Application of Electrovestibulography on Post-Concussion Syndrome: Diagnosis and Monitoring

By

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Abstract

Following a mild Traumatic Brain Injury (mTBI), there can be neuropathological changes in the brain resulting in permanent or transient neurological symptoms and signs of a functional disturbance. The persistence of these symptoms for more than one month is usually referred to as Post-Concussion Syndrome (PCS). PCS severity usually increases when comorbid depression exists. Moreover, the diagnosis of PCS might be overlooked in favour of a diagnosis of depression due to the overlap in the symptoms of the two pathologies. This study, for the first time, presents staged research to evaluate a novel technology, called Electrovestibulography (EVestG), that holds the potential to objectively and cost-effectively be utilized as an assistive tool to diagnose PCS, its comorbid depression and quantitatively measure the recovery from PCS and its sequelae following a treatment.

In the first stage of this research, two EVestG features were extracted from the recorded signals to distinguish PCS from age and gender-matched healthy controls. These two features resulted in an unbiased classification accuracy of 84% and 79% for separating healthy controls from PCS sufferers and for separating long (>3 months) and short-term (<3 months) PCS sufferers, respectively.

Secondly, it was shown that the calculated accuracy for separating PCS from healthy controls can be affected when comorbid depression exists. By adding an EVestG depression-specific feature from a previous study, the calculated accuracy was improved from 83% to >90% for those with moderate/severe depression.

Then, it was shown EVestG features could monitor recovery following repetitive Transcranial Magnetic Stimulation (rTMS) treatment for PCS with and without comorbid depression. Additionally, the EVestG features used have shown the potential to robustly detect and monitor
changes, relatively independently, in both persistent PCS and in depression when comorbid PCS-depression present.

Finally, the effect of mTBI on other sensory systems, in particular, that closely linked visual system was examined. Given the prevalence of convergence insufficiency (CI) among the mTBI population, as well as the link between the vestibular and oculomotor system, the effect of the mTBI on the CI was investigated and found to be significantly correlated with the EVestG features and PCS clinical assessment.
Acknowledgements

It is a genuine pleasure to express my deep sense of thanks and gratitude to my mentors and guides, Prof. Zahra Moussavi and Prof. Brian Lithgow, who have been extremely supportive and helpful in the development of this body of work. Their timely advice, meticulous scrutiny, scholarly advice and scientific approach have helped me to a very great extent to accomplish this work as well as gaining the characters of a good researcher.

I would like also to acknowledge Dr. Behzad Mansouri, a neuro-ophthalmologist, for referring the patients for EVestG recordings and for his clinical advices through the course of this work.

Financial support was received from MITACS, Neural Diagnostics Pty. Ltd., Manitoba Public Insurance (MPI) and Riverview Health Center Foundation.

I would like to thank the Riverview Health Center also for giving us space for the EVestG lab where we conducted all the recordings.

A special thank you goes to all the volunteers who participated in this study and were patient for completing the experiments.

Finally, I would like to extend my heartfelt gratitude to my family for being understanding and encouraging throughout this work.
Dedication

To my lovely parents: Ibrahim and Montaha Suleiman

To my siblings: Ameer, Mona and Anwar Suleiman

To my lovely fiancé: Asma Mahajnah

To my brother and sisters in law: Haytam Zoabi, Latefah and Nedaa Suleiman

To my nephews and nieces: Majd, Roaya, Ward, Ghena and Ibrahim
Data Availability

The data that support the findings of this study is available from Neural Diagnostics Pty. Ltd. but restrictions apply to the availability of this data, which was used under license for the current study, and so is not publicly available. Data is however available from the authors upon reasonable request and with permission of Neural Diagnostics Pty. Ltd.
Contribution of the Co-Authors on the Papers

This thesis consists of four individual papers combined into a “sandwich thesis”. Two papers (Chapter 3-4) have been published in a peer-reviewed journal. The third and fourth manuscripts (Chapters 5-6) will be submitted to peer-reviewed journals soon.

Mr. Abdelbaset Suleiman was the main contributor and first author of all the manuscripts presented in the thesis. Mr. Abdelbaset Suleiman’s contribution to this work includes recruiting participants, gathering data by conducting the EVestG recordings, signal and statistical analysis, investigating the research questions, writing all manuscripts, and responding to reviewers’ comments. Prof. Zahra Moussavi was principal investigator of this study. She contributed to the conception and design of the studies, obtaining ethics approval, reviewing the papers, and help with the submission and review process.

Prof. Brian Lithgow contributed in the study design, scientific advice and reviewing the papers and responding to the reviewers’ comments.

Dr. Behzad Mansouria examined and referred the patients and contributed to the discussion of the results. He also reviewed the manuscripts and helped to respond to the reviewers’ comments.

Dr. Zeinab Dastghieb contributed to data analysis in Chapter 3.

Mehrangiz Ashiri contributed to the introduction and discussion of Chapter 6.
List of Publications

The following is a list of the publications, submitted manuscripts and abstracts obtained from this thesis work:

**Peer-Reviewed Full Papers Published:**


**Submitted To Peer-Reviewed Journal:**


**Peer-Reviewed Published Abstracts:**


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<td>PCS</td>
<td>post-concussion syndrome</td>
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<tr>
<td>EVestG</td>
<td>Electrovestibulography</td>
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<tr>
<td>TBI</td>
<td>traumatic brain injury</td>
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<tr>
<td>mTBI</td>
<td>mild traumatic brain injury</td>
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<tr>
<td>SPCS</td>
<td>short-term PCS</td>
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<tr>
<td>LPCS</td>
<td>long-term PCS</td>
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<tr>
<td>rTMS</td>
<td>repetitive Transcranial Magnetic Stimulation</td>
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<tr>
<td>SCAT5</td>
<td>sports concussion assessment tool-5</td>
</tr>
<tr>
<td>BESS</td>
<td>balance error scoring system</td>
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<tr>
<td>ImPACT</td>
<td>immediate post-concussion assessment and cognitive testing</td>
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<tr>
<td>RPQ</td>
<td>Rivermead post-concussion questionnaire</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<td>SWI</td>
<td>susceptibility weighted imaging</td>
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<td>Electrocochleography</td>
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<td>Abbreviation</td>
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<tr>
<td>EVS</td>
<td>efferent vestibular system</td>
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<td>MADRS</td>
<td>Montgomery–Asberg depression rating scale</td>
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<tr>
<td>AP-area</td>
<td>action potential area</td>
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<td>interval histogram</td>
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<td>frontal eye field</td>
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<td>inter nuclear ophthalmoplegia</td>
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Chapter I – Introduction

1.1 Overview

Annually, an estimated number of 42 million people worldwide suffer a mild traumatic brain injury (mTBI), also known as concussion\(^1\). mTBI may occur following a transient alteration of consciousness that can be caused by biomechanical forces to the head (e.g. sports injuries, falls, motor vehicle accidents (MVA), etc.). mTBI can be followed by some transient or permanent neurological symptoms and signs. If symptoms of a head injury such as cognitive, somatic and emotional symptoms sustain for an extended period of time, the condition is referred to as post-concussion syndrome (PCS). While PCS usually resolves within three months after the injury\(^2\), 5 to 15\% of the concussed population may carry their symptoms and functional impairment for months to years\(^3,4\). Persistent PCS impacts individuals’ personal and social life such as entertainment activities and employment\(^5,6\).

Why and how PCS develops and changes over time has remained controversial for decades. Some researchers think that the persisting symptoms are the result of neurogenic factors (e.g., neurological residuals of the original mTBI)\(^7\), while others think it is psychogenic factors (e.g., comorbid psychopathology such as depression)\(^8-10\). Some researchers take the more balanced perspective that these two types of factors are complementary and can be integrated\(^9\). However, it is certain that persistent PCS occurs due to pathophysiological changes occurring after a mTBI\(^11\).

The major problems confounding treatment for PCS root from the fact that there are no clinically accepted objective tools for early detection of both the presence of PCS and its comorbid depression as well as monitoring recovery from PCS (with or without comorbid depression).
The most commonly and traditionally applied diagnostic tools for PCS are the neuropsychological tests. The application of neuropsychological assessments in PCS evaluation can play an important role in clinical decision-making\textsuperscript{12,13}, however, it is not recommended to use these assessment outcomes as the sole basis for clinical decision making, as they may be biased by several confounding factors\textsuperscript{14}. Therefore, it is recommended that these assessments should be included in conjunction with other clinical domains\textsuperscript{15}.

The most used objective techniques for assessing head injuries, in general, are neuroimaging techniques\textsuperscript{16,17}, such as computed tomography (CT) or susceptibility weighted (SWI) magnetic resonance imaging (MRI)\textsuperscript{18}. While these techniques have been shown to be sensitive for identifying moderate/severe TBI where lesions and structural fractures are more likely to exist\textsuperscript{15,18}, they provide little contribution to PCS evaluation as the injury to the neural tissues is micro-structural and is usually not detected with imaging\textsuperscript{17}.

Electrovestibulography (EVestG)\textsuperscript{19–21} is a novel technique that provides a quantitative indirect measure of activities in various brain regions and neural pathways, particularly in the vestibular nucleus and vestibular peripheral apparatus. There is support for the use of EVestG features in PCS studies given that the prevalence of the dizziness or imbalance in the mTBI population is 23-81\%\textsuperscript{34,35}, and that vestibulopathy after mTBI can have a central axonal injury component\textsuperscript{36} (for review see Chapter 2).

1.2 Goals and Objectives

In this study, the overall goal was to investigate the feasibility of the EVestG technique to be used as an assistive tool for screening PCS, its depression comorbidity, and monitoring their recovery.

The specific objectives of this study were:
1. To recruit participants with PCS and age and gender-matched healthy controls and assess them with neuropsychological tests and EVestG.

2. To derive the characteristic features from the EVestG recordings (of both short-term PCS (SPCS) and long-term PCS (LPCS) groups) specific to PCS comparative to controls.

3. To find the correlation of the extracted features respect to the duration of the PCS since the head injury.

4. To test the validity of our previously developed EVestG classifier for PCS detection on a blind dataset.

5. To investigate the comorbidity of depression in PCS using an additional EVestG feature for depression (from a previous study) for improving the detection of PCS when moderate/severe depression is present.

6. To investigate the statistical correlation between the EVestG PCS-specific and EVestG depression-specific features as well as their correlation with the neuropsychological assessment tools used in this study (the Rivermead Post-concussion Questionnaire (RPQ) and Montgomery–Åsberg Depression Rating Scale (MADRS)).

7. To investigate the EVestG feature changes in PCS individuals with and without comorbid depression following rTMS treatment.

8. To investigate whether EVestG features can independently monitor PCS and depression changes following rTMS.

9. To investigate whether rTMS treatment applied to PCS patients with a depression comorbidity improve both PCS and depression.

10. To investigate whether the EVestG extracted features characterizing the PCS population are correlated with the severity of convergence insufficiency (CI).
1.3 Report Organization

This document is composed of seven chapters. This first chapter introduces the key concepts, motivation, rationale, and objectives of the thesis. The second chapter includes background information about PCS definition, PCS current assessments, EVestG, and the rationale of using EVestG for PCS diagnosis and monitoring. The following chapters (3-6) consist of individual manuscripts, while each chapter also includes a synopsis of the whole chapter. Chapter 3 (a peer-reviewed published journal paper) includes the EVestG extracted features, classification and its accuracy for separating PCS from healthy controls, and the physiological meaning of the extracted features. Chapter 4 (a peer-reviewed published journal paper) discusses how the EVestG-PCS classifier obtained from chapter 3 is affected by co-morbid depression. We showed that increasing the dimension of the classifier by adding a third feature specific for depression results in increasing the classification accuracy when co-morbid depression exists. We also calculated the correlation between the EVestG extracted features and the neuropsychological assessments. Chapter 5 (will be submitted to a peer-reviewed journal soon) presents the investigation of whether rTMS treatment applied to PCS patients with a depression co-morbidity can improve both PCS and depression. Chapter 6 (has been submitted to a peer-reviewed journal) presents a review of the neuropathways between brain regions controlling vergence eye movements and the vestibular system. We also investigated whether the EVestG extracted features characterizing the PCS population were correlated with the severity of CI. Chapter 7 includes a summary of all the studies in this thesis, a summary of this thesis contributions, and suggestions for future projects related to this work.
1.4 Summary of the contributions

Overall the contributions of this thesis can be summarized as the followings.

1- The work presented in this thesis is the first to quantitatively diagnose and monitor PCS by measuring vestibular static and dynamic responses. The objective tool used to measure the vestibular response was EVestG. Features characterizing PCS derived from EVestG are shown here to be related to known channelopathies known to occur after a head injury. (This work has been published in Nature Scientific Report journal, 2017).

2- Using EVestG PCS and depression features together, it was shown that the accuracy for detecting PCS with comorbid depression was markedly improved. Moreover, EVestG was shown to independently monitor PCS and its comorbid depression (This work has been published in Nature Scientific Report journal, 2018).

3- We showed EVestG is capable of monitoring the efficacy of rTMS treatment on the PCS and comorbid PCS/depression populations. (This work will be submitted to Brain Injury Journal)

4- It was shown that vergence eye movements are affected in PCS. It was shown that the EVestG PCS feature was significantly correlated with the CI measure. (This work has been submitted to Annals of Biomedical Engineering (BMES) Journal).
References


Chapter II – Background

2.1 Post-Concussion Syndrome (PCS)

2.1.1 Definition

According to the 5th international conference on concussion in sport, concussion was defined as: “A transient alteration of brain function caused by a biomechanical force to the brain or elsewhere. Concussion may result in neuropathological changes, which usually are followed by some permanent or transient neurological symptoms and signs which reflect a functional disturbance rather than a structural injury.” In this thesis, the terms mild Traumatic brain injury (mTBI) and concussion are used interchangeably.

In North America, the estimated annual incidence of TBI is 1299 per 100,000 individuals. The spectrum of severity of TBI varies, but about 70% of the reported cases of TBI are mTBI.

Following a mTBI, patients usually experience one or more of the following clinical symptoms: headaches, dizziness, depression, memory loss, confusion, blurred vision and balance problems that may occur with or without loss of consciousness. The persistence of these symptoms for more than one month is usually referred to as post-concussion syndrome (PCS). These symptoms except headache and dizziness are often reported for a few days and up to weeks following the injury. The headache and dizziness symptoms typically occur immediately as well as later in the recovery period.

Persistent PCS has been reported in 20-30% of concussed individuals and comprises incomplete recovery which include somatic (e.g. headaches, dizziness), cognitive (e.g. poor concentration), mood (e.g. depression), visual (e.g. convergence insufficiency, poor accommodation) and behavioural (e.g. irritability) problems. These symptoms can be dominant in the first few hours
or days right after the impact but may also persist for weeks, months or even years\textsuperscript{11,12}. Neuropsychological assessments following a concussion have shown that the cognitive function mostly recovers within 1-3 months’ post-injury\textsuperscript{13}. Additionally, some improvement can take place during the first two years but some patients may remain symptomatic for longer than 2 years\textsuperscript{13}.

Generally (and in this thesis), the PCS individuals who reported symptoms after 7 days but within 3 months of their injury are referred as SPCS, while PCS individuals with much longer (>3 months) persisting symptoms\textsuperscript{6} are referred to as LPCS\textsuperscript{7,9,12}. However, in the last part of this thesis, where we tested the PCS recovery following repetitive transcranial magnetic stimulation (rTMS) treatment, due to limited sample size and the need to have workable group sizes, the SPCS individuals were referred to those with head injury < 1 year and LPCS to those with head injury >1 year.

2.1.2 PCS-depression comorbidity

A recent systematic review of the psychiatric sequelae of mTBI has reported an increased incidence of psychiatric disorders and psychological symptoms in the mTBI population compared to healthy controls\textsuperscript{14}. Depression and/or anxiety are considered as some of the most concerning psychiatric symptoms after a head injury. The estimated rate of depression in the first year following a mild to severe TBI ranges from 26 to 53\%\textsuperscript{15–18}. Individuals with mTBI have three times higher risk for lifetime depression, even after adjusting for factors such as poor-to-fair parental mental health, older age, and socioeconomic status\textsuperscript{19}.

Studies show an association between PCS severity, depression severity\textsuperscript{20,21} and neurocognitive impairment\textsuperscript{22} after a mTBI. However, still little is known about the nature of depression in PCS population. One potential way to explain is using the Kay model\textsuperscript{23}, in which there is described a dysfunctional feedback loop that may result in the maintenance of numerous symptoms even after
medical symptoms resolve; when cognitive impairments during the early stage of recovery co-occur with pain symptoms or emotional factors, a feedback loop may develop whereby the chronic pain and symptoms start to elicit secondary cognitive complaints. Over time, the interaction between these symptoms strengthens to the point that persisting symptoms will continue to elicit cognitive and emotional complaints such as depression even after the primary complaints/symptoms have resolved. Based on this model, persistent PCS may be aggravated by depression and vice versa.\textsuperscript{23}

Moreover, depression after mTBI might be due to structural changes rather than psychiatric sequelae. It has been shown that individuals with clinical depression exhibit structural and morphological changes of the brain’s mood centres such as hippocampus\textsuperscript{24}, amygdala and prefrontal brain regions which may also be affected after mTBI\textsuperscript{25,26}.

Major problems confounding treatment or recovery planning for PCS patients derive from the fact that there is no universally and clinically accepted tool with the ability to detect and diagnose the presence of PCS soon after the impact. The most commonly and traditionally applied tools for PCS assessment include neuropsychological and quantitative tests described in the following sections.

2.2 PCS Assessments

2.2.1 Neuropsychological Assessments for PCS

The neuropsychological assessments assess the cognitive and functional deficits resulting from the neurological disorder or injury. The application of neuropsychological assessments in PCS evaluation can play an important role in clinical decision-making\textsuperscript{27,28}. The most commonly and traditionally applied diagnostic tools for concussion and PCS include the Sports Concussion Assessment Tool-5 (SCAT5)\textsuperscript{2}, Balance Error Scoring System (BESS)\textsuperscript{29}, the Immediate Post-
Concussion Assessment and Cognitive Testing (ImPACT)\textsuperscript{30}, Rivermead Post-Concussion Questionnaire (RPQ)\textsuperscript{31,32}, and computerized cognitive tests such as CogSport\textsuperscript{33}.

Despite the importance of these assessments, they are still subjective and rely on self-reported symptoms and have a limited ability to accurately detect the presence of PCS mostly due to confounding factors/variables such as intelligence, age, education, depression and malingering\textsuperscript{34}. Therefore, it is recommended that clinical assessment not be on the basis of neuropsychological questionnaires alone\textsuperscript{1}. Rather, they should be seen as an aid to the clinical decision-making process in conjunction with a range of assessments of different clinical domains and investigational results\textsuperscript{1}.

2.2.2 Quantitative measurement for PCS

Most objective tools used for mTBI evaluations are brain imaging techniques, such as computed tomography (CT) or susceptibility weighted (SWI) magnetic resonance imaging (MRI)\textsuperscript{35}. However, they provide little contribution to PCS evaluations and are best utilised when there is suspicion of an intracerebral or structural lesion (skull fracture)\textsuperscript{1,35}.

Other imaging modalities such as functional magnetic resonance imaging (fMRI) can provide additional insight into pathophysiological mechanisms. It has been shown that the activation pattern of the brain provided by fMRI is correlated with symptom severity and recovery in concussion\textsuperscript{36–39}.

More advanced techniques such as positron emission tomography (PET), diffuse tensor imaging (DTI), magnetic resonance spectroscopy and functional connectivity are showing promise. However, these techniques are still in the early stages of development and cannot yet be recommended other than in research settings\textsuperscript{1}. 

1
Other quantitative approaches, such as quantitative electroencephalogram (qEEG)\textsuperscript{40–42} and robotic-assisted test battery\textsuperscript{43}, are investigating PCS severity and its recovery with some very positive diagnostic outcomes\textsuperscript{42,44,45}. However, some studies question qEEG’s clinical usefulness\textsuperscript{46,47}.

