T-score)	Oppositional	50.7	50.6	8.6	11.7	0.99	
	Cognitive Problems/ Inattention	46.3	51.7	4.5	13.1	0.26	
	Hyperactivity	46.2	49.8	2.4	6.5	0.13	
	ADHD Index	47.4	49.1	3.0	8.0	0.57	
Child Behavior Checklist (T-score)	Activities	45.6	50.5	8.0	12.8	0.31	
	Social	49.6	53.3	6.2	8.9	0.28	
	School	53.4	52.1	3.7	4.6	0.48	
	Total Competence	48.9	52.7	6.5	13.4	0.42	
	Anxious/ Depressed	52.9	55.1	5.0	7.1	0.43	
	Withdrawn/ Depressed	53.1	55.8	5.0	7.9	0.37	
	Somatic Complaints	56.6	61.9	4.5	9.4	0.12	
	Social Problems	52.6	53.3	3.3	4.2	0.66	
	Thought Problems	52.2	55.8	2.3	7.1	0.14	
	Attention Problems	52.5	53.1	2.9	4.3	0.72	
	Rule-breaking Behavior	51.9	51.9	3.1	3.3	0.99	
	Aggressive Behavior	52.2	52.8	4.3	3.5	0.74	
	Internalizing Problems	50.4	56.0	8.6	10.4	0.19	

	Externalizing Problems	48.5	47.8	5.7	7.4	0.82	
	Total Problems	48.8	50.8	6.3	8.4	0.54	
Child Behavior Checklist DSM-IV (T-score)	Affective Problems	54.5	56.1	6.6	7.4	0.61	
	Anxiety Problems	52.9	53.6	5.0	5.7	0.77	
	Somatic Problems	56.9	60.4	6.3	8.3	0.29	
	ADH Problems	52.3	53.0	3.1	4.1	0.66	
	Oppositional Defiant Problems	54.1	52.9	3.8	2.6	0.40	
	Conduct Problems	51.3	52.1	3.1	3.8	0.60	

Table 2. Summary of neuropsychological testing, Beck Youth Inventories, and parental questionnaires.

With respect to the task performance during the fMRI, the concussed subjects performed worse than the control subjects with respect to the number of trials completed and time to complete trials (table 3). Neither measure reached statistical significance but this could be due to the fact that there was a higher inter-subject variability in the concussed group.

Test	Mean		Standard deviation		T-test
	Control	Concussed	Control	Concussed	p-value
Number of trials completed	17.1	15.6	4.8	5.1	0.42
Time to complete trials (s)	540	594	85	128	0.29

Table 3. Summary of navigational memory task performance data.

5.5.3. Activation during navigational memory task.

Significant peak activations comparing the mean percentage of BOLD signal change between the navigation task and the control task are summarized in table 4 and their t-maps are shown in figure 2. The regions in the control group that demonstrated significantly increased bilateral activation included the posterior parahippocampal gyri, retrosplenial, premotor, superior parietal, primary visual, precuneus, dorsolateral prefrontal, lingual, and thalamic areas. The concussed group activation in some of the regions listed above did not reach a level of significance but they demonstrated an additional significant peak in the right middle temporal gyrus.

