Antisaccadic Eye Movements as a Correlate for Response Inhibition and White Matter Integrity in Mild Traumatic Brain Injury

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science

Institute of Medical Science
University of Toronto

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Abstract

Antisaccades are thought to involve higher level inputs from neural centers involved in rapid eye movement inhibition and control. Previous work has demonstrated that performance on the antisaccade task can help in assessment of injury in acute and/or chronic Mild Traumatic Brain Injury (mTBI). We validated antisaccade performance in mTBI assessment against gold standard assessments of symptom burden, diffusion tensor imaging, and a neuropsychological test of response inhibition. Significant deficits in antisaccade median latency and prosaccade mean duration were found between patient groups and controls, despite overall integrity of the pupillary light reflex; the former was correlated with loss of white matter integrity in the splenium of the corpus callosum in acute mTBI. Furthermore, antisaccade median latency was also associated with poor performance on executive functioning tasks, and greater symptom burden in the acute patients. This research suggests that the antisaccade task is useful as a neurological marker for mTBI.
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For giving their all so that their children could achieve more;

And my Sister:
For being the awesome person she is.

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Contributions

PAC Members: Revisions and intellectual contributions to the improvement of this work; Stanley Zhang: Excellent assistance in study administration and guidance throughout this work; Anthony Sheen: Magnetic resonance technologist at St. Michael’s Hospital, conducted the MRI scans for all participants; Anthony Wong: Assistance in recruiting and testing three PTS patients, proofread literature review tables for accuracy; Iryna Pshonyak: Assistance in recruiting and scheduling one PTS patient, scoring and data entry of testing files. Dr. Ruiwei Jing: Exploratory Power Analysis; Jocelyn Lee: Assistance in backing up testing files; Harrish Nithianandan, Iryna Pshonyak, Jocelyn Lee: Double data entry of all the data collected.
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1 CHAPTER 1: LITERATURE REVIEW AND INTRODUCTION

1.1 Traumatic Brain Injury as a Global Health Problem

Mild Traumatic Brain Injury (mTBI) is a significant global health problem. A recent population-based study from New Zealand reported an incidence of 749 cases per 100,000 person-years (Feigin, Theadom et al. 2013). This number is probably an underestimate of the true incidence of mTBI and concussion, given the large proportion of cases which do not require hospitalization, or those mild enough that the patient only seeks medical advice from the family physician (hence undetected in hospital medical records) (Cassidy, Carroll et al. 2004). However, the qualifier ‘mild’ is often unrepresentative of the protracted sequelae after injury. Recent work has characterized TBI as a silent epidemic (Rusnak 2013), as many injuries are “invisible” and not captured in statistics, and have significant effects on quality of life and well-being.

Diagnosing an mTBI can be challenging for clinicians and currently the gold standard is self-report of the event and ongoing symptoms. There are few objective assessment tools available to aid the clinician in assessment from both an emergency standpoint and in clinic follow-up. It is essential for good healthcare that validated, effective tools exist and be developed that can be helpful in the accurate diagnosis and treatment of mTBI. This study will explore the potential of using a type of rapid eye movement called the antisaccade to understand and measure the effects of mTBI.

1.2 Clinical Course of Mild Traumatic Brain Injury

Most patients who sustain mTBI make a complete apparent recovery in the days to weeks following injury but a significant portion continue to experience psychological, somatic, or neurological symptoms several months to years after brain trauma. Persistent Post-Traumatic Symptoms (PTS) occur in patients when the acute sequelae due to their injury continue to be present greater than 3 months after their injury. Up to 30% continue to experience symptoms following injury after one year (Waljas, Iverson et al. 2015). PTS is associated with prolonged
suffering, health care costs and the loss of productivity resulting in significant personal and societal burden.

For these patients, the symptoms (for example, headaches, dizziness, sensitivity to noise/light, nausea, cognitive/emotional difficulties, and more) can be constantly debilitating, having negative consequences for their quality of life and perceived capability to accomplish activities of daily living (ADLs) (Emanuelson, Andersson Holmkvist et al. 2003, Sveen, Bautz-Holter et al. 2010). The source of these symptoms has been hard to pinpoint – it is difficult to distinguish between PTS arising from the psychological trauma associated with some instances of TBI, as opposed to brain dysfunction (Motzkin and Koenigs 2015).

1.3 Assessment of Level of Consciousness and Symptom Burden after Mild Traumatic Brain Injury

The emergency assessment of patients with all forms of TBI requires an assessment of the level of consciousness (LOC). The Glasgow Coma Scale (GCS) is the most widely used measure of LOC and was originally developed for coma assessment in all severities of TBI. It requires an assessment of eye opening, verbalization and motor movements so that the patient can be assigned a score ranging from 3 (deep coma) to 15 (“normal”). GCS assessment by the medical professional is insufficiently sensitive for mTBI since many patients, although clearly symptomatic, score at the highest level (GCS 15). The current study measured the GCS score and illustrated the ceiling effect of using this scale in mTBI assessment.

Alternatively, symptom burden can be determined using symptom assessment scales that have been developed for use after concussion in sports, and injuries from different etiologies (like falls, athletics, and motor vehicle accidents, to name a few). These comprehensive scales systematically seek to identify or the collective cognitive, emotional and somatic symptoms after mTBI. The Rivermead Post-Concussion Symptoms Questionnaire (RPQ) has been used to track recovery of symptoms in mild TBI patients up until 3 months post-injury (Lundin, de Boussard et al. 2006). An older version of the Sports Concussion Assessment Tool (SCAT2) has been used to assess athletics-related mild TBI (Guskiewicz, Register-Mihalik et al. 2013, Luoto, Silverberg et al. 2014). The SCAT3 represents the most recent iteration of the SCAT scale but its use has been primarily in the sports environment. These questionnaires are well-validated, easy to
administer, and do not take long compared to more detailed assessment scales such as the Neuropsychological Impairment Scale (NIS). Our team has conducted an analysis focusing specifically on the performance of the SCAT3 and RPQ in acute mTBI and PTS assessment (Cusimano 2015). There is a direct link between the psychological effect of trauma and subjective symptom burden, which will be explored next.

Notably, the psychosocial effect of trauma can increase symptom burden and contribute towards PTS. Mild TBI is also a significant predictor of post-traumatic stress disorder (PTSD), which can increase subjective cognitive, somatic and emotional difficulties and detrimentally affect quality of life (Yurgil, Barkauskas et al. 2014, Haagsma, Scholten et al. 2015, Xue, Ge et al. 2015). Since the symptoms of PTSD can overlap with those caused by mTBI, it is often difficult to distinguish PTS from PTSD. Depressive symptomatology (which can be measured using the Center for Epidemiologic Studies Depression Scale (CES-D)) may also be a confounding factor. Symptom scales such as the RPQ and SCAT3 can be confounded by these overlapping conditions. Moreover, questionnaires are quite susceptible to reporting bias by injured patients, who may report elevated symptom burden for secondary gain (i.e., financial/legal benefit if litigation is pending) (Sweet, Goldman et al. 2013). Most importantly from a neuroscience perspective, little is known about the relationship of symptom load to measures like white matter integrity and other objective measures of brain function.

1.4 Neuropsychological Assessment of Executive Functions after Mild Traumatic Brain Injury

A more objective marker of cognitive dysfunction is neuropsychological testing. Executive functioning deficits after TBI encompasses domains such as attention (Mathias and Wheaton 2007), working memory (Vakil 2005, Kumar, Rao et al. 2013), response inhibition (discussed below), and other cognitive sub-systems involved in the control and regulation of behavior due to frontal lobe susceptibility to injury. Exploring these executive deficits is important to understand how acute sequelae and PTS arises because cognition is a fundamental contributing factor to apparent symptom load after injury.

The effect of mTBI on verbal fluency has been summarized in a meta-analysis (Henry and Crawford 2004). Findings from Henry et al.’s study suggest that the phonemic fluency subtask of verbal fluency, in which participants are asked to say words starting with a certain letter, is a
more sensitive tool to screen for TBI than the Wisconsin Card Sorting Task (WCST), a popular executive functioning assessment tool. The left frontal lobe and temporal lobe is involved in verbal fluency (Strauss E. 2006, Zakzanis, McDonald et al. 2011). Verbal fluency scores tap into mental speed and attention (Strauss E. 2006) and results indicate verbal fluency is a suitable test for patients with diffuse axonal injury (discussed later) following moderate to severe TBI (Zaninotto, de Paula Guirado et al. 2014). The neuropsychological tests used in this study included a measure of attention and visuomotor speed, the Trail Making Tests (A and B). TMT-B has been related to the set switching (the ability to switch from one cognitive mode of attention to another) component of executive functioning (Strauss E. 2006).

The Stroop Color and Word Test is a common measure of a specific type of executive functioning known as interference control. Generally, this involves suppressing a commonly trained or the easiest response in favour of a different response, based on certain instructions (a type of response inhibition). A meta-analysis of Stroop interference deficits found an overall statistically significant detrimental effect of TBI on inhibitory control (Dimoska-Di Marco, McDonald et al. 2011). They acknowledged that there was large variation of the individual effects for this measurement. This meta-analysis included traumatic brain injury studies of all severities, and did not focus on studying effects purely in the milder cases. It is likely that a different result would have been found if the authors of this study focused on only mTBI.

Neuropsychological testing suffers similar problems as questionnaire assessment. Patients who are looking for secondary gain after injury can malinger poor performance on some neuropsychological measures. There are some tests specifically designed for detecting purposeful poor performance, but there is a fine distinction between malingering and exaggeration of performance, as reviewed by Iverson et al. (Iverson and Binder 2000). A neurological marker has potential to be much more robust compared to this type of assessment.

Two main unresolved questions arise from this discussion so far: (1) why do response inhibition deficits arise at all, and often persist in some patients for such a long time after injury? (2) what is the relationship between executive functioning and neurological functioning in acute mTBI and PTS patients? Understanding the physics of impact in mTBI is very important to answering these questions, since the injury directly affects the structure of white matter after injury. The relationship between mTBI and the inhibitory control deficits can only be connected through a
brief digression into the physics of injury, the neurometabolic cascade which is at the root of neuroanatomical damage, and a discussion of neuroimaging which measures this damage after mTBI.

1.5 Impact Dynamics Affect White Matter Structure after mTBI

In order to further understand how an insult to the brain can result in acute symptoms and overt PTS, one needs to consider the dynamics of impact. The etiology of injury dictates the angle and momentum of the head relative to the source at time of impact, affecting the nature of injury incurred. Although mTBI can damage any area of the brain, evidence in laboratory models of brain injury suggest that damage is inflicted from rotational acceleration/deceleration forces upon impact, in addition to translational forces (McCrea 2008). Modeling has shown that for focal injuries, rotational and translational force of one primary impact alone (60 g weight, dropped from one meter onto a surgically fixed steel disk on the skull surface) is sufficient to damage major white matter tracts in the brain, such as the corpus callosum in mice (Xu, Nguyen et al. 2014). For obvious reasons the same types of experimental design cannot be applied to humans, limiting translatability of the findings, but animal models have great value in recapitulating different types of injury and allowing scientists much greater control over the nature of the impact. Recent work has tried to model impact dynamics of human traumatic brain injury, to improve protective equipment and to better understand the physics of trauma (Meaney, Morrison et al. 2014, Post and Blaine Hoshizaki 2015, Post, Kendall et al. 2015). Informing clinical care by modeling the biomechanics of injury is also making progress (Namjoshi, Good et al. 2013). The physics of injury generates a neurometabolic cascade which will be discussed next.

1.6 The Neurometabolic Cascade Connects Impact Dynamics to Diffuse Axonal Injury Measurable with Diffusion Neuroimaging

The white matter integrity of neural networks is directly related to injury effects at the cellular level. The injury triggers a neurometabolic cascade (a series of chemical and biological changes that occur on the neuronal level post-insult) (Giza and Hovda 2014) immediately altering the neurochemistry of the brain. The acute neurochemical changes associated with brain trauma have been studied (Giza and Hovda 2014). Briefly, the mechanical forces of impact disrupt the
phospholipid bi-layer membrane of neurons, which acutely disrupts the physiological ion balance. This leads to over-compensation from sodium potassium ATPase, and consequent excitotoxicity. The acute cascade resolves over a few days, but the toxic effects on the tissue and organ levels can persist for long periods of time. Overdrive ATPase action depletes glucose stores over two to four weeks, and eventually results in lactic acid buildup, tissue inflammation and edema (Dominguez and Raparla 2014). The latter can be indirectly measured with an advanced type of neuroimaging called diffusion tensor imaging, and is mechanistically relevant to why abnormalities in diffusion neuroimaging are observed at all after injury.

Adding to the persistent stress on the brain, previous work has shown there are significant detrimental effects of recurrent concussions on rate of recovery (Slobounov, Slobounov et al. 2007). Many of the neurochemical changes in the neurometabolic cascade take place on a subcellular to molecular level. It is very difficult to directly measure these changes in clinic, without access to advanced neuroimaging resources. There needs to be a more convenient neurological biomarker for the objective assessment of concussion severity and for prognosis of outcome. Although a biophysical and biochemical understanding is important, it needs to be complemented with neuroimaging to improve our understanding of what happens inside the brain on the level of white matter structure after injury.

1.7 Neuroimaging and Mild Traumatic Brain Injury

For more objective assessment of mTBI, one can use neuroimaging. In the milder cases, neuroimaging is used to rule out more severe head injuries causing hematomas or skull fractures. Computerized tomography (CT) neuroimaging has become a core part of care for severe and moderate traumatic brain injuries. It uses pulses of ionizing radiation (x-rays) to generate an image of brain tissues. The Canadian CT head rule is a validated guideline system used by clinicians to determine whether a mTBI patient requires a CT scan. The rule has been invaluable in clinical care to determine whether a patient requires diagnostic CT neuroimaging completed for their concussion (Stiell, Wells et al. 2001).

The magnetic resonance image (MRI) is the product of variable relaxation times of hydrogen nuclei when subjected to a rectangular electromagnetic pulse. For an excellent review of magnetic resonance imaging and the principles involved, see *MRI: Basic Principles and*
Applications, Third Edition (Brown 2003). Traditional clinical MRI findings of multifocal hyperintensities and cortical contusions are poor predictors of clinical outcome (Niogi and Mukherjee 2010). More recently, advanced MRI techniques such as diffusion tensor imaging have been developed to analyze diffuse and traumatic axonal injury in mTBI. These diffusion based procedures have not been integrated into clinical care for mTBI as more empirical evidence is needed to verify its utility. However, there is a growing field of research supporting its use for clinical applications.

**Diffusion Tensor Imaging (DTI)**

The diffusion of water molecules in an unbounded environment can be said to be isotropic, i.e., equally probable in all directions. It can also have a degree of anisotropy, a preferred direction of diffusion. Complete isotropy means the likelihood of diffusion in all directions is equal, while varying degrees of anisotropy indicate that this diffusion is not completely free and uniform (Mori 2007). The motion of water molecules in the cerebrospinal fluid (CSF) can be said to be isotropic, but motion along white matter fibers and tracts are usually anisotropic as the inherent structure of the axon and neuron favors the diffusion of water in one direction along the cell body and intracellular compartments (Alexander, Lee et al. 2007, Mori 2007). A breakdown in the axon and neuronal necrosis will increase the apparent diffusivity of water through that space (Alexander, Lee et al. 2007). This effect mainly originates from the increased diffusion when membrane layers are spaced farther apart (Alexander, Lee et al. 2007). The movement of water molecules in a particular direction causes a signal differential and reaction to the radiofrequency pulse from the magnetic resonance machine, which is received as a diffusion signal (Mori 2007).

A mathematical construct called the diffusion tensor (Basser, Mattiello et al. 1994) can be generated from the DTI scan. The diffusion tensor is sufficiently represented in a 3 x 3 symmetric matrix (Mori 2007). The nature of the 3 x 3 symmetric matrix allows for mathematical diagonalization, which collapses the matrix into three eigenvalues and their three associated eigenvectors (the minimum to mathematically describe the diffusion ellipsoid). Together, these measurements estimates the likelihood of diffusion of water molecules in each particular voxel (Mori 2007). In order to determine the apparent diffusion coefficients (ADC) which are necessary for including into the diffusion tensor during processing, there must be one image obtained with no diffusion weighting (b = 0).
Usually in diffusion tensor imaging sequences, more than the minimum of six diffusion directions are used. This allows for over-determination of the diffusion tensor, further increasing its accuracy. However, this also requires specialized processing software with the ability to fit all of the diffusion directional information into the standard diffusion tensor.

Several metrics can be calculated from the diffusion model. Two common reported values are the fractional anisotropy and mean diffusivity.

Fractional anisotropy (FA) is defined as (Mori 2007)

\[
FA = \sqrt{\frac{1}{2} \sqrt{\frac{\left(\lambda_1 - \lambda_2\right)^2 + \left(\lambda_2 - \lambda_3\right)^2 + \left(\lambda_3 - \lambda_1\right)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}}
\]

*Equation 1. Fractional Anisotropy Equation.*

\(\lambda_1, \lambda_2\) and \(\lambda_3\) represent the three eigenvalues of the diffusion tensor generated from the scan processing. Axial and radial diffusivity can be calculated from these values as well, and the represent different aspects of diffusion flow. Clinically FA decreases when the white matter membranes are damaged, and can arise from a change in axial diffusivity or radial diffusivity, which are component measures of diffusion that are parallel and perpendicular to the main fibre pathway, respectively (Alexander, Lee et al. 2007). Astrogliosis (abnormal proliferation of astrocytes and accompanying neural death) has been connected to FA increases on DTI scanning, one aspect of neuropathology which may arise after traumatic injury (Budde, Janes et al. 2011).

Mean diffusivity is defined as (Mori 2007)

\[
\frac{\lambda_1 + \lambda_2 + \lambda_3}{3}
\]

*Equation 2: Mean Diffusivity Equation.*

Where \(\lambda_1, \lambda_2\) and \(\lambda_3\) represent the three eigenvalues of the diffusion tensor. Clinically the MD is related to the degree of edema and inflammation in the brain white matter (Alexander, Lee et al. 2007).
Cytotoxic edema after more severe TBI has been linked to decreased MD (Barzo, Marmarou et al. 1997).

In summary, FA and MD reflect different aspects of the brain white matter structure: one purported to measure directionality of water flow, and the other the mobility of water within the brain white matter, respectively (Croall, Cowie et al. 2014). These measurements help scientists to determine how intact the white matter tracts in the brain are. Specifically for mTBI patients, traumatic axonal injury (focal or diffuse damage to the white matter due to physical trauma, 

*diffuse/traumatic axonal injury*) is thought to be a result of damage to the neurofilament organization and axolemma on a neuronal level (Niogi and Mukherjee 2010). In the current study, both FA and MD were studied to get a more comprehensive view of the white matter microstructure than with one metric alone. An important logistical problem is that not all health care centers have capacity for advanced neuroimaging for clinical and research use, and even if the resources are available, they cannot be realistically scheduled for clinical examinations in the emergency time periods hours to days after the injury (Niogi and Mukherjee 2010) (conventional clinical MRI investigations require weeks to months of advance booking, by which time the acute phase has passed). Thus a neurological measure that is much less expensive, and can be completed on the day of injury, but still correlated with these measures will significantly advance clinical assessment.

**1.8 Diffusion Tensor Imaging as a Biomarker for Mild Traumatic Brain Injury**

Diffusion tensor imaging is a significant step forward compared to CT scanning or T1 imaging in neuroimaging assessment. Niogi et al. summarized DTI findings after mTBI (Niogi and Mukherjee 2010). Reviewing the studies to date (2010), they found that the frontal association white matter tracts, namely the anterior corpus callosum, superior longitudinal fasciculus, anterior corona radiata and uncinate fasciculus were common areas of white matter damage in mTBI. Not only are these tracts involved in frontal inhibition, they connect the frontal areas to the rest of the brain. The corpus callosum is of interest because it is one of the largest white matter pathways in the brain connecting the two cerebral hemispheres and is especially susceptible to damage in mTBI.
The corpus callosum is important for many cognitive functions; relevant to our discussion, it is involved in bilateral information transfer and coordination between the prefrontal cortices (Mendoza 2010). A sufficient number of studies have focused on corpus callosum damage after mTBI, summarized by a meta-analysis focusing on this structure as well as the internal capsule in mTBI patients (Aoki, Inokuchi et al. 2012). The meta-analysis determined there were significant effects of mTBI on the corpus callosum structure (Aoki, Inokuchi et al. 2012). Namely, significant effect sizes of FA reductions and MD increases were found across studies in the whole corpus callosum and the splenium portion of this structure. This meta-analysis has demonstrated (1) there is statistical consensus that diffusion tensor imaging of the corpus callosum reveals significant changes after DTI; (2) the splenium of the corpus callosum specifically can be used as a sensitive marker in mTBI. This work is an important piece of the puzzle for specifying regions of interest to focus on for the diffusion tensor imaging analysis and further discussion in this study.

Traumatic axonal injury is revealed by DTI and translates into structural network disintegration at the systems level in the brain. This is related to overall cognitive impairment post-injury. Recent work using network graph theory has linked this disconnection in the brain to deficits in executive functioning of traumatic brain injury patients (Fagerholm, Hellyer et al. 2015). The link between neuroimaging and executive functioning after mTBI has been directly investigated, and is important to discuss because it links neuroanatomy with some of the cognitive sequelae that patients experience after mTBI.

1.9 Neuroimaging Findings are Related to Executive Functioning after Mild Traumatic Brain Injury

There has been a cluster of research investigating executive functioning and its relationship to white matter integrity in patients with traumatic brain injury. The reader is referred again to Niogi et al., Table 1 for an excellent summary of the subject (Niogi and Mukherjee 2010). Croall et al. conducted a longitudinal study in mild TBI patients who were scanned acutely and returned for a chronic assessment, correlating structural integrity with neuropsychological performance, and found significant increases in fractional anisotropy and mean diffusivity in acute mTBI patients (on average 6 days post-mTBI) compared to controls in the anterior and posterior corpus callosum, respectively (Croall, Cowie et al. 2014). Executive functioning tests revealed that in
the acute patients, lower verbal fluency score regressed negatively with increased FA. There was a different pattern in chronic mTBI patients, who had a significant decrease in FA in the anterior forceps but an increase in MD in the posterior corpus callosum and associated fibers and a significant negative regression with verbal fluency scores. These findings suggest that (1) FA and MD measure different aspects of microstructural integrity post-injury; (2) are likely to fluctuate over time as the mTBI patient moves from acute to chronic phases; and (3) there are demonstrated relationships with the verbal fluency aspect of executive functioning in acute and chronic mTBI. However, this study did not investigate inhibitory control processes, a critical component of the executive functioning domain.

A similar question was investigated by Kraus et al. (Kraus, Susmaras et al. 2007), who conducted DTI on a series of patients with mild, moderate and severe TBI, all at least six months from their injury. The mild TBI patients exhibited significant deficits in structural integrity in the sagittal striatum, corticospinal tract and superior longitudinal fasciculus. Kraus et al. suggested that these findings, combined with the normal readings of axial and radial diffusivity in their mild TBI patients indicated that the myelin structure is relatively intact, but axonal damage persisted six months after the injury. The mild TBI group did not differ from controls in neuropsychological testing scores, but there were significant correlations between the number of regions of interest with significant differences in FA and normalized scores of executive functioning, memory and attention. In this study all patients were tested greater than six months post-injury, which did not include acute mTBI patients who had just sustained a head injury.