2.3 Electrovestibulography (EVestG)

Herein we present a new quantitative technology which can be used as an assistive tool for PCS diagnostics. The EVestG signals are recorded from the external ear in response to a vestibular stimulus. EVestG detects specific vestibulo-acoustic and more particularly vestibular field potentials (FP)\textsuperscript{48–50}, potentially, providing a quantitative indirect measure of activity in brain regions and neural pathways associated with neuropsychiatric disease\textsuperscript{51}. The EVestG system records spontaneous and driven signals whilst stationary (no stimulus) and during a whole body tilt (stimulus)\textsuperscript{52}. EVestG signal analysis has shown great promise for diagnosis and separation of Parkinson’s\textsuperscript{53}, Depression\textsuperscript{50,54} (Unipolar or Bipolar) and Meniere’s Disease\textsuperscript{55} from Controls (healthy age-matched subjects).

There is a substantial link between the Vestibular Nucleus (VN) and many brain regions including the limbic regions\textsuperscript{56}. Substantial research suggests an association between vestibular function and psychiatric and cognitive symptoms\textsuperscript{51}. There are a number of substantial neurobiological links between the brain process regulating vestibular activity in the brainstem and other brain regions implicated in the neurobiology of psychiatric symptoms which encourage the usage of the vestibular response measurement as an assessment tool for many neurodegenerative disorders\textsuperscript{51}. In mammalian species, the peripheral afferent response can be affected by the spontaneous activity of the efferent vestibular system (EVS)\textsuperscript{57}. The EVS receives input from (1) the peripheral afferent nerve\textsuperscript{58}, (2) VN\textsuperscript{59}, and (3) and other systems such as somatosensory
systems. Thus, the EVestG recorded signal is a mix of peripheral and brainstem, consisting of spontaneously and driven vestibulo-acoustic potentials modulated by the EVS, VN and more cortical regions including limbic regions.

2.3.1 Why EVestG should be sensitive to the brain changes due to mTBI? In order to answer this question, one should review the following issues:

1- The Neuroanatomical changes after mTBI

A mTBI is often caused by some rotational or stretching forces. According to Bigler, any biomechanical forces such as stretching or rotational forces to the brain may result in damage to the upper brainstem, hypothalamic-pituitary connections, the internal carotid, the amygdala, and the hippocampus. This damage can include slight mechanical deformation such as stretching or twisting to the nerves of these area and or to the vascular system connected to these areas. Accordingly, neurophysiological changes of these damaged nerves are expected and this might explain some of the behavioural and emotional impairment following a head injury.

2- The Neurophysiological changes after mTBI

Recently, the understanding of the pathophysiology of PCS has been expanded significantly. As mentioned above, one of the hallmarks of mTBI is that neurological signs and symptoms result in from biomechanical forces to the brain, and that can potentially cause micro-structural injuries to neural tissues. The functional injury is usually due to the perturbation of cellular or physiological changes including ionic shifts, metabolic changes, or impaired neurotransmission. In the early stage of injury, an indiscriminate release of excitatory amino acids glutamate and a massive influx and efflux of sodium potassium ions is observed. This is followed by the more persistent Ca influx, mitochondrial dysfunction
with decreased oxidative metabolism, diminished cerebral glucose metabolism, reduced cerebral blood flow, and axonal degeneration. The summation of these changes is thought to underlie both the short- and long-term symptomatology seen in mTBI (SPCS and LPCS).

Moreover, studies which were conducted in vitro\textsuperscript{64,65} have shown that when axons were stretched, the sodium gates are perturbed resulting in the sodium influx and subsequent depolarization with calcium influx through voltage-sensitive calcium channels and the subsequent reversal of the sodium-calcium exchangers. They also showed that mechanical trauma or deformation of the axons triggered Na\textsuperscript{+} influx through sensitive voltage-gated sodium channels (NaChs); that would result in an increase in Ca\textsuperscript{2+} influx and subsequent degradation of the NaCh α-subunit followed by a more persistent elevation of Ca\textsuperscript{2+}. Consequently, the changes in the permeability of the nerve membrane result in changes in the response pattern of the nerve.

To summarize, we hypothesize that EVestG signals are basically a measure of vestibular and brainstem activity. A recent study suggests that the vestibular system is a potential window for identifying psychiatric disorders (see Gurvich (2013)\textsuperscript{51} for review). Specifically, it was shown that vestibular nuclei have a bidirectional link to the raphe nuclei and locus coeruleus which both have reciprocal connections to the amygdala, cerebellum, hypothalamus, and prefrontal cortex. Additionally, there is a direct connection between the vestibular system and hippocampus\textsuperscript{51}. Based on the (1) neuroanatomy of the amygdala, cerebellum, hypothalamus, prefrontal cortex, and the hippocampus explained above, and (2) the neurophysiological changes that resulted from the deformation of the nerves, we hypothesize that the waveform of the FP, as well as the firing pattern of the vestibular response, will be different between individuals with mTBI and healthy controls.
3- Vestibulopathy changes after mTBI

Dizziness is considered one of the major symptoms after a head injury. It is reported that 23-81% of mTBI cases experience dizziness and balance problems in the first days after injury. The prevalence of persistent dizziness after mTBI varies widely from 1.2% at 6 months to 32.5% at 5 years.

The source of persisting dizziness may include unilateral (or bilateral) vestibular nerve injury or damage to the otolithic organs the utricle and or saccule. Some dizziness after a head injury may be not associated with a direct injury to the vestibular labyrinth but it may be related to structural or microstructural central nervous system changes. A head injury may lead to diffuse axonal injury (DAI), and traction on or bruising of the brainstem and or cerebellum which may disrupt the vestibular and the postural reflex pathways, also in part, accounting for dizziness.

As mentioned above, biomechanical forces may lead to a stretching local to the upper brainstem (midbrain). The midbrain region of the upper brainstem is comprised of the cerebral peduncles, which house all of the major ascending and descending white matter pathways connecting the cerebrum with the periphery of the body (including the vestibular periphery) and the connection between cerebrum and cerebellum. Thus, the biomechanical forces to the midbrain can result in a deformation of these white matter pathways. DAI commonly affects gray-white matter junctions, the brainstem, corpus callosum, cerebral and cerebellar peduncles, basal ganglia and thalamus, frontal and temporal lobes white matter. DAI refers to injury to axons within the white matter fibre tract due to stretching which damages the axon and impairs axoplasmic transport. Moreover, using DTI, the vestibulopathy in mTBI patients has been found to have a DAI component. Therefore,
due to changes in the vestibular pathways and in particular the DAI in the vestibulopathy, changes in the EVestG signals are expected.

2.3.2 EVestG Recording Procedure

1. Placing the electrodes: the ear canal wick electrode was placed in each ear canal close to the ear drum (TM-EcochGtrode, Bio-logic, France (Fig. 1A)). Identical reference electrodes were placed on each ipsilateral ear lobe close to the ear canal (Fig. 1B). One common ground (Biopac EL258S) electrode was placed on the forehead (Fig. 1C).

2. After placing the electrodes, the participant was positioned in an acoustically attenuated (>30dB) and electromagnetically shielded chamber, and seated in a stationary hydraulic chair, with their head supported by a headrest (Fig. 1C). Participants were instructed to close their eyes closed during the recordings.

3. The signals of both ears were recorded using Spike2™ with a sampling rate of 41,666 Hz for compatibility with previous studies.

Figure II-1: (A) Ear electrode; (B) electrodes placement; (C) participant connection.
The recorded EVestG signal is a combination of FPs buried in noise, environmental artefacts, and biological signals such as; electromyogram (EMG), Electronystagmography (ENG), Electrooculogram (EOG). Figure 2 shows an example of a recorded EVestG signal from both ears.

EVestG repeatability studies\textsuperscript{108} show there is a small diurnal variation but elsewise re-recordings across days at the same time are repeatable if the same signal to noise ratio (SNR) conditions are also repeated. Most of the recordings herein were scheduled between 9am and 12 noon with some who were recorded between noon and 4:00 pm. The SNR of the EVestG signals did vary between the recordings but not significantly. The SNR can be affected by different factors including: (1) the placement of the active electrodes inside the ear, i.e., how far it is from the ear drum (2) the placement of the reference electrodes (3) good contact between the electrodes and the skin (4) wax inside the ear can affect (5) participants’ movement artefact during the recording. Each signal was filtered using a 300 Hz high-pass filter to particularly remove muscle artifacts. Moreover, we
follow an established protocol of the EVestG recording to maintain an adequate SNR recording. The SNR of a recording is always examined by its power spectrum and a relative comparison between its high and low frequency bands; if the SNR is above 20 dB (ratio between the low and high frequency amplitudes above 10), then we proceed to the actual recording; otherwise, the electrodes positioning and connecting cables are examined to bring the SNR to the accepted level.

2.4 PCS follow up recovery

The current standard guidelines recommend a period of cognitive and physical rest in the early days of head injury\(^2,81,82\). However, prolonged rest can lead to physical deconditioning\(^83\), metabolic disturbances\(^84\), and secondary symptoms such as fatigue and depression\(^85\). Moreover, there is no scientific evidence that prolonged rest is beneficial for PCS\(^86\).

The current treatment for PCS includes psychological treatments\(^87,88\) and medications\(^89\). In a study, it was shown that psychological interventions reduced PCS symptoms when applied at 3-6 months after injury\(^88\). However, a recent systematic review of psychological interventions for PCS concluded that there was only limited evidence of benefit and that the studies which conducted these experiments were not well designed\(^90\).

In terms of medication treatment, the most common medications prescribed for PCS are antidepressants\(^89\). Selective serotonin reuptake inhibitors are used for head injury associated depression\(^83,91,92\). Amitriptyline\(^93\) is often used to aid sleep and headaches in PCS patients. Although these medications may improve PCS symptoms, there is no scientific evidence that these medications speed up recovery. This was confirmed by a recent controlled clinical trial that tested the efficacy of sertraline on depression following mTBI\(^92\).
Vestibular rehabilitation is another approach for PCS treatment. Vestibular dysfunction is very common following a mTBI and can delay PCS recovery\(^66\). Thus, some studies have used vestibular rehabilitation and show a reduction in dizziness and improvement in gait and balance\(^94,95\).

In recent years a few studies have considered applying Transcranial Magnetic Stimulation (TMS) as a treatment for PCS/mTBI\(^96–98\). TMS is a popular and well-tolerated, neuromodulation method that is emerging as a therapeutic tool for a variety of neurological conditions particularly depression\(^99–101\). TMS is a non-invasive procedure based on the principles of electromagnetic induction, whereby a magnetic field pulse (generated by the coil placed on the scalp) induces electrical currents in the underlying neural tissue of the brain. The repetitive application of TMS (rTMS) pulses can modulate cortical excitability, either increasing (by high-frequency pulses) or decreasing it (by low-frequency pulses), impacting periods of time well beyond the duration of stimulation. This has shown to have a therapeutic effect for several neurodegenerative diseases, i.e. Parkinson and psychiatric disorders such as depression\(^102–104\).

rTMS pulses are commonly applied to the dorsolateral prefrontal cortex (DLPFC). This site is near the surface of the brain and reachable using a typical figure-8 coil. The DLPFC is known for its involvement in the executive functions, which is an umbrella term for the management of cognitive processes\(^105\), including working memory, cognitive flexibility\(^106\), and planning\(^107\). The DLPFC has primary and secondary association areas including posterior temporal, parietal, and occipital areas, and is described in the pathophysiology of concussion\(^28\). In addition, the DLPFC has a significant role in acetylcholine and dopamine production and modulation; these neurotransmitters have a significant role in restoring normal cognitive function\(^28\). There is evidence that stimulation of DLPFC area can be an effective treatment for depression\(^29\), Alzheimer’s\(^30\) and PCS\(^16\). Moreover, a recent small open-label study of high-
frequency rTMS applied to the DLPFC of mTBI participants supports the tolerability of rTMS treatment in a PCS population and showed a significant improvement in PCS Symptom Scale score\textsuperscript{96}.
References


102. Fitzgerald, P. B. *et al.* Transcranial magnetic stimulation for depression after a traumatic brain


Chapter III – Quantitative Measurement of Post-Concussion Syndrome Using Electrovestibulography

3.1 Synopsis

This study was done to answer the following questions:

1) Whether the characteristic features of the EVestG signals representing the vestibular system can classify PCS individuals from healthy controls.

2) Whether these features are correlated with the duration time between the injury and the recording dates.

3) Whether these features can be related to known physiological changes in PCS.

To answer these questions, EVestG signals were recorded from a group of concussed individuals with PCS as well as a control group of age and gender-matched healthy individuals. The extracted field potentials (FPs) from the EVestG signals of the two groups were compared. Based on this comparison, characteristic features were extracted followed by an unbiased classification routine to investigate whether the features have a diagnostic classification power to differentiate between PCS and healthy control as well as between the short and long-term symptomatology sufferers.

This paper was published in Scientific Reports-Nature Journal, Nov. 2017. Article number: 16371 (2017), 10 pages. **Note:** The manuscript style was designed according to the journal style. **Authors:** Abdelbaset Suleiman, Brian Lithgow, Zeinab Dastghieb, Behzad Mansouri and Zahra Moussavi. **DOI:** 10.1038/s41598-017-15487-2.
3.2 Abstract

In this study, a noninvasive quantitative measure was used to identify short and long-term post-concussion syndrome (PCS) both from each other and from healthy control populations. We used Electrovestibulography (EVestG) for detecting neurophysiological PCS consequent to a mild traumatic brain injury (mTBI) in both short-term (N=8) and long-term (N=30) (beyond the normal recovery period) symptomatic individuals. Peripheral, spontaneously evoked vestibuloacoustic signals incorporating and modulated by brainstem responses were recorded using EVestG, while individuals were stationary (no movement stimulus). Tested were 38 individuals with PCS in comparison to those of 33 age-and-gender-matched healthy controls. The extracted features were based on the shape of the averaged extracted field potentials (FPs) and their detected firing pattern. Linear discriminant analysis classification, incorporating a leave-one-out routine, resulted in (A) an unbiased 84% classification accuracy for separating healthy controls from a mix of long and short-term symptomatology PCS sufferers and (B) a 79% classification accuracy for separating between long and short-term symptomatology PCS sufferers. Comparatively, short-term symptomatology PCS was generally detected as more distal from controls. Based on the results, the EVestG recording shows promise as an assistive objective tool for detecting and monitoring individuals with PCS after normal recovery periods.
3.3 Introduction

Mild traumatic brain injury (mTBI), also known as concussion, is defined as a transient alteration of brain function caused by a biomechanical force to the brain after a head injury, which is usually followed by some permanent or transient neurological symptoms and signs. mTBI is more frequent in teenagers, young adults, males and people who are engaged in high impact physical activities (e.g. soldiers, contact sports players). Following a mTBI, patients usually experience one or more of the following clinical symptoms: headaches, dizziness, depression, memory loss, confusion, blurred vision and balance problems that may occur with or without loss of consciousness. The persistence of these symptoms for more than one week is usually referred to as post-concussion syndrome (PCS). These symptoms except headache and dizziness are often reported for a few days and up to weeks following the injury. The headache and dizziness symptoms typically occur immediately as well as later in the recovery period. The observable neural dysfunction resulting from a head injury in most cases is temporary; however, the symptoms can last for days, weeks (short-term PCS (SPCS)), and sometimes much longer with persisting symptoms which is referred to as long-term PCS (LPCS). Permanent, undiagnosed and untreated symptoms may disable the affected person and, in some cases, it may be fatal.

Persistent PCS has been reported in 20-30% of concussed individuals and comprises incomplete recovery which include somatic (e.g. headaches, dizziness), cognitive (e.g. poor concentration), mood (e.g. depression), visual (e.g. convergence insufficiency, poor accommodation) and behavioural (e.g. irritability) problems. These symptoms can be dominant in the first few hours or days right after the impact but may also persist for weeks, months or even years. Neuropsychological assessments following a concussion have shown that the cognitive function
mostly recovers within 1-3 months’ post-injury\textsuperscript{11}. Additionally, some improvements can take place during the first two years but some patients may remain impaired longer than 2 years\textsuperscript{11}.

The major problems confounding treatment for concussed patients derive from the fact that there is no universally and clinically accepted tool\textsuperscript{12} with (A) the ability to early detect and diagnose the presence of PCS soon after an impact, or (B) the ability to monitor full recovery.

The most commonly and traditionally applied diagnostic tools for concussion and PCS, such as the Sports Concussion Assessment Tool (SCAT3)\textsuperscript{13}, Balance Error Scoring System (BESS)\textsuperscript{14}, and computerized cognitive tests such as CogSport\textsuperscript{15} and ImPACT\textsuperscript{16} are subjective, and rely on self-reported symptoms. Other objective tools, like most of the brain imaging techniques, i.e. computed tomography (CT) or susceptibility weighted imaging (SWI) - magnetic resonance imaging (MRI)\textsuperscript{17}, cannot reliably diagnose mTBI. In addition, the more reliable ones such as diffuse tensor imagining (DTI) have several limitations (e.g. cost, availability, resolution, etc.\textsuperscript{13}). These imaging techniques are more reliable for detecting lesions as the case in SWI-MRI\textsuperscript{17} or skull fracture by CT\textsuperscript{13}. However, in the majority of patients with PCS, the clinical neuroimaging findings are found to be normal\textsuperscript{18, 19}. Such disagreement between the imaging finding and the existence of cognitive impairments in the PCS group might be due to a damage in the neural network rather than a focal lesion site likely to be detected by imaging techniques.

Neuropsychological assessments after mTBI, on the other hand, have a limited ability to accurately detect the presence of PCS mainly due to the fact that the scores can also be affected by several other confounding factors such as intelligence, age, education, depression and malingering\textsuperscript{20}. Thus, having an objective and reliable assessment technique for PCS, such as the one investigated in this study, would be of great interest. There have been some quantitative approaches, such as quantitative EEG (qEEG)\textsuperscript{21-23} and robotic-assisted test battery\textsuperscript{24}, investigating
PCS and its recovery with some very positive outcomes including, when studying different post-concussion times using qEEG, a reported 77.8%-92.3% accuracy in detecting short-term and long-term TBI\textsuperscript{23}. However, there are also some studies that question qEEG’s clinical usefulness\textsuperscript{12, 25} but recent publications do continue to support its utility\textsuperscript{26, 27}. The search for a universally clinically acceptable, quantitative PCS assessment tool continues.

Electrovestibulography (EVestG)\textsuperscript{28} has shown promise as a diagnostic assist tool for neurological and neuropsychiatric conditions such as Meniere’s\textsuperscript{29}, Depression\textsuperscript{30} and Parkinson’s Disease\textsuperscript{31}. EVestG signals are recorded in the external ear. The recorded signal is a combination of acoustic and vestibular generated field potentials (FPs)\textsuperscript{28}. The EVestG technology can detect the vestibular response, which is hypothesized to be driven predominantly from the utricle\textsuperscript{28}. The utricle has tonically active (spontaneously firing) hair cells that can be modulated by linear acceleration\textsuperscript{32-34}. There is support for a vestibular change following mTBI. One of the major symptoms after mTBI is dizziness with about 23-81% of mTBI cases experiencing balance problems and reporting dizziness days after injury\textsuperscript{35}. The prevalence of persistent dizziness after mTBI varies widely from 1.2% at 6 months to 32.5% at 5 years\textsuperscript{36-39}. EVestG signal analysis can detect the changes in the balance/vestibular system\textsuperscript{28}. Using DTI, the vestibulopathy in mTBI patients was found to have a central axonal injury component\textsuperscript{40}. In addition, vascular compression of the 8\textsuperscript{th} cranial nerve has been shown to lead to dysfunction of the auditory and vestibular systems\textsuperscript{41}. Herein, this study presents staged research to evaluate EVestG as an assistive tool to measure and monitor both short and long-term symptoms of PCS.

We hypothesize that one of the many reasons that mTBI patients experience balance problems is due to stretching, compressing or twisting of the vestibular nerve leading to a change in the vestibular response, which may also be persistent. Likewise, if the damaged regions of the brain
that are bruised, swollen or have bled are connected to the vestibular system, their electrical signals related to balance might have changed. Further, during the metabolic cascade following mTBI, the sensory systems including the peripheral vestibular system will be impacted. Thus, the EVestG signals will likely change following a concussive impact, and that may prove to be indicative of a concussion and its continuing symptomatology. This paper reports on the use of EVestG addressing the above hypotheses.