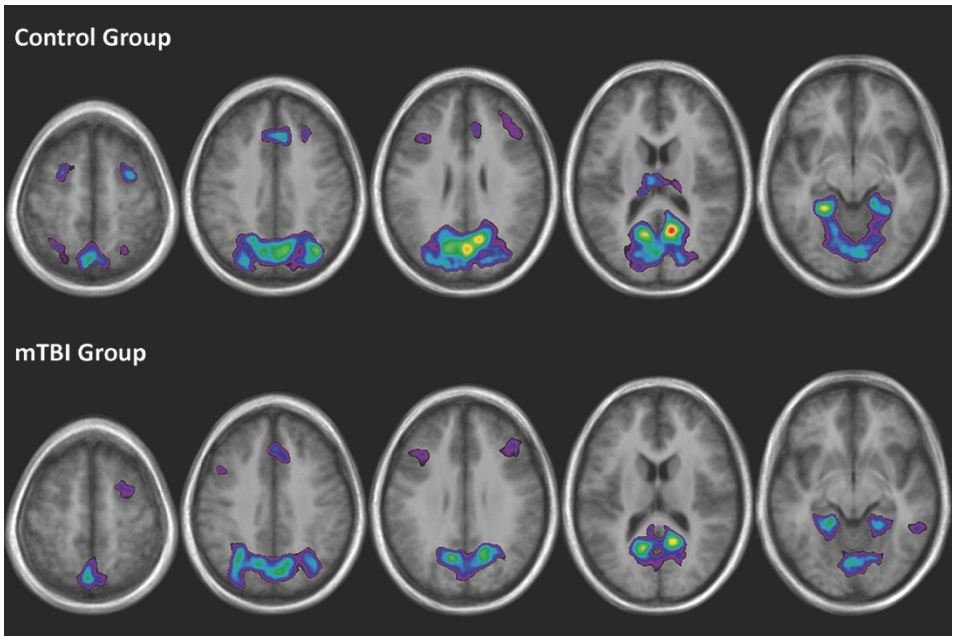

Figure 2. Functional MRI t-maps of controls and mTBI subjects during navigational memory task.

Navigation Memory Activation Peaks

	Control				mTBI			
Region/Gyrus	**x**	**y**	**z**	**t**	**x**	**y**	**z**	**t**
Left posterior parahippocampal	-26	-44	-6	9.4	-18	-40	-8	7.5
Right posterior parahippocampal	18	-38	-10	7.2	22	-42	-10	6.5
Left retrosplenial	-10	-64	16	8.8	-12	-62	18	8.9
Right retrosplenial	12	-60	16	10.3	16	-58	24	9.4
Left premotor	-26	6	58	5.9	-46	12	38	5.1
Right premotor	32	2	54	7.1	26	6	54	5.0
Right SMA	6	24	42	7.4	8	20	44	5.7
Left superior parietal	-26	-80	36	7.2	-30	-64	40	7.9
Right superior parietal	36	-72	40	8.7	30	-68	48	7.0
Left precuneus	-16	-74	26	8.5	-12	-68	42	7.6
Right precuneus	8	-70	32	9.3	4	-74	44	8.3
Left lingual	-2	-76	2	7.5	-4	-78	-12	7.8
Right lingual	8	-82	-8	6.8				
Left dorsolateral prefrontal	-36	22	26	4.6	-38	22	26	4.3
Right dorsolateral prefrontal	48	24	32	4.2	38	24	32	4.2
Right dorsolateral prefrontal	28	32	42	4.9				
Left thalamus	-6	-18	14	6.6				
Right thalamus	8	-10	12	5.0				
Right middle temporal gyrus					56	-46	-8	4.3

Table 4. Activation peak coordinates with respective t-values.

5.5.4. Control versus concussed subjects.

Significantly higher mean percent BOLD activation changes were found in the retrosplenial, parahippocampal, and thalamic areas bilaterally in control subjects compared to the concussed group. The right dorsolateral prefrontal and left precuneus areas also showed significantly higher activation in the control group (figure 3a, table 5). In contrast, the concussed group had significantly higher percent BOLD signal change in the left hippocampal and right middle temporal areas (figure 3b, table 5).