A data-driven, tract-based spatial statistics (TBSS) neuroimaging approach to determining correlations of executive functioning with FA and MD in TBI patients was conducted (Kinnunen, Greenwood et al. 2011). Kinnunen et al. found significantly lower FA in the mTBI compared to control groups in the corpus callosum, bilateral cingulum bundle, corticospinal tracts, bilateral anterior thalamic radiations, bilateral forceps major and minor, left superior longitudinal fasciculus, the inferior fronto-occipital fasciculi, left external capsule, and right interior capsule. MD was elevated in the mTBI groups compared to the control groups in similar areas, including the internal and external capsule. The authors of this study did not find significant correlations between executive functioning and FA. Although this study used a full TBSS approach and localized changes in white matter structure after mTBI, the patient group
only contained eight mTBI patients (small sample size) and all were several months post-injury at testing; as above, no conclusions could be drawn about acute mTBI patients.

In summary, there does not seem to be any studies directly correlating a subset of executive functioning, namely interference control performance to FA/MD changes in both acute mTBI and PTS patients at the same time. These findings are necessary because the antisaccade task is a test of interference control (this statement will be explained in Section 1.12). Let us now move to the neurological markers which can be used to assess mTBI.

1.10 Pupillometry after TBI and Neurophysiology of the Pupillary Light Reflex

The pupils regulate the amount of light that falls on the retina. They constrict in bright environments, and expand in dark environments to ensure effective exposure of the retina to the environment for vision. Pupillometry (distinct from saccadometry discussed below) is the quantitative measurement of the pupillary light response using a specially-designed machine. The pupillometer can measure several clinically relevant variables within a few seconds. It combines all relevant measurements into a scalar value indicating pupillary reactivity (the NPi Score), an easy-to-interpret number for clinicians and nurses caring for TBI patients or patients with limited consciousness. The minimum NPi score for a normal pupillary response is 3.0 (Chen, Gombart et al. 2011). Neurophysiology of the pupillary light reflex (PLR) is composed of a well-defined reflex arc, consisting of an afferent arm, an interneuron arm, and an efferent arm (Kaufman and Alm 2003). Pupillary dilation and constriction is concerted in healthy eyes. Deficiencies in pupillary light reflex may indicate defects in the functioning of the third and sixth cranial nerves which are also important for horizontal saccades. Quantitative measurement of the pupillary light reflex helped to screen for neurological effects of any medications that participants might be taking, and more severe damage to the brain; this also allows the researcher to measure sympathetic and parasympathetic responses in participants. This work will be presented at a conference abstract further examining pupillometry in mTBI assessment (Ting 2015). However, the PLR is a relatively simple system that involves little of the cortical regions and little work has been done investigating the PLR in mTBI. Abnormal pupillary light reflex in acute mTBI and PTS patients during a manual penlight exam is rare, creating a ceiling effect. To
assess the functioning of the higher control areas of the brain in a more sensitive manner, we need to turn to the saccadic system, and antisaccades can potentially be useful in this regard.

1.11 Saccadic Eye Movements

1.11.1 Saccades

Saccades are very rapid eye movements that move the fovea to interesting places in the field of vision and hold it there. A saccade rotates the eye from its current orientation to one in which the fovea is fully aligned with the object of interest. They are indispensable for effective visual analysis and visual memory of our environment (Kaufman and Alm 2003). Remarkable neural coordination between the control circuitry of the brain, from the higher cortical areas down to the brainstem nuclei is involved in saccades (Figure 1). Saccades can be generated as a reflexive response towards a novel stimulus, generated to a memorized location, or as a response to cognitive control (Wong 2008). Saccade measurements have been used to assess diverse neurological conditions, from Alzheimer Disease to spinocerebellar ataxias (Marx, Respondek et al. 2012, Chen, Todd et al. 2014, Molitor, Ko et al. 2014, Moscovich, Okun et al. 2014, Saleh, Marcus et al. 2014).

1.11.2 Neurophysiology of the Human Saccadic System

Generating a saccadic eye movement requires coordinated action between many control centers (Muri and Nyffeler 2008). Figure 2 depicts a simplified version of the human saccadic model. A
saccade signal can be split into two components: a pulse and step signal. The pulse signal triggers an immediate jerk of the eye to the target location. The step signal is generated using a mathematical integration of the velocity of the oculomotor muscle contraction over time, which dictates the final position for the muscles to enable stable and fixed gaze on the target of interest. The final combined command for a horizontal saccade is dispatched from the paramedian pontine reticular formation (PPRF) in the pons (Wong 2008).

A fixation trigger from the cerebral cortex inhibits omnipause neurons in the pons, which release their tonic inhibition on excitatory burst neurons (EBNs) in the PPRF and allows the latter to emit a pulse signal concordant with the desired final eye destination. The EBN signal bifurcates and one end stimulates inhibitory burst neurons (IBNs), which inhibits antagonistic eye muscle contraction and inhibits the omnipause neurons until the saccade is completed (see Figure 2).

Figure 2: Simplified Schematic of Human Saccadic Eye Movement Physiology. The pulse and step signal is combined into the final saccadic eye movement. From Wong, AMF. Eye Movement Disorders. New York: Oxford University Press; 2008. Adapted and redrawn with permission from Oxford University Press. Abbreviations: EBN = Excitatory Burst Neurons; IBN = Inhibitory Burst Neurons.
An efference copy of the EBN signal is replicated and fed into the neural integrator, which is located in different areas depending on the saccade type generated; the neural integrator for horizontal saccades is located in the nucleus prepositus hypoglossi and medial vestibular nucleus straddling the medulla oblongata and caudal pons, while for vertical and torsional saccades the rostral interstitial nucleus performs a similar function (Wong 2008). The pulse and step are then combined to generate the characteristic pulse-step neural signal which is translated into a saccade. Figure 3 illustrates the pulse and step signal for saccade generation. Later, I will discuss how different neuropathology can affect this finely tuned system and the connections between higher level cortical inputs to the brainstem saccade generator, explaining the deficits in saccades that result.

Figure 3: Pulse and Step of Innervation Generates a Saccade (Dell'Osso 2006.).

1.11.3 Measurement of Saccadic Eye Movements and Factors Contributing to Saccade Deficits

Operationally, saccade performance can be defined by many different characteristics. Saccadic latency (time from presentation of stimulus to initiation of saccade), amplitude (gross displacement of fovea from the previous fixation point), and saccadic trajectory in space are easily measured using machines and chip-based devices.

Damage to any area along this network alters parameters of the resulting saccade. An abnormality can signal underlying pathology in the brain, or differences in arousal and motivation (Leigh RJ. 2006).

Lesion studies have identified changes in saccadic parameters, especially latency and accuracy (Heide and Kompf 1998). Since the generation of a saccade requires the calculation of many different signals between nuclei, cortical processing time originating from the integration of signals contributes to saccadic latency (Leigh RJ. 2006). Lesions of the frontal eye fields
(FEFsac) and/or superior colliculus have been shown to increase the saccadic latency (Wong 2008). The frontal eye fields are particularly important for making saccades in the correct and volitional direction for antisaccades (defined and further discussed later).

Other aspects of saccadic eye movement which are readily measured using a machine include peak velocity, and time to peak velocity (Wong 2008). Saccadic velocity (tied to duration and hypo/hypermetria) follows a main-sequence relationship (a mathematical relationship of the peak velocity and amplitude required for the saccade) (Wong 2008), and deficits in saccadic velocity may signal deficits in the functioning of the brainstem saccade generation system (Leigh RJ. 2006). Lesions to the omnipause neurons, for example, may alter the membrane properties of EBNs, changing saccadic velocity (Miura and Optican 2006).

Finally, saccade landing point accuracy is tied to the functioning of the cerebellum (which performs calibration) and brainstem. Information about a saccade’s accuracy is used to correct for performance in the next similar saccade. Damage to the saccade integrator can produce post-saccadic glissades, which are sliding movements of the eye after the pulse has completed (due to a mismatched pulse and step signal) (Leigh RJ. 2006). Saccadic accuracy is also readily measured using portable hardware, with millisecond precision and to tenths of a degree (Herczyński 2013, Instruments 2013, Ltd. 2013).

From a clinical research perspective, different saccadic variables can be compared between groups, or correlated with neuroimaging and neuropsychological variables to validate its use against current gold standard assessments.

The PPRF is critical to horizontal saccade generation (Wong 2008) (Figure 4). Impairment in reflexive saccades (natural saccades towards a new stimulus) may represent impairment of function in the parietal eye fields in the posterior parietal cortex or the frontal eye fields in the prefrontal cortex (Muri and Nyffeler 2008). Volitional saccades may be generated in response to a visual cue, in anticipation to a potential target, in memory of a previously presented saccadic destination, or made to a position opposite to a previously presented saccadic target (the antisaccade) (Leigh RJ. 2006).

A recent review described some of these tasks in terms of their clinical utility (Ventura, Balcer et al. 2014). Saccade circuitry can be damaged by rotational force that can lead to diffuse axonal injury from concussion and other types of TBI. Each task can be modified based on stimulus presentation duration, location, and characteristics, requiring different cognitive functions to successfully complete each task. The antisaccade task requires cognitive inhibition and is more taxing than others like the visually guided saccade task (which does not require this additional...
layer of cognitive processing). Many different saccade tasks have been used to assess TBI in the acute stages, but the antisaccade is especially interesting as it involves special inhibitory processes not tested in the other tasks.

1.12 What the Antisaccade Task Is, How to Measure it, and Why It Matters

Completing the antisaccade task requires making a rapid eye movement to the mirror opposite location to where a saccade target appears, relative to a central fixation point as soon as possible. Figure 5 illustrates this:

Figure 5: An illustrated schematic of the antisaccade task. From Everling S., Fischer B. The antisaccade: a review of basic research and clinical studies. Neuropsychologia, 36(9):885-99. Reproduced with permission from RightsLink, Elsevier Systems. Abbreviations: PS = Prosaccade; AS = Antisaccade.

The saccade system is programmed to directly track interesting objects that appear or move in the field of vision. It is easiest for participants to make a reflexive prosaccade error, towards a novel stimulus (for example, the yellow star in Figure 6).
It takes significantly more cognitive processing to (1) inhibit the incorrect reflexive saccade; (2) program an antisaccade in the mirror-opposite location; and (3) carry out the antisaccade as quickly as possible, to a location where there is no stimulus or target at all.

The antisaccade task was a potent choice because it involves inhibitory processes that are not needed in the visually guided saccade task, but was also easier to perform in acutely injured patients and patients with persistent symptoms compared to the more involved countermanding task (requiring last minute inhibition of a pre-programmed saccade response). The antisaccade task is easy to perform, and could potentially be useful as a marker for executive functioning and deteriorated white matter integrity determined through neuropsychological testing and neuroimaging after mTBI, respectively. Furthermore, acquisition of the necessary resources and training to operate a magnetic resonance machine is costly compared to a rapid eye movement measuring machine, the latter which is much more portable, easier to use, and easier to understand given the right measurements to look for. Hence, a rapid eye movement-based measurement can provide significant advantages over a neuroimaging-based marker for immediate assessment of mTBI severity at clinical sites with limited resources, and can serve as a potentially useful addition for larger sites.

Symptom load assessment and neuropsychological testing suffer from inherent problems in reporting bias. These are difficult to completely eliminate in a patient population which often seeks litigation for their injuries. Neuroimaging can be prohibitively expensive for clinicians who may not work at the large healthcare centers with such resources, or coaches who need to make return-to-play decisions on the field. Many currently used tests are subjective, relying on patient self-report, and a neurological based marker can provide an additional dimension of injury that cannot be captured using these methods alone. To some extent, functioning of the visual system is heavily connected to functioning in the central nervous system. Other biomarkers, such as genetic testing or blood sample testing, are already several degrees removed from brain function. Hence it is important to study the visual system, because it gives us a good
idea of how well the brain is coping after mTBI. This knowledge is directly important for better care of acute mild TBI patients, since an effective, non-invasive marker that can be easily measured, in the form of the antisaccade, will be an invaluable asset to clinicians -- highlighting a pressing need for this to be studied.

On the Stroop interference component of the Stroop Color and Word test, participants must give a verbal response indicating the color ink in which the word is printed, but not what the word says. The parallels to the antisaccade demands are clear -- it is easiest for participants to read the actual word on the page. It takes a lot more cognitive processing to (1) inhibit this trained and natural process to read the word content; (2) program a verbal response that says the color in which the word is printed; and (3) carry out this verbal response as quickly as possible. The word stimulus interferes with the correct verbal response, in much the same way that the prosaccade error target interferes with the correct antisaccade response.

Critical for our discussion, the interference component of the Stroop test assesses the dorsolateral prefrontal cortex and anterior cingulate cortex functioning (Levy, Mendell et al. 2004). Not only are these areas important for good performance on the Stroop, they are also critical for effective performance on the antisaccade task. In other words, both of these tests assess functioning in the cortical prefrontal networks, but one is neuropsychological in nature and the other is neurological. Hence, performance on these two tasks should be correlated to some extent.

Measurement of the antisaccade can be done manually with fingers. Manually, the examiner extends one index finger from each hand and holds them together. The patient is instructed to look at the two fingers in the center of their field of vision. Then, one finger (for the sake of argument let us say the right index finger) is moved quickly to the periphery, the patient’s left. The patient is instructed to look as quickly as possible to their right hand side, in the mirror opposite location to where the finger moved. Observing a consensual response in both eyes and stable gaze after the saccade, the right index finger is moved back to center and the process is repeated for the patient’s right side.

Eye trackers and chip-based saccadometers can replace the role of the examiner, and substitutes the finger stimuli with lights or graphical targets. The primary advantage of using machines over manual antisaccade testing is that they can track the rapid eye movement from beginning to end with high accuracy and precision. They can also be programmed to administer the antisaccade
task with different characteristics – whether the center “finger” target stays “on” before the prosaccade error target appears, the precise duration of the antisaccade stimulus, and so on. These small changes can have a big impact on antisaccade performance, irrespective of the underlying effect of injury. A machine can deliver the exact same test over hundreds of trials, save results, and average antisaccade performance over all of these trials, something that humans can only approximate. Useful measurements to record in antisaccade tests include the correct antisaccade and incorrect prosaccade error rate, and their corresponding latency, velocity, duration, and amplitude.

The antisaccade task matters because it can be used to test the functioning of higher level control networks. Patients who are unable to successfully inhibit the prosaccade error are likely to have deficits in neuropsychological testing because there is some degree of cognitive impairment, and this translates into increased symptom burden and cognitive symptoms that are clinically significant for mTBI patients, their caregivers, and the physicians caring for these patients.

1.13 Neurophysiology of the Antisaccade Task

Successful completion of interference tasks requires well-functioning response inhibition control systems from the higher cognitive systems in the frontal and parietal areas of the brain, which are common to both neuropsychological and antisaccade performance. For the antisaccade task specifically (Munoz and Everling 2004), it is thought that the higher cortical regions in the frontal (Guitton, Buchtel et al. 1985) and parietal lobes, namely the frontal eye fields (Pierrot-Deseilligny, Muri et al. 2005, Leigh RJ. 2006), supplementary eye fields, dorsolateral prefrontal cortex (Pierrot-Deseilligny, Muri et al. 2005, Ploner, Gaymard et al. 2005, Leigh RJ. 2006), posterior parietal cortex, and parietal eye field (Leigh RJ. 2006) are responsible for the inhibiting the reflexive prosaccade error (Everling and Fischer 1998). This signal is relayed to subcortical structures, namely the anterior cingulate cortex and basal ganglia (Condy, Rivaud-Pechoux et al. 2004), which feed all the signals to the superior colliculus (Everling and Fischer 1998).

Nevertheless, there are direct connections between some higher cortical areas to the superior colliculus as well (Leigh RJ. 2006). Acting as an integrator of all these signals (Leigh RJ. 2006), the superior colliculus then directs the brainstem saccade generator to create an antisaccade pulse in the right direction and to the right location, after which a step signal keeps the eye trained at
the final position. The final steps leading to saccade generation were described in section 1.10.2. An illustration of the neuroanatomy involved in antisaccades is in Figure 7 below.

**Figure 7:** Higher cortical areas thought to be involved in antisaccade generation. The interconnections between these areas and the subcortical/brainstem structures traverse much of the telencephalon and mesencephalon. From Everling S., Fischer B. The antisaccade: a review of basic research and clinical studies. Neuropsychologia, 36(9):885-99. Reproduced with permission from RightsLink, Elsevier Systems. Abbreviations: PFC = Prefrontal Cortex; FEF = Frontal Eye Field; SEF = Supplementary Eye Field; SC = Superior Colliculus; PPC = Posterior Parietal Cortex; BG = Basal Ganglia.

Importantly, the corpus callosum is involved in interhemispheric communication between the frontal cortices (Mendoza 2010). Several other white matter tracts are responsible for inter-regional communication, susceptible (to varying degrees) to mTBI. Diffuse axonal injury in mTBI affects information conduction within other white matter tracts, perhaps adversely affecting antisaccade performance after injury. The latter is discussed next.

**1.14 What is known about Antisaccade Performance after mTBI**

Many central nervous system (CNS) disorders, including mTBI, involve disruptions in one or more of the systems involved in antisaccades, impairing performance. Table 2 summarizes the primary literature to date which investigated antisaccades after TBI. All severities of TBI were included. If a study included both acute (< 3 months) and chronic (≥ 3 months) assessments, the
entry was duplicated and entered into both sections so that each section could be reviewed independently.

1.14.1 Assessment in Acute TBI (< 3 months)

Research has shown that participants with mTBI exhibit deficits in saccadic generation and difficulty inhibiting a planned saccadic eye movement within two days of injury (DeHaan, Halterman et al. 2007). Antisaccade latency returns to normal after one week post-mTBI (Heitger, Anderson et al. 2004, Heitger, Jones et al. 2006), but accuracy differences have been documented up to a month post-injury (Heitger, Jones et al. 2006). Motor function and oculomotor rapid eye movements within one week post-TBI have been investigated as a prognostic marker for neuropsychological and functional outcome (Heitger, Jones et al. 2007). The investigators found that motor function within the first week (oculomotor and upper limb movement combined) was a better predictor of recovery at three and six months post-injury than neuropsychological function or health state (assessed using self-reported scales) within the first week. Some studies have shown that antisaccade accuracy (number of directional errors, and presence of any hypo/hypermetria) appears to be impaired in the acute stages, with patients making more errors than controls (Crevits, Hanse et al. 2000, Heitger, Anderson et al. 2004, Johnson, Zhang et al. 2014); others indicate better performance (Heitger, Jones et al. 2006, Phillipou, Douglas et al. 2014). Very little is known about the acute effects of mTBI on antisaccades in children, but one study reported paradoxically better performance in the number of antisaccade directional errors in mTBI children compared to healthy controls (Phillipou, Douglas et al. 2014). This study illustrates that children may have a different neurological response to mTBI, manifest by their interesting antisaccade performance. Only one study was found to relate acute antisaccade performance to neuroimaging, and the focus was mainly on functional performance (Johnson, Zhang et al. 2014). In this study, task-based fMRI was used to scan acute mTBI participants as they completed a battery of saccade tests (including the antisaccade task). mTBI participants had significantly worse performance on antisaccade directional error, gain, and positional error compared to healthy controls. Functional hyperactivation was observed in the cerebellum and V5 / V1 cortical areas, but not the frontal areas during the antisaccade task, contrary to their initial predictions. Their study is very unique in that acutely injured mTBI patients were scanned at the same time as conducting the antisaccade task, obtaining a real-time picture of performance during antisaccades after mTBI.
However, PTS patients were not tested, and the sample size was relatively small. Furthermore, the sample was selected from sports concussion patients, all college students. A gap in our understanding remains from direct assessment of patients sourced from the emergency department. Considering the current literature base, no study performed longitudinal antisaccade comparisons in both acute mTBI patients and healthy controls to determine the course of antisaccade recovery. This is critical to identify (1) the evolution of antisaccade performance in acute mTBI, and (2) the normal variation in antisaccade performance in healthy controls, who did not have an intervening mTBI.

1.14.2 Persistent Post-Traumatic Symptoms (≥ 3 mo)

The utility of antisaccade eye movement measurement extends beyond the acute stages after injury although some parameters are expected to return back to normal levels. In the head injury clinic, it can be used for assessment of long term recovery of function. Antisaccade performance in TBI patients with chronic symptoms several years after injury has been investigated (Kraus, Little et al. 2007). Kraus et al. found significant increases in the number of prosaccade errors (saccade towards stimulus) and latency in the antisaccade task in TBI patients compared with healthy controls but no differences between TBI patients of different severities (mTBI vs. moderate/severe TBI). This suggested there was low specificity of the antisaccade task in differentiating between mTBI and more severe head injuries in the chronic stages, but their use of electro-oculography (a trace-based method of eye tracking) limits the precision of antisaccade measurement compared to the chip-based measurement methods employed in our study.

An interesting component of Heitger et al. (2009)’s study found hypermetric gain in the PTS group compared to a similar non-PTS group on the antisaccade task (Heitger, Jones et al. 2009). This indicated the antisaccade task can be used to distinguish between PTS and non-PTS patients, an important responsibility for the head injury clinician. Antisaccade parameters such as latency and accuracy appear to resolve by the time several months have elapsed according to one study (Heitger, Jones et al. 2006), but other characteristics such as the amplitude error, time to peak velocity and the duration of the antisaccade continue to have persistent deficits (Heitger, Jones et al. 2009). Further investigation is needed to verify these earlier results. In addition to the knowledge gaps for acute patients discussed previously, studies to date in PTS patients have not (1) correlated antisaccade performance with gold-standard structural neuroimaging; (2)
conducted tests of the pupillary light reflex and the pathways essential to it, which also play a role in effective antisaccade generation; and (3) directly correlated antisaccade performance with symptom burden measured on the same day.


Several studies have manually done saccade tests in clinic with TBI patients of all severities. However, these were approximate and qualitative appraisals of eye movement, and the stimulus was often conducted using movement of the fingers within the patient’s field of view. Although this illustrates its feasibility to integrate into standard workup (Ciuffreda, Kapoor et al. 2007, Brahm, Wilgenburg et al. 2009, Alvarez, Kim et al. 2012, Goodrich, Flyg et al. 2013), one of the aspects of our study was to establish the feasibility of fully automated and chip-based measurement of antisaccades so that it can eventually replace manual finger-based antisaccade appraisals in the clinic (see the benefits of this approach over manual methods in section 1.11).