The objectives of this study were to 1) investigate the EVestG detected spontaneous FP changes between concussed individuals with PCS and healthy controls, and 2) investigate whether the features representing those changes have diagnostic classification power between the two groups of PCS and controls.

To accomplish the first objective, we recorded EVestG signals from a group of concussed individuals with PCS as well as a control group of age and gender-matched healthy individuals. We compared the extracted field potentials (FPs) from the EVestG signals of the two groups. To achieve the second objective, we extracted two characteristic features that had shown sensitivity to PCS in our previous study followed by an unbiased classification routine to investigate whether the features have a diagnostic classification power.

3.4 Results

All patients who presented to neuro-ophthalmology clinic and were diagnosed with PCS, met the inclusion criteria for the study and did not have the exclusion criteria, were referred to the research assistant to be included in this study. All referred patients had a diagnosis of PCS, which was made based on the history and the positive examination findings such as convergence insufficiency and abnormal balance, determined by the neuro-ophthalmologist collaborator of the study (4th author).
The inclusion criteria for PCS group were: 1) being over 15 years of age, 2) having at least one head trauma with or without loss of consciousness in the last 10 years, 3) having a Glasgow Coma Scale (GCS) > 13 within 10 minutes after the head trauma, 4) having continued symptoms and signs of concussion one month after the head trauma at the time of neurological examination (e.g. blurred/double vision, vertigo, headache, imbalance, mood/cognitive/sleep abnormalities, convergence insufficiency, eye misalignment, cerebellar/vestibular abnormality, cognitive abnormality as examined by a neurologist/neuro-ophthalmologist (4th author), and 5) having normal hearing. The healthy control group’s inclusion criteria were: 1) being over 15 years of age, 2) have no history of head trauma, ear infection/injury, any psychiatric and/or neurological disorder, and 3) having normal hearing.

Out of 45 referred patients, tested were 38 individuals (19 males, 43±13.5 years) with PCS, out of which 8 were short-term PCS (SPCS- concussion <3 months prior to testing) and 30 patients were long-term PCS (LPCS- concussion >3 months prior to testing). As per International Classification of Headache Disorders-3, post-traumatic headache is called “persistent” if it lasts more than 3 months\(^4\). Therefore, 3 months is the best reasonable criterion to identify short-term from the long-term post-concussion syndrome. That criterion has been extensively used in concussion literature as well.

We also recruited 33 healthy age-and-gender-matched individuals (13 males, 42.5 ±16.2 years) with no history of concussion as the control group of the study.

The duration between the majority of LPCS patient’s mTBI’s and the recording date was between 5 months to 5 years with the exception for two patients remaining symptomatic for more than 10 years prior to the EVestG recording. The aim was to test PCS patients with ongoing symptomology during (1 week to 3 months) and post (>3 months) normal recovery periods to...
investigate whether we could identify any malingering or psychogenic symptomology with the EVestG assessment. Data were recorded at the Neural Diagnostic Laboratory, Riverview Health Center, Winnipeg, Manitoba. Demographic details of the PCS participants can be found in the supplementary Table S1 online (Appendix B).

3.4.1 Signal Analysis

The EVestG-evoked response FPs and their firing pattern from stationary (BGi) segments of the signals were extracted using a wavelet-based signal processing technique called the Neural Event Extraction Routine (NEER)\textsuperscript{28}. The muscle artifacts were removed from the recorded signals by a high pass filter set to 300Hz. Nevertheless, if a signal was corrupted by muscle artifacts, poor electrode placement, movement or contact of the electrodes, that signal was removed from the analysis. Approximately 7\% of signals were excluded. The NEER algorithm detects a series of FPs to produce an average FP plot like the one shown in Fig. 1A. The extracted FPs have the same fundamental shape as the vestibular and acoustic compound action potentials \textsuperscript{45, 46} (Fig. 1B).
However, the cochlear and vestibular periphery have major differences: vestibular axons have a broad spread of thickness and are on average thicker; cochlear axons have a narrow spread and are thinner; this means the averaged vestibular FPs are likely wider than acoustically evoked compound action potentials 47, 48.

Characteristic features of the signals representing the vestibular system and PCS were extracted with the following procedure. Using the FP curve as well as the FP firing pattern, we extracted two types of features as elaborated below.

**Feature Type 1** - After normalizing the FPs with the absolute value of the action potential (AP) point (Fig. 1A), we calculated the area between the baseline and the AP point, which was basically the area of the AP curve below the baseline. This feature type was found significantly different among controls and PCS subjects in a previous study that used a subset of data of this study42. However, when we looked at each subject’s AP area individually, we found in some cases there were differences in the descending part of the AP, the ascending part of AP, or on both sides of the AP. The choice of the AP area as our feature can take into account all three differences. Further investigations are needed to see why some have a difference in the ascending part while some others in the descending part and some in both parts of the AP.

**Feature Type 2** - Beside the FP, the NEER algorithm also provides the time of occurrence of each detected FP. It was shown in 49 that vestibular efferent spontaneous activity is usually seen in the range 10-50 spikes/s. Thus, we also looked for the low frequency (modulated) spontaneous FP interval activity (~ 10 Hz). Since the average measured time gap that NEER algorithm detects between two FPs is ~ 3.3 ms, a 33 FP gap corresponding to about ~100 ms (10 Hz) 30 was used (Fig. 2a). Therefore, the average interval histograms based on 33rd (IH33) FP gap during the no movement (BGi) phases from the signals of study participants was generated. A significant
(p<0.05) difference was found between both groups as the average distribution of the PCS group was shifted towards the right (lower frequencies and longer gaps) as shown in Fig.2b. Feature Type 2 comprised the total percentage of the response intervals with bin value more than 90 ms.

Figure 3 shows the mean ±95% confidence interval of the AP area (Feature Type 1) of the two groups of concussed patients and healthy controls extracted from the right (Fig.3A) and left (Fig.3B) ears. As can be seen, the averaged AP area of patients with PCS was found smaller than that of the healthy controls. In addition, among all the PCS patients, the ones with SPCS-concussion the AP area was the smallest.

Thirteen out of the 38 PCS participants had a lateral head impact either from left or right. In our previous pilot study\textsuperscript{42}, we observed an asymmetry between left and right ear in lateral-impact PCS participants. Indeed, the AP area was always narrower on the coup side, while it was either wider or similar to healthy control response in the contra-coup site. Therefore, we calculated the minimum AP area for left and right ear signals and used this smaller value as a characteristic feature.
Figure III-2: (a) The generation process of finding the gap between 33 FPs and generating the interval histogram. (b) Interval Histogram for an FP gap equal to 33 FPs during static (no motion) phase (BGi). The blue and red solid lines represent the healthy controls (n=32) and PCS (long and short-term PCS, n=38) encased by dashed 95% confidence interval lines respectively.

Figure III-3: Average response for control (n=32) and PCS (long and short-term PCS, n=38) groups. The marked circles/arrows show significant (P< 0.05) difference in the AP area between control and concussed during static segment (BGi) extracted from (A) right ear and (B) left ear.
Figure 2b shows the average interval histograms of the time between detected 33 FPs for both groups during static phase (BGi). We can see that the concussed histogram is shifted to the right of the curve of the healthy controls; this is indicative of an increase in time between IH33 intervals and hypothetically may be related to a reduction or slowing of efferent input. For the same reasons mentioned above on the asymmetry between left and right ear signals for laterally impacted PCS participants, we calculated the maximum IH33 interval difference for left and right ears and used the maximum as our second characteristic feature.

3.4.2 Classification

We applied linear discriminant analysis (LDA)\textsuperscript{50} as the classification routine for separating healthy controls from concussed patients. LDA is a standard approach for supervised classification; it estimates the membership probability of each class as a Gaussian distribution assuming identical covariance matrices for all classes. Due to small size of our dataset and yet keeping the training and testing separate for an unbiased classification and also to avoid the overfitting problem, a leave-one-out routine was applied for classification, in which one subject’s data was left out for testing and the rest used as training; this routine was repeated until all subjects were used as test once. In each fold, the two features were tested individually using LDA for classifying the two groups and the resultant accuracy was calculated. The same routine was repeated for the combination of the two features and the resultant accuracy calculated.
Figure III-4: Combination of features 1 and 2 for separating the groups of Control (n=32) vs. long-term PCS (LPCS- concussion >3 months prior to testing, n=30) plus short term PCS (SPCS- concussion <3 months prior to testing, n=8). Feature 1 is the calculated AP area during static phase (BGi). Feature 2 is derived from the IH histogram using a gap equal to 33 FPs.

Figure III-5: (A) Ear electrode; (B) electrodes placement; (C) participant connection.
Tables 1-4 summarize the LDA classification results with a leave-one-out routine of each of the two features as well as their combination. Feature 1 (the AP area) showed a leave one out test accuracy of 81%, while feature 2 (the IH33) showed a 73% testing leave one out routine accuracy when each is considered for classifying the groups separately. The combination of the two features increased the leave one out accuracy to 84% with an 81.6% sensitivity and 87.5% specificity (Table 1).

The two features used are highly correlated (R=-0.7), however, we are using the IH33 feature as well as the AP area feature as both are potentially physiologically meaningful features (see discussion). To give a visual representation of the selected features among the two groups of controls and concussed (SPCS and LPCS), the scatter plot of the features for the two groups of concussed and controls are shown in Fig. 4. Additionally, this two-feature combination was also able to separate the SPCS and LPCS subgroups of concussed individuals with a 79% accuracy using a leave one out routine. In addition, the LDA classification accuracy of separating LPCS from healthy control was 77% (Table 2), while its accuracy for separating SPCS from healthy control was 95% (Table 3).

The classification accuracy can be improved using other nonlinear classification techniques or a support vector machine (SVM). However, in small sample size studies, LDA is more reliable than other non-linear classification methods because LDA is more robust to the variance. In other words, if a technique shows a reasonable accuracy using the LDA, then we can be confident that the method will perform better using nonlinear classification methods. For comparison, we did run the classification using SVM. The classification accuracy using SVM for separating LPCS and
Tables 1-4: LDA classification accuracies using a leave-one-out routine for features 1 and 2 and their combination. Accuracy, Sensitivity and Specificity were calculated. For comparison SVM classification accuracy for combined features are also presented. AUC represents Area under the ROC curve.

Table III-1: Testing accuracy, sensitivity and specificity of classification between PCS (n=38) and Healthy control (n=32).

<table>
<thead>
<tr>
<th>ACCURACY (%)</th>
<th>SENSITIVITY (%)</th>
<th>SPECIFICITY (%)</th>
<th>AUC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEATURE 1</td>
<td>81.4</td>
<td>84.2</td>
<td>78.1</td>
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<tr>
<td>FEATURE 2</td>
<td>73.2</td>
<td>73.6</td>
<td>72.7</td>
</tr>
<tr>
<td>FEATURE 1&amp;2</td>
<td>84.3</td>
<td>81.6</td>
<td>87.5</td>
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<tr>
<td>FEATURE 1&amp;2(SVM)*</td>
<td>84.3</td>
<td>88.6</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Table III-2: Testing accuracy, sensitivity and specificity of classification between LPCS (n=30) and Healthy control (n=32).

<table>
<thead>
<tr>
<th>ACCURACY (%)</th>
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<th>SPECIFICITY (%)</th>
<th>AUC (%)</th>
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</thead>
<tbody>
<tr>
<td>FEATURE 1</td>
<td>77.4</td>
<td>80.0</td>
<td>75.0</td>
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<tr>
<td>FEATURE 2</td>
<td>73.1</td>
<td>73.3</td>
<td>72.7</td>
</tr>
<tr>
<td>FEATURE 1&amp;2</td>
<td>77.4</td>
<td>76.6</td>
<td>78.1</td>
</tr>
<tr>
<td>FEATURE 1&amp;2 (SVM)*</td>
<td>82.3</td>
<td>85.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Table III-3: Testing accuracy, sensitivity and specificity of classification between SPCS (n=8) and Healthy control (n=32).

<table>
<thead>
<tr>
<th>ACCURACY (%)</th>
<th>SENSITIVITY (%)</th>
<th>SPECIFICITY (%)</th>
<th>AUC (%)</th>
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</thead>
<tbody>
<tr>
<td>FEATURE 1</td>
<td>95.0</td>
<td>87.5</td>
<td>96.8</td>
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<tr>
<td>FEATURE 2</td>
<td>87.8</td>
<td>100.0</td>
<td>84.8</td>
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<tr>
<td>FEATURE 1&amp;2</td>
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<td>87.5</td>
<td>96.8</td>
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<tr>
<td>FEATURE 1&amp;2(SVM)*</td>
<td>97.5</td>
<td>100.0</td>
<td>96.9</td>
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</table>

Table III-4: Testing accuracy, sensitivity and specificity of classification between LPCS (n=30) and SPCS (n=8).

<table>
<thead>
<tr>
<th>ACCURACY (%)</th>
<th>SENSITIVITY (%)</th>
<th>SPECIFICITY (%)</th>
<th>AUC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEATURE 1</td>
<td>76.3</td>
<td>76.6</td>
<td>75.0</td>
</tr>
<tr>
<td>FEATURE 2</td>
<td>55.2</td>
<td>50.0</td>
<td>75.0</td>
</tr>
<tr>
<td>FEATURE 1&amp;2</td>
<td>78.9</td>
<td>80.0</td>
<td>75.0</td>
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<tr>
<td>FEATURE 1&amp;2(SVM)*</td>
<td>92.4</td>
<td>85.7</td>
<td>93.5</td>
</tr>
</tbody>
</table>

* Comparison LDA with SVM
SPCS groups improved to 92% (Table 4) (c.f. 79% for LDA). Also, SVM classification for separating LPCS from controls improved to 82% (Table 2) (c.f. 77% for LDA), and 97.5% for separating SPCS from controls (Table 3) (c.f. 95% for LDA).

The area under the receiver operating characteristic curve (AUC) was calculated (Tables 1-4) as an additional indicator of the diagnostic ability of the features used in binary classification. The SVM showed a better performance than LDA, especially in for the classification of LPCS and SPCS.

3.5 Discussion

Following a mTBI, the most common symptom after headache is dizziness\(^51,52\) that can cause balance problems. Poor balance and postural instability have been reported in many studies after mTBI\(^53-55\) and have been correlated with dysfunction in sensory integration\(^56,57\). Some symptoms including vertigo and dizziness can be due to neurovascular compression of the 8\(^{th}\) cranial nerve\(^58\). Considering this fact and the link between dizziness and the abnormal function of the vestibular apparatus, the representative results of this study support the hypothesis that vestibular activity is perturbed following mTBI. This study has shown that the EVestG evoked averaged FP responses of the two groups of PCS individuals and healthy controls have classification power to separate PCS individuals from controls.

Vestibular\(^45\) and acoustic compound action potentials have similar characteristic shape and both are comparable with the extracted vestibular FP of the recorded signals. Many studies discuss the generation of the acoustic compound action potentials (Fig. 1B) and the generation of its different components\(^46,59-61\), which may help explain the differences seen in the extracted FP between healthy controls and PCS patients in our study. In our results, the significant differences were in the AP part of the FP. According to studies\(^46,60\), the AP region of the signal corresponds
predominantly with the N1 and P1 component (Fig. 1B) of the acoustic compound action potentials. The N1 negative peak is generated by the flow of Na$^+$ current through the voltage-gated Na$^+$ channels into the primary afferent neuron$^{46, 59}$. The P1 component has been shown to be generated by the K$^+$ efflux from primary afferent nerve through voltage-gated K$^+$ channels$^{61}$. The generation of the P1 peak is still a controversial topic, and it is not clear where it is generated. Some studies have shown that after removing the cochlear nucleus (CN) the P1 peak is reduced$^{60}$. Another study$^{46}$ showed the P1 peak was recovered one hour after removing the CN; this indicates the P1 peak might be produced (at least partially) in the 8th nerve, and may not be entirely of CN origin. Brown et al.$^{46}$ showed that applying pressure to the 8th cranial nerve lead to a decrease in the P1 amplitude; that implies any physical change to the 8th cranial nerve results in an AP shape change.

The changes in the AP area observed in the vestibular responses following mTBI in this study can also be partly explained by the fact that after a head trauma, a cascade of neurochemical and neurometabolic events occur$^3$. A physical change of the neuronal cell membrane or the axons leads to the indiscriminate flux of ions through ion gates. This abnormal process increases the release of the excitatory neurotransmitters like glutamate, resulting in further ionic flux. Then, in order to maintain an ionic balance, the Na/K ATP-dependent channels become activated, and that increases the glucose metabolism. This mismatch of energy supplies and demand leads to further cell injury and dysfunction$^3$. Linking the process of the development of the AP after stimulation with the generation of N1 and P1 peaks of the acoustic compound action potentials (Fig. 1B), the abnormal movement of the ions in and out the cell membrane would produce a change the AP shape. However, this change is likely to be short-term, lasting less than 3 months.
To explain our results supporting the long-term symptomatology of our PCS participants, we need to consider the possibility of a more permanent damage that might have occurred in the 8th nerve region. In studies\textsuperscript{62, 63} it was shown that when axons were loaded in vitro, the sodium gates became perturbed resulting in the sodium influx and subsequent depolarization with calcium influx through voltage-sensitive calcium channels and the reversal of the sodium-calcium exchangers. They also showed that post mechanical trauma and deformation of the axons triggered Na\textsuperscript{+} influx through sensitive voltage-gated sodium channels (NaChs); that would result in an increase in Ca\textsuperscript{2+} influx and subsequent proteolysis of the NaCh α-subunit. As a result of the α-subunit degradation, the α-subunit promotes persistent elevation in Ca\textsuperscript{2+}, which helps explain the narrower AP area in long-term PCS. In another study\textsuperscript{64} plaques composed of amyloid β (Aβ) were found in the damaged axons following a brain trauma in humans. In the same study, the authors described the accumulation of Aβ in the damaged axons as well as in a limited number of neurons of the cortex, hippocampus and cerebellum 3 and 7 days and 6 months after trauma in the TBI mice. As mentioned before, the extracted FP curve was always narrower for PCS individuals compared to healthy controls. We speculate that this change is due to an increase influx and efflux of sodium-potassium ions that might be explained by the accumulation of intra-axonal Ca\textsuperscript{2+} \textsuperscript{62-65}. The ability of the Aβ to form neurofibrillary tangles could be the consequence of its ability to increase the Ca\textsuperscript{2+} influx into neurons\textsuperscript{65}.

Following Brown’s\textsuperscript{46} observation that the generation of N1 and P1 peaks of the acoustic compound action potentials were entirely by the 8th nerve, we believe the changes we observe in the AP area (Fig.3A-B) of the PCS individuals arise from the 8th cranial nerve changes. The difference in the AP area during a static phase was observed to be significantly different between both groups, i.e. narrower. This indicates that the PCS individuals’ vestibular system responded
differently from that of the healthy controls. The difference in the AP area among the two groups was observed in both peaks (N1 and P1); this implies more influx of the Na\(^+\) and more K\(^+\) efflux in the PCS group compared to those of the healthy controls, which in turn implies a difference in the depolarization and repolarization mechanism of both groups.

Using the two-feature combination, there were seven misclassified PCS patients. It is of interest to note that most (5 out of 7) of the misclassified patients had the head trauma more than 1.5 years prior to our recording. Thus, considering the plasticity factor of the nerves, one may speculate that those patients had more time to recover. However, one of the two patients who had the impact more than 10 years prior to recording, was classified as healthy, while the other patient was classified as PCS. This suggests PCS individuals’ symptoms may persist for a long time and recovery depends on their brain’s plasticity or other confounding influences like anxiety.

Accurate diagnosis and prognosis of the TBI consequence are essential for patient care and long-term rehabilitation. In a recent study\(^{24}\), using a robotic-assisted assessment of neurological function, they investigated if PCS following mTBI can be predicted during the initial presentation to an emergency department. However, they only validated their prediction accuracy over a short (3 weeks post-injury) duration.