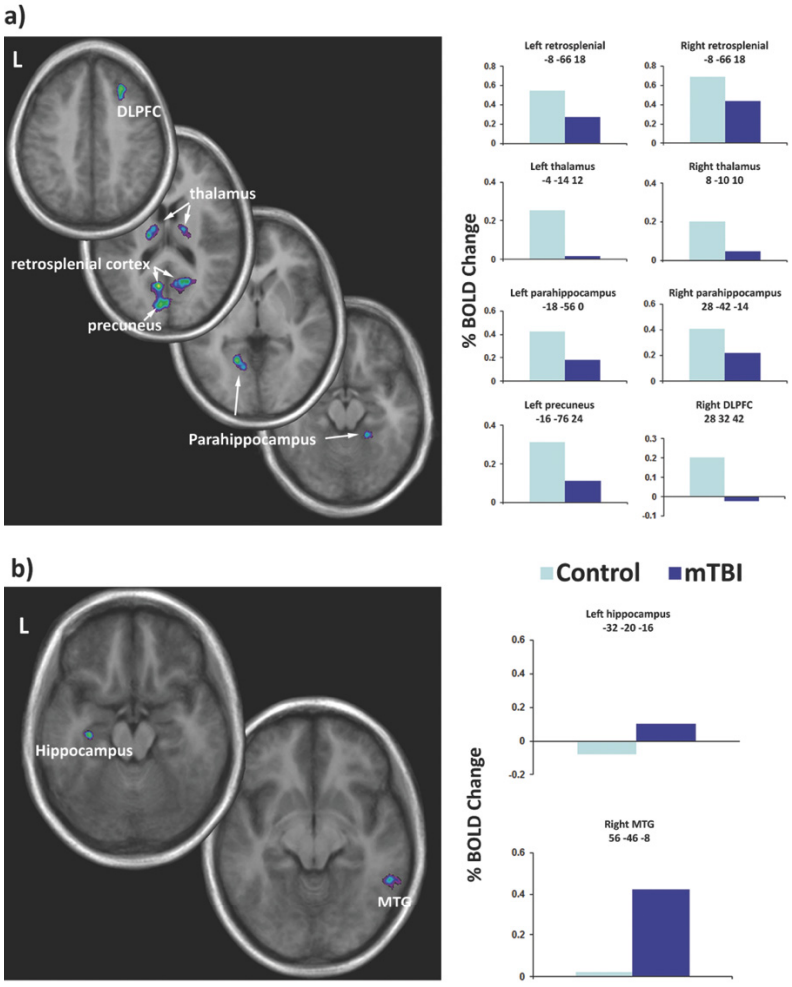

Figure 3. Subtraction analysis. Panel (a) demonstrates areas where the activation in the controls > concussed along with graphic representation of the % BOLD signal changes in those areas. Panel (b) shows the areas where activation in concussed > controls.

Control > Concussed					
Region	**x**	**y**	**z**	**t**	**p**
Right dorsolateral prefrontal	28	32	42	3.6	0.002
Left precuneus	-16	-76	24	3.3	0.002
Left retrosplenial	-8	-66	18	3.4	0.003
Right retrosplenial	14	-60	18	3.0	0.014
Left thalamus	-4	-14	12	3.6	<0.001
Right thalamus	8	-10	10	3.0	0.016
Left parahippocampal	-18	-56	0	3.2	0.004
Right parahippocampal	20	-42	-14	2.9	0.020
Concussed > Control					
Region	**x**	**y**	**z**	**t**	**p**
Left hippocampus	-32	-20	-16	3.2	0.007
Right middle temporal gyrus	56	-46	-8	2.9	0.011

Table 5. Subtraction analysis activation peak coordinates with t-values.

5.5.5. Regression Analyses.

Percent BOLD signal change was found to be negatively correlated with PCS score, indicating that decreased self-reporting of symptoms was associated with increased signal change in the left superior parietal lobule, inferior parietal lobule, prefrontal area, and parahippocampal area (figure 4). A regression analysis of performance versus BOLD signal change was performed which demonstrated negative correlations mainly in areas outside of the regions of peak activation and were, overall, non-contributory.