1.14.4 Symptom Burden, Executive Functioning and their Relationship to Antisaccade Performance in Acute mTBI and PTS

Symptom burden is incredibly important to the patient, who uses the cognitive, somatic and emotional sequelae after injury as their own gold standard for recovery after their mTBI. A useful antisaccade marker should be related to the symptoms that patients are experiencing. Symptom burden directly affects quality of life and the ability to complete activities of daily living. Heitger et al. correlated saccade deficiencies in the acute phase with quality of life in the PTS phase (Heitger, Jones et al. 2007). Importantly, oculomotor performance (including antisaccade tasks as well as other saccade test results) one week after injury was significantly predictive of greater symptom burden on the RPQ and certain domain scores of the short-form-36 quality of life questionnaire at 3 and 6 months following mTBI in a multiple regression. Hence, deficits in specific saccadic parameters were associated with impairment in domains of quality of life and increased symptom burden several months after injury. Heitger et al.’s study is unique in the literature, assessing a predictive relationship between saccade performance and outcome. Not only is this important from a scientific perspective, it shows that antisaccades have some predictive value for symptom burden in the PTS phase. This study expanded upon Heitger et al.’s earlier results but performed correlations within the same visit. Heitger et al. (2007) used a composite score combining several different types of rapid eye movements, which may have
masked significant effects (but antisaccade task performance has not been correlated specifically with symptom burden only).

Antisaccade functioning has also been directly associated with executive functioning in mTBI patients, but to my knowledge only in the PTS phase. Kraus et al. (2007) correlated antisaccade performance with a cluster of executive functioning tests and found a significant negative correlation between antisaccade latency and a pooled executive functioning score for a pooled sample of TBI subjects of all severities (Kraus, Little et al. 2007). Only considering the mTBI patients, they found nonsignificant trends. As mentioned earlier, their eye movement recordings were conducted using electro-oculography which can be further improved upon by using chip-based saccadometer measurement. Also important is their use of pooled scores for their correlational analyses, which may have masked direct correlations between specific executive functioning tests and antisaccade performance.

It is important to understand the relationships of symptom burden and executive functioning to antisaccade performance in acute mTBI and PTS, but antisaccades’ relationship to diffusion tensor imaging has not been investigated until now.

1.14.5 The Relationship of Diffusion Tensor Imaging to Antisaccade Performance in Acute mTBI and PTS is Unknown

We do not know whether the major white matter pathways damaged in mTBI (corpus callosum, superior longitudinal fasciculus, uncinate fasciculus, and anterior corona radiata) are necessarily common to the white matter pathways necessary for antisaccade performance (projections from the dorsolateral prefrontal cortex, anterior cingulate cortex to the superior colliculus) in mTBI, or are related to their function in some way. This is a large gap in the current literature. A study directly correlating diffusion tensor imaging with saccade performance in acutely and chronically injured patients is required. The focus of this study will be on confirming differences in white matter integrity as expected with mTBI, then identifying correlations with significant differences in saccade performance variables, to identify associations that can be further explored in prospective studies focusing on these areas.

The saccadic eye movement system is heavily integrated into the neurological functioning of the nervous system. We know that sensitive neurological damage characteristic of mTBI can be
detected by diffusion tensor imaging, so establishing the relationship between the two is critically important for validation of saccades in mTBI. Importantly, there is no literature investigating whether there is any correlation between acute structural integrity (measured by DTI) and performance on the antisaccade. However, it is expected that there would be some relation between them, especially because both methods assess different dimensions of neurological white matter integrity. This study will explore these relationships.

Table 2 summarizes the antisaccade literature that is relevant to this study.

1.14.6 Literature Table

Table 1: Literature Results for Antisaccadic Eye Movements Measurement after Traumatic Brain Injury: Grouped by Evaluation Time After Injury

<table>
<thead>
<tr>
<th>Study Class: Study Design</th>
<th>Reference (Year)</th>
<th>Authors’ Conclusions Supporting (+), Disputing (-) or Neutral (N) to Clinical Utility of Antisaccadic Measurement^</th>
<th>Sample Size x number of times repeat testing (as applicable)</th>
<th>Saccade Outcome Tests (computerized unless otherwise noted)</th>
<th>Measurement Time After Injury (mean [SD] unless otherwise specified)</th>
<th>Significant Saccade Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>P: Prospective</td>
<td>(Phillipou, Douglas et al. 2014)</td>
<td>+</td>
<td>33 mTBI (26 with only one mTBI) x 3; 29 HC</td>
<td>VGS, AS; SPS</td>
<td>2 wk, 3 mo, 6 mo</td>
<td>mTBI group, 2 wk and 3 mo: fewer corrected and total antisaccade errors than HC; mTBI group, 6 mo: significantly increased prosaccade latency, correct antisaccades, corrected</td>
</tr>
<tr>
<td>P: Prospective</td>
<td>(Johnson, Zhang et al. 2014)</td>
<td>+</td>
<td>9 mTBI; 9 HC</td>
<td>VGS, AS, MGS, SPS, SPEM</td>
<td>≤ 7d</td>
<td>mTBI group: significantly greater prosaccade error latency, AS and MGS directional errors + gain + positional errors than HC; significantly smaller number of SPS than HC</td>
</tr>
<tr>
<td>P: Prospective</td>
<td>(Heitger, Jones et al. 2007)</td>
<td>+</td>
<td>37 mTBI × 3</td>
<td>VGS, AS, MGS, SPS</td>
<td>1 wk, 3 mo, 6 mo</td>
<td>Saccadic performance at 1w regressed as significant predictors of recovery of quality of life and symptom load 3 mo and 6 mo post-injury</td>
</tr>
<tr>
<td>P: Prospective</td>
<td>(Heitger, Jones et al. 2006)</td>
<td>+</td>
<td>37 mTBI (mCHI) × 4, 37 HC</td>
<td>VGS, AS, MGS, SPS</td>
<td>1 wk, 3 mo, 6 mo, 12 mo</td>
<td>AS saccadic latency for mTBI &gt; HC on 1 wk visit but not on subsequent visits; MGS directional errors for mTBI &gt; HC on 1 wk &amp; 3 mo visit but not on subsequent visits; AS saccade</td>
</tr>
<tr>
<td>P:Prospective</td>
<td>(Heitger, Anderson et al. 2004)</td>
<td>+</td>
<td>30 mTBI; assume 30 HC</td>
<td>VGS, AS, MGS, SPS, SPEM</td>
<td>Session 1: 4.23 [1.79] d; Session 2 (different tests): 6.46 [3.3] d</td>
<td>AS: mTBI saccadic latency of directional errors &gt; HC; SPS: mTBI intra-saccadic intervals &gt; HC; MGS: mTBI # directional errors &gt; HC; Both AS and MGS: mTBI poorer spatial accuracy (hypermetria) of saccades compared to HC</td>
</tr>
<tr>
<td>P:Prospective</td>
<td>(Crevits, Hanse et al. 2000)</td>
<td>+</td>
<td>AS: 31 mTBI (25 non-alcohol intoxicated/6 alcohol intoxicated), 27 HC; MGS: 29 mTBI (23 non-alcohol intoxicated/6 alcohol intoxicated), 27 HC</td>
<td>AS, MGS</td>
<td>&lt; 24 h</td>
<td>mTBI + alcohol intoxication: MGS latency &gt; HC, AS prosaccade errors &gt; HC</td>
</tr>
<tr>
<td>Study Class: Study Design</td>
<td>Reference (Year)</td>
<td>Authors’ Conclusions Supporting (+), Disputing (-) or Neutral (N) to Clinical Utility of Saccadic Measurement^</td>
<td>Sample Size x no. repeat testing (as applicable)</td>
<td>Saccade Outcome Tests (computerized unless otherwise noted)</td>
<td>Measurement Time After Injury (mean [SD] unless otherwise specified)</td>
<td>Significant Saccade Findings</td>
</tr>
<tr>
<td>--------------------------</td>
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</tr>
<tr>
<td>P: Prospective</td>
<td>(Phillipou, Douglas et al. 2014)</td>
<td>+</td>
<td>33 mTBI (26 with only one mTBI) x 3; 29 HC</td>
<td>VGS, AS; SPS</td>
<td>2 wk, 3 mo, 6 mo</td>
<td>mTBI group, 2 wk and 3 mo: fewer corrected and total antisaccade errors than HC; mTBI group, 6 mo: significantly increased prosaccade latency, correct antisaccades, corrected antisaccade errors than HC</td>
</tr>
<tr>
<td>P: Prospective</td>
<td>(Heitger, Jones et al. 2009)</td>
<td>+</td>
<td>72 mTBI: 36 PCS, 36 non-PCS</td>
<td>VGS, AS, MGS, SPS, SPEM</td>
<td>PCS 140.3 [51] d; non-PCS 163.2 [48] d</td>
<td>AS and MGS: PCS # of directional errors and final</td>
</tr>
</tbody>
</table>
amplitude error > non-PCS, time-to-peak velocity and duration of saccade in PCS > HC; AS: hypermetric gain in PCS compared to non-PCS; MGS: final amplitude error in PCS > non-PCS; SPS: PCS peak saccadic velocity < HC, PCS saccadic duration > HC

<table>
<thead>
<tr>
<th>Study</th>
<th>Tasks/Groups</th>
<th>Duration</th>
<th>Measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>P: Prospective</td>
<td>(Heitger, Jones et al. 2007)</td>
<td>+</td>
<td>37 mTBI x 3</td>
<td>VGS, AS, MGS, SPS 1 wk, 3 mo, 6 mo Saccadic performance at 1 w regressed as significant predictors of recovery of quality of life and symptom load 3 mo and 6 mo post-injury</td>
</tr>
<tr>
<td>P: Prospective</td>
<td>(Kraus, Little et al. 2007)</td>
<td>+</td>
<td>20 mTBI; 17 M/S TBI; 19 HC</td>
<td>VGS, AS mTBI 65.20 [18.13] mo; M/STBI 107.12 [22.04] mo VGS Task: in overlap condition, latency M/STBI &gt; mTBI; AS Task: # prosaccade errors in HC &lt; mTBI &amp; M/STBI, but no difference between patient groups. All TBI patients: AS overlap latency</td>
</tr>
<tr>
<td>Study Type</td>
<td>Authors</td>
<td>Design</td>
<td>Sample Size</td>
<td>Measures</td>
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</tr>
<tr>
<td>Prospective</td>
<td>(Heitger, Jones et al. 2006)</td>
<td>+</td>
<td>37 mTBI x 4, 37 HC</td>
<td>VGS, AS, MGS, SPS</td>
</tr>
<tr>
<td>Prospective</td>
<td>(Mulhall, Williams et al. 1999), including erratum</td>
<td>-</td>
<td>19 STBI, 5 retested; 26 HC, 12 retested</td>
<td>Manual (VGS, MGS, AS, SPS)</td>
</tr>
<tr>
<td>Prospective</td>
<td>(Williams, Ponsford et al. 1997)</td>
<td>+</td>
<td>16 STBI, 9 retested; 12 HC, 5 retested</td>
<td>SF, VGS, MGS, SPS, AS</td>
</tr>
</tbody>
</table>
refixations < HC; VGS: STBI accuracy < HC, STBI # of steps > HC; MGS and AS: STBI # prosaccade errors > HC; at retest, STBI showed significant improvements in saccadic latency in MGS and AS

Key: Study Classes: P=Primary Research Study. Abbreviations: mTBI=Mild Traumatic Brain Injury (mCHI, mild closed head injury and concussions subsumed under this category); MTBI = Moderate Traumatic Brain Injury; STBI=Severe Traumatic Brain Injury; BR=Blast-Related TBI; NBR=Non-Blast Related TBI; PCS=Post-Concussive Syndrome; HC=Healthy Control; SF=Saccadic Fixation Task; VGS=Visually Guided Saccade Task; MGS=Memory Guided Saccade Task; SGS=Self-Guided Saccade Task; SPS=Self-Paced Saccade Task; AS=Antisaccade Task; AD=Adaptive Saccade Task; CM=Countermanding Task; PRED=Predictive Saccade Task; SPEM=Smooth Pursuit Eye Movement Task; ^ Study authors’ conclusions of evidence, appraising utility of antisaccadic eye movement measurement post-TBI. +/-, supporting or not supporting, respectively.

1.14.7 Sensitivity and Specificity of Antisaccade Measurement

Experimental factors such as the nature of verbal instruction given (Mosimann, Felblinger et al. 2004), any visual pre-target clues (Fischer and Weber 1998), directional probability (Koval, Ford et al. 2004), and whether there are cognitive tasks competing for attention (Kristjansson 2007) will dictate whether any measurable deficit is detected. The sensitivity of saccadometry is a more important quality, although it may not be able to differentiate between patients with PTS and non-PTS mTBI patients (Kraus, Susmaras et al. 2007). Saccadometry has been recently combined with more specific measures such as functional magnetic resonance neuroimaging
In Johnson et al.’s study, concussed athletes conducted functional magnetic resonance imaging at the same time as completing an antisaccade task. Although there were no negative correlations between task-based functional activity and antisaccade performance, the mTBI group did have antisaccade performance disadvantages compared to the healthy controls on antisaccade directional error, gain, and position error. Functionally the antisaccade task required greater activation in the left primary visual cortex (V1), right visual cortex (V5/MT) and left cerebellum in the acutely concussed athletes compared to controls. As seen here, antisaccade performance can be coupled with more specific tools such as functional or diffusion neuroimaging to localize areas of damage, leading to conclusions with high sensitivity and relatively good specificity to injury. Having identified the gaps in the literature as discussed above, this study attempts to correlate antisaccade performance with structural integrity in regions of interest following injury, which has not been investigated to date.
2  CHAPTER 2: RATIONALE AND HYPOTHESES

2.1 Rationale

The current gold-standard markers discussed earlier (symptom burden assessment, neuroimaging, neuropsychological assessment) are helpful but insufficient for diagnosing mTBI. Here, the analysis of rapid eye movements can be an excellent measurement to take after injury, either alone or as an adjunct to the measures previously covered. Although I have reviewed several eye movements that may be useful for more severe disorders of consciousness inflicted by moderate to severe TBI (Ting, Perez Velazquez et al. 2014), the lack of validated rapid eye movements in the mTBI and concussion literature present a unique clinical challenge and a pressing research opportunity.

Previous work has demonstrated that performance on the antisaccade task may be a valuable assessment marker of injury in acute and chronic mTBI. However, we do not understand enough about how performance on antisaccade tasks changes over time after an mTBI and how antisaccade performance is related to white matter tract integrity, symptom load, and neuropsychological functioning. These relationships need to be explored if antisaccades are to be useful as a neurological marker for mTBI. This study addressed each of these gaps in the literature by a systematic consideration of the following three aims:

2.2 Summary of Aims

2.2.1 Aim 1

To confirm that components of antisaccade performance are affected by acute mTBI and those with PTS when compared to controls;

2.2.2 Aim 2

To identify what changes of white matter integrity are related to changes in antisaccade performance in acute mTBI and PTS;
2.2.3 Aim 3

To identify changes in executive functioning after mTBI and to relate these to changes in antisaccade performance.

2.3 Detailed Rationale and Hypotheses for Each Aim

2.3.1 Aim 1

Confirming that components of antisaccade performance are affected by acute mTBI and those with PTS compared to controls, required achieving the following goals:

2.3.1.1 Goal 1.1: Determine that patients had normal bilateral pupillary light reflex

First I wanted to ascertain the integrity of the midbrain structures, optic nerve/tract and cranial nerve III through quantified measurements of the pupillary light reflex using the NPi score.

2.3.1.2 Goal 1.2: To determine the effect of injury and time since injury on antisaccade performance

The second goal was to detect differences in saccadic performance on the antisaccade task between patients and controls, as reflected in the literature. We hypothesized that antisaccade performance should be most affected early after acute mTBI and that it should improve as the patient recovered from the acute mTBI, eventually reaching the same performance as non-injured control subjects. Furthermore, we hypothesized that if patients had persistent symptoms post-mTBI, then antisaccade performance would also be affected in these individuals. The following specific comparisons were made to assess these hypotheses:

- Hypothesis 1: There will be significantly different performance between the study groups on one or more antisaccade performance variables as noted below, with the following planned contrasts (A1 = Acute mTBI First Visit, on average less than a week after injury; P1 = PTS First Visit, greater than or equal to 3 months after injury; C1 = Healthy Control First Visit):
  - Number of Correct Antisaccades: A1 and P1 < C1;
• Hypothesis 2: Recovery of antisaccade performance will be represented by significant within-group difference in antisaccade performance in the acute group between first (A1) and second (A2) visit (two to four weeks after injury) as time post-injury increases, on one or more antisaccade variables, such that:
  o Number of Correct Antisaccades: A1 < A2;
  o Number of Prosaccade Errors: A1 > A2;
  o Antisaccade Median Latency: A1 > A2;
  o Antisaccade Latency Rate: A1 < A2;
  o Antisaccade Mean Duration: A1 > A2;
  o Antisaccade Mean Amplitude: A1 > A2;
  o Antisaccade Mean Peak Velocity: A1 > A2;
  o Prosaccade Median Latency: A1 > A2;
  o Prosaccade Latency Rate: A1 < A2;
  o Prosaccade Mean Duration: A1 > A2;
  o Prosaccade Mean Amplitude: A1 > A2;
  o Prosaccade Mean Peak Velocity: A1 > A2.

To have confidence in antisaccade performance measures, I assessed the test-retest reliability of the antisaccade task in normal controls at separate visits two weeks apart. Specifically, the null hypothesis was:
Hypothesis 3: There will be no significant within-group differences in antisaccade performance over a two week period in healthy controls.

In the next set of aims, I tried to understand the mechanisms and underlying basis for these deficits in terms of white matter integrity and neuropsychological functioning.

2.3.2 Aim 2

To identify what changes of white matter integrity are related to changes in antisaccade performance in acute mTBI and PTS, I sought to identify the neuroanatomical basis of any difference on the antisaccade performance between patients and controls. This was explored in two parts.

2.3.2.1 Goal 2.1: To determine the effect of injury on white matter integrity

Previous literature has shown there are distinct effects of mTBI on white matter (WM) integrity. The first goal in this aim was to confirm the differences in our patient sample of acute mTBI and PTS by quantifying FA and MD. These values were selected instead of more detailed measures of diffusivity like axial and radial diffusivity because they provide information about the overall movement of water molecules in the brain, and there is a robust literature base supporting their use in acute mTBI and PTS.

- Hypothesis 1: There will be lower fractional anisotropy in acute and PTS patients’ scans compared to control patients’ scans in defined white matter regions of interest due to diffuse axonal injury after mTBI. Specific areas of interest derived from the literature review (see section 1.9) included the corpus callosum and all its component areas (genu, body, splenium); uncinate fasciculus; superior longitudinal fasciculus; internal capsule (part of the anterior corona radiata).

- Hypothesis 2: There will be higher mean diffusivity in acute/PTS scans compared to control scans in the same white matter regions of interest described above.
2.3.2.2 Goal 2.2: To correlate white matter integrity and antisaccade performance

I tested the following hypothesis by correlating the areas with significantly affected FA and MD to antisaccade performance in both acute mTBI and PTS patients.

- Hypothesis 1: Antisaccade performance will be positively correlated with WM structural integrity as measured by FA and MD.

2.3.3 Aim 3

The final aim linked concepts in the first two aims, and connected our results back to the patient’s experiences (symptom load) after mTBI. From the patient’s perspective this aim is often the most pressing issue to resolve after injury.

2.3.3.1 Goal 3.1: To determine the effect of injury on executive functioning and response inhibition

In order to better understand the neuropsychological basis of antisaccade performance after TBI, I assessed a variety of neuropsychological tests in the participants. Since antisaccades involve inhibitory processes mediated by the frontal and prefrontal cortices, I hypothesized that patients with poor antisaccade performance would also show poor performance on neuropsychological tests of response inhibition. Also included were tests of task switching and working memory/attention.

- Hypothesis 1: injury will detrimentally affect executive functioning:
  - Stroop Color Score: A1 = P1 = C1;
  - Stroop Word Score: A1 = P1 = C1;
  - Stroop Color-Word Score (number of items covered in 45 seconds) : A1 and P1 < C1;
  - MoCA Phonemic Fluency Score: A1 and P1 < C1;
  - TMT-A Time: A1 = P1 = C1;
  - TMT-B Time: A1 and P1 > C1;
2.3.3.2 Goal 3.2: To characterize symptom load and relate it to performance on the antisaccade performance and response inhibition tasks

To achieve this goal, I first hypothesized that measures of symptom load improve after mTBI. Interpreting the results of this study required determining whether the acute patients recovered in symptom load from a few days after injury to several weeks after injury. Identifying the recovery course of these different assessment markers is also very important.

- Hypothesis 1: There will be a significant within-group difference in symptom reporting in the acute group between the two visits as time post-injury increases, such that:
  - SCAT3 Symptom Severity Score: A1 > A2;
  - RPQ Symptom Load: A1 > A2.

Next I related these symptom burden findings to antisaccade performance.

- Hypothesis 2: Poorer antisaccade performance will be correlated with impaired executive functioning, and greater symptom load (based on the SCAT3 and Rivermead) on the first visit in acute mTBI patients.
3 CHAPTER 3: METHODS

I investigated saccadometry as a tool for mTBI assessment in a depth and breadth of analysis greater than previously published work by validating against neuropsychological tests, standardized symptom burden assessments, and state-of-the-art neuroimaging modalities. This study uniquely combined all these important tools for a comprehensive assessment of mTBI both in cross-section between different patient groups, and within the same participants over time, demonstrating the potential for antisaccade performance to be a biomarker for diagnosis of mTBI.

3.1 Study Initiation

A pilot study was previously completed by our research team investigating the step saccade task in a small number of mTBI patients, recruited from the emergency department at St. Michael’s Hospital (SMH) (Mullen, Yucel et al. 2014). However, upscaling the study required understanding, adapting and ultimately changing the culture around research in the minor area of the SMH emergency department (ED), the location where mTBI patients are directed after triage. Getting the study up and running provided unique challenges and learning opportunities that are important to discuss.

3.1.1 Roadblocks to Study Initiation and Solutions Thereof

I understood that the only way to successfully integrate research recruitment into this environment would be to minimize the disruption to normal flow and be clear about any additional responsibilities placed upon staff and resources in the department. I decided to implement a multi-pronged approach to increase awareness – creating and posting signs around the emergency department and especially in the ED minor area, making an effort to both introduce ourselves and the purpose of our research to the staffing clericals who would be helping with the project, and taking time to have several meetings and in-service sessions for staff who were interested and available to learn more. I was able to assess the typical route of a mTBI patient through the unit and when/where the best time would be to discuss research with potential participants and conduct enrolment for the study. A plan was needed to attain a circle-of-care introduction from a staff member on duty before approaching potential participants to
discuss about research opportunities. The ED research liaison suggested that I ask the Rapid Assessment Zone (RAZ) patient flow coordinator on duty to conduct this introduction because the staff were likely to be busy with clinical care. As the study began, I involved all the RAZ coordinators rotating within the emergency department, and they were very helpful in recruitment for the study.