In another study, using qEEG, it was claimed that qEEG analysis, independent of other assessments, could predict the severity of the injury with high accuracy in a post-trauma period ranging from months to 8 years\(^{22, 23}\). However, qEEG has not shown a clear ability to differentiate between SPCS and LPCS\(^{22, 23}\) as both SPCS and LPCS show increased delta and reduced alpha band power\(^{27}\), while PCS within the recovery time and recent to the injury is expected to be different than PCS beyond the recovery time. The authors in\(^{27}\) continue, “EEG/qEEG findings in mTBI have been hypothesized to be related to the known pathophysiology of mTBI, and in some
cases have also been corroborated with other investigations such as neuroimaging or histopathology” but also state, “Although the literature indicates the promise of qEEG in making a diagnosis and indicating prognosis of mTBI, further study is needed to corroborate and refine these methods” 27. In other study25 it was indicated that qEEG provides, at best, an imperfect assessment of mTBI and reports the high specificity of qEEG evaluations of TBI must be interpreted with care questioning qEEG’s disease specificity. However, these authors also state “The published literature does indicate, however, that it (qEEG) can be an important complement to other assessment procedures”25.

In this current study, we showed the use of a quantitative physiological measure of the vestibulo-acoustic response (EVeStG signals) has promising potential to identify SPCS and LPCS both from each other and from healthy control populations. EVeStG is shown to have the potential to monitor the PCS within the non-persisting symptom recovery time and also differentiate it from persistent PCS beyond the normal recovery time. However, according to the literature, the recovery time might be within the first days, weeks, up to 3 months after the injury. Thus, it is possible that some of our SPCS participants were beyond the non-persisting symptom recovery time, and appeared only with persistent symptoms (which may be a reduced form of those in LPCS). That may explain why two out of eight SPCS were classified in the LPCS group (Fig. 4). This is considered a study limitation. The main limitations of this study are: 1) The overall sample size, particularly the SPCS sample size, was small; 2) There were 3 times as many LPCS as SPCS, and 3) detailed symptomatology was not recorded and graded at regular time intervals. Future studies should address these issues. Overall, the results of this study suggest the AP area of the responses during the static phase is a promising feature with a sensitivity to post-concussion
symptoms. The results of this study are encouraging the use of EVestG analysis for screening and monitoring PCS patients (SPCS and LPCS) and the recovery from LPCS-concussion.

3.6 Methodology

Data used in this study was collected through two separate but related projects. One was to investigate the feasibility of the EVestG technology for diagnosis of concussion, and the second was a clinical trial (ClinicalTrials.gov Identifier: NCT02426749) for treatment and recovery monitoring (using EVestG) of post-TBI Syndrome. Both studies were approved by the University of Manitoba Biomedical Research Ethics Board, and all the participants signed an informed consent (Appendix A) prior to the experiment. All experimental procedures were performed in accordance with the protocol approved by the Biomedical Research Ethics Board and its regulations.

3.6.1 EVestG Recording Procedure

4. Placing the electrodes: the ear canal wick electrode was placed in each ear canal close to the ear drum (TM-EcochGtrode, Bio-logic, France (Fig. 5.A)). Identical reference electrodes were placed on each ipsilateral ear lobe close to the ear canal (Fig. 5B). One common ground (Biopac EL258S) electrode was placed on the forehead (Fig. 5C).

5. After placing the electrodes, the participant was positioned in an acoustically attenuated (>30dB) and electromagnetically shielded chamber, and seated in a stationary hydraulic chair, with their head supported by a headrest (Fig. 5C). Participants were instructed to close their eyes closed during the recordings.

6. The signals of both ears were recorded using Spike2™ with a sampling rate of 41,666 Hz for compatibility with previous studies.
The vestibulo-acoustic system is highly spontaneously active\textsuperscript{32-34}. In this study, in order to minimize any artifacts caused by body movement which may corrupt the recorded signal and to, at least initially, consider the ability of features based only on the spontaneous activity of the vestibule-acoustic system to discern PCS, the analyzed EVestG recorded signals are only from the stationary phase (BGi) recordings \textsuperscript{28}. The analyzed segment was the average of three BGi recordings.

References


Chapter IV – Investigating The Validity And Reliability Of Electrovestibulography (EVestG) For Detecting Post-Concussion Syndrome (PCS) With And Without Comorbid Depression

4.1 Synopsis

In this chapter, the Chapter 3 classifier which resulted in 84% accuracy of separating between PCS and healthy controls, was validated using a new blind dataset. As the diagnosis of PCS might be overlooked in favour of a diagnosis of depression due to the overlap between the symptoms, the EVestG-PCS classifier was tested whether it can be influenced when co-morbid depression present. By including previously defined depression feature (Lithgow, 2015), the classifier accuracy improved significantly when co-morbid depression present. The correlation between the EVestG features and the standard neuropsychological assessments used to assess PCS and depression was calculated.

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4.2 Abstract

Features from Electrovestibulography (EVestG) recordings have been used to classify and measure the severity of both persistent post-concussion syndrome (PCS) and major depressive disorder. Herein, we examined the effect of comorbid depression on the detection of persistent PCS using EVestG. To validate our previously developed EVestG classifier for PCS detection, the classifier was tested with a new blind dataset (N=21). The unbiased accuracy for identifying the new PCS from controls was found to be >90%. Next, the PCS group (N=59) was divided into three subgroups: PCS with no-depression (n=18), PCS with mild-depression (n=27) and PCS with moderate/severe-depression (n=14). When moderate/severe depression was present, PCS classification accuracy dropped to 83%. By adding an EVestG depression feature from a previous study, separation accuracy of each PCS subgroup from controls was >90%. A four and three-group (excluding mild-depression subgroup) classification, achieved an accuracy of 74% and 81%, respectively. Correlation analysis indicated a significant correlation (R=0.67) between the depression feature and the MADRS depression score as well as between the PCS-specific feature and Rivermead Post-Concussion Questionnaire (RPQ) (R=0.48). No significant correlation was found between the PCS-specific feature and the MADRS score (R=0.20) or between RPQ and the depression feature (R=0.12).

The (PCS-specific and depression-specific) EVestG features used herein have the potential to robustly detect and monitor changes, relatively independently, in both persistent PCS and its depression comorbidity. Clinically, this can be particularly advantageous.
4.3 Introduction

Individuals, who sustain a head injury, are usually affected by a cluster of cognitive, somatic and emotional symptoms for periods of time. These symptoms usually include headaches, dizziness, fatigue, irritability, reduced concentration, sleep disturbance, memory loss, sensitivity to noise or light, double or blurred vision, nausea, anxiety and depression\textsuperscript{1,2}. These symptoms, except headache and dizziness, are often reported to last for a few days and up to a few weeks following the injury. When these symptoms persist more than a month or so, it is called persistent post-concussion syndrome (PCS). Why and how PCS develops and changes over time has remained controversial for decades. However, it is certain that persistent PCS occurs due to pathophysiological changes occurring after a mild traumatic brain injury (mTBI)\textsuperscript{3} (commonly called a concussion).

One of the most concerning symptoms or psychiatric diagnosis after a head injury is post-concussive depression. The estimated rate of depression in the first year following a mild to severe traumatic brain injury (TBI) ranges from 26 to 53\%\textsuperscript{4-7}. Many studies have shown an association between PCS severity and depression severity after mTBI\textsuperscript{8,9}. Moreover, it was shown that the diagnosis of PCS might be overlooked in favour of a diagnosis of depression\textsuperscript{10}.

There are several diagnostic tools for concussion diagnosis, which are used either alone or in combination. These include the neuropsychological assessments, such as the Rivermead Post-Concussion Questionnaire (RPQ)\textsuperscript{11,12}; The Glasgow Coma Scale (GCS)\textsuperscript{13}, the Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT)\textsuperscript{14}, the Sports Concussion Assessment Tool and the Sport Concussion Assessment Tool version 5 (SCAT5)\textsuperscript{15}. However, it is not recommended to use these assessment outcomes as the sole basis for clinical decision making, as
they may be biased by several confounding factors such as intelligence, age, education, depression and malingering.16

The most used objective techniques are neuroimaging techniques15,17, such as computed tomography (CT) or susceptibility weighted (SWI) magnetic resonance imaging (MRI)18. While these techniques have been shown to be sensitive for identifying moderate/severe TBI where lesions and structural fractures are more likely to exist18,19, they provide little contribution to PCS evaluation as the injury to the neural tissues is micro-structural and is usually not detected with imaging15. More advanced techniques such as positron emission tomography (PET), diffuse tensor imaging (DTI), have more positive outcomes19. However, these techniques still in the early stages of development and cannot be recommended other than in research settings19.

Quantitative electroencephalogram (qEEG)20–22 is another tool which has been used for PCS detection. It has shown a positive outcome in predicting the severity of head trauma and can also provide information on the long-term prognosis. The accuracy obtained for detecting PCS using qEEG is as high as 95.6% for short-term TBI22 and 77% for predicting the existence of the PCS one year after injury23. However, qEEG is not clinically used yet and still some studies question its clinical usefulness24,25.

Electrovestibulography (EVestG)26–28 that measures vestíbulo-acoustic predominantly vestibular response changes29–31. The recorded signal is a combination of acoustic and vestibular generated field potentials (FPs)32. EVestG measures the predominantly vestibular response either statically or in response to passive whole body tilts from the external ear (Fig. 1). It is used for PCS diagnosis because vestibular deficiencies commonly occur after a head injury; these include dizziness33,34, imbalance and vertigo35,36. Up to 81% of the PCS population experienced dizziness
within the first three months after the injury and 23% of the cases continued experiencing dizziness beyond 6 months\textsuperscript{33,34}.

In a recent study\textsuperscript{26}, two features of the average FPs of the EVestG were shown to identify PCS individuals from age-and-gender-matched healthy individuals with an 84\% leave-one-out cross-validated test accuracy when compared to their clinical diagnosis. The two EVestG features were: 1) the area under the baseline and the action potential (AP) point of the average FP signal (Fig. 1D) called AP-area, and 2) relative to controls, the distribution wise change of the FP firing pattern (called IH33). The AP area feature predominantly measures the influx and efflux of sodium and potassium ions of the afferent vestibular nerve, while the IH33 feature is hypothesized to represent the spontaneous activity of the efferent nerve and or α band activity\textsuperscript{26}.

An EVestG feature extracted from post potential trough (PPT) region of the average FP (Fig. 1D), is known to be sensitive to depression. It has been previously applied with two other Depression features as an aid to diagnose major depressive disorder with an \textasciitilde87\% accuracy\textsuperscript{32}. It was similarly described in a depressive phase bipolar disorder study\textsuperscript{37}. Given the common comorbidity of depression with PCS, it is necessary to question whether the PCS classifier is, at least partially, affected by the presence of depression and whether the EVestG PCS features used herein and in\textsuperscript{26} for PCS classification are correlated with the PPT region depression feature. These questions are addressed in this paper.

The goals of this study were two-fold: 1) is to test the validity of our previously developed EVestG classifier for PCS detection\textsuperscript{26} using a new blind dataset, and 2) to test whether the EVestG-PCS classifier is affected by co-morbid depression. Thus, we used the PCS classifier trained and cross-validated on 38 PCS and 33 healthy age-gender-matched controls data from our previous study\textsuperscript{26} and tested it on a newly recorded PCS dataset of 21 individuals. Next, we combined the
two PCS datasets (N=59) and grouped them based on their comorbid depression level based on their Montgomery–Åsberg Depression Rating Scale (MADRS) score. Three groups were formed: PCS with no current depression (MADRS ≤ 6, n=18), PCS with mild depression (7 ≤ MADRS ≤ 19, n=27), and PCS with moderate/severe depression (MADRS ≥ 20, n=14). Then, we used the PPT feature (Fig. 1D) of EVestG shown to be sensitive to depression to help differentiate the above three PCS groups and investigate the correlation of the depression-specific feature with the two PCS specific features. We then determined the correlations of EVestG depression and PCS features (IH33 and AP-area) with the standard neuropsychological assessments used in this study as well as with each other as an indicator of their independence.

4.4 Results

The inclusion/exclusion criteria for PCS group were: 1) being over 15 years of age, 2) having at least one head trauma with or without loss of consciousness in the last 10 years, 3) having a GCS scale > 13 within 10 minutes after the head trauma, 4) having continued symptoms and signs of concussion one month after the head trauma at the time of neurological examination (e.g. blurred/double vision, vertigo, headache, imbalance, mood/cognitive/sleep abnormalities, convergence insufficiency, eye misalignment, cerebellar/vestibular abnormality, cognitive abnormality), and 5) having normal hearing. The healthy control group’s inclusion criteria were: 1) being over 15 years of age, 2) have no history of head trauma, ear infection/injury, any psychiatric and/or neurological disorder, and 3) having normal hearing.

All participants were referred from the neuro-ophthalmology clinic after being diagnosed with PCS and met the inclusion/exclusion criteria for the study. The diagnosis of PCS was conducted by the study neuro-ophthalmologist (author BM).
Table 1 shows demographic information and duration of the injury of the data adopted from the previous study and the new data recorded in this study. Table 2 shows the demographic information of the PCS and depression subgroups. All data were recorded at the Neural Diagnostic Laboratory, Riverview Health Center, Winnipeg, Manitoba, Canada. All participants signed an informed consent (Appendix A) approved by the Biomedical Research Ethics Board of University of Manitoba prior to recording.

Table IV-1: Demographics table of PCS, LPCS, SPCS and healthy controls.

<table>
<thead>
<tr>
<th>Descriptive variables</th>
<th>PCS (n=59)</th>
<th>LPCS (n=44)</th>
<th>SPCS (n=15)</th>
<th>Control (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female</td>
<td>38</td>
<td>30</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Age (SD)</td>
<td>43.3 (12.9)</td>
<td>43.42 (12.48)</td>
<td>42.8 (15.6)</td>
<td>42.5 (16.2)</td>
</tr>
<tr>
<td>Time since injury (years (SD))</td>
<td>2.2 (4.4) [5 months - 5 yrs]*</td>
<td>2.9 (4.8) [1 week – 3 months]</td>
<td>0.1 (0.09)</td>
<td>N/A</td>
</tr>
<tr>
<td>MADRS (SD)</td>
<td>12.6 (8.1)</td>
<td>14.3 (8.4)</td>
<td>7.7 (4.7)</td>
<td>4.1 (1.3)</td>
</tr>
</tbody>
</table>

*4 participants were > 8 yrs.

Table IV-2: Demographics table of PCS when they were divided into subgroups based on their MADRS score (PCS with no depression, PCS & mild depression and PCS & moderate/severe depression).

<table>
<thead>
<tr>
<th>Descriptive variables</th>
<th>PCS with no depression (n=18)</th>
<th>PCS &amp; mild depression (n=27)</th>
<th>PCS &amp; moderate/severe depression (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female</td>
<td>11</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Age (SD)</td>
<td>42.5 (15.8)</td>
<td>41.4 (13.9)</td>
<td>48.1 (10.2)</td>
</tr>
<tr>
<td>Time (years (SD))</td>
<td>0.8 (0.6)</td>
<td>2.1 (3.4)</td>
<td>3.5 (5.3)</td>
</tr>
<tr>
<td>MADRS (SD)</td>
<td>4.6 (1.6)</td>
<td>11.0 (3.5)</td>
<td>24.7 (2.6)</td>
</tr>
<tr>
<td>SPCS (n)</td>
<td>9</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>LPCS (n)</td>
<td>9</td>
<td>21</td>
<td>14</td>
</tr>
</tbody>
</table>
Figure 1D shows an average FP plot, extracted from the EVestG signal using the wavelet-based signal processing technique called the Neural Event Extraction Routine (NEER)\textsuperscript{29}, with areas marked locating where both the PCS and depression features are extracted. An average FP plot can be extracted by the NEER algorithm from all static (stationary—no movement) and dynamic (movement evoked) segments of an EVestG recording. In this current study, however, we analyzed only the static segments (no movement) to be congruent with our method in the previous study. In general, static segments have the least number of movement artefacts.

Figure IV-1: (A) Ear electrode; (B) electrodes placement; (C) participant connection (D) A typical normalized field potential (FP) (Horizontal scale 41.6 samples=1ms). AP-area: the bounded area between the baseline and the AP peak (marked area). PPT area: the bounded area between the samples (571: 619) which represents the PPT range of control population.
**4.4.1 EVestG PCS Features** - *Action potential (AP) area* represents the area bounded between the baseline and the AP point (Fig.1D) of the normalized FP. This feature was found significantly different between controls and PCS participants in previous studies\textsuperscript{26,27}. It showed a high classification accuracy (feature ROC=0.84) between controls and PCS groups. Similar to our previous studies, the AP area was extracted from the static segments; the average AP area was considered an EVestG PCS-specific feature. Figure 2A shows the average FP with marked AP-area for 33 healthy controls and all 59 PCS data.

Beside the FP curve, the NEER records the time of occurrence of each detected FP. The average experimentally measured time gap that NEER detects between two successive FPs is \( \sim 3.3 \) ms. Therefore, a 33 FP gap corresponds to about \( \sim 100 \) ms (10 Hz)\textsuperscript{32}. *The IH33 feature is* a measure of the low frequency (\( \sim 10 \) Hz) modulations of spontaneous (and driven) FP interval activity. It has been hypothesized the low-frequency activity occurs in response to efferent and or \( \alpha \) band activity\textsuperscript{32}. Spontaneous vestibular efferent activity is seen at 10-50 spikes/sec\textsuperscript{38}, and the \( \alpha \) band is 8-13 Hz. The average interval histogram based on the 33\textsuperscript{rd} (IH33) FP gap during the average static segments was then generated. This IH33 feature was also used in a previous PCS study\textsuperscript{26} and showed promise as a feature for separating controls and PCS (ROC=82\%). Figure 2B shows the IH33 histogram with 95\% confidence intervals for the two groups of healthy controls and PCS dataset. As can be seen, the IH33 is shifted right for PCS individuals compared to healthy controls. This shift is indicative of an increase in time between IH33 intervals, and hypothetically may be related to a reduction or slowing of efferent input. Therefore, the calculated feature comprised the total percentage of the interval histogram with bin values of more than 90ms.

Figure 3 shows the scatter plot of PCS individuals (n=59) versus healthy controls (n=33) using the same two PCS features (AP area and IH33) as in\textsuperscript{26}. Similar to our previous study, for this figure
we show the clusters of PCS individuals with short-term injury (concussion<3 months prior to recording), called SPCS, and PCS with long-term injury (concussion>3 months prior to recording), called LPCS, versus controls. Congruent to the previous results, the new PCS individuals (both SPCS and LPCS) were found within the PCS cluster. Moreover, the new SPCS individuals were classified with the SPCS cluster, which was always more distal from controls compared to the LPCS.

When applying the same classifier trained with the previous study’s data (38 PCS and 33 healthy participants) to the new 21 PCS data (14 LPCS and 7 SPCS) using the same AP area and
IH33 features a blind test accuracy of 95% was achieved. This was even higher than the 84% test accuracy reported previously. This increase in accuracy was likely due to the increased ratio of SPCS to LPCS in the new population.

4.4.2 EVestG Depression feature- Within a population of Major Depressive Disorder (MDD) patients, Lithgow et al. showed using 3 features one of which was the post potential trough (PPT) region of the FP signal of the EVestG (Fig.1D) could separate between MDD and healthy controls with high leave-on-out-cross-validated accuracy (~87%). The best classifier feature between controls and MDD (feature ROC=0.75) was found to be the left side PPT region of the FP. Herein, within our PCS population, we compared the average FP of non-depressed PCS and depressed PCS groups using this PPT area feature extracted from static segments (Fig.1D). The

Figure IV-3: Scatter plot of healthy controls (n=33) versus PCS group (n=59) using AP-area and IH33 features. The two subgroups of short- and long-term PCS (SPCS, n=15 and LPCS, n=44) are shown with different colors and markers.
PPT area is defined as the area bounded by samples (571:619) based on the average healthy control FP curve (Fig.1D). As shown in Fig. 4, this area was found marginally significant (p=0.06) different between PCS with no depression (n=18) and PCS with moderate/severe depression (n=14) and also when comparing each of these two groups with the healthy control group (p=0.08). On the other hand, no significant difference was found between these two groups (PCS with no depression and PCS with moderate/severe depression) and PCS with mild depression.