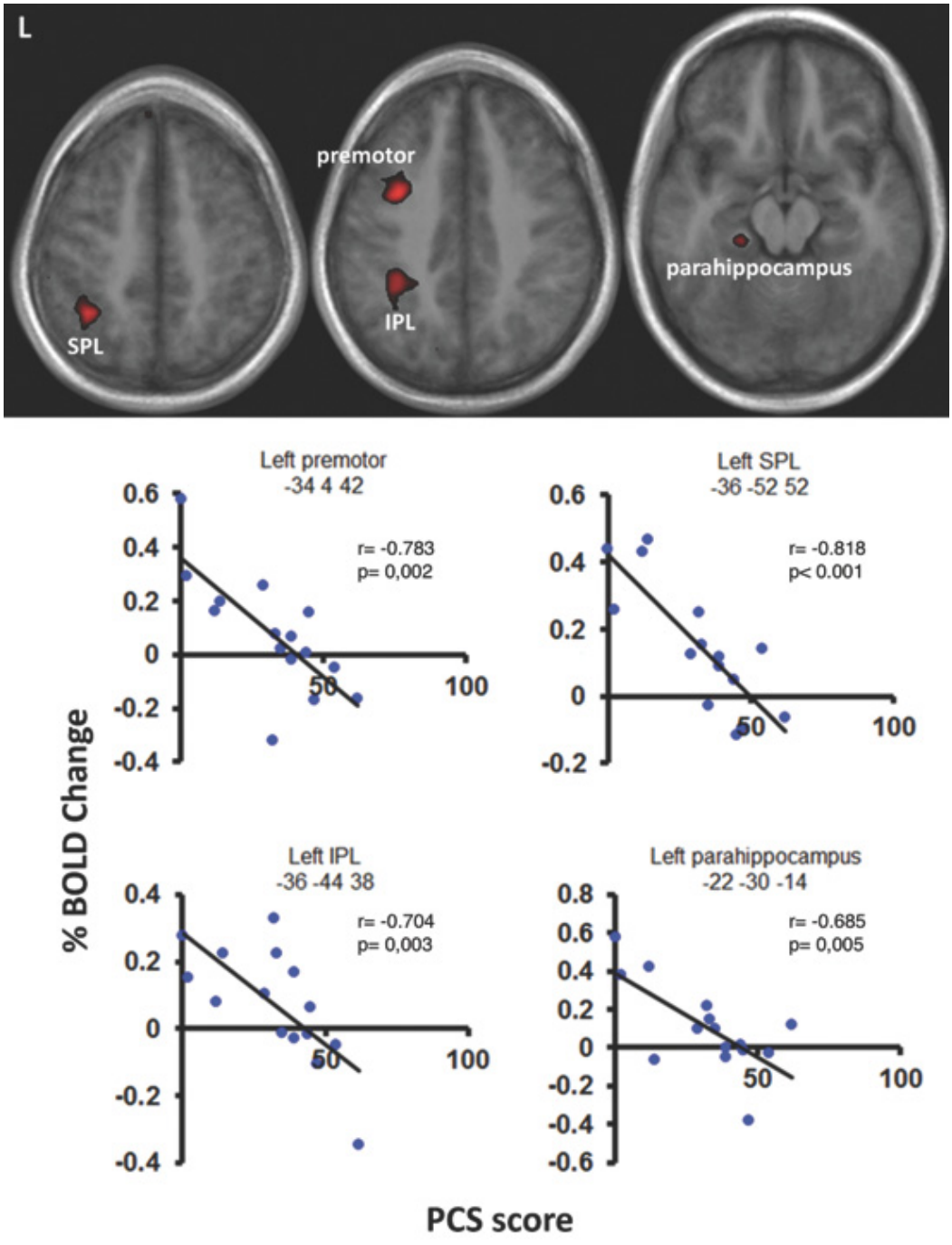

Figure 4. Regression analysis. Correlation between % BOLD signal change and PCS.

5.5.6. Scatter-plots.

To explore the clinical applicability of this test on an individual level, a scatter-plot analysis was performed to examine the BOLD signal activation of each individual in the concussed group compared to the entire control population (n=35). In the left retrosplenial region, 13 out of 15 concussed subjects had % BOLD signal changes below the normal range; in the right retrosplenial region it was 11/15; left parahippocampus 12/15; right parahippocampus 7/15; left precuneus 12/15; left thalamus 10/15; right thalamus 11/15; and right DLPFC 11/15 (figure 5a). In the case of the left hippocampus and the right middle temporal gyrus, 12/15 and