3.2 Study Protocol

All participants provided written informed consent before being prospectively enrolled in the study, and this study was approved by the Research Ethics Board at St Michael’s Hospital. Acute mTBI patients were asked to come in for two visits to SMH: the first within a week of injury, and a second visit two to four weeks after the injury. Patients with PTS recruited from the head injury clinic were invited to SMH once to participate in all study activities. Healthy control participants who had no history of concussion or TBI were recruited and invited to come into SMH twice, two to four weeks apart. The control group was matched to the patient groups based on age (±5 years), sex and years of education (±5 years) preferentially to the acute mTBI participants. Due to scheduling difficulties, for some participants the scheduling was several days off the planned timeframe but they were still allowed to participate. The experimental design is illustrated below. All participants were tested at St. Michael’s Hospital under as similar testing conditions as possible.
3.2.1 Acute Mild Traumatic Brain Injury

3.2.1.1 Operational Acute mTBI Definition

Concussions are a form of mTBI without abnormalities on standard neuroimaging. We used a modified version of the World Health Organization definition of mTBI (Carroll, Cassidy et al. 2004) (additions to the WHO definition are underlined), with some elements extracted from the American College of Rehabilitation Medicine recommendations (Ruff, Iverson et al. 2009).

MTBI is an acute brain injury resulting from mechanical energy to the head from external physical forces. Operational criteria for clinical identification include: (i) 1 or more of the following: Any alteration in mental state at the time of the injury (confusion, disorientation, dazed, slowed thinking, etc.), loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours or for events immediately before (retrograde amnesia), and/or other transient/persistent neurological abnormalities/deficits such as weakness, loss of balance, change in vision, dyspraxia paresis/plegia [paralysis], sensory loss, aphasia, etc; other focal signs, seizure, and intracranial lesion not requiring surgery; (ii) Glasgow Coma Scale score of 13–15 after 30 minutes 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, Cassidy et al. 2004)

We included concussions within our group of mTBI patients. We chose to focus on mTBI and not purely concussion, as positive neuroimaging should not preclude antisaccade measurement if this tool were to be eventually used in the clinic environment. Further elaborating on this point, the antisaccade marker should be sensitive to less severe head injury (in the form of concussions) and to more serious insult (mTBI). One of the primary motivations for validating a saccadic biomarker is to determine the sensitivity of antisaccade performance on a broad spectrum of similar patients. Future work may focus more with a subgroup analysis and identify markers specific to each type of injury. On the flip side, a more inclusive definition also presents limitations on the conclusions that can be drawn from the study, as one cannot say that the findings were specific to one type of mTBI patient or another. Overall I think I have struck a good balance between inclusiveness and exclusiveness for this study.

3.2.1.2 First Assessment

Due to the acute nature of their injury, we accommodated these patients depending on their availability and clinical state. If an acute patient was able to stay for 2.5 hours after they were discharged from the ED, and if we had the necessary testing resources available, we tested them immediately on the same day as their ED visit. If the patient was unable to stay, we advised them to come in for the first visit within a week of their injury (up to eight days). In this case we advised the patient that we would be trying to schedule the MRI scan within their first visit. This procedure minimized the latency between injury and the first visit.

During the first visit the participant completed an antisaccade task for 100 trials, a series of neuropsychological tests and the MRI scan if scheduling allowed. Participants also completed pupillometry, and we obtained demographics information and injury characteristics through questionnaires. The neuropsychological battery included a test of global cognitive functioning, namely the Montréal Cognitive Assessment (MOCA) and tests of multiple domains of executive functioning, namely the Stroop Color-Word test (targeting response inhibition/interference processes), and task switching/attention (the Trail Making Test (TMT-A and TMT-B)).
Participants also completed the Center for Epidemiological Studies – Depression (CES-D) scale, reporting information about their affective state. The results used in this analysis were part of a larger battery of neuropsychological tests, and only a subset is reported here. Participants were asked to complete the Rivermead Post-Concussion Symptoms Questionnaire (RPQ) and SCAT3 Symptom Scale, both self-report questionnaires used to assess their symptom burden.

The saccadometry and neuropsychological testing required a closed quiet room with dimmable lights, one table and two chairs, and the room needed to be large enough to accommodate a 1.5 – 3.5 m distance between the participants' forehead and a white matte wall. Due to lack of space within the emergency department this was not feasible, so an alternate room outside of the ED was designated for participant testing.

3.2.1.3 Second Assessment

The second assessment was carried out between two to four weeks after the participant’s mTBI. Participants were asked to have an MRI scan if the MRI scan could not be scheduled within the first visit. During the second assessment, we carried out the same tests as we did in the first assessment to assess recovery of function after mTBI on both neuropsychological testing and antisaccade performance.

3.2.2 Persistent Post-Traumatic Symptoms

3.2.2.1 Operational PTS Definition

For the purposes of this study, we defined patients with persistent post-traumatic symptoms (PTS) as the following:

1. Sustained mTBI at least three months prior to testing.

2. Reported persistent post-traumatic symptoms 3 months or longer after their injury.

3. Had a self-reported score of two or higher on three or more items (Babcock, Byczkowski et al. 2013) on the Rivermead Post-Concussive Symptoms Questionnaire (RPQ).
3.2.2.2 First Assessment

We recruited a group of patients who had a history of mild traumatic brain injury presenting to the ambulatory Head Injury Clinic at SMH. The circle of care introduction was made by the nurse practitioner in the head injury clinic before and after patient appointments. Testing conditions were kept as similar as possible between participants and between visits. These were patients who had experienced a brain injury greater than three months prior and continued to experience clinically documented symptoms (see operational definition). These patients were asked to come in once to St. Michael's Hospital and complete one MRI scan, neuropsychological testing, and saccadometry as above. Pupillometry, symptom load assessments and demographics data were all collected on the same day.

3.2.3 Healthy Controls

3.2.3.1 First Assessment

Healthy controls were recruited from participants’ friends and family and relatives. Controls were matched to acute mTBI and PTS participants based on age (± 5 years), sex, and years of education (± 5 years). Priority was given to matching with the acute mTBI group. Controls were asked to complete two testing sessions. A first visit was carried out soon after recruitment whereby they completed an MRI scan if applicable, neuropsychological testing, and eye movement testing as described above under as similar conditions as possible.

3.2.3.2 Second Assessment

Control participants completed a second visit two to four weeks later whereby they completed the same tests and an MRI scan (the latter only if they did not complete it during the first assessment). Control MRI scans were conducted on the first visit or second visit depending on the participant that the control matched (if participant did their scan on the second visit, the control completed their scan on the second visit).
3.2.4 Study Inclusion and Exclusion Criteria

For acute mTBI patients, approximately 215 mTBI patients from an electronic record system were initially screened, 77 were approached for recruitment, and 29 mTBI patients consented. For the PTS group, approximately 352 head injury clinic patients were eligible for recruitment, 89 were approached, and 28 were consented for the study. I aimed for at least 10 participants tested for their first visit in all three groups, a sample size which is generally sufficient to power prospective neuroimaging studies (Tom Schweizer, personal communication, 2015). Three consort diagrams outlining the approximate recruitment stage numbers for each of the three participant groups is outlined over the next three pages (some records were incomplete). The consort diagram template was obtained from the CONSORT group website (Group 2010).
Saccades Study CONSORT Flow Diagram

**ACUTE mTBI**

**1.1 Enrollment**
- Assessed for eligibility (n= 215)
  - Excluded (n= 138)
    - Not meeting incl/excl criteria (n= 56)
    - Declined to participate (n= 24)
    - Other reasons (n= 58) (missed, lost to follow up, left without being seen, etc)

**1.2 Allocation**
- Allocated to intervention (n= 29) CONSENTED
  - Received allocated intervention (n= 11) GROUP TOTAL
  - Did not receive allocated intervention (consented but declined afterwards or terminated) (n= 18)

**1.3 Follow-Up**
- Lost to follow-up (did not get back to me for 2nd visit) (n= 2)
- Discontinued intervention (N/A) (n= 0)

**1.4 Analysis**
- Analysed (n= 8)
  - Excluded from MR analysis (technical difficulties) (n= 0)
Saccades Study CONSORT Flow Diagram

PTS

1.5 Enrollment

Assessed for eligibility (n= 352)

Excluded (n= 263)
- Not meeting inclusion/exclusion criteria (n= 3)
- Declined to participate (n= 38)
- Other reasons (n= 222 (No show, cancelled appt, busy with other research, advised not to approach, no introduction possible, recruiter skipped clinic due to schedule conflict, missed.))

1.6 Allocation

Allocated to intervention (n= 28)
- Received allocated intervention (n= 15)
- Did not receive allocated intervention (consented but declined afterwards or terminated) (n= 13)

1.7 Follow-Up

(For Subsequent Visit)

Lost to follow-up (N/A: No Follow-up) (n= 0)
Discontinued intervention (terminated saccadometry) (n= 1)

1.8 Analysis

Analysed (n= 14)
- Excluded from MR analysis (technical difficulties) (n= 2)
Saccades Study CONSORT Flow Diagram

Healthy Control

1.9 Enrollment

Assessed for eligibility (n=29)

Excluded (n=16)
- Not meeting inclusion criteria (n=1)
- Declined to participate (n=5)
- Other reasons (n=10) (Lost to initial followup)

1.10 Allocation

Allocated to intervention (n=13) CONSENTED
- Received allocated intervention (n=10) GROUP TOTAL
- Did not receive allocated intervention (consented but declined afterwards or terminated) (n=3)

1.11 Follow-Up
(for subsequent visit)

Lost to follow-up (n=1)
Discontinued intervention (adverse event terminated MRI scan) (n=1)

1.12 Analysis

Analysed (n=8)
- Excluded from MR analysis (technical difficulties) (n=1)
3.2.4.1 Inclusion Criteria

Inclusion criteria for the study were age greater than or equal to 16 years; able to provide informed consent; acute group: sustained an acute mild traumatic brain injury according to the modified WHO criteria; PTS group: experiencing persistent post-traumatic symptoms as a result of traumatic brain injury at least three months prior to the recruitment date; adequate verbal English language skills as all the study assessments required a working understanding of the language and effective communication. All patients required a basic history to rule out horizontal diplopia, severe loss of visual acuity or history of visual diseases such as glaucoma or cataracts. Every subject needed a detailed assessment of the pupillary light reflex.

3.2.4.2 Exclusion Criteria

Acute mTBI, PTS and healthy control participants were excluded if they were unable to provide informed consent, medically unstable or intoxicated (by drugs or alcohol) at the time of recruitment, had medical comorbidities of multiple sclerosis, prior incisional brain surgery, prior brain irradiation, prior hydrocephalus, a history of stroke, early dementia, neurodegenerative disease including Alzheimer’s disease, and uncontrolled diabetes. In terms of optic pathology participants were excluded if they had a history of eye disease (lazy eye or glaucoma) or direct orbital trauma due to presenting injury. Participants were excluded from the saccadometry analysis if they were taking medications known to interfere with saccadic eye movement function (benzodiazepines and antipsychotics).

3.2.5 Acquisition of Data and Preparation for Statistical Analysis for the Three Aims

All neuropsychological tests, questionnaires and pupillometry data were scored, then independently entered by other students on the project (see contributions) in a separate Microsoft Access 2010 database with the same formatting and structure as the main database, and any discrepancies were detected by an automated visual basic algorithm in Microsoft Excel (a macro, code listed in Appendix 1). This two-pass verification system ensured that any inadvertent errors in data entry were detected in an unbiased fashion and corrected prior to analysis. The students were undergraduate/recent graduate volunteer research students who were trained on all
procedures necessary for data entry and scoring on the project, as well as the applicable compliance regulations for patient privacy and research ethics. Although pairs of students worked together, the two-pass verification was completed between pairs of different students.

3.2.5.1 Aim 1

The goals of the first aim were to determine the characteristics of oculomotor integrity, antisaccade performance and executive functioning in the patient groups compared to controls.

3.2.5.1.1 Goal 1.1: Determine that patients had normal bilateral pupillary light reflex

3.2.5.1.1.1 Data Acquisition

We used the NeurOptics NPi-100 pupillometer, an automated non-invasive device (Chen, Vakil-Gilani et al. 2014) currently used for clinical purposes in the Trauma and Neurosurgery Ward at St. Michael’s Hospital. We described characteristics of pupillary response in all participants during their respective visits, obtaining information about the integrity of the pupillary light reflex in each eye (both left and right pupils). We used the NPi score under photopic conditions as a screening measure for oculomotor integrity. If a participant had an NPi score less than 3.0 in any eye, this indicated a ‘sluggish’ pupillary light response. The NPi score is a summary score integrating the measurements of minimum pupil diameter, maximum pupil diameter, pupil constriction latency, and the other variables measured by the pupillometer. As a result, the NPi score can be applied to the measurement of basic oculomotor integrity in our participants.

Though we did not measure the level of ambient illumination, the light level was kept well-lit and consistent for all participants. No participant received eye drops prior to the testing or had previous incisional eye surgery. The pupillometer shone a white light into the subject’s eye, and onboard cameras measured several pupil response characteristics, namely constriction velocity, dilation velocity (after light is turned off), maximum pupil diameter, minimum pupil diameter, response latency, constriction/dilation velocity, average and maximum constriction velocity as well as a trace of pupil response dynamics. These measurements were completed once per visit for every participant and automatically calculated into a NPi score. A complete measurement in one eye took a few seconds, and automated algorithms detected if the subject blinked or not. If
the subject blinked during the measurement, pupillometry was retaken in both eyes as several variables could not be reliably measured with an intervening blink.

3.2.5.1.2 Goal 1.2: To determine the effect of injury and time since injury on antisaccade performance

3.2.5.1.2.1 Data Acquisition

Our research team ordered a portable saccadometer (Plate 1) from Ober Consulting in Poland, the Saccadometer Advanced. This machine was designed specifically for research purposes. The saccadometer unit consisted of a headband connected to low-powered laser lights on an integrated circuit board, connected to a display with an on board chip which drives the target locations for the saccadic tasks. The tasks themselves were projected onto the wall with three red low-power laser lights and one green low-power laser light (programmable in the center position). The lights were shone onto a matte white wall 1.5-3.5 meters away from the participant’s eyes. All saccadometry was conducted under darkened conditions as per a published pilot study by our research group (Mullen, Yucel et al. 2014). The saccadometer automatically adjusted for minor deviations in distance between the participant’s forehead and the distance to the wall via in-built programming, so long as it was within the range defined above. It also automatically marked and excluded intervening blinks.
Plate 1: Saccadometer worn on forehead. The saccadometer was driven by a hand-held electronic device about the size of a palm.

All participants were guided through a calibration trial prior to the antisaccade task. In the calibration phase they were asked to look as accurately as they could at the lights as they jumped 10 degrees horizontally from a center reference point. A red center light first appeared, which then jumped ten times to the left, followed by ten times to the right, returning to center each time. This allowed the saccadometer to automatically calibrate its readings to each individual participant. Participants who were myopic or hyperopic were asked to wear contacts to ensure corrected visual acuity for the saccade tests. If they did not have any prescription contacts available, all participants were asked to ensure they could see all three saccadic targets clearly and without horizontal diplopia before proceeding, as a brief screen to ensure sufficient visual acuity for assessment.

The antisaccade task was pre-programmed into the saccadometer by the manufacturer. Participants were shown a red center light, after which it jumped either to the left or to the right (fixed probability 50%) with 10 degrees horizontal displacement, after a fixed fore-period of 1000 ms and a random fore-period with a maximum of 1000 ms. They were instructed to look at the center light. When it jumped to the left or to the right, participants were instructed to look in the mirror-opposite location as accurately and quickly as they could. The waiting time for each
saccade was 2000 ms, and the trial break time was 1000 ms. We performed the gap antisaccade task (in which the center light turned off before presentation of the antisaccade target), although a good adjunct for future studies would be to conduct the overlap antisaccade task as well (in which the center light stays on for a period of time after the antisaccade target appears). Prior to the data recording phase of the task, an interactive tutorial was given to each participant, with a manual step-by-step walk-through of the expected eye movement at each stimulus. Each participant reported seeing all three lights clearly, and demonstrated full understanding of the expected antisaccade task before recording of the data started.

Raw saccadometry data was transferred to a computer with LatencyMeter Software version 5.2 via the included optic-fiber cable. The software automatically conducted pre-processing on the raw data then segregated each trial into graphed traces of eyeball location and velocity (Figure 9) and displayed the parameters used for each set of trials. The software and integrated hardware automatically accounted for eye blinks and head movement during its pre-processing. The saccadometry data were exported to coded excel files, which contained all the dependent variables used in the antisaccade analysis.
Figure 9: Sample antisaccade session latency profile (eyeball position as a function of time), recorded from the author’s eyes. Only correct responses are displayed. Note the normal variation in antisaccade performance. This data has not been pre-processed for analysis yet, showing outlier trials that would have been excluded based on specific criteria described below. Picture exported from LatencyMeter Software 5.2.

3.2.5.1.2.2 Data Pre-Processing

Saccades with latencies less than 50 ms (anticipatory saccades) and greater than 600 ms (increased latency due to inattention/possible attempt at malingering) were excluded from the analysis, forming the lower and upper bounds of antisaccade latency, respectively. *A priori*, I believed that this would make for a more homogeneous and reliable subset of antisaccade trials.

Each antisaccade trial was then categorized into one of the following bins:
1. Correct antisaccade, in-bounds latency 50-600 ms

2. Correct antisaccade, out-of-bounds latency < 50 ms or > 600 ms

3. Incorrect antisaccade (prosaccade error), in-bounds latency 50-600 ms

4. Incorrect antisaccade (prosaccade error), out-of-bounds latency < 50 ms or > 600 ms

Each antisaccade trial was split into these four groups and variables were tabulated for categories 1 and 3. Detailed analyses for this work were only conducted on trials that fit within these two categories (the accepted antisaccade trials). The number of trials which fit into categories 2 and 4 were too low for useful statistical comparisons (the not accepted antisaccade trials).

3.2.5.1.2.2.1 Antisaccade Accuracy

The antisaccade directional accuracy was operationally defined by the number of accepted antisaccades in the correct (anti-) direction for each session. This was calculated for each participant, across all visits.

3.2.5.1.2.2.2 Antisaccadic Latency and Latency Rate

Antisaccadic latencies were measured for each participant. After individual saccadic trials were screened based the criteria above, intra-participant median latencies were calculated across trials for each participant at each visit. The inter-participant means of these median values were used for statistical comparisons between groups. Literature has modeled that the antisaccadic latency distribution is skewed, which could be remediated by taking the reciprocal of the latency for each saccade trial (Noorani and Carpenter 2013). To explore this further, we also calculated the reciprocals of the median latency for each participant (the latency rate, 1/latency (ms)) as a separate dependent variable, but kept the original raw latency measurements as well. The inter-participant means of the reciprocal latencies were used in statistical comparisons, since there was no reason to assume that the distribution of the medians of the latencies for the population were skewed.
3.2.5.1.2.2.3 Antisaccadic Mean Duration, Amplitude, and Maximum Velocity

For each participant, the intra-participant mean duration, amplitude and maximum velocity across all their accepted antisaccadic trials were calculated. The inter-participant means of these values were used in statistical comparisons.

3.2.5.1.2.2.4 Prosaccade Performance

An analogous set of dependent variables (prosaccade accuracy, prosaccade latency and latency rate, prosaccade mean duration, and prosaccade amplitude and maximum velocity) were calculated for incorrect prosaccades for all three groups across all visits (see details in sections 3.2.5.1.2.2.1-3 above).

3.2.5.2 Aim 2

The goal of the second aim was to identify the differences in structural fine white matter in our patient groups compared to controls, and correlate these with antisaccade variables.

3.2.5.2.1 Goal 2.1: To determine the effect of injury on white matter integrity

3.2.5.2.1.1 Data Acquisition

We used a 2.4 x 2.4 x 3.0 mm voxel interleaved echo-planar acquisition, on a 3 Tesla SIEMENS Skyra Magnetom (204 coil elements), with 12 non-collinear diffusion directions and one $b_1 = 0$ reference scan at 3.0 mm slice thickness. The $b_2$ factor $= 1000$ s/mm$^2$. The date of the MRI scan was matched to a particular patient visit. The DTI acquisition took approximately 6-8 minutes, and was part of a larger neuroimaging protocol with T1 and T2 scans. For acute patients, the majority of the MRI scans were conducted on the second visit (nine out of eleven participants). For PTS patients, all MRI scans are conducted on the first study visit. Finally, for control participants the majority of MRI scans were also conducted on the second visit (seven out of ten participants), to match the acute patients. Prior to the MRI scan participants completed a screening questionnaire ensuring they could undertake the scan safely.
3.2.5.2.1.2 Data Pre-Processing

DTI sequences were obtained from the St. Michael’s Hospital Siemens 3T scanner in anonymized DICOM format on CD-ROMs. Images were converted to the FSL compatible NIFT1 format using the DCM2NII tool of the MRICRON software package. This automatically generated one corresponding .nii.gz file, one .bval file and one .bvec file for each input scan. Diffusion MR images were pre-processed and analyzed using the FMRIB FSL Toolbox (Jenkinson, Beckmann et al. 2012).

Raw .nii.gz images were inspected slice by slice for any gross abnormalities generated during acquisition. Scans that developed excessive gross distortions due to severe head motion or dental braces during acquisition, or incomplete scans were excluded from further analyses.

3.2.5.2.1.3 Eddy Correction, Mask Generation and Fitting to the Diffusion Tensor

Images were eddy-corrected using the EDDY program in the FSL toolbox to reduce the effect of gradient distortion. Scans were skull-stripped using the Brain Extraction Tool (BET) from the FSL toolbox and the first non-diffusion weighted image, removing non-brain voxels. The threshold for brain extraction was set at 0.2 or 0.3, depending on the results of the brain extraction.

Using FSLVIEW, each scan was manually checked for eddy correction and brain extraction errors. Binary masks were manually drawn for each scan using FSLVIEW, cycling through each acquired slice for the z axis. These modified binary masks were used for diffusion tensor fitting in the following steps.

Following this, images were fitted to diffusion tensors using the DTIFIT program in FSL. The parameters included the original eddy corrected diffusion weighted image, the manually drawn binary mask, and the eigenvectors and eigenvalues files, respectively.

Fitting of the diffusion tensor generated several files for each scan, including three eigenvector files, the corresponding three eigenvalues files, the mean diffusivity files, and several other logs.
3.2.5.2.1.4 Excluded Scans

Four scans could not be included in the DTI analysis. Two PTS participants were excluded due to severe distortion during acquisition of the magnetic resonance image; this could not have been predicted beforehand from their MRI screening form responses. A control participant was not included in the analysis because the accompanying .bval and .bvec files could not be generated from the accompanying DTI scan. Finally, another control participant could not be included in the DTI analysis because of an adverse event, prompting early termination of their scan before the diffusion sequence was acquired.

3.2.5.2.1.5 Generation of Fractional Anisotropy Maps and Mean Diffusivity Maps

After preprocessing, the final number the scans that could be used for analysis were: nine acute patients, thirteen PTS patients, and seven control patients. We used Tract-Based Spatial Statistics (TBSS) to generate the fractional anisotropy and mean diffusivity scans necessary for further analysis and calculation of the ROI-based values.