Figure 5 shows the scatter plot for the three different PCS depression severity groups using a combination of AP-area and PPT features. The AP-area ideally classifies PCS, whilst the PPT region area classifies depression. However, these two features extracted from the PCS individuals were significantly correlated (R=-0.28, P=0.03). On the other hand, no significant (R=0.05, P=0.7) correlation was found between these two features when the healthy control population was included (Table 3). As can be seen in Fig. (5A-5B), the PPT feature provided good separation.
between the three PCS depression severity groups. Moreover, a significant correlation was found between the EVestG depression feature (PPT area) and the MADRS score (R= 0.67, P<0.01), while no significant correlation was found between the EVestG PCS feature and the MADRS score (R=0.20, P=0.12). Figure 6C shows an example of the association between the PPT area and the MADRS score. As can be seen, the larger the PPT area (trough area becomes more negative compared to healthy control Fig.4), the higher the MADRS score which in turn implies an increase in depression level.

Using linear discriminant analysis (LDA) incorporating a leave-one-out routine, we calculated the accuracy of separating each of the three PCS depression groups from healthy controls. The features used for classification were: the AP-area, IH33 plot and the PPT area. Table 4 shows the resultant accuracy using each feature alone, while Table 5 shows the resultant accuracy of the combinations of two and three features. As expected, the best combination of the features resulted in higher accuracies, namely, 100% for separating PCS with no depression from healthy controls, and ~94% for separating PCS with moderate/severe depression from healthy controls. Using LDA with a leave-one-out routine, we also calculated the 4 group (3 PCS and Healthy) classification accuracy as well as 3-groups classification (PCS with mild depression subgroup excluded) accuracy as 74% and 81.5%, respectively (Table 6).

We also investigated the correlation between the EVestG features (AP-area and IH33) and the RPQ scores (Table 3). It should be noted that we had RPQ scores for only 26 study participants; the rest were recorded in the first study wherein RPQ was not included in that study’s assessment. The AP-area showed significant correlation with RPQ (R=-0.48, p=0.003) and RPQ13 (R=-0.45, p=0.004) but not with RPQ3 (R=-0.22, p=0.20). No significant correlation was found between IH33 and the RPQ scores (Table 3). Figures 6A and 6B show examples of the association between
the AP-area versus RPQ (Fig. 6A) and RPQ13 (Fig. 6B), respectively. As can be seen, the narrower the AP-area, the higher was the RPQ/RPQ13 score; that implies a decrease in AP-area represents an increase in PCS symptoms severity.

Table IV-3: Calculated correlation between the neurophysiological assessments scores including RPQ and MADRS versus the PCS and the depression-specific features (AP-area and PPT area respectively) (n=26). The AP-area was extracted from the average static segment (BGi) while sitting upright, while the PPT area was extracted from the static segment while sitting in a supine position. All the correlation were calculated without including control data except if it is mentioned.

<table>
<thead>
<tr>
<th></th>
<th>AP-AREA</th>
<th>PPT AREA</th>
<th>IH33</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPQ</td>
<td>-0.48*</td>
<td>0.07</td>
<td>0.21</td>
</tr>
<tr>
<td>RPQ3</td>
<td>-0.22</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>RPQ13</td>
<td>-0.45*</td>
<td>0.12</td>
<td>0.24</td>
</tr>
<tr>
<td>MADRS SCORE</td>
<td>0.16</td>
<td>0.67*</td>
<td>0.20</td>
</tr>
<tr>
<td>PPT AREA</td>
<td>-0.28*</td>
<td>--------</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(without healthy control)</td>
<td></td>
<td>(without healthy control)</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>--------</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>(including healthy control)</td>
<td></td>
<td>(including healthy control)</td>
</tr>
<tr>
<td>AP-AREA</td>
<td>--------</td>
<td>--------</td>
<td>-0.57*</td>
</tr>
</tbody>
</table>

*P<0.05

Table IV-4: LDA classification accuracies using a leave-one-out routine for three features: AP-area, PPT area and IH33. Testing accuracy of each feature for separating each of the three PCS groups (PCS (n=18), PCS& mild depression (n=27) and PCS& moderate/severe depression (n=14)) from healthy controls (n=33).

<table>
<thead>
<tr>
<th></th>
<th>AP-AREA</th>
<th>PPT AREA</th>
<th>IH33</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCS (NO DEPRESSION) VRS. CONTROL</td>
<td>92%</td>
<td>62%</td>
<td>86%</td>
</tr>
<tr>
<td>PCS &amp; MILD DEPRESSION VRS. CONTROL</td>
<td>88%</td>
<td>61.7%</td>
<td>81.7%</td>
</tr>
<tr>
<td>PCS &amp; MODERATE/SEVERE DEPRESSION VRS. CONTROL</td>
<td>83%</td>
<td>85%</td>
<td>72%</td>
</tr>
</tbody>
</table>
Figure IV-5: Combination of the PCS feature (AP-area) and the depression feature (PPT region) for separating the groups of (A) PCS with no depression (MADRS<6, n=18), PCS with moderate/severe depression (MADRS>20, n=14) and healthy control (n=33). (B) When PCS with mild depression (MADRS: 7-19, n=27) was included.

Figure IV-6: (A) The correlation (R=-0.48) between RPQ total and the PCS specific feature (AP-area). (B) The correlation (R=-0.45) between RPQ13 and the PCS specific feature (AP-area). (C) Correlation (R=0.67) between MADRS score and the depression specific feature (PPT area).
Table IV-5: LDA classification accuracies using a leave-one-out routine for three features: AP-area, PPT area and IH33. Testing accuracy of two and three features combination for separating each of the three PCS groups (PCS & no depression (n=18), PCS & mild depression (n=27) and PCS & moderate/severe depression (n=14)) from healthy controls (n=33).

<table>
<thead>
<tr>
<th></th>
<th>AP AREA &amp; IH33</th>
<th>AP AREA &amp; PPT AREA</th>
<th>AP AREA, PPT AREA AND IH33</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCS (NO DEPRESSION) VRS. CONTROL</td>
<td>100%</td>
<td>94%</td>
<td>94%</td>
</tr>
<tr>
<td>PCS &amp; MILD DEPRESSION VRS. CONTROL</td>
<td>90%</td>
<td>83.3%</td>
<td>90%</td>
</tr>
<tr>
<td>PCS &amp; MODERATE/SEVERE DEPRESSION VRS. CONTROL</td>
<td>83%</td>
<td>93.6%</td>
<td>89.4%</td>
</tr>
</tbody>
</table>

Table IV-6: The calculated accuracy of LDA with leave-one-out classification using 3 features for separating PCS with no depression versus PCS & mild depression versus PCS & moderate/severe depression versus healthy control. The 3 group classification accuracy was calculated as well using 3 and 2 features when the PCS & mild depression subgroup was excluded.

<table>
<thead>
<tr>
<th></th>
<th>ACCURACY</th>
<th># FEATURES</th>
<th>FEATURES</th>
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<tbody>
<tr>
<td>4 GROUPS CLASSIFICATION</td>
<td>73.9%</td>
<td>3</td>
<td>AP-area, PPT area and IH33</td>
</tr>
<tr>
<td>3 GROUPS CLASSIFICATION*</td>
<td>81.5%</td>
<td>3</td>
<td>AP-area, PPT area and IH33</td>
</tr>
<tr>
<td>3 GROUPS CLASSIFICATION*</td>
<td>80%</td>
<td>2</td>
<td>AP-area and PPT area</td>
</tr>
<tr>
<td>3 GROUPS CLASSIFICATION*</td>
<td>75.4%</td>
<td>2</td>
<td>PPT area and IH33</td>
</tr>
<tr>
<td>3 GROUPS CLASSIFICATION*</td>
<td>64.6%</td>
<td>2</td>
<td>AP-area and IH33</td>
</tr>
</tbody>
</table>

*Without including PCS & mild depression subgroup
4.5 Discussion

Brain injury can affect how neurons process and transmit information between cells. While affected cells usually recover after an injury, some may degenerate and die\textsuperscript{41}. The neurological changes are identified acutely in the first week post-injury and for individuals with persistent PCS even much later\textsuperscript{3}.

The results of this study indicate that the classifier accuracy based on the two PCS-specific features only produce a high accuracy in classifying PCS from healthy control. However, the performance of this classifier reduces when depression is comorbid. Consequently, we improved the classifier by increasing its dimension from two to three with the addition of an EVestG derived depression feature. This resulted in a better accuracy (from 64\% to 81\%) for separating the PCS with depression group from the healthy control group.

In the previous study\textsuperscript{26}, EVestG was shown to have potentials as a reliable diagnostic assistive tool for PCS and have the ability to provide a measure for recovery from PCS. In addition, the EVestG signal analysis was shown to classify both short- and long-term PCS (SPCS and LPCS) from healthy controls and also from each other with high accuracy. Herein, we tested our previously developed classifiers on a new set of recorded data. The calculated accuracy increased from 84\% to 95\% when blind tested with new data. This accuracy improvement is likely due to a larger percentage of SPCS rather than LPCS subjects who are more likely to be correctly classified. This indicates that our developed classifier for separating PCS and healthy control is likely valid and reliable. It is noteworthy too that of the 21 new set only one was misclassified. As shown in Fig. 3, using the same calculated PCS features as in\textsuperscript{26}, we still see two clear clusters of SPCS and LPCS participants. The responses of the AP-area and IH33 features for LPCS are closer to healthy controls compared to SPCS (Fig. 2C and 2D).
To diagnose PCS, it is imperative for clinicians to systematically evaluate and eliminate the possible contribution of co-morbidities and/or socio-psychological factors that may cause or maintain self-reported symptoms after a mTBI. Depression and anxiety are considered common clinical conditions that often occur after a mTBI which may be due to chronic pain such as headaches and neck pain that might be caused by whiplash.

Depression is considered as one of the most persistent and confounding differential diagnoses for the PCS. It has been reported that PCS can become more severe when comorbid with depression. In this study, the average time between the mTBI event and the EVestG recording was 3.5 ± 5.3 yrs for the “PCS & moderate/severe depression”, 2.1 ± 3.4 yrs for the “PCS & mild depression” and 0.8 ± 0.6 yrs for the “PCS with no depression” (Table 2). This indicates that depression score may increase with time since the injury.

Given that EVestG technology is also sensitive to depression and perhaps mood disorders, we investigated the effect of the comorbid depression on PCS and whether that can be teased out using the EVestG technology. The answer to this question can be seen in Fig. 5B. The three subgroups of PCS with depression were cluster-wise clearly identifiable along the X-axis (the depression (PPT) feature). Of particular note is having the PCS with moderate/severe depression clustered more distal compared to both PCS with mild depression or PCS without depression (Fig. 5A, 5B). The PCS feature (AP-area), as presented in this figure, showed a significant correlation (R=-0.28, p=0.03) with the depression. As a result, the AP-area tends to become slightly wider as depression severity increases (Fig. 4A) potentially confounding the PCS detection.

In our previous study on PCS population, the neurophysiological changes that may take place post injury and could lead to a narrowing of the AP-area were hypothesized. In summary, the
narrowing of the AP-area was argued to be due to an excessive of influx and efflux of sodium potassium ions through the membrane, and this change has been argued to be due to the accumulation of calcium ions (Ca\(^{+2}\)) inside the injured nerves\(^{26}\). On the other hand, the PPT region of the FP is more likely generated as a combination of peripheral and brainstem response activity\(^{46-48}\), and likely corresponds to the repolarization mechanism. This is based on the hypothesis that the PPT region is comparable with the N2 component of the acoustic compound action potentials (Fig.1D). Traditionally, the acoustic N2 peak was thought to be generated in the brainstem and this view was primarily based on the observation that when the cochlear nucleus (CN) was removed, or the central end of the cochlear nerve was sectioned, the N2 peak was abolished\(^{47-50}\). Later, it was shown also that sectioning the cochlear nerve produced only a reduction in the N2 peak amplitude\(^{46}\). Considering the fact that Vestibular\(^{51}\) and the acoustic compound action potentials have similar characteristic shape and both are comparable with the extracted vestibular FP of the recorded signals. We believe that the PPT region of the vestibular FP is also a combination of peripheral and brainstem response activity. Lastly, in a study on depressed population\(^{32}\), it was shown that the average repolarization mechanism in depression was slower than in healthy controls.

Herein, when comparing PCS with no depression and PCS with moderate/severe depression, the repolarization mechanism and in particular the P1 and N2 (see Fig. 1D) peaks appear to occur with longer latencies (Fig.4). We hypothesize the result is a significant FP waveform difference in the PPT region. The generation of P1 and N2 peaks (see Fig. 1D) of the FP corresponds to the efflux of the potassium ions (K\(^{+}\))\(^{26,46}\). It was argued in\(^{26}\), that PCS individuals are characterized as having increased potassium ions (K\(^{+}\)) current efflux, and this was one reason behind the narrowing of the AP-area for PCS individuals. This repolarization mechanism appears to continue being
faster in the PPT region (P1 and N2 peaks). Herein, we further hypothesize this increased flux can also help explain why the generation of the P1 peak is faster for PCS with no depression compared to healthy controls.

When there is comorbid depression associated with PCS, the depression appears to slow this mechanism, P1’s and N2’s latencies increase to become more control like and depression like, respectively. Though not significant (P=0.2), the AP-area of the PCS with moderate/severe depression (red line) is wider than for PCS with no depression (Fig. 4A, black line); i.e. the mechanism of the influx and efflux of sodium (Na⁺) potassium (K⁺) ions may also slow with depression. This decrease continues to be observed in the PCS population with moderate/severe depression group in the PPT region as the potassium ions (K⁺) efflux has a slowed depression component thus, we hypothesize it to be acting in opposition to the faster PCS component and potentially confounding the PCS measures.

From the previous 26,28 and current studies, the AP-area is considered as a robust feature for separating PCS from healthy control. However, it is not as true when it comes to the classification of PCS with comorbid depression versus healthy controls (Table 4-5). Comorbidity of PCS and depression can result in a slightly wider AP-area closer to AP-area of healthy individuals. The presence of depression resulted in a decrease in classification accuracy from 100% in PCS with no depression to 83% for PCS with moderate/severe depression (Table 5). By adding the third (depression) feature, the PPT area, to the previous features, the calculated accuracy improved to 89% for classifying PCS with moderate/severe depression from healthy controls (Table 5). The AP and PPT features have the least correlation between them (Table 3) and interestingly, the use of these two features alone improved this classification accuracy to 93% (Table 5). Therefore, the presence of depression in a person with a history of brain injury can make it potentially more
challenging to diagnose persistent PCS, given the interplay of symptoms. However, the overall results show that the combination of the AP-area, IH33 and PPT area feature resulted in the best accuracy for four and three (excluding PCS with mild depression subgroup) way classifications (Table 6). Thus, using the PCS (AP-area and IH33) and depression (PPT area) specific features we may be able to assist in the detection of someone having symptoms resulted from a head injury, depression or both.

To test the association between the AP-area extracted feature and the severity of the PCS, we calculated the correlation between the AP-area and the RPQ scores. The resultant correlations were significant between the AP-area and RPQ13 (R=-0.45, p=0.004) but not RPQ3 (R=-0.22, p=0.20). This indicates that AP-area is more likely associated with the symptoms which are common to during later stages of the injury as characterized in the RPQ13 score and less so with RPQ3 and the symptoms characterizing the early stage of the injury.

In this study, we showed that when depression and PCS are comorbid in a PCS group, the EVestG features could be used to detect both conditions with two different and relatively independent neurophysiological mechanisms that can be applied simultaneously.

The main limitation of this study is its relatively small sample size. The main finding of this study is that EVestG has the potential to and appears is a reliable tool for assisting in the diagnosis of PCS with and without the comorbidity of PCS and depression.

4.6 Methodology

All the methods and experimental procedures of this study were approved by the University of Manitoba Biomedical Research Ethics Board, and all the participants signed an informed consent
prior to the experiment. All experimental procedures were performed in accordance with the protocol approved by the Biomedical Research Ethics Board and its regulations.

4.6.1 EVestG recording. A typical EVestG signals recording is conducted on a hydraulic chair inside an electromagnetically shielded and sound attenuated (> 30 dB) chamber with eye closed and head supported to minimize the muscle artifacts. The placement of the electrodes includes two electrodes resting close to the tympanic membrane of each ear (Fig.1B), reference electrodes on each ipsilateral earlobe, and a ground electrode on the forehead. The recordings were made whilst the chair was static and moving.

4.6.2 Neuropsychological assessments. Besides the EVestG assessment, participants also completed two neuropsychological assessments: MADRS\textsuperscript{39,40} and RPQ\textsuperscript{11,52}.

MADRS is a commonly used instrument in depression research to measure the severity of depression\textsuperscript{40}. It contains 10 diagnostic questions with a total score of 60. Herein, we used MADRS to measure the depression severity among our PCS population. Based on the MADRS score, the PCS population was divided into three subgroups: 1) PCS with no depression (MADRS score < 6, n=18), 2) PCS with mild depression (7 < MADRS score < 19, n=27), and 3) PCS with moderate/severe depression (MADRS score > 20, n=14).

The RPQ score was used for calculating the severity of the PCS\textsuperscript{11,52}. This questionnaire consists of 16 post-concussion symptoms, and for each symptom, there is a score from 0 to 4 as an indication of the severity of that specific symptom. In this study, we divided the RPQ score into two sub-scores: 1) RPQ-3, which is the score of the first three symptoms of RPQ (headaches, dizziness and nausea) which are particularly common in the early stage post-injury\textsuperscript{11} and 2) RPQ-13, the score of the other thirteen symptoms that are mostly cognitive and emotional symptoms and particularly common as later PCS symptoms\textsuperscript{11}. 
References


50. Sellick, P., Patuzzi, R. & Robertson, D. Primary afferent and cochlear nucleus contributions to


Chapter V - Vestibular Changes After Brain Stimulation Of Individuals With Persistent Post-Concussion Syndrome (PCS)

5.1 Synopsis

The studies presented in Chapters 3 and 4 showed that EVestG has the potential to be used as an assistive tool for detecting both PCS and its comorbid depression. In this chapter, it was investigated whether that EVestG has the potential to monitor follow up recovery after a therapy, in particular, rTMS. It is shown that rTMS is potentially an effective therapy for improving PCS and its comorbid depression and that this can be teased out using the EVestG extracted features from chapters 3 & 4.

This manuscript is a follow up for another paper which has been submitted to Nature Scientific Reports journal. Once that paper is accepted, this manuscript will be submitted to Brain Injury journal. The authors of this manuscript are: Abdelbaset Suleiman, Brian Lithgow, Behzad Mansouri, and Zahra Moussavi.

5.2 Abstract

Electrovestibulography (EVestG) has been separately shown to be a promising diagnostic assist tool for Post-concussion syndrome (PCS) and depression. Herein we investigate if the EVestG features can separately monitor both PCS and depression recovery following rTMS treatment when PCS and depression are comorbid.

Eighteen individuals (9 Males, age=49.5±12.4 years) with PCS were treated with rTMS. Eight were short-term PCS (SPCS- concussion < 1 year prior to baseline assessment) and ten were long-term PCS (LPCS- concussion > 1 year prior to baseline assessment). Half of each subgroup (SPCS
and LPCS) received active rTMS, whilst the other half received sham rTMS. An established EVestG feature for diagnosing PCS as well as an established EVestG feature for diagnosing depression were compared at baseline, post-treatment as well as 1 and 2 months following the end of treatment for efficacy and independence. Quantitative comparative measures were the Rivermead post-concussion questionnaire (RPQ) and the Montgomery Asperg Depression Rating Scale (MADRS).