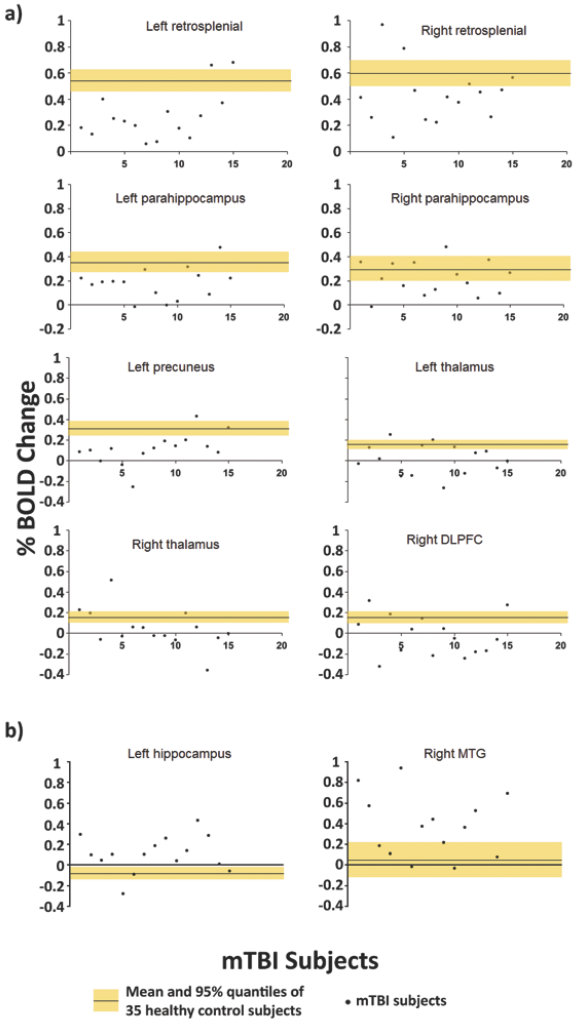

Figure 5. Scatter-plot showing % BOLD signal changes in concussed subjects as compared to the 35 control subjects (horizontal line represents the mean and the shaded area represents the 95% quantile) in the areas where (a) controls > concussed and (b) concussed > controls.

9/15 had changes above that of the normal range respectively (fig. 5b). As can be seen, the majority of the points fall below the normal range in the first eight scatter-plots, and above the range in the last two plots.

5.6. Discussion

Our group recently performed a scoping review of studies examining the neural impact of concussions in children using structural and functional MRI techniques and found a dearth of literature. Of the five studies reviewed, only two used fMRI techniques (Keightley et al., 2012; Talavage et al., 2014; Yang et al., 2012). In both of these studies, only modest differences were found between concussed and control children and youth. We have recently published findings using a working memory task that demonstrated significant differences between the two groups (Keightley et al., 2014). These results taken together with those of the current study on navigational memory, add valuable knowledge to a relatively scarce literature on functional neuroimaging in children. In particular, whereas other studies in the literature focus on frontal-lobe related tasks, the present study, in addition to showing significant differences between concussed and control subjects, is among the first to demonstrate differences using a spatial navigation memory task. Given the type of BOLD activation patterns observed, these data imply that the hippocampal/parahippocampal regions may be susceptible to injury from concussion.

The need for more robust and objective tests to diagnose concussions is once again underscored by the results on the neuropsychological testing battery. As demonstrated in our previous study (Keightley et al., 2014) as well as in others (Bohnen et al., 1992; Lovell and Collins, 1998; Newcombe et al., 1994; Stuss et al., 1985), neuropsychological testing here shows only mild differences between concussed and control groups with none of the tests reaching

statistical significance. This is in spite of the presence of significant differences in the reported PCS scores. Although neuropsychological testing has an important role in identifying specific cognitive deficits at an individual level, its contribution in the diagnosing of concussions is limited. fMRI acquisitions during a navigational memory task, on the other hand, may hold promise.

The normal controls in our study showed increased task-specific activation in the posterior parahippocampal gyri, retrosplenial, premotor, superior parietal, primary visual, precuneus, dorsolateral prefrontal, lingual, and thalamic areas bilaterally. These findings are concordant with previous studies performed in normal adults (Iaria et al., 2007; Spiers and Maguire, 2007) and they underline the important involvement of these areas in navigational memory.

In contrast, the BOLD signal changes seen in our concussed subjects are significantly lower in the posterior parahippocampal gyri, retrosplenial regions, and thalami bilaterally, along with the left precuneus and right DLPFC. Thus, it seems that the areas normally involved in navigational memory remain involved but to a lesser extent following concussion. These alterations are noted despite the fact that the performance on the task is not significantly different between the two groups. These findings are similar to those seen in adults using a working memory task where significant differences were seen in activation but not performance (Chen et al., 2007).

Another finding that has very interesting implications is that the concussed subjects have significantly increased activation in the left hippocampus and right middle temporal gyrus. Given the lack of difference seen in performance, this suggests that the concussed individuals may

recruit additional brain regions to help carry out the navigation task. Although it is possible that the uptake in the right middle temporal gyrus represents simply compensatory activation to make up for the diminished activation in the normally activated areas, the activation in the left hippocampus may have another significance. From previous studies, the left hippocampus appears to play an important role in the acquisition of the cognitive map needed to navigate a virtual environment (Iaria et al., 2007; Spiers and Maguire, 2007). The persistent activation in this region by the concussed group implies that, during the testing phase of the study, these subjects may have still been trying to formulate a cognitive map of the virtual environment they were required to learn, but with difficulty. Additional behavioural data examining the formation of the cognitive maps and routes taken by participants may be useful in further studying this aspect of the findings.