3.2.5.2.1.6 Tract-Based Spatial Statistics (TBSS)

The TBSS pipeline consisted of three distinct steps, conducted using three scripts packaged with FSL. The first step was TBSS preprocessing, conducted using the TBSS_1_preproc script. This script zeroed the end slices and eroded the fractional anisotropy images, and created the necessary folders after for further analysis. This script generated a webpage (slicesdir.html) allowing for the screening of any overt problems with preprocessing. Next, direct nonlinear registration was run by aligning all the fractional anisotropy images to a FMRIB58_FA standard space image. The final script performed affine alignment to the 1x1x1 mm Montreal Neurological Institute 152 template transforming the original fractional anisotropy image into MNI space. Finally, all the subjects’ fractional anisotropy images were combined into one 4D image volume and skeletonized. The skeletonized image was viewed in FSLVIEW to observe the results of the TBSS processing.
3.2.5.2.1.7 Calculation of Region of Interest-Based FA and MD Values

We did not conduct voxel wise statistics on the fractional anisotropy data and instead opted to calculate the mean fractional anisotropy values for 27 predetermined Region of Interest (ROI) white matter tracts from the Johns Hopkins University (JHU) DTI Atlas provided by Dr. Susumu Mori (NeuroDebian-Team 2014). These ROI-based mean FA and MD values were then used in further statistical comparisons. This procedure was used for DTI analyses in publications currently being prepared in-house in other mTBI neuroimaging studies (Caroline Lewis, personal communication, 2015).

Mean diffusivity maps were generated in a similar fashion to fractional anisotropy, by running the three TBSS scripts, but the final processing step was completed using a custom TBSS script which projected the mean diffusivity maps onto the original fractional anisotropy data in order to generate a skeletonized mean diffusivity map. MD ROI values were calculated in a similar fashion using the binarized templates from the JHU Atlas from FSL. This process was completed separately for an acute versus control comparison, and a PTS versus control comparison.

3.2.5.2.2 Goal 2.2: To correlate white matter integrity and antisaccade performance

See sections 3.2.5.1.2 and 3.2.5.2.1 for notes on data preparation.

3.2.5.3 Aim 3

The goal of the third aim was to correlate the significant antisaccade variables that we found in our Aim 2 analysis with symptom load and executive functioning.

3.2.5.3.1 Goal 3.1: To determine the effect of injury on executive functioning and response inhibition

To measure executive functioning, a core set of tests within a larger battery was used, including the Stroop Color and Word Test, the Trail Making Test, and the phonemic fluency score from the Montréal Cognitive Assessment, English Version 3. This version of the MoCA was used instead of version 1 to mitigate test-retest effects with assessments in the head injury/neurosurgery clinic and emergency department for the PTS and Acute mTBI groups, respectively.
3.2.5.3.1.1 Stroop Color and Word Test

The Stroop Color and Word Test was administered with modified instructions from Golden (1945), reviewed by Dr. Grant Killian (Killian 1985). Each card contained 100 items, organized with 10 items per row. For the word trial, the stimulus included 100 words printed in black ink naming several colors. Participants were instructed to read the words as quickly as they could across the row from left to right, and if they completed reading all the words before the tester said “stop”, to start again from the beginning. If they made any errors I would let them know by saying "no", prompting a correction before continuing. Participants were stopped after 45 seconds and the number of covered items was operationally defined as the Stroop Word Score.

For the color trial, the stimulus included 100 colored rectangles, organized with 10 items per row. Participants were tested for color blindness, by instruction to name the colors as quickly as they could down the row from left to right, and if they completed the trial before the tester said stop, to start again from the beginning. Same as the word trial, if they made any errors the participant was notified, and they were stopped after 45 seconds; the number of words covered was operationally defined as the Stroop Color Score.

For the interference trial, the stimulus included 100 color words printed in color ink, which were incongruent (i.e., the word specified did not match the ink color of the word). Participants were instructed with the same speed and accuracy directions as the previous two trials, but this time to say the ink color in which the word is printed, and not what the word said. Again, participants were notified if they made any errors, and were stopped after 45 seconds; the number of words covered in this timeframe was operationally defined as the Stroop Color-Word Score.

3.2.5.3.1.2 Trail Making Test

The Trail Making Test was administered according to standardized instructions. Briefly:

For Trail Making A, participants were asked to connect the numbers from 1 to 25 in ascending order. They were instructed to do this as quickly as they could, and not lift their pen up from the paper once they began. A separate sample trial was performed prior to the full task (with numbers 1 to 5) to ensure participants understood what was expected. Corrections were made as necessary using standard instructions without stopping the timer. I operationally defined Trail
Making A performance as the time (in seconds) needed for participants to complete the task successfully. This task does not involve set switching.

For Trail Making B, participants were instructed to connect the numbers and letters in ascending order, from 1 to A, A to 2, 2 to B, and so forth until the end. They were instructed to do this as quickly as they could, and not lift their pen up from the paper once they began. A separate sample trial was performed prior to the full task (with numbers and letters up to “C”) to ensure participants understood the task. Corrections were made as necessary without stopping the timer. We operationally defined Trail Making B performance as the time (in seconds) needed for participants to complete the task successfully. We also calculated the difference in performance between TMT B and TMT A.

3.2.5.3.3 Phonemic Fluency

Participants’ phonemic fluency score was operationally defined as the number of “B” words that they could generate within one minute, as part of the MoCA English Version 3. Participants were given standard instructions.

3.2.5.3.2 Goal 3.2: To characterize symptom load and relate it to performance on the antisaccade performance and response inhibition tasks

3.2.5.3.2.1 Sport Concussion Assessment Tool

Means and standard deviations of all SCAT3 symptom assessment scale items were calculated. The symptom severity score was calculated by totaling the individual ratings for each SCAT3 item, for a maximum score of 132 (the standardized and recommended method of summarizing symptom burden on this questionnaire).

3.2.5.3.2.2 Rivermead Post-Concussion Symptom Questionnaire

Next, symptom load was characterized using the RPQ, a reliable and standardized questionnaire of symptom burden used for clinical care in our head injury clinic. We calculated a total symptom load score by summing the ratings on all 16 Rivermead items and ratings on two “other” Rivermead questions where participants were asked to name any other sequelae they
were currently experiencing, and to rate them on the same scale. Subscale scores for each participant were calculated on the Rivermead, including somatic, emotional, and cognitive subscores. The Rivermead somatic subscore was calculated using the sum rating of items: headache, dizziness, nausea or vomiting, sensitivity to noise, sleep disturbance, fatigue, blurred vision, light sensitivity, and double vision. The Rivermead emotional subscore was calculated using the sum rating of items: irritability, frustration or impatient, depressed or tearful, and restlessness. The Rivermead cognitive subscore was calculated using the sum rating of items: forgetfulness or poor memory, taking longer to think, and poor concentration. The RPQ and these subscores were previously used to summarize symptom load in traumatic brain injury patients (Smith-Seemiller, Fow et al. 2003) and have been validated in a confirmatory factor analysis. See sections 3.2.5.1.2 and 3.2.5.3.1 for the data preparation necessary for the correlational analyses.

3.2.6 Analysis Software and Hardware

All statistical analyses were conducted in GNU R 3.0.3 “Warm Puppy” for Windows (R-Core-Team. 2014). The same software was used to generate all data figures in this work. Drawings were created in Inkscape Vector Graphics Software for Windows 0.91. Neuroimaging analysis was completed on a 27-Inch iMac, with OS X 10.7.5. The custom R script that I wrote for the following statistical analyses is available upon request.

3.3 Aim 1 Statistical Design

My conclusions on this aim were obtained from cross-sectional analyses of pupillometry data between patients and controls, as well as consideration of the minimum NPi score.

3.3.1 Goal 1.1: Determine that patients had normal bilateral pupillary light reflex

Each participant’s NPi score was checked that it was above 3.0 bilaterally, indicating normal pupillary light reflex.
3.3.2  **Goal 1.2: To determine the effect of injury and time since injury on antisaccade performance**

I made the conclusions necessary for this aim from statistical analyses of antisaccade latency, accuracy, prosaccade error rate, and false error latency amongst other metrics.

All three groups were compared at once: Acute V1 (A1) vs. Control V1 (C1) vs. PTS V1 (P1), using the one-way ANOVA (or its non-parametric equivalent, the Kruskal-Wallis Test), with planned contrasts. The statistical analysis was conducted separately for right pupil. If homogeneity of variance could not be assumed with the results of Levene’s test, a one-way Welch’s F test was used. The contrasts were designed to first test for differences between the injury and control groups; then, a second contrast was designed to test for differences between the acute and PTS groups. This ensured valid statistical inference while doing group-wise comparisons (Field 2012). Categorical data was analyzed with Chi-Square tests and Fischer exact tests for cross-sectional comparisons, and McNemar tests for within-group comparisons as applicable.

To test antisaccade performance over time, A1 was compared with Acute V2 (A2) longitudinally using the paired t-test (or its non-parametric equivalent). To assess reliability of antisaccade measurement in healthy controls, C1 was longitudinally compared with Control V2 (C2), using the paired t-test (or its non-parametric equivalent).

3.4  **Aim 2 Statistical Design**

3.4.1  **Goal 2.1: To determine the effect of injury on white matter integrity**

Results that arose from cross-sectional comparisons between groups in fractional anisotropy and mean diffusivity allowed for localization of the structural diffuse axonal injury (DAI) potentially responsible for the saccadic characteristics observed in our patient groups. This increased explanatory power for any differences in saccadic parameters identified in our Aim 1 analyses. Fractional anisotropy and mean diffusivity were first compared between the acute scans and control scans, using Welch’s t-tests. If the data were not normal, the Wilcoxon rank-sum test was used for this comparison. Then, fractional anisotropy and mean diffusivity were compared between PTS and control scans, using Welch’s t-tests.
3.4.2 Goal 2.2: To correlate white matter integrity and antisaccade performance

Correlations were conducted between fractional anisotropy/mean diffusivity values of patients and their antisaccade parameters. Pearson correlations (or their non-parametric counterpart, the spearman correlation) were used to identify relationships between significantly different continuous variables in antisaccade performance in our Aim 1 Section 2 analyses, and ROIs exhibiting differences in FA or MD identified in our Aim 2 Section 1 analyses. The majority of MRI scans were conducted during the second visit (see Table 3). Hence, the antisaccade performance measurements at the A2 visit were selected for our analyses using Pearson correlations. For PTS correlations, correlational analyses were conducted using antisaccade data from the only PTS visit using Pearson correlations and their corresponding DTI data.

3.5 Aim 3 Statistical Design

3.5.1 Goal 3.1: To determine the effect of injury on executive functioning and response inhibition

Analyses of neuropsychological test results, including the verbal fluency subtest of the Montreal Cognitive Assessment (MoCA), the Trail Making Test A and B, and the Stroop Color-Word Interference Task provided the information required to determine the effect of injury on specific domains of executive functioning.

All three groups were compared at once: Acute V1 (A1) vs. Control V1 (C1) vs. PTS V1 (P1), using one-way ANOVAs (or the Kruskal-Wallis Test for data that was not parametrically distributed), with planned contrasts. If homogeneity of variance could not be assumed with the results of Levene’s test, one-way Welch’s F test was used. A first contrast tested for differences between the injury and control groups; then, a second contrast was designed to test for differences between the acute and PTS groups.

3.5.2 Goal 3.2: To characterize symptom load and relate it to performance on the antisaccade performance and response inhibition tasks

In the first analysis I described the symptom reporting of all participant groups to better understand the multiple sequelae that the participants experience using the Sport Concussion
Assessment Tool (SCAT) Version 3 (Symptom Scale), and Rivermead Post-Concussive Symptoms Questionnaire (RPQ). Both of these questionnaires have been used in the field and in the head injury clinic, respectively, to help identify symptoms after mTBI. The means and standard deviations of all groups at the first visit were calculated for each item in the SCAT3 Symptom Scale and RPQ, and subscale scores were also calculated. For both acute and PTS patients, two sets of correlations were performed between symptom load, executive functioning and significantly different continuous antisaccade performance variables at the first visit. Pearson correlations (or Spearman correlations if the data was not normal) were conducted. Longitudinal comparisons of SCAT3 and RPQ within the acute group on the first and second visit contributed towards my conclusions on this goal as well, and the results were considered with the longitudinal comparisons of antisaccade performance evolution in acute mTBI and healthy controls. A1 was compared with Acute V2 (A2) using the paired t-test (or its non-parametric equivalent).
4 CHAPTER 4: RESULTS

Most consented participants completed the antisaccade task without any major problems, although some needed rest before or after the task. One PTS participant could not complete the antisaccade task because the sharp low-powered red light exacerbated symptoms. Our antisaccade task took approximately 10 to 15 minutes to complete. In combination with the MRI scan DTI sequence (approximately 10 minutes, plus 30 minutes setup/takedown), executive functioning battery and questionnaires, the whole process would practically take ~1.5 hours for a targeted antisaccade research study.

4.1 Participant Characterization and Descriptions

Basic demographic characteristics of our participant groups are in Table 2.

Table 2: Study Participant Demographic Characteristics of Acute mTBI, PTS and Healthy Control Groups

<table>
<thead>
<tr>
<th>Participant Group</th>
<th>N (1st visit)</th>
<th>Mean Age (SD)</th>
<th>Sex (M:F)</th>
<th>Education (full years from Grade 1) Mean (SD)</th>
<th>GCS Score</th>
</tr>
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<tbody>
<tr>
<td>Acute mTBI V1</td>
<td>11</td>
<td>36.5 (17) years</td>
<td>7:4</td>
<td>15.8 (5) years</td>
<td>All 15 in Emergency Department (ED) All 15 at recruitment</td>
</tr>
<tr>
<td>PTS V1</td>
<td>15</td>
<td>42.5 (15) years</td>
<td>5:10</td>
<td>14.9 (4) years</td>
<td>30 min post injury or later upon reassessment in ED (based on ED chart or clinical note describing injury): GCS 13: 1 GCS 14: 1 GCS 15: 13 All 15 at recruitment</td>
</tr>
<tr>
<td>Healthy Control V1</td>
<td>10</td>
<td>35.5 (21) years</td>
<td>5:5</td>
<td>15.4 (1) years</td>
<td>All 15</td>
</tr>
</tbody>
</table>
The sample sizes were the highest in the PTS group (N = 15), followed by the acute mTBI group (N = 11), and finally the control group (N = 10). The acute mTBI group all had a GCS score of 15 after their injury and subsequently upon presentation and recruitment in the emergency department. The PTS group was more varied, with one person having a GCS of 13, one 14, and the rest GCS 15 after their injury (determined by chart review). All participants in the PTS group were GCS 15 at the time of recruitment. Other participant characteristics are in Table 3.

Table 3: Participant Characteristics

<table>
<thead>
<tr>
<th>Participant Group</th>
<th>Acute mTBI</th>
<th>PTS</th>
<th>Healthy Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Visit 1: 11</td>
<td>Visit 1: 15</td>
<td>Visit 1: 10</td>
</tr>
<tr>
<td></td>
<td>Visit 2: 9</td>
<td></td>
<td>Visit 2: 8</td>
</tr>
<tr>
<td>Testing Latency (Mean Days between injury and testing)</td>
<td>Visit 1: 4.73 [Range = 0, 9]</td>
<td>Visit 1: 460.2 [64-1832 (5 years)]. Median 8.2 months</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Visit 2: 22.56 [7, 45]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI Conducted on First or Second Visit</td>
<td>Visit 1: 2</td>
<td>Visit 1: 15</td>
<td>Visit 1: 3</td>
</tr>
<tr>
<td></td>
<td>Visit 2: 9</td>
<td></td>
<td>Visit 2: 7</td>
</tr>
<tr>
<td>History of Previous Concussion</td>
<td>Yes: 7</td>
<td>Yes: 5</td>
<td>Yes: 0</td>
</tr>
<tr>
<td></td>
<td>No: 4</td>
<td>No: 9</td>
<td>No: 10</td>
</tr>
<tr>
<td></td>
<td>No sure: 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant Hand</td>
<td>Left: 0</td>
<td>Left: 2</td>
<td>Left: 2</td>
</tr>
<tr>
<td></td>
<td>Right: 11</td>
<td>Right: 12</td>
<td>Right: 8</td>
</tr>
<tr>
<td></td>
<td>Ambidextrous: 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Language</td>
<td>English: 9</td>
<td>English: 9</td>
<td>English: 3</td>
</tr>
<tr>
<td></td>
<td>Farsi: 1</td>
<td>Spanish: 2</td>
<td>Spanish: 2</td>
</tr>
<tr>
<td></td>
<td>French: 1</td>
<td>Russian: 1</td>
<td>German: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Greek: 1</td>
<td>Japanese: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Italian: 1</td>
<td>Chinese: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arabic: 1</td>
<td>Tamil: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ukrainian: 1</td>
</tr>
<tr>
<td>If First Language not English, Mean Age at which first started learning English</td>
<td>13.0</td>
<td>10.3</td>
<td>5.43</td>
</tr>
<tr>
<td>History of Eye Disease or Past Eye Injury</td>
<td>Yes: 0  No: 11</td>
<td>Yes: 1  No: 14</td>
<td>Yes: 0  No: 10</td>
</tr>
<tr>
<td>Presence of Other Medical Conditions (comorbidities)</td>
<td>Yes: 5  No: 6</td>
<td>Yes: 8  No: 7</td>
<td>Yes: 3  No: 7</td>
</tr>
<tr>
<td>Alcohol Consumption History (Current or past consumption)</td>
<td>Yes: 10  No: 1</td>
<td>Yes: 12  No: 3</td>
<td>Yes: 6  No: 4</td>
</tr>
<tr>
<td>History of Drinking Problems and Treatment</td>
<td>Yes: 0  No: 11</td>
<td>Yes: 0  No: 15</td>
<td>Yes: 0  No: 10</td>
</tr>
<tr>
<td>Affective Disorder History (anxiety or depression)</td>
<td>Yes: 2  No: 9</td>
<td>Yes: 11  No: 4</td>
<td>Yes: 0  No: 10</td>
</tr>
<tr>
<td>Mean CES-D Score</td>
<td>M: 13.09  SD: 7.92</td>
<td>M: 23.2  SD: 10.8</td>
<td>M: 6.90  SD: 4.46</td>
</tr>
<tr>
<td>Vision Correction History</td>
<td>Yes: 7  No: 4</td>
<td>Yes: 14  No: 1</td>
<td>Yes: 5  No: 5</td>
</tr>
<tr>
<td>Hearing Difficulty History</td>
<td>Yes: 0  No: 11</td>
<td>Yes: 2  No: 13</td>
<td>Yes: 0  No: 10</td>
</tr>
</tbody>
</table>

The mean test latencies for acute mTBI patients between injury and their first and second visits were 4.7 days and 22.6 days, respectively. A significant proportion (64%; N = 7) had a history of previous concussion or head injury, and about half had comorbid medical conditions. 91% (N = 10) acute mTBI participants reported a significant history of alcohol consumption, although none endorsed a drinking problem that needed treatment. The mean CES-D score for this group was
13.1 points, below the 16 point cutoff for depression symptomatology. In terms of NPi score the minimum score for the left pupil in this group was 3.5 and the right eye was 3.0. 64% (N = 7) of the acute group needed corrected vision due to myopia or hyperopia.

The PTS group had their single visit a median of 8.2 months after their mTBI. A third of the PTS patients endorsed a history of previous concussion or head injury; about half (N = 8) had comorbid medical conditions. One PTS participant endorsed a previous history of eye injury but this was not due to the presenting injury and they had made a full recovery prior to recruitment. 80% (N = 12) of our PTS group endorsed a current or previous history of alcohol consumption, but none reported having problems with their drinking and seeking treatment. 73% (N = 11) reported a history of affective disorder. The latter was reflected in a mean CES-D score of 23.2, well above the 16 point cutoff for depressive symptomatology. In terms of NPi score, the minimum NPi in the left eye was 3.2 and the minimum NPi in the right eye was 4.0. 93% (N = 14) of the PTS group needed corrected vision due to myopia or hyperopia. No participant in the control group suffered a mTBI in the interval between the first and second antisaccade measures.

4.2 Aim 1

4.2.1 Goal 1.1

4.2.1.1 Patients had normal bilateral pupillary light reflex

The NPi score was ≥ 3.0 for all participants at all visits, indicating normal pupillary light reflex.

4.2.2 Goal 1.2

4.2.2.1 Significant Effect of Injury on Antisaccade Number, Median Latency, and Prosaccade Error Mean Duration

Results of this comparison are in Table 4.