When compared to baseline, using univariate analysis, the SPCS subgroup who received active rTMS, showed a significant improvement in both the EVestG-depression and EVestG-PCS feature two months after treatment. The LPCS subgroup who received active rTMS showed a significant improvement only in the EVestG-depression feature. The EVestG-depression improvement was correlated with the MADRS score change (R=0.63, P=0.01). This EVestG-PCS improvement was correlated with RPQ score change (R=-0.78, P=0.001). The EVestG depression and PCS features are relatively independent measures (R = -0.17, P=0.16) each assistive in monitoring depression, PCS and comorbid PCS/depression changes. These results support rTMS treatment for both depression and PCS symptoms.
5.3 Introduction

There is evidence that neuropathological, neurophysiological and neurocognitive changes occur following mild traumatic brain injury (mTBI)\(^1\). Individuals who sustain a brain trauma, often complain of physical, emotional and cognitive symptoms including headaches, dizziness, irritability, memory loss, visual disturbance, sensitivity to noise, depression, and anxiety\(^2,3\). Headache and dizziness usually occur immediately after injury as well as later in the course of recovery\(^4\). These symptoms usually persist for months to years following a mTBI and are referred to as post-concussion syndrome (PCS)\(^2,5\). The symptoms normally occur within the first 3 months following the injury. However, 20-40% of cases continue to experience PCS at 6 months post-injury\(^6\) and 10-20% still have difficulties at 1 year and beyond\(^7\). The most commonly and traditionally applied diagnostic tools are brain imaging techniques\(^8,9\). However, a majority of mTBI patients do not show any acute intracranial findings in imaging\(^10,11\). Therefore, these techniques are not reliable enough to be used as a diagnostic tool as well as for monitoring recovery.

Recently, repetitive Transcranial Magnetic Stimulation (rTMS) has been suggested as an effective treatment\(^12,13\) of PCS, and Electrovestibulography (EVestG) suggested to detect PCS-induced vestibulo-acoustic predominantly vestibular\(^14\) changes for use in diagnosis\(^15\) and for measuring treatment efficacy\(^13\). rTMS is a promising, well-tolerated, non-invasive brain neuromodulation technique emerging as a therapeutic tool for a variety of neurological conditions. Two recent studies\(^12,13\) show it is safe and well-tolerated by most PCS patients and it may be considered as a promising PCS treatment\(^13\), however, rTMS can have serious side effects such as seizures, but these are extremely rare\(^16,17\). Other side effects such as transient headache, local pain, neck pain, toothache and paresthesia are more common\(^16\). EVestG\(^18\), on the other hand, is a non-
invasive technique that records neural activity particularly from the vestibular apparatus and vestibular nuclei. EVestG signals can be recorded from the external ear from spontaneous activity or in response to a vestibular stimulus. EVestG signal analysis demonstrates promise for diagnosis and separation of patients with Major Depression\textsuperscript{19}, Meniere’s disease\textsuperscript{20,21}, PD\textsuperscript{22,23} and PCS\textsuperscript{15,24,25} from healthy controls.

The big question is does rTMS improve PCS, depression or both? In our previous study in a PCS population\textsuperscript{15}, the extracted features from the EVestG signal could discriminate between PCS and healthy controls with good accuracy (84%). Moreover, using EVestG features, we were able to separate PCS individuals with recent injury (<3 months) and long-term injury (>3 months) from healthy controls as well as from each other with accuracies of 77%, 95% and 79% respectively. In a recent study\textsuperscript{26}, it was shown that rTMS is a tolerable treatment and an effective treatment option for individuals with an injury <1 year).

In a different study on a completely different depression population\textsuperscript{19}, EVestG extracted features resulted in an unbiased classification accuracy of 87% for separating major depression from healthy controls. Also, in chapter 4 it was shown that EVestG has the potential to robustly detect and monitor changes in depression and PCS relatively independently with >80% accuracy. Therefore, we hypothesize that EVestG can monitor the recovery from both PCS and its comorbid depression. The objectives of this study were: 1) to investigate the EVestG feature changes in PCS individuals following rTMS treatment when they do and do not have comorbid depression and, 2) to investigate whether EVestG features can independently monitor PCS and depression changes following rTMS, 3) answer the question “Can rTMS applied to PCS patients with a depression co-morbidity improve both PCS and Depression?
5.4 Methodology

The data used in this study was adopted from a recent study of rTMS treatment on PCS individuals\textsuperscript{13}. Added to that data are the results of subsequent active rTMS treatment for two of those subjects who initially received sham treatment. Below is a brief description of the previous study\textsuperscript{26} and data collection.

5.4.1 Study design

The research design was a randomized, placebo-controlled and double-blind clinical trial study to study the effects of rTMS on PCS. PCS participants were randomly assigned to one of two groups of active or sham rTMS treatment. The participants in the sham treatment group were given the opportunity to receive active treatment after the study was completed. Two participants from the sham group agreed to receive active treatment after completing the study.

This study was approved by the University of Manitoba Biomedical Research Ethics Board, and all the participants signed a consent form (Appendix A) at the beginning of the study. Patients were primarily assessed at the Adult Medical Clinic, Victoria General Hospital and Traumatic Brain Injury Clinic, Winnipeg and referred by coauthor BM. The rTMS treatment and the EVestG recordings were conducted in Riverview Health Center, Winnipeg, Manitoba.

5.4.2 Intervention

All PCS participants received 13 sessions of 750 either active or sham rTMS pulses at 20 Hz with 28.5 sec intertrain interval between the 25 trains of pulses, applied to the left dorsolateral prefrontal cortex (DLPFC) over 3 weeks. For more details of the treatment protocol see a recent study of rTMS treatment on PCS individuals\textsuperscript{26}. 
The inclusion criteria were: 1) being above 19 years old, 2) having at least one or more head trauma in the last 5 years, 3) Glasgow Coma Scale (GCS) range is 13-15, 4) Having continued symptoms (e.g. blurred/double vision, vertigo, headache, imbalance, mood/cognitive/sleep abnormalities) and signs (e.g. abnormal convergence insufficiency, eye misalignment, cerebellar/vestibular abnormality, cognitive abnormality) of PCS at least one month after the head trauma at the time of enrollment, and 5) having a normal hearing test.

Participants were excluded from the study if they had brain lesions, heart disease, alcoholism, pregnancy, a history of epilepsy or seizure, metallic objects or pacemakers in the body, active use of illicit drugs, or use of neuro- or psycho- active medications.

The 33 healthy control data used in this study, were taken from a previous study\(^\text{15}\)

5.4.3 Participants

As shown in the flowchart in Fig. 1, eighteen individuals (9 Males, age=49.5±12.4 years) with PCS were recruited from the pool of patients visiting their treating neurologist (Author B.M.). Eight participants were short-term PCS (SPCS, concussion < 1 year prior to baseline assessment) and ten were long-term PCS (LPCS, concussion > 1 year prior to baseline assessment). The duration between the brain injury and the baseline assessment of SPCS participants was 4.5 months to 11.5 months (8.8± 2.4 months), while for LPCS it was 1.2 years to 4.8 years (32.0 ± 14.0 months). The participants’ demographics are shown in Table 1.
Figure V-1: Flow chart of enrollments and treatment
5.4.4 Outcome Measures

All study participants were assessed at baseline, immediately after the treatment set and at two more follow up assessments each one month apart. The assessments included: EVestG, Montgomery Asberg Depression Rating Scale (MADRS), the Rivermead Post Concussion Symptoms Questionnaire (RPQ) and a screening hearing test.

Table V-1: demographics table

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (SD)</th>
<th>Years since injury (SD)</th>
<th>Gender</th>
<th>Handedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPCS-Sham (n=5)</td>
<td>56.8 (10.8)</td>
<td>2.5 (1.2)</td>
<td>3M, 2F</td>
<td>5 Right, 0 Left</td>
</tr>
<tr>
<td>SPCS-Sham (n=4)</td>
<td>49.3 (5.4)</td>
<td>0.7 (0.2)</td>
<td>1M, 3F</td>
<td>4 Right, 0 Left</td>
</tr>
<tr>
<td>LPCS&amp;SPCS-Sham (n=9)</td>
<td>53.0 (8.8)</td>
<td>1.6 (1.2)</td>
<td>4M, 5F</td>
<td>9 Right, 0 Left</td>
</tr>
<tr>
<td>LPCS-Active (n=5)</td>
<td>50.8 (17.4)</td>
<td>2.8 (1.3)</td>
<td>4M,1F</td>
<td>4 Right, 1 Left</td>
</tr>
<tr>
<td>SPCS-Active (n=4)</td>
<td>42.5 (15.4)</td>
<td>0.8 (0.2)</td>
<td>1M, 3F</td>
<td>4 Right, 0 Left</td>
</tr>
<tr>
<td>LPCS&amp;SPCS-Active (n=9)</td>
<td>46.7 (15.3)</td>
<td>1.8 (1.4)</td>
<td>5M, 4F</td>
<td>8 Right, 1 Left</td>
</tr>
</tbody>
</table>

5.4.5 EVestG measurement

All study participants (both sham and active groups) had four EVestG assessments: at baseline, immediately post-treatment and two follow-ups at 4 and 8 weeks after the end of treatment. For EVestG measurement, the participants sat in a hydraulic chair with their eyes closed and head supported; all recordings were made with no motion applied.

The recordings were made in an acoustically attenuated (>30dB) and electromagnetically shielded chamber at the EVestG Lab, Riverview Health Center. The recording electrodes were silastic wrapped silver wire with the tip covered in cotton wool soaked in a mixture of saline and
conductive gel to reduce interface impedance (Fig. 2A); they were placed in both ears proximal to the tympanic membrane and on the ipsilateral earlobes as reference (Fig.2B). A common ground electrode (Biopac EL258S) was placed on the forehead.

The left and right ears’ signals were recorded using Spike2 software via a CED-1902 amplifier (60Hz notch filter, 10k gain, 1Hz high pass filter) and digitized using CED1401 ADC board at a sampling rate of 41,666Hz. Additionally, the signals were filtered using a 300 Hz high-pass filter to particularly remove muscle artifacts. The wavelet-based signal processing technique, called the Neural Event Extraction Routine (NEER)\textsuperscript{18}, was used to detect spontaneous vestibular field potentials (FPs) buried in the noise similar to the one shown in Fig.2C.

![Figure V-2: (A) silastic wrapped silver wire with the tip covered in cotton wool soaked in a mixture of saline and conductive gel. (B) Electrodes placement. (C) A typical normalized FP. The bounded area between the baseline and the AP (marked area) is our calculated feature. (Horizontal scale 41.6 samples=1ms).](image-url)
Montgomery-Åsberg Depression Rating Scale (MADRS) - MADRS is one of the most commonly used instruments in depression research\textsuperscript{27}. It is a ten-item diagnostic questionnaire with a total score of 60 that is used to measure the severity of depression. Usually, a score >6 indicates depression with 7-19 being considered asymptomatic-mild and >18 moderate-severe. Inter-rater reliability of MADRS ranges from 0.89 to 0.97\textsuperscript{27}. Table 2 summarizes the MADRS score for each subgroup.

Rivermead Post Concussion Symptoms Questionnaire (RPQ)

RPQ is a self-reported and reliable measure of PCS. It was originally developed as a measure of the severity of the symptoms post injury\textsuperscript{21, 22}. The questionnaire consists of 16 post-concussion symptoms including headaches, dizziness, nausea, sensitivity to noise and light, sleep disturbance, fatigue, irritability, depression, feeling frustrated, forgetfulness, poor concentration, taking longer to think, blurry vision, double vision, and restlessness. Each symptom was rated by individuals a scale from 0 to 4 where 0 indicates that the symptom is not experienced at all, 1 indicates that it is no longer a problem, 2 indicates a mild problem, 3 indicates a moderate problem, and 4 indicates a severe problem. The “no longer was a problem (1)” response was changed to 0 by the assessor before summing the final score of all symptoms. It was shown in\textsuperscript{28} that the 16 questions making up RPQ should not be combined into single score but broken into RPQ-3 (headaches, dizziness and nausea) and RPQ-13 (others) to form a unidimensional construct. The first three symptoms of RPQ (headaches, dizziness and nausea) are referred to as RPQ-3 and are the early (immediate post-injury) symptoms associated with the PCS\textsuperscript{21}. The other thirteen symptoms are referred to as RPQ-13 and are the late symptoms associated with PCS\textsuperscript{21}. The RPQ-13 and RPQ-3 scales showed test-retest reliability coefficients of 0.89 and 0.72 (both p-values < 0.01)\textsuperscript{28}. Table 2 summarizes the RPQ-3 and RPQ-13 scores for each subgroup.
**EVestG Analysis**

In our previous pilot study\(^{15}\) we showed that the action potential (AP) area of the field potential (FP), extracted from the static recording, was narrower for PCS participants compared to healthy controls (Fig.3). We investigated the change in the AP area feature from baseline to (1) post-treatment, (2) one month (Follow-up 1 (FU1)) and (3) two months (Follow-up 2 (FU2)) after the end of treatment. Table 2 summarizes the calculated AP-area for each subgroup.

We analyzed data using a multivariate Analysis of Repeated Measures ANOVA with “Time” as a within-subjects factor with four levels (baseline, post-treatment, FU-1 and FU-2), “Treatment Group” (real and sham) as a between-subjects factor, and “Time Since Injury” (SPCS and LPCS) as a second between-subjects factor. We also investigated the correlation of the EVestG extracted feature to the RPQ, RPQ-3, RPQ-13 and MADRS scores presented in our previous paper\(^{13}\).

**Table V-2**: The average outcome measurements for all subgroups during the four assessments (baseline, Post, FU-1 and FU-2).

<table>
<thead>
<tr>
<th>Assessment</th>
<th>RPQ3 Mean (SD)</th>
<th>RPQ13 Mean (SD)</th>
<th>MADRS Mean (SD)</th>
<th>AP-area [EVestG] Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment time</td>
<td>Baseline</td>
<td>Post</td>
<td>FU-1</td>
<td>FU-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPCS Sham [n=5]</td>
<td>6.6 (3.8)</td>
<td>5.6 (2.6)</td>
<td>6.2 (3.6)</td>
<td>5.8 (3.5)</td>
</tr>
<tr>
<td>SPCS Sham [n=4]</td>
<td>8.9 (3.4)</td>
<td>6.8 (3.9)</td>
<td>5.5 (2.8)</td>
<td>4.3 (3.1)</td>
</tr>
<tr>
<td>LPCS/SPCS Sham [n=9]</td>
<td>7.6 (3.1)</td>
<td>5.1 (3.1)</td>
<td>6.9 (3.1)</td>
<td>5.1 (3.2)</td>
</tr>
<tr>
<td>LPCS-Active [n=5]</td>
<td>9.7 (2.0)</td>
<td>7.4 (2.9)</td>
<td>6.8 (2.5)</td>
<td>6.7 (2.0)</td>
</tr>
<tr>
<td>SPCS-Active [n=4]</td>
<td>10.3 (1.7)</td>
<td>7.0 (3.4)</td>
<td>5.0 (4.1)</td>
<td>3.8 (3.8)</td>
</tr>
<tr>
<td>LPCS&amp;SPCS-Active [n=9]</td>
<td>7.7 (3.0)</td>
<td>6.7 (2.9)</td>
<td>5.5 (2.9)</td>
<td>5.6 (2.9)</td>
</tr>
</tbody>
</table>
5.5 Results

5.5.1 EVestG PCS-specific feature (AP-area) changes due to treatment:

Figure 3 shows the average FP waveform with the standard error (SE) ranges overlaid of the SPCS and LPCS participants at baseline. These were compared to the average healthy control. As expected, congruent to our previous study, the AP-area of SPCS was narrower than LPCS and both of them were narrower than the healthy control.

Figure 4A and 4B show the change of the AP-area feature extracted from the average static segments (BGi) during the four assessments of SPCS and LPCS participants, respectively. The multivariate test did not show any significant differences overall between the assessments.

We also tested data using univariate tests. As can be seen in Fig. 4A, SPCS participants who received the active treatment showed a significant improvement (increase) in the AP area during the FU-1 and FU-2 assessments compared to baseline, while SPCS subjects who received the sham treatment showed a slight change at the follow-up assessments. No significant improvement was found in the LPCS group (Fig. 4B).

To give a visual presentation of the change of the FP after rTMS, Fig. 5 was generated. It shows the average FP±SE extracted from the EVestG signals during baseline assessment which was then compared to the FU-2 assessment. This comparison was done in 4 different groups: the two SPCS subgroups (active and sham) and the two LPCS subgroups (active and sham). Only SPCS subgroup who received active rTMS showed a significant difference (P< 0.05) between baseline and FU-2 assessments in the AP area Fig. (5A). Figure 6A and 6B were generated to show that there were no significant differences in the AP-area between the active and sham groups in SPCS (Fig. 6A) and LPCS (Fig. 6B), respectively, at baseline.
5.5.2 EVestG depression-specific feature changes due to treatment:

In a previous study, it was shown that EVestG has the potential for detecting depression and its severity. And in chapter 4, it was shown that EVestG has the potential for detecting PCS and its comorbid depression. Herein, the same depression feature (FP-shape) used in those studies was investigated in our PCS participants and we investigated whether this feature changed in response to rTMS treatment. The EVestG feature sensitive to depression was extracted from the post potential trough (PPT) region of the FP and it is potentially related to an altered brainstem response (See Fig. 5, depression feature). Figure (5A and 5B) show the marked area, which represents the depression feature before and after active rTMS in both SPCS and LPCS. This change (towards a control like or PCS without depression like PPT area response) was observed in PCS participants (SPCS & LPCS) who received active rTMS, but not in the sham group. When compared to the MADRS score, the improvement observed in the SPCS depression feature was consistent with the MADRS which showed a drop in its score (-11± (-4) SD) and it was significantly correlated.

Figure V-3: The average ± SE of the extracted FP of SPCS (n=8) (blue) and LPCS (n=10) (orange) groups during baseline and were compared to average healthy control (n=30) (grey).
(R=0.63, P<0.01) (Table 3A), however, the MADRS score of the LPCS subgroup showed a slight increase (3.5 ± (-8) SD) which is opposite to what the EVestG depression feature showed.

Figure V-4: (A-B) The average ± SE of the calculated feature (AP area) extracted from the EVestG signal during stationary segments in all four assessments. (A) Short-term PCS (SPCS) participants who received Real (n=4) and Sham (n=4) treatments. (B) Long-term PCS (LPCS) participants who received Real (n=5) and Sham (n=5) treatments. (C-F) Rivermead post-concussion questionnaire (RPQ) difference in each follow-up assessment from baseline. Each point is the average change ± standard error (SE). (C) RPQ-3 of SPCS participants who received Real (n=4) and Sham (n=4) treatments. (D) RPQ-3 of LPCS participants who received Real (n=5) and Sham (n=5). (E) RPQ-13 of SPCS participants who received Real (n=4) and Sham (n=4) treatments. (F) RPQ-13 of LPCS participants who received Real (n=5) and Sham (n=5).
5.5.3 Rivermead versus EVestG

To investigate the association between the RPQ and the EVestG AP-feature results, the correlation between these two assessments was calculated. The RPQ score was divided into two sub-scores: (1) RPQ-3: the score of the first three symptoms (headaches, dizziness and nausea) which are common in the early stage of concussion. (2) RPQ-13: the score of the rest thirteen symptoms which are more common as for late PCS symptoms. The correlation coefficients between the total RPQ, RPQ-3 and RPQ-13 scores and the AP area extracted from the average static segments (BGi) of the EVestG signal were calculated and shown in Table 3B. As can be seen in Table 3B, the AP area was significantly correlated with the RPQ (R=-0.35, P=0.003), RPQ3 (R=-0.53, P=0.000) and RPQ13 (R=-0.28, P=0.02). The correlation was higher in the SPCS subgroup who received active rTMS (Table 3B).

Figure V-5: The average ± SE of the extracted FP during Baseline and FU2 of (A) SPCS who received real rTMS (B) LPCS who received real rTMS (C) SPCS who received sham rTMS (D) LPCS who received sham rTMS.
Table V-3: Calculated correlation

A. Calculated correlation between the EVestG depression feature and the EVestG PCS-feature (AP-area) and MADRS for all PCS and SPCS data pooled across the four times (baseline, post, FU-1 and FU-2).

<table>
<thead>
<tr>
<th>Depression Feature</th>
<th>AP-area (correlation, p)</th>
<th>MADRS (correlation, p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All PCS (N=68), 2 tail test, *=0.05 and **=0.01 significance</td>
<td>-0.17 (0.16)</td>
<td>-0.01 (0.90)</td>
</tr>
<tr>
<td>SPCS Active subgroup (N=14), 2 tail test, *=0.05 and **=0.01 significance</td>
<td>-0.03 (0.91)</td>
<td>0.63** (0.01)</td>
</tr>
</tbody>
</table>

B. Calculated correlation between the EVestG feature (AP area) and the RPQ scores and MADRS for all the PCS and SPCS data pooled across the four times (baseline, post, FU-1 and FU-2).