In order to correlate these fMRI results with clinical findings, regression analyses were performed using PCS scores. With this analysis, inverse correlations were noted in the superior parietal lobule, inferior parietal lobule, and the posterior parahippocampal gyrus, all on the left side. These findings suggest that the more symptomatic the subjects are, the lower the activation in these regions. Again, we note that the left posterior parahippocampal gyrus activation appears to correlate importantly with symptom severity.

Taken together, these findings indicate that a difference exists between the activation patterns associated with a navigation task in concussed subjects versus those of controls. The question then arises as to how these findings apply to an individual patient who presents with a concussion. How do we determine whether an individual's altered activation pattern is consistent with a concussion? To help answer these questions, we performed a scatter-plot analysis whereby the mean activations and the 95% quantiles of 35 control subjects aged 10-17 were

shown and plotted with the activation values of each of the individual concussed subjects in our study. In each of the areas in question, the activation values of the concussed subjects fell below those of the larger control population, most strikingly in the retrosplenial regions bilaterally, along with left posterior parahippocampal gyrus and precuneus. Conversely, whereas the majority of subjects in the control population had negative BOLD signal values in the left hippocampus, most of the concussed subjects had positive values. These findings demonstrate that, for diagnostic purposes, a spatial navigation task carried out in an fMRI environment may be useful to objectively assess individuals with suspected concussions by comparing their t-map activation values to those of a normal control population.

One other study has examined fMRI testing using "spatial memory navigation tasks," but the task used appears to represent more a route-learning memory task than a navigational memory task (Slobounov et al., 2010). In that study, subjects were shown a route in a three-dimensional environment after which they were required to immediately replicate the route they were shown, essentially more of a spatial working memory type of task. In our paradigm, in contrast, subjects had to formulate their own cognitive maps of the virtual environment, then, in a delayed fashion, had to use this cognitive map to navigate from one location to another using their own routes. This task and its associated activation patterns are in keeping with previous studies on spatial navigation memory testing (Iaria et al., 2007; Spiers and Maguire, 2007). Given this, our paradigm may represent a robust method of assessing patients post concussion to help with diagnosis and prognosis.

The use of fMRI as a diagnostic tool for concussion has some important drawbacks. These include high cost, need for knowledgeable personnel to carry out testing and post-processing, and a lack of universal availability. However, to date, no other diagnostic tool has

had the objectivity of fMRI. The current reliance of clinicians on subjective symptoms raises secondary gain issues, making objective measures imperative. Although fMRI is unlikely to become a universal test for concussion, it still can play an important clinical role, particularly in borderline cases or those where significant secondary gain issues are suspected.

5.7. Conclusions

Objective assessments to diagnose concussions are scarce, particularly in children. The current study adds valuable data to the literature as it shows that spatial navigation memory fMRI demonstrates significant differences between concussed adolescents compared to controls, despite little difference seen in the actual performance on the task. The main limitation of this study is that we are, to date, uncertain if the activation patterns return to normal as symptoms resolve or if they remain permanently altered. We know though that in adults, atypical patterns return to normal following symptom resolution (Chen et al., 2008b). Another important issue that should be addressed is the fact that the current study includes subjects up to three months post injury, a relatively large time range. It would be important to determine if time since injury had any effect on fMRI activation patterns. In children, this type of information could, along with other measures, contribute to diagnosis in the acute phase and allow for assessment of recovery in the longer term. This would help determine when it is safe for the child to return to play or school. We have begun to address these issues in a longitudinal study that is currently underway. Nevertheless, this current study has demonstrated that fMRI associated with a spatial navigation memory task has the potential to become a useful tool to aid in the diagnosis and attainment of recovery from concussions in young individuals.

5.8. Acknowledgements

This study was supported by grants from the Canadian Institutes of Health Research. R.S.S. was a recipient of the Frank Litvack Fellowship for Clinician Scientists and an award from the Fonds de recherche du Québec - Santé for resident physician scientists. J-K.C. was a recipient of a postdoctoral award from Fonds de recherche du Québec - Santé. IG is supported by a career award from the Fonds de recherche du Québec - Santé. A.P. and I. G. are supported by CIHR grant #231257. The neuropsychological testing was performed by Dr. Gabriel Leonard at the Montreal Neurological Institute.