Table 4: Cross-Sectional Comparisons of Antisaccade Performance in the First Visit

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>Acute mTBI &lt;br&gt;Visit 1</th>
<th>PTS &lt;br&gt;Visit 1</th>
<th>Healthy Control &lt;br&gt;Visit 1</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>14</td>
<td>9</td>
<td>NA</td>
</tr>
<tr>
<td>Correct Antisaccade Number Accepted (Count of Trials)</td>
<td>Total Number Accepted: 571 Trials</td>
<td>Total Number Accepted: 770 Trials</td>
<td>Total Number Accepted: 650 Trials</td>
<td>S: Chi Square Analysis: $X^2 (2) = 96.59, p = 1.06276 \times 10^{-21}$. Odds ratio (acute/ctrl): 1.079/2.600 = 0.415; odds ratio (PTS/ctrl): 1.222/2.600 = 0.47.</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Median Latency (ms)</td>
<td>278.36 (28.8)</td>
<td>272.86 (26.1)</td>
<td>241.17 (45.2)</td>
<td></td>
</tr>
<tr>
<td>Latency Rate (ms⁻¹)</td>
<td>0.003626 (0.000358)</td>
<td>0.003695 (0.000345)</td>
<td>0.004276 (0.000785)</td>
<td>S: One-Way ANOVA with planned contrasts (normality passed, homogen variance passed); F(2, 31) = 3.648, p = 0.0378 (2 observations deleted due to missingness). Planned contrast 1: Injury vs. Control: S: t(31) = 2.689, p = 0.0114 (two-tailed); Planned contrast 2: Acute vs. PTS: NS: t(31) = 0.416, p = 0.6805 (two-tailed).</td>
</tr>
<tr>
<td>Mean Duration (ms)</td>
<td>Mdn = 89.89 [45.1]</td>
<td>Mdn = 57.10 [20.8]</td>
<td>Mdn = 65.66 [23.1]</td>
<td>NS: H(2) = 2.5871, p = 0.2743.</td>
</tr>
<tr>
<td>Mean Amplitude (deg)</td>
<td>15.48 (5.98)</td>
<td>13.04 (6.14)</td>
<td>12.58 (5.36)</td>
<td>NS: F(2, 31) = 0.747, p = 0.482. (2 observations deleted due to missing values)</td>
</tr>
<tr>
<td>Mdn =</td>
<td>Mdn =</td>
<td>Mdn =</td>
<td>NS: H(2) = 0.5767, p = 0.7495</td>
<td></td>
</tr>
</tbody>
</table>
Mean Peak Velocity (deg/s) | 395.77 [158.7] | 396.49 [137.2] | 368.79 [232.4] |
Incorrect Prosaccade Median Latency (ms) | 178.64 (1.36) | 182.29 (28.9) | 193.17 (39.3) | NS: F (2, 31) = 0.689, p = 0.51. |
Incorrect Prosaccade Latency Rate (ms-1) | 0.00563 (4.47e-4) | 5.61e-3 (8.77e-4) | 5.36e-3 (1.04e-3) | NS: F (2, 31) = 0.334, p = 0.719. (2 observations deleted due to missing values). |
Incorrect Prosaccade Mean Duration (ms) | 54.07 (11.3) | 45.96 (3.99) | 45.73 (9.10) | S: F(2, 31) = 3.63, p = 0.03823. (2 observations deleted due to missing values). Planned contrast 1: Injury vs. Control: NS: t(31) = 1.325, p = 0.1947 (two-tailed); Planned contrast 2: Acute vs. PTS: S: t(31) = 2.425, p = 0.0213. (two-tailed) |
Incorrect Prosaccade Mean Peak Velocity (deg/s) | Mdn = 433.87 [88.96] | Mdn = 408.68 [102.58] | Mdn = 382.59 [125.00] | NS: H(2) = 0.6217, p = 0.7328. |

Table 5: Results in Longitudinal Comparisons of Antisaccade Performance in Acute mTBI

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>Acute mTBI V1</th>
<th>Acute mTBI V2</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8 (3 excluded as they did not have complete visit series)</td>
<td>8</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Antisaccade Number Accepted (Count of Trials)</td>
<td>Total Number Accepted: 376</td>
<td>Total Number Not Accepted: (800 - 376) = 424</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Antisaccade Median Latency (ms)</td>
<td>286.5 (29.78)</td>
<td>271.63 (34.24)</td>
<td>NS: $t(7) = 2.2064, p = 0.06314, 95% CI [-1.067044 30.817044]</td>
</tr>
<tr>
<td>Antisaccade Latency Rate (ms-1)</td>
<td>0.00352 (0.000366)</td>
<td>0.00373 (0.000462)</td>
<td>NS: $t(7) = -2.2646, p = 0.05794, 95% CI [-4.264635e-04 9.213488e-06]</td>
</tr>
<tr>
<td>Antisaccade Mean Duration (ms)</td>
<td>76.05 (36.86)</td>
<td>74.98 (25.17)</td>
<td>NS: $t(7) = 0.1042, p = 0.9199. 95% CI [-23.26761 25.41262]</td>
</tr>
<tr>
<td>Antisaccade Mean Amplitude (deg)</td>
<td>14.63 (6.20)</td>
<td>12.45 (2.69)</td>
<td>NS: $t(7) = 1.1182, p = 0.3004. 95% CI [-2.435425 6.805021].</td>
</tr>
<tr>
<td>Antisaccade Mean Peak Velocity (deg / s)</td>
<td>444.84 (100.08)</td>
<td>388.63 (81.65)</td>
<td>NS: $t(7) = 1.2431, p = 0.2538. 95% CI [-50.71111 163.13222]</td>
</tr>
<tr>
<td>Prosaccade Median Latency (ms)</td>
<td>177.25 (15.62)</td>
<td>184.62 (22.81)</td>
<td>NS: $t(7) = -0.9304, p = 0.3831, 95% CI [-25.23262 10.98262]</td>
</tr>
<tr>
<td>Prosaccade Latency Rate (ms-1)</td>
<td>0.00568 (0.000512)</td>
<td>0.00549 (0.000636)</td>
<td>NS: $t(7) = 0.9191, p = 0.3886. 95% CI [-0.0002972417 0.0006752417].</td>
</tr>
<tr>
<td>Prosaccade Mean Duration (ms)</td>
<td>52.03 (11.2)</td>
<td>50.68 (7.59)</td>
<td>NS: $t(7) = 0.4653, p = 0.6558. 95% CI [-5.504922 8.202308].</td>
</tr>
<tr>
<td>Prosaccade Mean Amplitude (deg)</td>
<td>10.44 (1.24)</td>
<td>10.04 (3.46)</td>
<td>NS: $t(7) = 0.2664, p = 0.7976. 95% CI [-3.165897 3.969822]</td>
</tr>
<tr>
<td>Prosaccade Mean Peak Velocity (deg / s)</td>
<td>467.92 (49.15)</td>
<td>434.12 (128.86)</td>
<td>NS: $t(7) = 0.6576, p = 0.5318. 95% CI [-87.75107 155.36557].</td>
</tr>
</tbody>
</table>
Table 6: Reliability of Antisaccade Performance in Healthy Control Participants

<table>
<thead>
<tr>
<th></th>
<th>Control V1 N (two excluded due to not having the full series of measurements)</th>
<th>Control V2</th>
<th>Results</th>
</tr>
</thead>
</table>
| **Antisaccade Number Accepted (count of trials)** | Total Number Accepted: 643  
Total Number Not Accepted: (800 - 643) = 157 | Total Number Accepted: 549  
Total Number Not Accepted: (800 - 549) = 251 | $X^2 (1) = 216.55$, $p = 2.2e-16$. Odds ratio (V2/V1): 4.0955/2.1873 = 1.87 x. |
| **Antisaccade Median Latency (ms)** | 231.44 (36.8) | 218.81 (28.8) | NS: $t(7) = 2.104$, $p = 0.07344$, 95% CI [-1.564065 26.814065] |
| **Antisaccade Latency Rate (ms-1)** | 0.00442 (0.000704) | 0.00464 (0.000608) | NS: $t(7) = -1.868$, $p = 0.1039$, 95% CI [-5.020795e-04 5.885449e-05]. |
| **Antisaccade Mean Duration (ms)** | 61.54 (17.37) | 65.53 (15.24) | NS: $t(7) = -0.5362$, $p = 0.6084$. 95% CI [-21.58937 13.60785]. |
| **Antisaccade Mean Amplitude (deg)** | 11.42 (4.36) | 10.48 (3.07) | NS: $t(7) = 0.9097$, $p = 0.3932$. 95% CI [-1.499525 3.374743]. |
| **Antisaccade Mean Peak Velocity (deg / s)** | 417.65 (139.86) | 363.51 (90.51) | NS: $t(7) = 1.2356$, $p = 0.2565$. 95% CI [-49.4633 157.7322]. |
| **Prosaccade Median Latency (ms)** | 189.31 (40.18) | 168.38 (24.59) | $t(7) = 3.155$, $p = 0.01604$. 95% CI [5.245297 36.629703]. |
| **Prosaccade** | 0.00547 (0.00105) | 0.00605 | $t(7) = -4.3025$, $p = $
<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency Rate (ms⁻¹)</td>
<td>(0.000849)</td>
<td>0.003556, 95% CI [0.0008883058 - 0.0002581942].</td>
<td></td>
</tr>
<tr>
<td>Prosaccade Mean</td>
<td>45.77 (9.73)</td>
<td>50.35 (7.26)</td>
<td>NS: t(7) = -1.3971, p = 0.2051. 95% CI [-12.356823 % 3.178572].</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosaccade Mean</td>
<td>8.06 (1.87)</td>
<td>8.39 (1.31)</td>
<td>NS: t(7) = -0.8069, p = 0.4463. 95% CI [-1.302061 % 0.6395252].</td>
</tr>
<tr>
<td>Amplitude (deg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosaccade Mean</td>
<td>387.93 (90.0)</td>
<td>368.58 (51.97)</td>
<td>NS: t(7) = 0.4867, p = 0.6414. 95% CI [-74.67404 - 113.37809].</td>
</tr>
<tr>
<td>Peak Velocity (deg/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A chi square test of independence was used to determine the effect of injury on the number of correct antisaccades that participants produced. This relationship was significant ($X^2 (2) = 95.59,$ $p = 1.1 \times 10^{-21}$); acute participants were 42% as likely to have an antisaccade trial performed correctly during the task as control participants. PTS participants were 47% as likely to have an antisaccade trial performed correctly during the task compared to control participants.

There was a significant omnibus group-wise difference on median antisaccade latency, $F(2, 31) = 3.65, p = 0.04.$ An omnibus difference indicates there is a significant difference overall between the groups, but the statistic itself cannot determine which group is significantly different from the others. Planned comparisons revealed that there was a significant effect of injury leading to greater antisaccade latency compared to controls (Mean, $M = 241.17$ ms), $t(31) = 2.69, p = 0.01$ (two-tailed) on antisaccade median latency, but there was no significant difference between Acute mTBI ($M = 278.36$ ms) and PTS ($M = 272.86$ ms) groups, $t(31) = 0.42, p = 0.68$ (two-tailed). The boxplot of this data is depicted graphically in Figure 10.
Figure 10: Boxplot of antisaccade latency performance across participant groups. Injury groups had significantly greater antisaccade median latency than the control group, but there was no difference between injury groups.

There was also a significant omnibus group-wise difference in mean incorrect prosaccade duration, $F(2, 31) = 3.63, p = 0.038$. Planned comparisons identified no significant effect of injury compared to controls ($M = 45.73$ ms), $t(31) = 1.325, p = 0.1947$ (two-tailed), but significantly higher mean prosaccade duration in the Acute mTBI ($M = 54.07$ ms) group compared to the PTS group ($M = 45.96$ ms), $t(31) = 2.425, p = 0.0213$. (two-tailed). A boxplot of this data is shown in Figure 11.
Figure 11: Boxplot of prosaccade duration across participant groups. A significant omnibus difference was observed between groups but no difference between injury groups and control group. There was a significant difference between acute and PTS groups with the former having higher mean duration.

Group-wise cross-sectional comparisons on the first visit between acute mTBI, PTS and Control groups were not significant for correct antisaccade latency, mean duration, mean amplitude, and mean peak velocity. No significant differences were found in terms of incorrect prosaccade median latency, latency rate, mean amplitude, and mean peak velocity.

There were no significant differences within the same acute participants between the first and second visits on all the other antisaccade performance metrics, but our sample size was low so it is difficult to draw definitive conclusions.
4.2.2.2  Reliability of Antisaccade Performance in Healthy Control Participants on Most Parameters

There was a significant effect of time between visits on the number of accepted antisaccades in healthy control participants based on McNemar’s analysis ($X^2 (1) = 216.55, p = 2.2e-16$). Participants were 1.87 times more likely to have an accepted antisaccade trial on the first visit compared to the second visit. All other antisaccade performance metrics were not significant between the two visits.

In terms of prosaccade error measurements, control participants had significantly greater prosaccade error median latency on the first visit ($M = 189.31$ ms) compared to the second visit ($M = 168.38$), $t(7) = 3.16, p = 0.016$. They also had significantly lower prosaccade error latency rate on the first visit ($M = 0.00547$) compared to the second visit ($M = 0.00605$), $t(7) = -4.303, p = 0.004$. All other prosaccade error performance variables were not significant between the two visits.

4.3  Aim 2

4.3.1  Goal 2.1

4.3.1.1  Significant Effect of Injury on White Matter Integrity in the Corpus Callosum Splenium and Corticospinal Tract in Acute mTBI and PTS, Respectively

Detailed results of these comparisons are in Tables 7 and 8, respectively. There was a significantly greater mean diffusivity in the acute (Median, Mdn = 0.000805) group compared to the control group (Mdn = 0.000766) in the splenium of the corpus callosum, with a Wilcoxon rank sum test ($p = 0.03112$). There were no significant differences between groups in fractional anisotropy, and in mean diffusivity in the other regions of interest studied.
### Table 7: Effect of mTBI on acutely injured white matter, as measured by FA and MD

<table>
<thead>
<tr>
<th>White Matter Tract ROI (JHU Atlas) Mean Values Unless Otherwise Noted</th>
<th>Acute FA</th>
<th>Control FA</th>
<th>Significance</th>
<th>Acute MD (x10^-4)</th>
<th>Control MD (x10^-4)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Brain Average</td>
<td>0.419</td>
<td>0.419</td>
<td>0.9411</td>
<td>785</td>
<td>777</td>
<td>0.4654</td>
</tr>
<tr>
<td>Anterior Thalamic Radiation, Left</td>
<td>0.427</td>
<td>0.430</td>
<td>0.7626</td>
<td>783</td>
<td>782</td>
<td>0.9356</td>
</tr>
<tr>
<td>Anterior Thalamic Radiation, Right</td>
<td>0.427</td>
<td>0.426</td>
<td>0.9062</td>
<td>807</td>
<td>798</td>
<td>0.413</td>
</tr>
<tr>
<td>Anterior Segment, Left</td>
<td>0.444</td>
<td>0.433</td>
<td>0.3991</td>
<td>747</td>
<td>744</td>
<td>0.8535</td>
</tr>
<tr>
<td>Anterior Segment, Right</td>
<td>0.429</td>
<td>0.418</td>
<td>0.3332</td>
<td>749</td>
<td>737</td>
<td>0.4812</td>
</tr>
<tr>
<td>Corpus Callosum, Whole</td>
<td>0.659</td>
<td>0.664</td>
<td>0.7548</td>
<td>854</td>
<td>819</td>
<td>0.1383</td>
</tr>
<tr>
<td>Corpus Callosum, Body</td>
<td>0.599</td>
<td>0.610</td>
<td>0.6355</td>
<td>910</td>
<td>867</td>
<td>0.135</td>
</tr>
<tr>
<td>Corpus Callosum, Genu</td>
<td>0.673</td>
<td>0.678</td>
<td>0.7073</td>
<td>816</td>
<td>788</td>
<td>0.1949</td>
</tr>
<tr>
<td>Corpus Callosum, Splenium</td>
<td>Mdn = 0.735</td>
<td>Mdn = 0.726</td>
<td>0.7577</td>
<td>Mdn = 805</td>
<td>Mdn = 766</td>
<td>0.03112*</td>
</tr>
<tr>
<td>Cingulum, Left</td>
<td>0.471</td>
<td>0.474</td>
<td>0.8075</td>
<td>778</td>
<td>766</td>
<td>0.4493</td>
</tr>
<tr>
<td>Cingulum, Right</td>
<td>0.450</td>
<td>0.455</td>
<td>0.6307</td>
<td>792</td>
<td>764</td>
<td>0.09395</td>
</tr>
<tr>
<td>Corticospinal Tract, Left</td>
<td>0.503</td>
<td>0.505</td>
<td>0.8957</td>
<td>743</td>
<td>751</td>
<td>0.3912</td>
</tr>
<tr>
<td>Corticospinal Tract, Right</td>
<td>0.499</td>
<td>0.497</td>
<td>0.8690</td>
<td>751</td>
<td>760</td>
<td>0.2645</td>
</tr>
<tr>
<td>Forceps Major</td>
<td>0.668</td>
<td>0.678</td>
<td>0.6019</td>
<td>816</td>
<td>810</td>
<td>0.8059</td>
</tr>
<tr>
<td>Forceps Minor</td>
<td>0.522</td>
<td>0.525</td>
<td>0.8325</td>
<td>782</td>
<td>768</td>
<td>0.3797</td>
</tr>
<tr>
<td>Inferior Fronto-Occipital Fasciculus, Left</td>
<td>0.422</td>
<td>0.430</td>
<td>0.4808</td>
<td>789</td>
<td>789</td>
<td>0.971</td>
</tr>
<tr>
<td>Inferior Fronto-Occipital Fasciculus, Right</td>
<td>0.428</td>
<td>0.426</td>
<td>0.8858</td>
<td>803</td>
<td>795</td>
<td>0.5014</td>
</tr>
<tr>
<td>Inferior Longitudinal Fasciculus, Left</td>
<td>0.417</td>
<td>0.423</td>
<td>0.4973</td>
<td>Mdn = 807</td>
<td>Mdn = 817</td>
<td>0.2228</td>
</tr>
<tr>
<td>Inferior Longitudinal Fasciculus, Right</td>
<td>0.404</td>
<td>0.403</td>
<td>0.9626</td>
<td>819</td>
<td>812</td>
<td>0.5448</td>
</tr>
<tr>
<td>Internal Capsule</td>
<td>0.527</td>
<td>0.527</td>
<td>0.9822</td>
<td>766</td>
<td>765</td>
<td>0.9074</td>
</tr>
<tr>
<td>Long Segment, Left</td>
<td>0.483</td>
<td>0.481</td>
<td>0.8783</td>
<td>Mdn = 785</td>
<td>Mdn = 800</td>
<td>0.6333</td>
</tr>
</tbody>
</table>
Detailed results of the PTS comparison are in Table 8 below. There was a significantly lower mean diffusivity in the PTS (M = 0.000743) group compared to the control group (M = 0.000761) in the corticospinal tract, with a Welch’s t-test (p = 0.04917). There were no significant differences between groups in fractional anisotropy and in mean diffusivity in the other regions of interest studied.

Table 8: Effect of mTBI on chronically injured white matter, as measured by FA and MD

<table>
<thead>
<tr>
<th>White Matter Tract ROI (JHU Atlas)</th>
<th>PTS FA</th>
<th>Control FA</th>
<th>Significance</th>
<th>PTS MD (x10^-4)</th>
<th>Control MD (x10^-4)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Brain Average</td>
<td>0.423</td>
<td>0.422</td>
<td>0.8732</td>
<td>769</td>
<td>776</td>
<td>0.546</td>
</tr>
<tr>
<td>Anterior Thalamic Radiation, Left</td>
<td>0.436</td>
<td>0.436</td>
<td>0.9485</td>
<td>766</td>
<td>776</td>
<td>0.3946</td>
</tr>
<tr>
<td>Anterior Thalamic Radiation, Right</td>
<td>0.435</td>
<td>0.431</td>
<td>0.6248</td>
<td>785</td>
<td>789</td>
<td>0.699</td>
</tr>
<tr>
<td>Anterior Segment, Left</td>
<td>0.433</td>
<td>0.431</td>
<td>0.8676</td>
<td>732</td>
<td>746</td>
<td>0.3757</td>
</tr>
<tr>
<td>Anterior Segment, Right</td>
<td>0.427</td>
<td>0.423</td>
<td>0.6538</td>
<td>726</td>
<td>736</td>
<td>0.4983</td>
</tr>
<tr>
<td>Corpus Callosum, Whole</td>
<td>0.661</td>
<td>0.664</td>
<td>0.7922</td>
<td>829</td>
<td>819</td>
<td>0.6191</td>
</tr>
<tr>
<td>Corpus Callosum, Body</td>
<td>0.598</td>
<td>0.611</td>
<td>0.4872</td>
<td>886</td>
<td>867</td>
<td>0.4568</td>
</tr>
<tr>
<td>Corpus Callosum, Genu</td>
<td>0.682</td>
<td>0.678</td>
<td>0.7636</td>
<td>792</td>
<td>788</td>
<td>0.8533</td>
</tr>
<tr>
<td>Corpus Callosum, Splenium</td>
<td>0.728</td>
<td>0.725</td>
<td>0.7799</td>
<td>Mdn = 787</td>
<td>Mdn = 800</td>
<td>0.1773</td>
</tr>
<tr>
<td>Cingulum, Left</td>
<td>0.469</td>
<td>0.472</td>
<td>0.7342</td>
<td>760</td>
<td>765</td>
<td>0.7151</td>
</tr>
<tr>
<td>Cingulum, Right</td>
<td>0.445</td>
<td>0.448</td>
<td>0.7036</td>
<td>Mdn = 763</td>
<td>Mdn = 763</td>
<td>0.7212</td>
</tr>
<tr>
<td>Structure</td>
<td>FA1</td>
<td>FA2</td>
<td>SD</td>
<td>Median (Mdn)</td>
<td>Mdn ± SD</td>
<td>p-value</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>-------</td>
<td>--------------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>Corticospinal Tract, Left</td>
<td>0.508</td>
<td>0.507</td>
<td>0.8954</td>
<td>Mdn = 732</td>
<td>759</td>
<td>0.1905</td>
</tr>
<tr>
<td>Corticospinal Tract, Right</td>
<td>0.508</td>
<td>0.499</td>
<td>0.3835</td>
<td>Mdn = 743</td>
<td>761</td>
<td><strong>0.04917</strong></td>
</tr>
<tr>
<td>Forceps Major</td>
<td>0.670</td>
<td>0.670</td>
<td>0.9788</td>
<td>798</td>
<td>810</td>
<td>0.6443</td>
</tr>
<tr>
<td>Forceps Minor</td>
<td>0.525</td>
<td>0.522</td>
<td>0.8271</td>
<td>757</td>
<td>769</td>
<td>0.4397</td>
</tr>
<tr>
<td>Inferior Fronto-Occipital Fasciculus, Left</td>
<td>0.431</td>
<td>0.433</td>
<td>0.8461</td>
<td>776</td>
<td>789</td>
<td>0.3445</td>
</tr>
<tr>
<td>Inferior Fronto-Occipital Fasciculus, Right</td>
<td>0.435</td>
<td>0.426</td>
<td>0.4722</td>
<td>784</td>
<td>795</td>
<td>0.3996</td>
</tr>
<tr>
<td>Inferior Longitudinal Fasciculus, Left</td>
<td>0.421</td>
<td>0.425</td>
<td>0.6737</td>
<td>Mdn = 798</td>
<td>817</td>
<td>0.1041</td>
</tr>
<tr>
<td>Inferior Longitudinal Fasciculus, Right</td>
<td>0.411</td>
<td>0.403</td>
<td>0.4618</td>
<td>799</td>
<td>812</td>
<td>0.3026</td>
</tr>
<tr>
<td>Internal Capsule</td>
<td>0.533</td>
<td>0.527</td>
<td>0.6272</td>
<td>754</td>
<td>765</td>
<td>0.3183</td>
</tr>
<tr>
<td>Long Segment, Left</td>
<td>0.482</td>
<td>0.481</td>
<td>0.9464</td>
<td>784</td>
<td>797</td>
<td>0.4335</td>
</tr>
<tr>
<td>Long Segment, Right</td>
<td>0.372</td>
<td>0.375</td>
<td>0.7941</td>
<td>Mdn = 703</td>
<td>725</td>
<td>0.2749</td>
</tr>
<tr>
<td>Posterior Segment, Left</td>
<td>0.477</td>
<td>0.481</td>
<td>0.7163</td>
<td>796</td>
<td>814</td>
<td>0.2412</td>
</tr>
<tr>
<td>Posterior Segment, Right</td>
<td>0.482</td>
<td>0.476</td>
<td>0.5953</td>
<td>799</td>
<td>814</td>
<td>0.2923</td>
</tr>
<tr>
<td>Superior Longitudinal Fasciculus, Left</td>
<td>0.419</td>
<td>0.420</td>
<td>0.8868</td>
<td>759</td>
<td>770</td>
<td>0.4069</td>
</tr>
<tr>
<td>Superior Longitudinal Fasciculus, Right</td>
<td>0.400</td>
<td>0.398</td>
<td>0.8252</td>
<td>754</td>
<td>761</td>
<td>0.6396</td>
</tr>
<tr>
<td>Uncinate Fasciculus, Left</td>
<td>0.396</td>
<td>0.395</td>
<td>0.9385</td>
<td>747</td>
<td>764</td>
<td>0.2377</td>
</tr>
<tr>
<td>Uncinate Fasciculus, Right</td>
<td>0.399</td>
<td>0.394</td>
<td>0.7059</td>
<td>764</td>
<td>778</td>
<td>0.2971</td>
</tr>
</tbody>
</table>

As an exploratory estimate, a statistician on our research team (see contributions) conducted a power analysis to determine the sample size needed to achieve 80% power for the whole corpus callosum, measuring FA in PTS patients. The sample size required is 1216 in both groups (Dr. Rowan Jing, personal communication, 2015). Hence, if a targeted prospective study were to focus specifically on whole corpus callosum, a much larger sample size would be needed to successfully detect a significant effect of injury in that area (if a true effect really existed).
4.3.2 Goal 2.2

4.3.2.1 Significant Correlation Between Acutely Injured White Matter Integrity and Antisaccade Performance

A significant positive relationship was identified with the Spearman correlation between the mean diffusivity in the splenium of the corpus callosum and the median antisaccade latency at the second visit for acute mTBI patients ($\text{Rho (8)} = 0.9048, p = 0.00045$). This relationship is depicted graphically in Figure 12 below.