<table>
<thead>
<tr>
<th>Depression Feature</th>
<th>AP-area (correlation, p)</th>
<th>RPQ (correlation, p)</th>
<th>RPQ3 (correlation, p)</th>
<th>RPQ13 (correlation, p)</th>
<th>MADRS (correlation, p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All PCS (N=68), 2 tail test, *=0.05 and **=0.01 significance</td>
<td>-0.357** (0.003)</td>
<td>-0.529** (0.000)</td>
<td>-0.280* (0.021)</td>
<td>-0.022 (0.856)</td>
<td></td>
</tr>
<tr>
<td>SPCS Active subgroup (N=14), 2 tail test, *=0.05 and **=0.01 significance</td>
<td>-0.785** (0.001)</td>
<td>-0.780** (0.001)</td>
<td>-0.764** (0.001)</td>
<td>-0.545* (0.043)</td>
<td></td>
</tr>
</tbody>
</table>

Figure V-6: The average ± SE of the extracted FP during Baseline of (A) SPCS who received active and sham rTMS (B) LPCS who received active and sham rTMS.
5.6 Discussion

In our recent work, we showed that EVestG has the potential to diagnose PCS and also to separate short and long-term PCS\textsuperscript{15}. It was also shown that EVestG has the potential to detect comorbid depression in the PCS population (chapter 4). On the other hand, in two recent studies\textsuperscript{12,13}, it was shown that rTMS is effective for improving PCS that persists beyond the usual 3-months post-concussion recovery period. In this study, using data of the double-blind placebo-controlled rTMS on PCS population study\textsuperscript{13}, we investigated changes resulted from rTMS treatment in the EVestG PCS-specific feature as well as the EVestG depression-specific feature when comorbid depression exist.

The results shown in Fig. 4A-4B which represent the vestibular changes during the static segment, were very similar to the RPQ13 changes in the previous study\textsuperscript{13}. Both EVestG and the RPQ scores verify that the rTMS treatment was more effective for the SPCS rather than the LPCS group. The two diagnostic tools showed a significant improvement in the FU-2 assessment between the active and sham groups for the SPCS group. However, the RPQ13 showed this significant difference earlier, in the FU-1 assessment (Fig. 4C-4F).

There was no significant difference in vestibular responses of SPCS and LPCS groups at baseline (Fig. 4). This confirms that the observed changes in the SPCS group due to treatment was indeed due to the rTMS treatment. The EVestG analysis, congruent with RPQ results, indicate that the treatment was effective only for individuals with short-term injury (SPCS group) (Fig.4).

As rTMS with the same protocol is also used for depression treatment\textsuperscript{29} one may question whether the improvements seen in SPCS group of this study was due to improvement in their depression. It is also possible that the rTMS treatment improved both PCS and depression
symptoms in the SPCS group. The EVestG analysis results congruent with MADRS scores imply both were improved.

What might be the mechanism that caused the rTMS to have an effect on PCS and its comorbid depression? In a recent study on PCS\textsuperscript{15}, it was discussed that the narrowing of the EVestG-AP area after a mTBI, may correspond to an excessive influx and efflux of sodium potassium ions through the membrane of the injured nerves that could be as a result of the Calcium (Ca\textsuperscript{2+}) ions concentration’ increase. We hypothesize that rTMS caused a degree of regulation of ions movement and caused the influx and efflux of sodium potassium ions to slow. A common interpretation of the high-frequency rTMS effect on the nerves is that it modulates the cortical excitability via long-term potentiation (LTP). It was shown that the direction and magnitude of the synaptic plasticity depend on the postsynaptic Calcium (Ca\textsuperscript{2+}) flux; a high level of (Ca\textsuperscript{2+}) leads to LTP. Following on from our hypothesize in\textsuperscript{15}, the level of (Ca\textsuperscript{2+}) increases in the injured nerve after a head injury. Additionally, we hypothesize that the level of (Ca\textsuperscript{2+}) in the injured nerve during the early stage of the head injury (< 1 year) is more than its level > 1 year. Therefore, we hypothesize that this is one reason that rTMS made the AP area increase, i.e. a decrease in the sodium and potassium influx and efflux in the AP region of the response for the SPCS participants and not for the LPCS. We recognized that the location of the rTMS application in our study i.e. prefrontal cortex, is far from where we capture the treatment effect by EvestG i.e. vestibular nerve. Therefore the modulatory effect of rTMS over the ionic status of the neurons is not just a localized effect. Our results clearly show that the rTMS effect can be transferred to different brain areas through broad connections to and from prefrontal cortex, which is considered a “hub” in functional and anatomical brain connectivity field of research.
On the other hand, the depression feature which was calculated from the post potential trough (PPT) region of the FP is more likely generated as a combination of peripheral and brainstem response activity\textsuperscript{30} and it corresponds to the repolarization mechanism. Figure 5A and 5B show the change of the PPT region after receiving the active rTMS for both SPCS and LPCS participants.

In summary, our further examination of the plausible effect of rTMS treatment on depression indicates that active rTMS resulted in significant improvement in depression indices for SPCS and to a lesser extent LPCS. However, this may be partially dependent on the initial baseline depression level although no significant difference was found in the FP baseline between active and sham for SPCS (Fig. 6A) and LPCS (Fig. 6B) participants. EVestG features for depression and PCS show that compared to sham, the active rTMS treatment has resulted in improvement in depression and PCS of the SPCS group (Fig.4A). While for the LPCS group, EVestG features indicate a moderate improvement in depression and very little in PCS symptoms (Fig.5B). Although, EVestG features for depression and PCS are used for different diagnostic i.e. the AP area feature was extracted to differentiate between PCS and healthy control on a concussed population while the depression feature was extracted to differentiate between major depression and healthy control in a depressed population, these observations still do not answer whether the PCS improvement was due to primarily a depression improvement or vice-versa, and the question still remains to be investigated in a larger study perhaps by investigating which feature might show improvement first.

It is worth mentioning that two of the SPCS participants of the sham group, received an active rTMS after they completed the initial double-blind study as shams. The FP extracted from the last follow-up assessment of the sham treatment was considered as the baseline for the active rTMS treatment. The EVestG analysis of the two individuals’ data, shown in Fig.7, is also congruent with the results of the SPCS group of the active treatment. These results indicate that the rTMS
can have a positive effect on the vestibular response and that the AP area of the EVestG signal is a robust diagnostic assist and monitoring feature for PCS.

This study showed that the EVestG and in particular the AP area extracted feature has the potential to monitor the primarily PCS recovery and these findings were consistent also with the RPQ results\textsuperscript{13}. EVestG also has shown the potential to detect the depression comorbidity of PCS as well as each pathologies recovery. We acknowledge the small size of the study and it is considered the main limitation of this study. Future studies will be conducted on a larger group to confirm our findings. We are also aiming to investigate the possibility of using EVestG to determine/investigate whether from the baseline FP the PCS participants will respond well to the treatment or not.

![Figure V-7: Extracted FP of two participants before and after real rTMS treatment. The two participants completed first the sham treatment and after that they received the real rTMS. Circled part of the FP represents the AP and the change before and after real rTMS.](image)
References


Chapter VI – Association Between EVestG Extracted Features And
Convergence Insufficiency In Post-Concussion Syndrome

6.1 Synopsis

Studies presented in Chapters 3-5 show the vestibular system is affected in PCS. There is evidence that other sensory modalities are also affected by PCS. The visual system is commonly impacted in PCS and one test hypothesized to reflect this is convergence insufficiency (CI). As the link between the visual and vestibular systems is well established, this study aimed to explore whether there is an intimate relationship between these two systems in PCS. The effect of the mTBI on the CI was investigated and found to be significantly correlated with the EVestG features and RPQ.

This manuscript has been submitted to Annals of Biomedical Engineering (BMES) Journal. The authors of this manuscript are: Abdelbaset Suleiman, Brian Lithgow, Mehrangiz Ashiri, Zahra Moussavi, and Behzad Mansouri.

6.2 Abstract

The vestibular and visual systems are intimately linked, and they are commonly impacted by post-concussion syndrome (PCS). Electrovestibulography (EVestG) recordings have shown the vestibular response is different in PCS compared to healthy control groups. Herein, we investigate the relationship between the vestibular and visual systems within a PCS population using EVestG and convergence insufficiency (CI).

Forty-eight PCS individuals (44.5±14.9 yrs, 16 males) were tested using EVestG, out of which 20 (46.3±15.1 yrs, 8 males) also completed the Rivermead post-concussion questionnaire (RPQ).
The EVestG feature (FP-area) was extracted from the stationary (i.e. background recording) part of the EVestG signals. CI was measured at near vision with a cross-cover test using a prism bar by a neuro-ophthalmologist.

Results indicate the average Field Potential area (FP-area) and the CI value are significantly correlated in patients with PCS (R=-0.68, p<0.01). Also, there is a significant correlation between RPQ3 (Headache, Dizziness, and Nausea symptoms) and CI (R=0.70, p<0.01) and between RPQ3 and FP-area (R=-0.56, p<0.02). When the PCS population was sub-grouped based on their Montgomery Asberg Depression Rating Scale score, CI was highest in the PCS with no-depression (CI=7.5 prism diopter (PD) ± 1.2(SE)), moderate (CI=5.2 PD ± 1.0) in PCS with mild depression, and lowest in PCS with moderate/severe depression (CI=1.6 PD ± 0.7).

To the best of our knowledge, this is the first study that objectively demonstrates a correlation between PCS, its depressive severity and CI. This finding is advantageous for clinical decision-making and for PCS-rehabilitation.
6.3 Introduction

Studies show the vestibular system is affected in individuals with post-concussion syndrome (PCS)\textsuperscript{1–3}. There is evidence that other sensory modalities such as the visual system, are also affected in PCS\textsuperscript{4–6}. Studies have shown that this impact on the visual system might be reflected in the high prevalence of vergence eye movement abnormalities in PCS. A common vergence eye movement abnormality is convergence insufficiency (CI)\textsuperscript{7–11}. As the link between the visual and vestibular systems is well established\textsuperscript{12}, this study aimed to explore the intimate relationship between these two systems in PCS.

Annually, an estimated 42 million people worldwide suffer a mild traumatic brain injury (mTBI), also known as concussion\textsuperscript{13}. mTBI may occur following a transient alteration of consciousness that can be caused by biomechanical forces to the head (e.g. sports injuries, falls, motor vehicle accidents, etc.). mTBI can be followed by some transient or permanent neurological symptoms. Following a head injury, individuals may show a cluster of cognitive, somatic and emotional symptoms for an extended period of time after the accident referred to as PCS. While PCS usually resolves within three months after the injury\textsuperscript{14}, 5 to 15\% of the concussed population may carry their symptoms and functional impairment for months to years\textsuperscript{15,16}; in that case, they are diagnosed with persistent PCS. Persistent PCS impacts individuals’ personal and social life\textsuperscript{17,18}.

It has been suggested that patients with mTBI can experience acute and chronic visual symptoms because of disrupted cortical and subcortical visual pathways\textsuperscript{4}. In addition, the ocular sensory-motor function has been strongly linked to a number of cognitive functions including attention, visuospatial processing, working memory, speed processing and predictive behaviour\textsuperscript{12,19–21}. Of the four stereotyped kinds of the eye movements i.e. saccade, pursuit,
vergence, and vestibule-ocular reflex (VOR), vergence dysfunction is more prevalent and more severe among mTBI than other ocular sensory-motor dysfunctions.

After a head injury, mTBI individuals often experience, dizziness and imbalance as major symptoms. The cause of dizziness after head trauma is not completely understood. Plausible causes of dizziness after a head injury might include; direct damage to the vestibular labyrinth, unilateral vestibular nerve injury, structural central nervous system damage, or ocular sensory-motor abnormalities such as vergence dysfunction. Below is a brief review of the brain regions controlling vergence eye movement and their link to the vestibular system.

6.3.1 Brain regions controlling vergence eye movements

Recent primate studies on rhesus monkeys have shown that the pathways shown in Fig. 1 are involved in controlling vergence. Visual information originating from the retina reaches the primary visual cortex (striate cortex) through the lateral geniculate nucleus (LGN) of the thalamus. The primary visual cortex projects to the extra-striate cortex, wherein it branches into two brain regions responsible for producing vergence eye movements: 1) the parietal cortex, which in turn sends fibers to frontal eye field (FEF) areas and the nucleus reticularis tegmenti pontis (NRTP), and 2) the supraoculomotor area (SOA), which eventually controls some of the actions of the medial recti muscles that then generates convergence eye movements. In human studies, it has been shown that acquired cerebral lesions, e.g. parietal lobe, may cause fusional convergence abnormality. Furthermore, studies in stroke patients have shown that damage to the NRTP causes vergence dysfunction. It has also been shown that during convergence, neural activity increases in vergence-sensitive cells in the SOA and NRTP.
6.3.2 Brain areas that are connected to CI and their link to the vestibular system

The anatomical connections between the vestibular system and vergence eye movement are complex (Fig. 1, for details, see (Leigh and Zee 2015)\textsuperscript{12}). The FEF and SOA areas have direct and indirect connections to the cerebellar cortex; the cerebellum also plays an important role in vergence eye movement\textsuperscript{31}. The FEF sends and receives innervations to and from the several brain centers such as the pulvinar nucleus, supplementary eye field, thalamus, and superior colliculus which play a significant role in eye movements including vergence\textsuperscript{12,32,33}. The superior colliculus, in particular, plays an important role in encompassing a topographical map of the visual field of view\textsuperscript{34}. The superior colliculus makes connections with the vestibular nuclei through the medial longitudinal fasciculus (MLF) which contains burst-tonic fibers that change their firing activity in response to the vergence\textsuperscript{35}. The MLF plays a significant role in adduction ipsilateral eye movement (i.e. rolling the eyes in) and damage to the MLF causes loss of the ability to adduct eyes which is known as Inter Nuclear Ophthalmoplegia (INO)\textsuperscript{36}. Importantly, vestibular and ocular sensory-motor signals integrate within the superior colliculi.

The SOA along with other brain regions those are engaged in convergence eye movements, such as pulvinar and extraocular muscles’ nuclei (i.e. cranial nerves 3, 4 and 6), have neural links to the vestibular nuclei and other vestibular-processing brain regions e.g. the Flocculonodular lobe and immediately adjacent vermis part of the cerebellum\textsuperscript{37}. Finally, the Vestibular nuclei send secondary efferent signals via the efferent vestibular system to the vestibular periphery (semicircular canals and otolith organs) modulating hair cell firing and consequently afferent vestibular signals. Thus, there are neural pathways by which the vestibular nuclei can affect the eye movements including vergence (Fig. 1).
Based on the above review, we hypothesize that the vestibular function (measured by the features extracted from the Electrovestibulography (EVestG)\textsuperscript{38}) is correlated with CI in the PCS population as:

1. The vestibular system has input to the visual system. In particular, the cortical regions responsible for vergence eye movement are intimately linked to the vestibular system.

2. From an epidemiological point of view, there is evidence indicating the increased prevalence of convergence abnormality in PCS; 90\% of patients with mTBI had one or more oculomotor dysfunctions\textsuperscript{7} and the prevalence of vergence dysfunction and especially convergence insufficiency ranged between 40-42\%\textsuperscript{7,39} in PCS versus 5\% in the normal population. It has also been demonstrated that vestibular response is commonly affected in PCS\textsuperscript{3}.

3. Common symptoms of PCS relate to dizziness and balance, which are likely caused by mTBI effects to the vestibular/cerebellar systems and their connections which are then intimately linked with pathways which are ultimately responsible for ocular sensory-motor functions and particularly the vergence eye movements. The reverse pathway is also true. This close ocular-vestibulo relationship can explain the presence of some ocular and vestibular symptoms and signs in PCS particularly if common pathways are damaged in the mTBI.

Herein, we record the vestibular response by EVestG as it can measure the spontaneous and driven vestibulo-acoustic, predominantly vestibular activity. Recent studies have shown that EVestG\textsuperscript{40} can detect biomarkers for diagnosing PCS and its comorbidities such as depression\textsuperscript{3,41–43}. The objectives of this study are to investigate: (1) whether the EVestG extracted features characterizing the PCS population\textsuperscript{3} are also correlated with CI and its severity, (2) if the EVestG
features used to characterize PCS are different between PCS populations with and without CI and,
(3) the correlation between CI and RPQ and the correlation between CI and the EVestG FP-area
feature, (4) whether there is an association between CI and depression, which is possible given
EVestG has already been shown also to detect depression comorbidity in PCS population\textsuperscript{43}.

Figure VI-1: A diagram describes the brain regions and selected pathways involved in
generating a vergence eye movement along with associated vestibular connections. SOA, Supraoculomotor area; NRTP, Nucleus reticularis tegmenti pontis; DLPN, Dorsolateral pontine nucleus; AN, Abducens nucleus; VN, vestibular nucleus; SC, Superior colliculus (Dashed lines refer to indirect connections and solid lines refer to direct connections).
6.4 Methodology

6.4.1 Participants and Assessments

Concussed participants were recruited from the pool of patients visiting their treating neuro-ophthalmologist (author BM); they were diagnosed with PCS, which included the diagnosis of convergence insufficiency, vertigo or imbalance without any other neurological disorders that could explain or confound their diagnosis. Forty-eight individuals (16 males, 44.5±14.9 years) with PCS (Table 1) were tested, out of which 10 (3 males, 43.2 ± 18.5 years) had short-term PCS (SPCS- concussion <3 months prior to testing) and 38 patients (13 males, 44.8 ± 13.7 years) had long-term PCS (LPCS- concussion >3 months prior to testing).

The duration between the head injury and the recording date for the majority of LPCS patients was between 3 months and 5 years with the exception for two patients remained symptomatic for more than 10 years prior to testing. Data were recorded at the Neural Diagnostic Laboratory, Riverview Health Center, Winnipeg, Manitoba. The PCS participants completed comprehensive neuropsychological assessments by a neurologist and neuro-ophthalmologist in addition to the RPQ and Montgomery Asberg Depression Rating Scale (MADRS), Glasgow Coma Scale (GCS) test and a screening hearing test (all had normal hearing).

This study was approved by the University of Manitoba Biomedical Research Ethics Board, and all the participants signed an informed consent (Appendix A) prior to the experiment.

6.4.2 CI measure

The CI was measured at near vision with a cross-cover test using a prism bar by a neuro-ophthalmologist for all participants. Cross-cover test is an objective measurement and the gold
standard in measuring strabismus, i.e. ocular misalignment, or the vertical and horizontal deviation of the eye\textsuperscript{43} (for details see Appendix C).

Table VI-1 Demographics table

<table>
<thead>
<tr>
<th>Descriptive variables</th>
<th>PCS without CI (n=28)</th>
<th>PCS with CI (n=20)</th>
<th>PCS with no depression</th>
<th>PCS with mild depression</th>
<th>PCS with moderate/severe depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>32 (14.9)</td>
<td>46.0 (12.2)</td>
<td>43.3 (16.4)</td>
<td>38.4 (16.6)</td>
<td>45.7 (15.4)</td>
</tr>
<tr>
<td>Age (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.5 (14.9)</td>
<td>46.0 (12.2)</td>
<td>43.3 (16.4)</td>
<td>38.4 (16.6)</td>
<td>45.7 (15.4)</td>
<td>48.4 (9.7)</td>
</tr>
<tr>
<td>Time since injury (years (SD))</td>
<td>2.2 (3.8)</td>
<td>1.7 (1.4)</td>
<td>2.8 (5.1)</td>
<td>0.9 (0.6)</td>
<td>1.7 (1.7)</td>
</tr>
<tr>
<td>CI (SD)</td>
<td>27.0 (7.1)</td>
<td>25.4 (5.7)</td>
<td>28.6 (5.6)</td>
<td>41.0 (7.7)</td>
<td></td>
</tr>
<tr>
<td>FP-area (SD)</td>
<td>32.4 (10.2)</td>
<td>40.0 (8.9)</td>
<td>27.0 (7.1)</td>
<td>25.4 (5.7)</td>
<td>28.6 (5.6)</td>
</tr>
<tr>
<td>RPQ3 (SD)</td>
<td>7.3 (2.6)</td>
<td>5.0 (2.6)</td>
<td>8.4 (1.9)</td>
<td>7.5 (1.9)</td>
<td>7.4 (2.8)</td>
</tr>
<tr>
<td>RPQ13 (SD)</td>
<td>28.3 (11.2)</td>
<td>26.7 (14.3)</td>
<td>29.0 (9.4)</td>
<td>22 (11.4)</td>
<td>28.0 (9.4)</td>
</tr>
</tbody>
</table>

* Clinically extraocular motility < 4 prism diopter (PD) considered normal CI and its measure was set as zero. SD=standard deviation.