Chapter 6

Summary/Conclusions

Over the years, the objective assessment tool that has shown the most promise in patients with concussion is fMRI. A number of fMRI studies have shown its utility but the vast majority of these studies were performed in adults. Prior to the work presented here, very few studies using fMRI in pediatric concussion had been performed and the ones that had been published were somewhat mixed. As a result, the main purpose of the studies performed as a part of this thesis was to determine if fMRI techniques could be applied to the evaluation of concussion in children.

In order to carry out this task, the first step was to determine if the the fMRIs that were developed in adults could be applied to children and whether age had any effect on the fMRI findings with the tasks in question. As a result, study 1 (chapter 3) was carried out using verbal and visual versions of a working memory task and a novel navigational memory task in adults and children to see if there were any age related fMRI activation changes. In testing these two tasks in the same subjects, we were additionally able to test a prevailing hypothesis in the

literature that the functional development of the brain proceeds begins with simpler tasks and moves on to more complex tasks over time. The results of the study demonstrated that, overall, the pediatric group had similar peak activation patterns compared to adults with both the working memory and navigational memory tasks. Furthermore, with the working memory task, the strength of activation was not significantly correlated with age while controlling for performance in the vast majority of regions. On the other hand, with the navigational memory task, many more regions were correlated with age, likely related to its higher cognitive complexity. As a result, we demonstrated that both of these tasks could be validly applied to children with adequate age-matching and, at the same time, provided evidence to support the theory of progressive functional development of the brain.

In study 2 (chapter 4), we examined the use of fMRI in the assessment of children after concussion. In this study we found that children after concussion had attenuations of their fMRI activation intensities as compared to normal controls. The findings of this study were similar to those seen in adult athletes with a couple of important differences. First, the actual performance of the pediatric concussion subjects was significantly worse than that of the healthy controls whereas the performance of the task in adult athletes was not significantly different than normal controls (Chen et al., 2007; Chen et al., 2004). Secondly, the concussed adult athletes had additional peaks seen on the fMRI scans such as in the temporal lobe as compared to controls that were not seen in the pediatric concussion subjects. Taken together, these findings suggest that, unlike adult concussed subjects, children lacked the ability to recruit other brain regions to compensate of areas in a state of dysfunction with the working memory task.

In study 3 (chapter 5), we examined the use of a novel fMRI task that employed the more complex navigational memory task. This was a task that had never before been used in the

context of concussion, neither adults nor children. Using this task, we identified similar task-related attenuation of fMRI signal as seen in the working memory task. In this case however, the performance of the concussed pediatric subjects was not significantly different compared to healthy controls. Additionally, the concussed subjects had additional peaks seen, including one in the right hippocampus. This finding was interesting as the right hippocampus, was found, in previous studies, to play an important role in learning the environment and formulation of the cognitive map (Iaria et al., 2007; Spiers and Maguire, 2007). This suggested that, despite adequate performance on the task itself, the concussed subjects were still in the process of "learning" in the 3-dimensional environment.

Taken together, these studies have demonstrated that fMRI can be used as an evaluation tool in the context of pediatric concussion. In healthy controls, activation peaks on these tasks are similar between adults and children and, like adults, the pediatric concussed subjects demonstrated decreased fMRI activation signal as compared to normal controls using both fMRI tasks.

One future avenue that should be explored is to look at the longitudinal changes seen with fMRI in children post-concussion to determine if, like adults, the fMRI changes seen in concussed individuals improve with time. Indeed, this is a subject of study that our group has already undertaken, with the support of the Canadian Institutes of Health Research (CIHR). Preliminary findings, presented at the International Brain Injury Association's Eleventh World Congress on Brain Injury in March 2016, suggest that pediatric concussion subjects seem to display prolonged alteration of brain activation signals despite symptom resolution (Gagnon et al., 2016). An earlier study done with ERP had similar findings in adult athletes where both symptomatic and asymptomatic subjects had reduced amplitudes while performing an auditory

Oddball task, suggesting altered brain functioning may be present despite an absence of symptoms (Gosselin et al., 2006). These findings suggest that a period of prolonged brain impairment may be present even after the time of symptom resolution. If true, this has important implications on return-to-play guidelines and must be investigated further to determine if symptom resolution is sufficient to safely authorize return-to-play.

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