![Graph showing the significant positive correlation between anteriorly injured white matter integrity in the corpus callosum splenium and antisaccade latency performance.](image)

Figure 12: Significant positive correlation between acutely injured white matter integrity in the corpus callosum splenium and antisaccade latency performance.

The correlation between the prosaccade error mean duration and corpus callosum splenium mean diffusivity was not significant ($\text{rho (84)} = 0, p = 1$).
4.3.2.2  No Correlation Between White Matter Integrity in Persistent Traumatic Symptom Patients and Antisaccade Performance

There were no significant correlations between right corticospinal tract mean diffusivity and antisaccade median latency ($r = -0.129$, $p = 0.6896$)/prosaccade mean duration ($r = 0.239$, $p = 0.4534$) for PTS patients at their only visit.

4.4  Aim 3

4.4.1  Goal 3.1

4.4.1.1  Significant Effect of Injury on Response Interference, Attention Domains of Executive Functioning

Executive functioning results are found in Table 9. Stroop color performance was significantly impaired (covered less items in 45 seconds) between groups with a one-way ANOVA, $F(2, 33) = 4.666$, $p = 0.0164$. The first planned contrast revealed a significant effect of injury compared to controls, $t(33) = -2.93$, $p = 0.006$ with the injury groups having poorer performance than controls. However, the second planned contrast did not find significant differences between acute mTBI and PTS performance within the injury bracket, $t(33) = 0.632$, $p = 0.532$.

Impaired stroop color-word performance means that participants covered fewer items than controls in the same 45 second timeframe. There was a significant difference between groups on this measurement with a one-way ANOVA, $F(2, 33) = 6.587$, $p = 0.00392$. The first planned contrast revealed a significant effect of injury compared to controls, $t(33) = -3.601$, $p = 0.001$ with the injury groups having poorer performance (covering less items in 45 seconds) compared to the controls. The second planned contrast did not find significant differences between acute and PTS groups in injured bracket, $t(33) = 0.875$, $p = 0.875$. An extreme statistical outlier was detected in the stroop color-word score for the control group, but in double-checking the raw data there was no study-specific reason to take it out. A non-parametric Kruskal-Wallis Test (more robust to outliers and does not require assumptions of normality) was performed to verify the groupwise difference identified in the ANOVA. This was supported ($X^2 (2) = 8.42$, $p = 0.015$). This result is depicted graphically in Figure 13 below.
Figure 13: Stroop color-word score performance (number of items covered in 45 seconds) across participant groups. Injury groups performed significantly worse than the control group on this task, but there was no significant difference between injury groups.

Trail Making Test A performance was also significantly different between groups with a Kruskal-Wallis Test, $H(2) = 6.426$, $p = 0.04$. Full results of the executive functioning comparison results are in Table 9 below.
### Table 9: Cross-Sectional Comparisons of Executive Functioning in the First Visit

<table>
<thead>
<tr>
<th></th>
<th>Acute V1</th>
<th>PTS V1</th>
<th>Control V1</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Stroop Color Score</strong></td>
<td>75.82 (17.3)</td>
<td>72.4 (10.2)</td>
<td>89.0 (13.6)</td>
<td>(S: F(2, 33) = 4.666, p = 0.0164. Planned contrast 1: Injury vs. Control: S: t(33) = -2.93, p = 0.00615 (two-tailed); Planned contrast 2: Acute vs. PTS: NS: t(33) = 0.632, p = 0.532 (two-tailed))</td>
</tr>
<tr>
<td>(in 45 seconds)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stroop Word Score</strong></td>
<td>97.82 (21.5)</td>
<td>94.73 (15.2)</td>
<td>111.3 (10.8)</td>
<td>(NS: F(2, 33) = 3.222, p = 0.0527.)</td>
</tr>
<tr>
<td>(Number covered in 45 seconds)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stroop Number of Interference Items Covered</strong></td>
<td>39.73 (9.65)</td>
<td>39.13 (7.06)</td>
<td>52.10 (12.0)</td>
<td>(S: F(2, 33) = 6.587, p = 0.00392. Planned contrast 1: Injury vs. Control: S: t(33) = -3.601, p = 0.00103 (two-tailed); Planned contrast 2: NS: Acute vs. PTS: NS: t(33) = 0.875, p = 0.875 (two-tailed))</td>
</tr>
<tr>
<td>(in 45 seconds)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MoCA Phonemic Word Fluency</strong></td>
<td>(N = 7) 10.0 (4.90)</td>
<td>(N = 12) 12.2 (5.49)</td>
<td>(N = 9) 14.8 (4.97)</td>
<td>(NS: F(2, 25) = 1.703, p = 0.203.)</td>
</tr>
<tr>
<td>(Number of B-words in 1 minute)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trail Making Test B- Time to Completion (s)</strong></td>
<td>Mdn = 63.0</td>
<td>Mdn = 46.0</td>
<td>Mdn = 39.0</td>
<td>(NS: H(2) = 5.541, p =)</td>
</tr>
</tbody>
</table>


4.4.2 Goal 3.2:

4.4.2.1 Symptom Load Characterized using Two Self-Report Measures was Correlated to Performance on the Antisaccade Task and Response Inhibition in Acute mTBI

The mean and standard deviation scores for each item in the SCAT3 Symptom Assessment Scale are in Table 10. The SCAT3 Total score and Symptom Severity Score were also calculated for all three participant groups at the first visit. PTS patients consistently scored higher on the SCAT3 symptom severity score (M = 52.9), total score and individual items compared to acute patients (M = 31.2) and healthy controls (M = 2.30), based on qualitative comparison of their mean ratings. Table 10 below shows descriptive statistics for the SCAT3 questionnaire.

Table 10: Descriptive Statistics of SCAT3 Self-Report for all Three Groups at First Visit

<table>
<thead>
<tr>
<th>Item</th>
<th>Acute mTBI Visit 1</th>
<th>PTS Visit 1</th>
<th>Healthy Control Visit 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>SCAT3 Headache</td>
<td>Mean = 2.36, SD = (1.29)</td>
<td>2.20 (1.70)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td>SCAT3 &quot;Pressure in Head&quot;</td>
<td>2.27 (1.62)</td>
<td>2.80 (1.66)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SCAT3 Neck Pain</td>
<td>1.45 (1.21)</td>
<td>2.87 (2.03)</td>
<td>0.20 (0.63)</td>
</tr>
<tr>
<td>SCAT3 Nausea or Vomiting</td>
<td>0.36 (1.21)</td>
<td>0.60 (1.18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SCAT3 Dizziness</td>
<td>1.18 (1.47)</td>
<td>2.07 (1.67)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SCAT3 Blurred Vision</td>
<td>0.18 (0.40)</td>
<td>1.40 (1.76)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SCAT3 Balance Problems</td>
<td>1.18 (1.66)</td>
<td>1.40 (1.68)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SCAT3 Sensitivity to Light</td>
<td>1.82 (1.89)</td>
<td>2.67 (1.35)</td>
<td>0.30 (0.67)</td>
</tr>
<tr>
<td>SCAT3 Sensitivity to Noise</td>
<td>1.18 (1.60)</td>
<td>2.47 (1.85)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td>SCAT3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Feeling Slowed Down</td>
<td>1.73 (1.35)</td>
<td>2.93 (2.37)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Feeling like “in a fog”</td>
<td>1.09 (1.22)</td>
<td>2.27 (2.43)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>“Don’t Feel Right”</td>
<td>1.91 (1.45)</td>
<td>3.13 (2.07)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Difficulty Concentrating</td>
<td>1.55 (1.75)</td>
<td>3.33 (2.06)</td>
<td>0.30 (0.95)</td>
</tr>
<tr>
<td>Difficulty Remembering</td>
<td>2.27 (2.45)</td>
<td>2.87 (2.00)</td>
<td>0.30 (0.95)</td>
</tr>
<tr>
<td>Fatigue or Low Energy</td>
<td>1.91 (2.02)</td>
<td>3.20 (1.93)</td>
<td>0.60 (1.07)</td>
</tr>
<tr>
<td>Confusion</td>
<td>1.73 (1.56)</td>
<td>2.13 (2.26)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>1.45 (1.57)</td>
<td>2.13 (1.92)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Trouble Falling Asleep</td>
<td>1.00 (1.18)</td>
<td>2.20 (1.86)</td>
<td>0.30 (0.67)</td>
</tr>
<tr>
<td>More Emotional</td>
<td>1.09 (1.14)</td>
<td>2.73 (1.67)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Irritability</td>
<td>1.55 (1.81)</td>
<td>2.73 (1.62)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sadness</td>
<td>0.82 (1.25)</td>
<td>2.00 (1.77)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nervous or Anxious</td>
<td>1.00 (1.10)</td>
<td>2.73 (2.09)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td>Symptoms worse with Physical Activity?</td>
<td>Yes: 5. No: 6</td>
<td>Yes: 12. No: 3</td>
<td>Yes: 0. No: 10</td>
</tr>
</tbody>
</table>

| SCAT3 Total (/ 22) | 13.3 (4.76)  | 16.33 (5.91)  | 1.30 (2.26)   |
| SCAT3 Symptom Severity Score (/ 132) | 31.18 (21.6) | 52.87 (31.0)  | 2.30 (3.95)   |

The mean and standard deviation scores for each item in the RPQ are detailed in Table 11 below. The RPQ Somatic, Emotional and Cognitive Subscores, as well as total Rivermead Symptom Load were tabulated as well.
Table 11: Descriptive Statistics of RPQ Self-Report for all Three Groups at First Visit

<table>
<thead>
<tr>
<th></th>
<th>Acute mTBI</th>
<th>PTS</th>
<th>Healthy Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>11</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td><strong>RPQ Headaches</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, M = 2.27</td>
<td>2.67 (1.35)</td>
<td></td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td>Standard Deviation, SD = (0.90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RPQ Feelings of Dizziness</strong></td>
<td>1.45 (1.29)</td>
<td>2.33 (1.05)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>RPQ Nausea and/or Vomiting</strong></td>
<td>0.73 (1.19)</td>
<td>1.13 (1.25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>RPQ Noise Sensitivity</strong></td>
<td>1.09 (1.51)</td>
<td>2.53 (1.30)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Sleep Disturbance</strong></td>
<td>1.00 (1.41)</td>
<td>2.40 (1.35)</td>
<td>0.40 (0.84)</td>
</tr>
<tr>
<td><strong>RPQ Fatigue, tiring more easily</strong></td>
<td>2.00 (1.26)</td>
<td>2.93 (0.80)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Being Irritable, easily angered</strong></td>
<td>1.18 (1.25)</td>
<td>2.67 (1.05)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Feeling Depressed or Tearful</strong></td>
<td>0.45 (0.69)</td>
<td>2.13 (1.06)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Feeling Frustrated or Impatient</strong></td>
<td>1.09 (1.14)</td>
<td>2.53 (1.19)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Forgetfulness, Poor memory</strong></td>
<td>1.27 (1.42)</td>
<td>2.67 (1.29)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>RPQ Poor Concentration</strong></td>
<td>1.36 (1.29)</td>
<td>2.67 (1.23)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Taking Longer to Think</strong></td>
<td>1.36 (1.36)</td>
<td>3.07 (1.10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>RPQ Blurred Vision</strong></td>
<td>0.27 (0.65)</td>
<td>1.60 (1.45)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>RPQ Light Sensitivity</strong></td>
<td>1.27 (1.27)</td>
<td>2.73 (1.03)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Double Vision</strong></td>
<td>0 (0)</td>
<td>1.27 (1.60)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>RPQ Restlessness</strong></td>
<td>0.55 (0.93)</td>
<td>1.87 (1.19)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td><strong>RPQ Any Other Difficulties 1?</strong></td>
<td>Yes: 3. No: 8</td>
<td>Yes: 11. No: 4</td>
<td>Yes: 0. No: 10</td>
</tr>
<tr>
<td><strong>RPQ Other 1</strong></td>
<td>0.64 (1.12)</td>
<td>2.60 (1.72)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>RPQ Any Other Difficulties 2?</strong></td>
<td>Yes: 0. No: 11</td>
<td>Yes: 3. No: 12</td>
<td>Yes: 0. No: 10</td>
</tr>
<tr>
<td><strong>RPQ Other 2</strong></td>
<td>0 (0)</td>
<td>0.73 (1.53)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Rivermead Somatic</td>
<td>Rivermead Emotional</td>
<td>Rivermead Cognitive</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------</td>
<td>---------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>10.09 (5.09)</td>
<td>19.60 (6.98)</td>
<td>0.80 (1.93)</td>
</tr>
<tr>
<td></td>
<td>3.27 (3.10)</td>
<td>9.20 (3.86)</td>
<td>0.40 (1.26)</td>
</tr>
<tr>
<td></td>
<td>4.00 (3.52)</td>
<td>8.40 (3.31)</td>
<td>0.10 (0.32)</td>
</tr>
<tr>
<td></td>
<td>18 (10.3)</td>
<td>40.53 (14.0)</td>
<td>1.30 (3.47)</td>
</tr>
</tbody>
</table>

Pearson correlations suggested there was a significant positive correlation between antisaccade median latency and SCAT3 symptom severity score, $r^2 = 0.480$, $p = 0.02$ in the acute mTBI patients at their first visit. This relationship is graphically depicted in Figure 14.

**Figure 14**: Significant positive correlation between SCAT3 symptom severity score and antisaccade median latency performance in acute mTBI patients.
There was a significant negative correlation between antisaccade median latency and stroop color-word score, $r^2 = 0.439$, $p = 0.026$ in the acute mTBI patients at the first visit. This relationship is graphically depicted in Figure 15.

**Figure 15:** Significant negative correlation between Stroop Color-Word score and antisaccade latency in acute mTBI patients.

There were no significant relationships between antisaccade median latency and rivermead symptom load (although this relationship was trending towards significance), as well as between prosaccade error mean duration and all symptom burden and executive functioning variables in the acute mTBI patients at First Visit. Full correlation results are in the Table 12 below.
Table 12: Correlations between Selected Variables of Symptom Load and Antisaccade Performance, Acute mTBI at First Visit

<table>
<thead>
<tr>
<th>Symptom Load and Executive Functioning Selected Summary Scores / Selected Antisaccade Performance Variables</th>
<th>Antisaccade Median Latency (ms)</th>
<th>Prosaccade Mean Duration (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCAT3 Symptom Severity Score</td>
<td>$S: \ t = 2.8847, \ df = 9, \ p = 0.01804, \ r = 0.693, \ 95% \ CI [0.1595979, 0.9132689]$.</td>
<td>$NS: \ t = 2.0844, \ df = 9, \ p = 0.06678, \ r = 0.571, \ 95% \ CI [-0.04451121, 0.87199928]$.</td>
</tr>
<tr>
<td>Rivermead Symptom Load</td>
<td>$NS: \ t = 2.2052, \ df = 9, \ p = 0.05487, \ r = 0.592, \ 95% \ CI [-0.01178237, 0.87962795]$.</td>
<td>$NS: \ t = 1.3557, \ df = 9, \ p = 0.2082, \ r = 0.4118, \ 95% \ CI [-0.2497729, 0.8112701]$.</td>
</tr>
<tr>
<td>Stroop Color-Word Score (in 45 seconds)</td>
<td>$S: \ t = -2.6513, \ df = 9, \ p = 0.02642, \ r = -0.6622, \ 95% \ CI [-0.9032721, -0.1034386]$.</td>
<td>$NS: \ t = 0.6409, \ df = 9, \ p = 0.5375, \ r = 0.2089, \ 95% \ CI [-0.4469672, 0.7187237]$.</td>
</tr>
</tbody>
</table>

There were no significant relationships between symptom load scores on the SCAT3 and RPQ with antisaccade median latency or prosaccade error mean duration for the PTS group (see Table 13 below)

Table 13: Correlations between Selected Variables of Symptom Load and Antisaccade Performance, PTS Participants

<table>
<thead>
<tr>
<th>Symptom Load and Executive Functioning Selected Summary Scores / Selected Antisaccade Performance Variables (N = 14, one PTS participant who filled out questionnaires did not complete saccadometry)</th>
<th>Antisaccade Median Latency (ms)</th>
<th>Prosaccade Mean Duration (ms)</th>
</tr>
</thead>
</table>
There were no significant differences within the acute group across visit 1 and visit 2 in terms of SCAT3 symptom severity score and RPQ symptom load, though a larger sample size is needed. The mean score differences were 16 points and 6 points, respectively, and the mean scores were qualitatively greater in both symptom load measurements at the first visit (see Table 14 below).

**Table 14: Recovery of Symptom Burden in Acute mTBI Across Two Visits**

<table>
<thead>
<tr>
<th>Test Choice and Results</th>
<th>Acute mTBI V1</th>
<th>Acute mTBI V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9 (2 excluded as they did not have complete visit series)</td>
<td>9</td>
</tr>
<tr>
<td>SCAT3 Symptom Severity Score</td>
<td>Mean = 32; SD = (23.36)</td>
<td>16 (12.1)</td>
</tr>
<tr>
<td>Rivermead Symptom Load</td>
<td>18.67 (11.37)</td>
<td>12.67 (12.01)</td>
</tr>
</tbody>
</table>
5  CHAPTER 5: DISCUSSION

I have now investigated in three areas of analysis the role of saccadic eye movements, and antisaccade performance in particular, in patients with acute mild traumatic brain injury and persistent post-traumatic symptoms. The main finding of this study is that there are distinguishing characteristics of the antisaccade which are significantly affected after injury, and that antisaccade performance is correlated with white matter integrity, response interference, and symptom burden. I will now discuss our results and how they fit within the overarching goal of this work and the context of the existing literature. I will conclude with an integrated discussion synthesizing findings from all three aims, after which I will discuss possible future directions given this study’s limitations and new discoveries.

5.1  Aim One

5.1.1  Goal 1.1

It was expected that the minimum NPi score was 3.0 bilaterally, the minimum threshold value for a normal pupillary response. This was supported in our results. The variation in NPi score between the two pupils may be attributed to differences in autonomic nervous system dysfunction that may be reflected in small differences in pupillary reactivity (Moshe Eizenman, personal communication, 2015). Considering with the screening for eye disease during recruitment and the brief check for visual acuity during testing, the pupillary light reflex and vision of all participant groups could be considered sufficiently intact for saccadometry measurement.

5.1.2  Goal 1.2

I hypothesized that there would be significant differences on at least one antisaccade parameter after injury between groups. This is crucial if the antisaccade task is to be useful as an effective biomarker between patients with acute mTBI and PTS. This overarching hypothesis was partially supported in my analysis.

In terms of the overall number of accepted antisaccades, I saw a 42% reduction in the total number of accepted antisaccades in the acute in TBI group compared to the healthy control
group, and a 47% reduction in the PTS group compared to the healthy control. The number of accepted antisaccades is an easily quantifiable measurement, which can be used to distinguish between acute TBI patients, PTS patients, and healthy controls who never had a brain injury. This was potentially a valuable metric as it does not require extensive calculations and only requires the calculation of a simple odds ratio. A potential explanation for these results is that participants were more careful to pay attention during the first visit compared to the second visit. However the conclusions using this variable are tempered given some of the longitudinal results in healthy controls.

In terms of longitudinal comparisons in the control group, there were several unexpected findings. In the first instance, any given antisaccade trial was approximately 1.7 times more likely to be accepted (antisaccade or prosaccade between 50 and 600 ms latency) in the first visit when compared to the second visit. Other areas of significant difference included the prosaccade median latency, and the prosaccade latency rate. The results suggest that some antisaccade variables might not be stable over several weeks, even in healthy controls. Hence, the metric that is used must be selected very carefully, because if a measurement exhibits normal variations over time with no intervening head injury, it will be difficult to isolate the effect of injury for diagnostic and assessment purposes. Further studies are needed to determine whether these variables are stable or unstable over longer time periods and further delineate the factors determining these components of antisaccade performance. The difference in antisaccade median latency in the pairwise control comparisons was not significant, suggesting stable measurement over time on this specific antisaccade variable. The prosaccade error mean duration was also similar between both visits. These findings suggest that any conclusions made for evolution of antisaccade performance in the acutely injured groups should be taken in the context that not all measurements are reliable indicators of change in clinical state.

The median antisaccade latency at the first visit was significantly greater in the patient groups compared to controls at the first visit, but we found no significant difference between acute mTBI and PTS groups at the first visit. We expected that there would be a significant effect of injury, but also that there would be significant differences between injury groups. Our analysis instead suggested that antisaccade latency is a good metric for distinguishing injury from non-injury, but not between types of mTBI. To some extent this supports what has been found in the literature in cross-sectional comparisons between injury and control groups. Heitger et al. (2006)
identified significant increases in antisaccade latency in acute mTBI groups within one week of injury compared to control groups (Heitger, Jones et al. 2006), and Williams et al. (1997) also identified similar greater antisaccade latency in severely injured TBI patients compared to controls (Williams, Ponsford et al. 1997). Regrettably, the experimental design with only one control group prevented us from setting up a parallel planned contrast between acute mTBI/PTS and separate control groups directly (which would increase statistical power). A separate control group matched specifically to the PTS participants would also help to control for other demographics differences and differences in affective state, both of which may affect neuropsychological or antisaccade performance, respectively. This was an inherent limitation of our design, although in theory post-hoc comparisons may be able to determine differences between these two groups with resultant loss of power. There was also a greater spread in latency scores within the control group compared to the injury groups. This could mean that there is a natural variation, or normal range of antisaccade latency in our control sample (similar to a normal range of blood pressure or body weight in humans). For practical reasons this is important to investigate further in future studies, if antisaccades are to be used as a diagnostic marker for mTBI. Overall, as the antisaccade task involves response inhibition of a prosaccade error, increased latency in the antisaccade task reflects greater cortical processing time needed to generate the correct oculomotor command.

There was also a significant omnibus group-wise difference in mean duration of their incorrect prosaccade trials, with no significant differences between the injury and control groups, but a significant effect between the acute and PTS patients. The duration of a saccade is related to its amplitude and velocity. We found that the average amplitude for all three participant groups were larger than the target amplitude of 10 degrees on the correct antisaccade responses, but overall were smaller than the target amplitude on the prosaccade error responses. It was interesting that a deficit was observed in prosaccade mean duration, but not antisaccade duration. It is noted that our results in the patient group do not conform to the typical main sequence relationships between amplitude and velocity (Moshe Eizenman, personal communication, 2015). Heitger et al. (2009) attributed their observed deficits in PTS patients in prosaccade and antisaccade duration to damage in subcortical processing (Heitger, Jones et al. 2009). Future work will need to elucidate the mechanisms behind this difference. The current study’s results suggest that prosaccade mean duration can potentially be used to distinguish between acute
mTBI and PTS, though a validation study focusing on this metric is needed before any definitive conclusions can be made.

Clearly the prosaccade duration measurement is of limited use to the emergency clinician, who is most interested in distinguishing between acute injury and non-injury. With a basic history the clinician will easily be able to differentiate between a patient who just had a mTBI and a patient who had their injury 3 months prior and are still experiencing symptoms. Our study team is continuing to collect data on the acute mTBI patients in a longitudinal fashion, who come back for a third visit three to seven months after their injury. Based on the literature, a majority are expected to make a full recovery. An interesting future analysis would compare prosaccade mean duration on the antisaccade task for acute mTBI patients at the post-3 month stage and who are PTS negative, compared to PTS positive patients, as they appear to react differently to mTBI in the chronic stages.

From a neurophysiological perspective, reflexive (prosaccades) saccades and antisaccades ultimately are fed through the same brain stem saccade generator, and the same extraocular muscles (Leigh RJ. 2006). It was surprising to see that there are differences in the ballistic characteristics of the prosaccade duration in acute mTBI participants, but not in PTS participants or healthy controls. Ultimately a larger sample size and experimental design including reflexive saccade data will be needed to verify these results and ensure that there is no underlying pathology in the brainstem saccade generator responsible for the deficits that were observed.

We used the latency range of 50 ms to 600 ms for our initial screening and identification of “accepted” saccades, and these upper and lower bounds were obtained from a previous paper that investigated the step saccade task in mTBI (Mullen, Yucel et al. 2014). However, close to half of the antisaccade trial responses ended up falling out of these boundaries, further tempering the validity of our conclusions. Given our results, it is possible that this latency range will need adjusting based on the distribution of antisaccade latency for these particular type of patients.
5.2 Aim Two

5.2.1 Goal 2.1

The significant difference we found in mean diffusivity of the acute group’s corpus callosum splenium compared to the control group was in line with our hypotheses that there would be decreased white matter integrity post-injury in the corpus callosum. Significant microstructural damage and change in the corpus callosum in TBI patients over time has been identified (Wu, Wilde et al. 2010, Arenth, Russell et al. 2014). As discussed, a recent meta-analysis specifically focusing on the corpus callosum found that DTI analysis of this area, was a sensitive marker for microstructural damage after mild TBI (Aoki, Inokuchi et al. 2012). Our findings confirm these results, suggesting that corpus callosum microstructural alterations are present several weeks after injury in acute mTBI patients compared to healthy controls. Finding that mean diffusivity was significantly elevated compared to controls supports the hypothesis that there might be increased edema and inflammation in the splenium component of this major white matter bundle.

There were no significant differences in the genu and body of the corpus callosum, which was surprising as previous studies have identified vulnerability of these areas (Aoki, Inokuchi et al. 2012). Further analysis stratifying the impact location for acute mTBI and PTS patients (i.e., frontal, temporal, parietal or occipital) may provide some mechanistic explanations for the negative findings in these areas, and the DTI analysis as a whole.

We did not find significant differences elsewhere. Inhibitory processes are mainly the domain of the frontal and prefrontal cortices, and any significant differences in FA or MD would be likely in the white matter bundles affected after mTBI (e.g., the uncinate fasciculus, superior longitudinal fasciculus). However, traumatic brain injury is a complex biophysical process often characterized by rotational injury as well as blunt trauma (Post and Blaine Hoshizaki 2015). There are likely variations in the severity and nature of white matter injury in the mTBI patients that were scanned, which could be further characterized by localizing the area of injury and a review of the structural images for distribution of focal white matter hyperintensities and/or infarcts.
Contrary to our hypothesis, there were no significant differences in fractional anisotropy between the acute and control groups in all the ROIs studied. An increase in MD would be expected to be mirrored by a correspondent decrease in FA. However, FA measures a different aspect of diffusion flow and white matter integrity compared to mean diffusivity and further studies are needed to examine why some differences were seen in MD, and why FA was left unaltered post-injury.

For the PTS group versus control group comparison, lower mean diffusivity in the PTS group’s right corticospinal tract compared to the control group was contrary to our predictions, although longitudinal studies of diffusion tensor imaging have found fluctuations in mean diffusivity differences from the days to a year following injury (Croall, Cowie et al. 2014). It is understood that FA and MD will rise and fall over time from the acute stages to chronic stages, and my findings may reflect these natural fluctuations in the corticospinal tract. Even in the acute stages there is little consensus as to whether FA and MD will increase or decrease, as different findings have been identified between studies (Alexander, Lee et al. 2007).

Overall, the neuroimaging findings may reflect limitations of the analysis methods. I averaged the FA and MD values over the whole white matter region of interest (ROI). FA and MD values are local, voxel-based measurements of water diffusion. This means that each 2.4 x 2.4 x 3.0 mm voxel has its own FA and MD measurement. The JHU atlas specifies which voxels belong to a particular ROI. To calculate the average of the whole ROI, all the FA and MD values are combined to generate one representative FA and MD number for the ROI as a whole. Thus, it is possible that any individual differences at specific points along the tracts were masked. An exploratory power analysis conducted by a team member (see contributions) for the whole corpus callosum in PTS vs Controls, for FA revealed a required sample size of over one thousand in both groups. This limitation was touched upon by Niogi et al. in their review (Niogi and Mukherjee 2010), highlighting the large number of participants needed to adequately power a ROI-based inquiry of DTI changes after mTBI. A side-by-side comparison study with advanced voxel-based and tractography methods would be interesting to see the differences between these approaches.
5.2.2  Goal 2.2

As expected, we identified a significant positive correlation between median latency on the antisaccade task at the acute mTBI second visit and mean diffusivity in the splenium of the corpus callosum. There appeared to be a connection between the time necessary for inhibitory processes to take place, generating a successful antisaccade, and integrity of this white matter bundle. This correlation did not hold for PTS patients. Similar relationships between antisaccade performance and corpus callosum integrity have been documented in patients with fetal alcohol spectrum disorder, in studies where the patients completed antisaccade tasks as well as diffusion MRI using voxel based DTI analysis methods (Paolozza, Treit et al. 2014). As the authors mention in their discussion, and as I have discussed in the introduction, the antisaccade task requires much greater recruitment of cerebral resources compared to a simple prosaccade task. It is possible that diffuse axonal injury of the many connections serviced by the corpus callosum splenium plays a significant role in the execution of an efficient antisaccade. Unresolved questions are whether these findings have any relation to the frontal and prefrontal areas; I could not identify significant group-wise differences in our analysis, and the focus of our analysis did not look at individual variation.

5.3  Aim Three

5.3.1  Goal 3.1

In terms of executive functioning, I found significant differences in the Stroop color score with poorer performance in the injury groups. This was interesting as the color score may be used as a baseline condition to ensure there is no color naming difficulty; though there have been reports of impaired color score differences in patients with post-concussive syndrome compared to controls (Strauss E. 2006).

In line with our hypotheses, there was a significant difference between the injured and control groups on the Stroop color-word score, with injured participants covering fewer items in the same amount of time as controls (hence scoring more poorly). The results suggested that the injured groups had significantly impaired interference control as tested by the Stroop interference trial compared to healthy control groups. There are methods of integrating both the color, word
and color-word score into one Stroop metric, which may isolate the interference effect from deficits in reading and color-naming performance. This could be further explored in future studies. We did not see significant differences between the acute and PTS group, suggesting similar levels of response inhibition impairment.

There were no significant differences in phonemic word fluency on the MoCA, and also no significant differences on the Trail Making Test B score and corresponding Trail Making Test B-A score, although TMT-A performance was altered. TMT-A does not involve task switching but taps into cognitive processing speed (Strauss E. 2006). Both these tests assess different subsets of executive functioning compared to the Stroop, with phonemic fluency testing attention monitoring and suppression processes, while the Trail Making Test B score assessing attentional switching (Strauss E. 2006). The results suggest there is a specific aspect of executive functioning impairment in the participant groups detected by the Stroop interference task. The finding that differences in TMT-A were observed indicates there are differences in psychomotor speed between groups. White matter structural damage may be the common denominator of deficits in psychomotor speed as the deficits observed in antisaccade latency performance and response inhibition, but our results do not immediately support this conclusion.

5.3.2 Goal 3.2

By characterizing symptom load using the SCAT3 and RPQ, I identified the mean scores across all three groups and found that PTS patients rated consistently higher mean scores on most symptom items and summary subscores compared to acute mTBI patients and control patients. This was expected, given that the cardinal feature of the PTS group is high symptom load. The unique aspect was that we followed up using correlations with antisaccade performance soon after.

The correlational analyses identified several associations between antisaccade performance and symptom load/executive performance that were clinically significant. I found a positive correlation between antisaccade median latency and SCAT3 symptom load, and a negative correlation between antisaccade median latency and Stroop interference performance. The first finding made sense in that poorer antisaccade performance was correlated to increased symptom burden on the SCAT3. Increased antisaccade latency is a disorder of saccadic initiation. The time
required for the integration and calculation of the antisaccade signal reflects the time needed to process this task involving higher brain structures and interhemispheric connectivity (Leigh RJ. 2006), in part mediated by the corpus callosum. Intact white matter architecture and functioning of the higher cerebral centers are necessary for effective saccadic initiation and normal cognitive functioning. Impaired cognitive functioning, and specifically an effective way to deal with response interference may push symptom burden higher in acute mTBI and PTS patients. It remains to be determined why this relationship was only identified for the acute mTBI group and not the PTS group, even though the PTS group reported significantly greater symptom burden on the RPQ and the SCAT3 on almost every question and symptom category.

This reasoning was supported with the second finding. Since there was a significant negative association between antisaccade performance and Stroop interference score, a potential mechanistic explanation is that antisaccade performance reflected this executive neurocognitive impairment, which in turn was associated with increased symptom burden on the SCAT3. Currently there is no validated subscaling system for the symptom assessment scale of the SCAT3. Future work could do factor analyses on the SCAT3 symptom scale to identify a stable cognitive performance subscale on the SCAT3 (similar to the RPQ), and use this subscore to correlate with antisaccade performance and executive functioning. Task interference inhibition was impaired in acute mTBI patients, who were instructed to say the color in which the word was printed, instead of the printed word (the easiest verbal response) in the Stroop interference test. A similar cognitive process is needed in the brain to generate an effective antisaccade: the participant must inhibit the easiest rapid eye movement, and interfering one moving towards the stimulus (committing a prosaccade error), and cognitively program an antisaccade, instead rapidly moving the eyes to the mirror opposite location. The interference deficit between the acute mTBI patients and controls suggest the antisaccade task could potentially be a marker for this specific neurocognitive domain and provides a neuropsychological link to the larger implications of this work.

Similar connections have been found in past research in PTS patients, between antisaccade performance and executive functioning (Kraus, Susmaras et al. 2007). We could not confirm this relationship in our results. This was surprising, given that the level of executive functioning impairment in PTS (measured by the Stroop color-word score) was similar to the acute group. One possible explanation is that in PTS patients, the changes in microstructure leading to
sustained impairment in antisaccade performance were different compared to acute patients. This explanation is supported by the fact that the differences in mean diffusivity were found in the corpus callosum in the acute patients, but not in the PTS patients; likewise, significant MD differences were identified in the right corticospinal tract in the PTS patients, but not the acute patients. Thus, a difference in the neuroanatomy of PTS may be an explanation for the negative findings here. As discussed in the introduction, the most drastic effects of the neurometabolic cascade happen acutely after injury, but they can ease into permanent damage in the chronic phases. Perhaps these changes are reflected in our neuroimaging results, but the study’s small sample size limits the strength of this conclusion.

Finally, statistical tests of symptom load recovery in the acute patients showed no statistically significant changes between visit one and visit two, although the mean symptom load ratings were smaller at the second visit. The first visit was completed within one week of injury, and the second visit was completed between two to four weeks after injury. As a result, the latency between the first and second visit was between one and three weeks. Although our experimental design and timing of visits catered to overall clinical course for recovery in mTBI patients and the expected course of recovery in saccadic eye movements, it is possible that this timeframe was not long enough for significant resolution of symptoms in the acute mTBI patients. The variance was also large, and a much larger sample size is needed to adequately power this analysis in future work.

This new hypothesis will be readily tested in the completion of the larger project, where we have three longitudinal visits for the acute mTBI patients (a third visit 3-7 months from injury). Within-group comparisons of these scales across the three visits may identify trends of recovery when considering longer timeframes. Recovery itself can be defined in many different levels. Our findings may reflect the different rates of change in these different domains of brain function, from the microstructure, to the neurocognitive, to the outward symptom burden in these patients after mTBI.

5.4 General Discussion

The main goal of this thesis was to determine whether the antisaccade task is a useful tool in the assessment of mTBI and PTS. Taken as a whole, the results demonstrate some potential but there
were some results that need to be further examined. There appears to be a unique characteristic in the antisaccade reflected in its latency which is related to impaired inhibitory functioning, diffuse axonal injury in the corpus callosum, and subjective symptom load. Aim 1 confirmed significant characteristics of antisaccade functioning (despite functional pupillary light reflex) which were affected in injury patients. Aim 2 confirmed significant structural differences in the brain after injury and identified relationships to antisaccade performance. Finally, Aim 3 brought all the findings back to the patient, confirming deficits in specific types of executive functioning, and we have seen that there are significant relationships between antisaccade performance and symptom load.

First, our findings indicate that it is potentially possible to distinguish between mTBI participant groups and controls using antisaccades, both in the acute and chronic phases. This is a crucial characteristic for a good biomarker. However, there are several findings that need further investigation. First, the reliability of other antisaccade performance metrics such as the number of accepted antisaccades, prosaccade latency and latency rate does not seem to be acceptable. An antisaccade metric that will be used for clinical purposes needs to be selected carefully and cannot demonstrate natural fluctuations over time when there is no intervening head injury (as was the case for the control participants). This limits the potential for using some antisaccade measurements as a metric for recovery after injury or distinguishing between different groups. More longitudinal research is needed in healthy controls to confirm whether antisaccades are reliable. The findings also indicate that it is difficult to discriminate between acute mTBI and PTS (in some respects, patients more severe TBI) with stable antisaccade characteristics.

Nevertheless, this study is one of the first steps to identify the qualities of the antisaccade which are correlated with currently used assessment methods for these patients, and have identified several metrics that should be further explored in future work.

The crux of this study rested upon the relationships identified between antisaccade functioning and measurements that are currently being used in research and the clinic to assess mTBI. The findings here suggest that antisaccades could be a good tool to use in the assessment of acute mTBI. More research needs to be completed to determine whether the same could be said of PTS patients, as many correlations in the PTS group were not significant and the results were quite different from that of the acute mTBI group.
A possible reason for the negative findings in PTS patients included the selection strategy for variables used for the correlations. I only selected the most promising variables based on my hypotheses, the previous literature, and some of the group-wise comparison findings in the previous sections to correlate, in order to reduce the number of statistical correlations required. There might be hidden relationships between other variables that may not be significantly different in group-wise comparisons, but nevertheless are clinically significant to explore.

Finally, some remarks about the larger implications of the results in this study. Realistically speaking, for the average mTBI patient, these early exploratory results are unlikely to translate immediately into improved care. The analysis and results need to be verified in a larger sample, conducted using more robust statistical analysis techniques (see below), and several studies need to point towards the same consistent recommendations before clinical practice guidelines will be modified to include antisaccade assessment. Any primary research study is unlikely to change clinical practice immediately; such is progress. Nevertheless, the findings suggest some potential value of certain parameters of the antisaccade task that might help clinicians in assessment, which will lead to better and more thorough care in the years following.
6 CHAPTER 6: CONCLUSION

It is shown in this pilot study that there are significant differences in antisaccade median latency and prosaccade mean duration between patient groups and controls, despite pupillary light reflex within normal limits. These measures were related to loss of structural integrity in the splenium of the corpus callosum; antisaccade median latency was also associated with poor performance on executive functioning tasks and greater symptom load. This study suggests that the antisaccade task has potential to be useful as a neurological marker for acute mTBI, but further investigation is needed to confirm and extend the findings identified herein.
7 CHAPTER 7: FUTURE DIRECTIONS

In terms of the experimental design, a larger number of trials could be used in each antisaccade session to increase intra-participant statistical power. We did not include a separate task to account for malingering on the neuropsychological testing and inflated symptom reporting in the acutely or chronically injured patients. However, all participants were informed and understood prior to written consent that their results would be used for research purposes only, minimizing the incentive for malingering to receive legal benefit (there was none) or financial benefit (beyond standard reimbursement for participation). A review by Everling and Fischer at the cusp of the new millennium (Everling and Fischer 1998) further supports a point raised earlier – that deficits in antisaccade performance should be studied at the same time with performance on reflexive saccades, i.e. performance on a step saccade task whenever possible so that a deficit in antisaccade performance can be isolated to the sole contribution of the higher inhibitory control centers. In a pilot study our research team previously demonstrated that acute mTBI patients have deficits in step saccade latency (Mullen, Yucel et al. 2014). It took longer for acute mTBI patients to generate a reflexive saccade. Although it was beyond the scope of this study, the results could be supplemented with data on the step saccade task in order to make more robust conclusions. In terms of the research protocol, as noted by a reviewer the time difference between the first and second visit in the acute group were different for each participant, varying from 2-4 weeks. Unfortunately this was due to scheduling difficulties with the participants, but future work may include the time since injury and between visits into the statistical model to take this factor into account. In terms of study recruitment, this study recruited directly from the emergency department, which I think is a more representative source of mTBIs in the general population than previously published studies which for the most part focused on recruitment of sport concussion participants.

The statistical design could be improved. With the relatively simple statistical designs employed here, I often needed to conduct several sets of one-way ANOVAs or pair-wise comparisons for each general hypothesis. Eye tracking studies are similar to genomics studies in that many different parameters can be generated for each participant, and consequently multiple comparisons are possible for each hypothesis testing the effect of injury by group category. Although care has been taken to minimize the number of multiple comparisons within each dependent variable, the experiment-wise error was still inflated. Hence, it is possible that any one
A statistically significant difference may not be meaningful and could have arisen due to chance. A similar group-wise multiple comparison approach has been used in the saccade literature (Heitger, Jones et al. 2009), but there are more elegant methods of statistical inference, such as feeding several hypotheses into a general linear model (GLM), which limits the experiment-wise error rate and increases the statistical power available for finding true experimental effects between and within study groups. Given that this problem is common to many similar studies in the literature, it is perhaps prudent to establish a common protocol for antisaccade measurement in the mTBI patient group after conducting a meta-analysis of the existing studies, thereby reducing the number of exploratory variables that will need to be collected and analyzed for each participant in future prospective studies.

An important issue to discuss is the reduced stability of certain characteristics of the antisaccade in healthy control participants. Generally speaking, one would like a marker which is relatively constant (or varies within normal limits) in healthy participants who do not have an intervening head injury between visits. There were some characteristics, such as the antisaccade latency which were stable in the longitudinal comparisons for controls, but exhibited significant differences in the acute mTBI group as they recovered. These are the best qualities of the antisaccade that holds potential for clinical practice. Potential sources of the differences observed between groups in healthy controls on the antisaccade test include differences in motivation, attention or alertness.

The diffusion tensor modeling could be conducted differently. Future studies will need to check the T1 scan for any focal abnormalities and conduct a voxel-wise comparison of FA and MD between groups, after which a statistical inference can be carried out with greater robustness than has been done here. Fitting to a GLM will also correct for multiple comparisons in the neuroimaging data, as described above. Our team is conducting analysis on resting state functional MRI in the same participants, which will allow us to obtain a better picture of functional connectivity and a new layer of information for understanding the effect of injury on antisaccade functioning.

In our correlation analyses, I did not account for depression score and other factors which may affect the relationships studied (age and time since injury, for example). This pilot work sought to identify relationships that could be further investigated in targeted prospective studies with
larger sample sizes. Future work will need to perform the correlations with these variables in mind to determine if the relationships identified still stand with the variance in these measurements accounted for, especially when the values of interest are so sensitive. Furthermore, we did not exclude patients who had a comorbid history of post-traumatic stress disorder (PTSD), which affects the psychological aspect of neurocognitive functioning and symptom burden. With a larger sample size, cross-sectional comparisons can be done to isolate the effect of PTSD and psychological trauma in antisaccade performance.

In this analysis I did not account for history of previous concussion due to low sample sizes. There is literature suggesting that a positive history is a risk factor for future concussions (Slobounov, Slobounov et al. 2007), but no literature has investigated the effect of previous head injury on saccade functioning, and antisaccade performance specifically. Future large scale studies could investigate this further by stratifying participants based on the number of subconcussive and concussive hits they experienced prior to saccadometry testing.

Of note, correlation does not imply causation. Although significant correlations were found between antisaccade performance and microstructural integrity, executive functioning, and symptom load, the results do not imply any causative relationship between these factors. One cannot conclude, for example, that poorer executive functioning caused poorer antisaccade performance and vice versa. Causation is a difficult aspect to pinpoint, especially in prospective research studies such as this one, and studies where the injury has already occurred.

Taking a step back, a larger data set will allow us to feed the results identified in significant aspects of antisaccade functioning, structural integrity and executive functioning into a unique regression model, to predict whether somebody who sustains a mild traumatic brain injury will experience a full recovery or will continue to experience the debilitating sequelae in PTS. An important aspect of validation is the calculation of sensitivity and specificity of this regression model in diagnosing injury and prognosis. An effective regression model for predicting PTS will be a powerful tool for clinicians who may wish to use saccadometry in the clinic. The successful modeling of these relationships will be a big step forward for clinical integration and is something to look forward to after more data are collected.

In the end, the eyes are a wonderful microcosm ripe with unique, well-defined behaviors and characteristics that reflect underlying neuropathology and neurological function. Antisaccade
performance is only a small sub-component of the full functionality of the eyes and associated brain structures which has potential to reflect changes after concussion, and this environment has so many different aspects which can be further explored to help clinicians better diagnose and ultimately treat mTBI.


Appendices

Appendix 1: Excel VBA macro used in the comparison of the double data entry, to ensure any differences and errors were identified and corrected prior to analysis.

Code obtained without modification from (Zemens 2014):


User: David Zemens. Usage notes are my own.

Option Explicit
Sub compare2sheetsex() 'and highlight the difference
    Dim wb1 As Workbook, wb2 As Workbook, sh1 As Worksheet, sh2 As Worksheet
    Dim rCount As Long, cCount As Long
    Set wb1 = Workbooks(InputBox("enter b1"))
    Set wb2 = Workbooks(InputBox("enter b2"))
    Set sh1 = wb1.Sheets(InputBox("enter s1"))
    Set sh2 = wb2.Sheets(InputBox("enter s2"))
    rCount = sh1.UsedRange.Rows.Count
    cCount = sh1.UsedRange.Columns.Count
    Dim r As Long, c As Integer
    For r = 1 To rCount
        For c = 1 To cCount
            If sh1.Cells(r, c) <> sh2.Cells(r, c) Then
                sh2.Cells(r, c).Interior.ColorIndex = 6
            End If
        Next c
    Next r
    Set sh1 = Nothing
    Set sh2 = Nothing
End Sub

'Usage Notes: 'both worksheets must be open in the same workbook
'b1 and b2 are both the same workbook, rename without space
's1 and s2 are the worksheet names, also rename without spaces
'the differences will be highlighted in yellow in the second sheet
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