6.4.3 EVestG recording

A typical EVestG recording procedure comprises placing active and references electrodes. Active electrodes (TM-EchoGtrode, Bio-logic, France (Fig. 2A)) were rested close to the tympanic membrane of each ear (Fig. 2B). Identical reference electrodes were placed on the entrance of each ear canal. One common ground electrode (Biopac EL258S) was placed on the forehead. The EVestG is conducted with eyes closed and head supported to minimize the muscle artifacts on a hydraulic chair inside an electromagnetically shielded and sound attenuated (> 30 dB) chamber (see Appendix C).
The recording was performed whilst the chair was stationary and moving. However, we only analyzed the stationary segments (no movement) to minimize the body movement artefacts resulted from the tilting. From our previous study\(^3\), we know that the use of the stationary segment data can result in good separation of PCS and healthy control populations.

To produce an average FP plot, shown in (Fig. 3), the wavelet-based signal processing technique called the Neural Event Extraction Routine (NEER)\(^40\) averages the detected spontaneous and driven FPs buried in the noise.

![Image](image.png)

**Figure VI-2:** (A) Ear electrode; (B) electrodes placement; (C) participant connection.

### 6.4.4 Neuropsychological assessments

*Rivermead Post-Concussion Questionnaire (RPQ)* score was used for calculating the severity of the PCS\(^35,36\). The questionnaire consists of 16 post-concussion symptoms and for each symptom, there is a score from 0 to 4 as an indication of the severity of this specific symptom. In this study, we divided the RPQ score into two sub-scores to achieve unidimensional construct\(^35\): 1) RPQ-3 is the score of the first three symptoms of RPQ (headaches, dizziness and nausea). They are categorized as most common symptoms after a concussion\(^35\). 2) RPQ-13 is the score of the other thirteen symptoms and they are particularly
common as later symptoms associated with PCS\textsuperscript{35}. RPQ-13 includes 4 questions particularly focused on depression and mood.

**Montgomery–Åsberg Depression Rating Scale (MADRS)** - MADRS is a test used to measure the severity of depression\textsuperscript{37}. It contains a ten-item diagnostic questionnaire with a total score of 60. Score <6 indicate no depression, score=7-19 being considered asymptomatic-mild depression and score >19 is moderate/severe depression.

### 6.5 Results

Screening test of hearing showed that all patients had normal hearing. Figure 3 is an example of an extracted averaged FP extracted from EVestG signal during the background phase. The average extracted amplitude of the FP is normalized to -1 as it can differ according to the electrode contact and placement (i.e. how close it is to the ear drum) facilitating comparison between participants. In our previous study\textsuperscript{3}, comparison of PCS and healthy control average FPs resulted in a significant (p<0.05) difference between PCS and control groups in the action potential (AP) area (see Fig. 3). AP area represents basically the area bounded between the baseline and the AP point as shown in Fig.3. In the current study, in addition to the AP area as a characteristic feature for separating PCS and healthy controls, we also calculated the post-AP area (area bounded between baseline and the post-AP peak) of the average FP (see Fig. 3). Then, the association between the combined areas and CI was calculated and called FP-area. By adding the post-AP area we arguably include an increased representation of the brainstem activity (see discussion section).

Figure (4A) illustrates how FP-area and the extraocular motility scale for CI measured with a cross-cover test using a prism bar are related. A significant (p<0.05) correlation (R=-0.68) was found between these two features for the PCS population including all short and long-term
injuries (Fig. 4A). When the PCS population was divided into SPCS and LPCS subgroups, the correlation between FP-area and CI was still significant in the SPCS (R=−0.94, p<0.01) and the LPCS (R=−0.66, p<0.01) subgroup (Fig. 4A).

Beside the FP, the NEER algorithm also provides the time of occurrence of each detected FP. Vestibular efferent spontaneous activity is usually seen in the range 10-50 spikes/s\textsuperscript{47}. Thus, we also looked for modulations of efferent spontaneous FP interval activity in this low-frequency range (~10 Hz). Since the average measured time gap that NEER algorithm detects between two FPs is ~3.3 ms, a 33 FP gap corresponding to about ~100 ms (10 Hz)\textsuperscript{48} was used. Therefore, the average interval histograms based on 33\textsuperscript{rd} (IH33) FP gap from the signals of study participants were generated. Figure (5) shows the IH33 histogram of the “PCS without Cl” and “PCS with Cl” dataset with 90% confidence bars. As it can be seen, the IH33 is shifted to the right in individuals suffering “PCS with Cl” (longer time interval between firing)

![Diagram](image_url)

**Figure VI-3:** A typical normalized FP extracted from EVestG signal during background phase. AP area: the bounded area between baseline and AP peak. Post AP area: area bounded between baseline and Post AP peak. The sum of the AP area and post AP area was used as a characteristic feature and called the FP-area. (Horizontal scale 44.1 samples=1ms)
compared to those experiencing “PCS without CI” (shorter time interval between firing). This shift is hypothetically related to a reduction or slowing of efferent activity.

Previous studies have shown that eye movement impairment after a head injury can vary between adolescent males and females\(^4^9\), with females being more likely to have eye movement impairment after a head injury. It was also shown that there is an association between abnormality of eye movement and depression as well as other mood disorders\(^5^0\). Therefore, in order to test different factors which might lead to CI development in PCS patients, we divided our PCS data into subgroups: (a) PCS with and without CI, (b) PCS with no depression (n=14), mild depression (n=19), and moderate/severe depression (n=15) (the depression level was determined based on their MADRS assessment); (c) SPCS and LPCS, and (d) Male and Female; we considered each subgroup as an independent variable. Then, we used the Univariate analysis to test subgroup-wise each of the measurements we used in this study: (1) CI measure (prism diopter (PD)), (2) EVestG AP area feature, (3) MADRS, and (4) RPQ3 and RPQ13 scores, respectively.

For univariate tests, Mauchly’s test indicated that the assumption of sphericity had not been violated for any of the dependent variables. Univariate analysis showed that EVestG feature was significantly (p=0.01) different between PCS with and without CI groups (F (1, 32) =12.45, \(\eta^2=0.28\)). A significant (p=0.04) difference was also obtained for the EVestG feature between the SPCS and LPCS groups (F (1, 32) =4.36, \(\eta^2=0.12\)). No main effect for depression and gender on the EVestG feature was obtained. However, post-hoc analysis showed a significant difference in the EVestG feature between PCS with no depression and PCS with moderate/severe depression (p<0.01), and between PCS with mild depression and PCS with moderate/severe depression (p<0.01).
No main effect was found for each independent variable (depression, SPCS/LPCS, gender groups) for the CI measure. However, post-hoc analysis showed a significant difference in the CI measure between PCS with no depression and PCS with mild depression (p=0.02), PCS with no depression and PCS with moderate/severe depression (p<0.01), as well as for PCS with mild depression and PCS with moderate/severe depression (p<0.01).

Of the total of 48 participants listed in our database, only the last 20 completed the RPQ test as the rest were recorded in another study\(^3\) wherein RPQ was not included in that study’s assessment. For this sample, RPQ3 showed a significant correlation (R=0.70, p<0.01) with CI and FP-area (R=0.56, p<0.02) (Fig. 4B). No significant correlation was found between RPQ13 and the CI or FP-area. However, when the PCS population was sub-grouped based on their MADRS score into three subgroups of PCS with different levels of depression, RPQ13 showed

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**Figure VI-4:** The calculated area of the extracted FP from EVestG signal during background recording versus the extraocular motility scale (prism diopter (PD)) for (A) All PCS participants (n=48). PCS (Blue regression line), LPCS (Green regression line) and SPCS (Orange regression line). (B) The calculated score of the first three symptoms of RPQ (RPQ3) versus the extraocular motility scale (PD) for concussed participants (n=20).
a significant correlation with CI (R=0.8, p=0.03) and FP-area (R=-0.8, p=0.03) in the PCS with no depression subgroup (Table 2).

Figure 6 shows the correlation between CI and RPQ13, CI and RPQ13 when only the 4 RPQ depression questions were used in the calculation, FP-area and RPQ13, and FP-area and RPQ13 when only the depression symptoms were used in the calculation. This figure shows

<table>
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<th>Table VI-2: Calculated correlations between the four measurements (CI, FP-area, RPQ3, RPQ13) of all the PCS population (n=48), PCS with no depression, PCS with mild depression, PCS with moderate/severe depression.</th>
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<td>FP-area (n=15)</td>
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*p < 0.05,      **p< 0.01
how the calculated correlation between RPQ13 and CI or FP-area increases when the PCS population was sub-grouped based on their depression score.

6.6 Discussion

CI is common in individuals with mTBI affecting their quality of life and functional abilities\textsuperscript{7-10}. In the introduction, the complex neural network that connects the vestibular system to the brain centers that control vergence eye movement was described. PCS can manifest with vestibular, visual and ocular sensory-motor dysfunction. It is not clear whether CI is the direct consequence of a vestibular abnormality or whether the vestibular and CI are each consequent to the mTBI. Vergence dysfunction and especially CI are considered the most common ocular sensory-motor dysfunction after a mTBI\textsuperscript{7}. There are many studies supporting the idea of using ocular sensory-motor dysfunction assessment and in particular CI for diagnosis of patients with PCS\textsuperscript{8,24}, however, there are some authors who disagree with using CI as a tool in the assessment of PCS patients\textsuperscript{51}. In the current study, we investigated the association between CI and PCS using our novel technology i.e. EVestG.

Our study results show a significant association (R=-0.68, p<0.01) between CI and the corresponding FP-area. Our previous study\textsuperscript{3} showed that the AP-area can be used as a robust feature for differentiating between PCS patients and healthy controls (the AP-area of the PCS patients was always narrower than healthy control). In this study, no significant association was found between CI and the AP-area; however, a significant association was found when the AP-area and post-AP area were combined (Fig.3). This can be explained by the fact that different areas of the FP are created as a result of the activity within the vestibular periphery, vestibular nucleus (brainstem) and the efferent vestibular system (EVS) loop. Hypothetically, the AP-area region of the FP is generated as a result of the influx and efflux of sodium and
potassium ions through the membrane of the peripheral nerve\textsuperscript{52} while the post-area region is generated as a combination of the peripheral and brainstem response activity\textsuperscript{52-54}. Therefore, having a significant association only when the post-AP is added to the AP-area is justifiable as the post-AP area includes a measure of that brainstem (vestibular nucleus) response activity.

In addition, it was shown in the previous study\textsuperscript{3} that when the PCS participants were divided into two subgroups of SPCS and LPCS (short and long-term), the AP-area of the SPCS subgroup on average was shown to be narrower than that of the LPCS subgroup\textsuperscript{3}. Herein, separating the PCS sample into the SPCS and LPCS also produced a significant correlation between the FP-area and the CI measure for both subgroups (Fig. 4A). As shown in Fig. 4A, the SPCS subgroup still shows a smaller FP-area compared to LPCS. Moreover, the correlation coefficients found herein between the FP-area and the CI measure were negative; this indicates that larger FP-area tend to be associated with smaller CI measure. This is consistent with the previous study\textsuperscript{3}, where the PCS population (mostly LPCS) with a larger FP-area (c.f SPCS) were shown to cluster closer to healthy control.

The reduction or slowing of efferent activity (right shift in Fig. 5) between “PCS with CI” and “PCS without CI” is in agreement with a previous study\textsuperscript{37}, in which activation of the brainstem oculomotor control nuclei was compared between PCS patients and healthy controls. Using fMRI, they showed a reduction in the signals in areas that mediate vergence eye movements such as the superior colliculi, the oculomotor nuclei, the abducens nuclei, and in the supra-oculomotor area (SOA).

Our results showed a significant correlation between CI and RPQ3 scores but not between CI and RPQ13 when the entire PCS population were considered. While it was shown in another study, the more convergence insufficiency, the higher severity of double vision/blurry vision
at near range, headaches and dizziness symptoms\textsuperscript{55}. Thus, a significant correlation between CI and the RPQ3 (headaches, dizziness and nausea) is expected. These results are also consistent with the results from a previous study\textsuperscript{24} in which vestibular/ocular motor screening (VOMS) was used to effectively identify PCS. VOMS measure was positively correlated with the total score of the post-concussion symptom scale (PCSS) (R=0.65, P<0.03).

A significant correlation between RPQ13 and CI or FP-area was observed only in the PCS with no depression subgroup. Although these correlations were not significant in the “PCS and mild depression” and “PCS and moderate/severe depression”, RPQ13 was positively correlated with CI (i.e. an increase in CI corresponded to an increase in the symptoms severity) and negatively correlated with the FP-area (increased FP-area is closer to healthy control loci and corresponds to reduced symptom severity) as expected (Fig. 6).

![Interval Histogram for an FP gap equal to 33 FPs.](image)

Figure VI-5: Interval Histogram for an FP gap equal to 33 FPs. The green and red solid lines represent the PCS without CI (n=20) and PCS with CI (n=28) encased by dashed 90\% confidence interval lines, respectively. The 95\% confidence interval did not show any significant difference.
Unlike several studies\textsuperscript{50,56,57} that have shown depression and other mood disorders are positively associated with abnormalities in eye movements and visual processing, herein in our PCS sample, the CI measure and depression were negatively correlated (Fig. 6B). We found a significant difference between the PCS and comorbid depression subgroups, wherein PCS with no depression subgroup had the highest CI measure (CI=7.5 PD ± 1.2(SE)), PCS with mild depression subgroup showed moderate CI measure (CI=5.2 PD ± 1.0(SE)) and PCS with moderate/severe depression subgroup had the lowest CI measure (CI=1.6 PD ± 0.7(SE)). Likewise, the average FP-area increases with depression (see Table 1). The cause of this negative correlation between CI or FP-area and depression is unclear. While another study has shown that depression post-mTBI may influence the patients’ perception of PCS and that depressed PCS individuals usually report greater severity of symptoms in the RPQ score compared to non-depressed PCS individuals, further investigations should be conducted to explain how depression has a “positive” effect on both the FP-area and the CI measures.

Although the cause of CI after mTBI is still unclear, the high prevalence of CI among mTBI population is expected for the following reasons: (1) regardless of the site of the head injury, it has been reported that the frontal cortex and sub-frontal white matter, the deeper midline structures including the basal ganglia and diencephalon, the rostral brain stem, and the temporal lobes, including the hippocampi are the most vulnerable brain regions to damage in mTBI\textsuperscript{58}; (2) From our EVestG results which show a difference in the efferent vestibular response of the PCS patients with CI. Considering the feedback between the vestibular nuclei (brainstem) and the vestibular periphery by the efferent vestibular nerve, we may hypothesize that the change in the vestibular response is more likely resulted from damage to the brainstem or periphery. Given that convergence centers, FEF and SOA, are located in frontal cortex and
midbrain but the relaying centers are in the brainstem (see the introduction for details). (3) From an evolutionary point of view, there is anecdotal evidence that vergence eye movement developed later in time in humans compared to other types of eye movements\textsuperscript{59,60}. As a result, the neural connections of vergence may not be as robust and vigorous as other eye movements (VOR, OKN, saccade and pursuit). Therefore, convergence may be more vulnerable in general to impact and neural damage in PCS.

Given the prevalence of CI among PCS population and associated functional impairment and symptoms, screening CI is becoming a common practice among clinicians as a part of a comprehensive clinical PCS examination. The general/standard tool used for screening the vestibular and ocular sensory-motor impairment and symptoms is the VOMS. The VOMS has shown good consistency and significant correlation with PCS, however, more research is needed to be conducted on its usage as an appropriate screening tool for vestibulo-ocular symptoms\textsuperscript{23}. Therefore, the search for a reliable and valid tool for screening vestibular and ocular impairments and symptoms is ongoing.

Our results show that EVestG can be used as a complementary clinical tool for detecting a vestibulo-ocular impairment associated with a head injury. It also might prove useful in detecting comorbid depressive/mood symptoms and their potential confounding effects. The main limitations of this study are: 1) The overall sample size was small, and 2) the EVestG signals, as well as the CI of healthy controls, were not included for comparison with the PCS population. Using EVestG technique can be advantageous to target rehabilitation strategies prescribing to reduce the severity of impairment and symptoms and to expedite the recovery time. However, more research should be conducted to investigate whether the EVestG signal changes after CI recovery.
Figure VI-6: the calculated correlations between (A) FP-area and RPQ13, (B) CI and RPQ13, (C) FP-area and RPQ13 when only the depression symptoms were considered (D) CI and RPQ13 when only the depression symptoms were considered.
References

17. Ponsford, J., Draper, K. & Schönberger, M. Functional outcome 10 years after traumatic brain...


35. Gamlin, P. D., Gnadt, J. W. & Mays, L. E. Abducens internuclear neurons carry an


52. Taghdiri, F., Varriano, B. & Tartaglia, M. C. Assessment of Oculomotor Function in Patients


Chapter VII – Conclusions

7.1 Summary of Findings

Many studies indicate that following a head injury, cerebral pathophysiology can be adversely affected for days, weeks and even much longer (> 1 year) with chronic neurobiological changes. Following a mTBI, patients usually experience one or more clinical symptoms which include somatic (e.g. headaches, dizziness), cognitive (e.g. poor concentration), mood (e.g. depression), visual (e.g. convergence insufficiency, poor accommodation) and behavioural (e.g. irritability) symptoms. When these symptoms persist for a prolonged period of time, it is called persistent post-concussion syndrome (PCS). The most common symptom after headaches in PCS population is dizziness and balance problems. Further, individuals with PCS are more likely to have vestibular response change even if the peripheral vestibular apparatus is not damaged. This was confirmed in the first part of this thesis, which was shown the EVestG evoked averaged FP responses, representing the vestibular responses, were different in PCS individuals compared to healthy controls (Chapter 3-4).

During the first stage of this thesis, the aim was to extract features from the EVestG signals which have a classification power in separating the PCS group from healthy control. The search for robust features resulted in two features: the AP-area and interval histogram (IH33). When these two features were combined, an 84% classification accuracy of separating PCS group from healthy control was obtained. Moreover, these two features were able to identify the SPCS and the LPCS subgroups and classify them from each other as well as from healthy controls with 79% accuracy for SPCS versus LPCS, 95% accuracy for SPCS versus controls and 77% for LPCS versus controls (Chapter 3).
Our observations indicate that in general, the AP-area of the PCS individuals was narrower than healthy controls. Furthermore, the AP-area of the SPCS group was narrower than LPCS. This finding has an important implication as it is representative of where the neurophysiological changes that may take place post-injury. In summary, the narrowing of the AP-area was argued (Chapter 3) to be due to channelopathies namely an excess of influx and efflux of sodium potassium ions through the membrane, and this change has been argued to be due to the accumulation of calcium ions (Ca^{+2}) inside the injured nerves.

On the other hand, the average interval histograms of the average time between the detection of 33 FPs (IH33) for both PCS subgroups shows that the PCS individuals’ histogram is shifted to the right of healthy controls; this is indicative of an increase in time between IH33 intervals and hypothetically can be related to a reduction or slowing of efferent input.

After introducing the AP-area and the IH33 as main features for detecting PCS, they were then applied on PCS with comorbid depression. The review of the literature indicates an increased incidence of psychiatric disorders and psychological symptoms in mTBI population. Depression and anxiety are considered the most concerning symptoms or psychiatric diagnosis after a head injury. Moreover, it was shown that the diagnosis of PCS might be overlooked in favour of a diagnosis of depression. Therefore, the EVestG-PCS classifier was tested whether it is affected by co-morbid depression (Chapter 4). The presence of depression resulted in a decrease in classification accuracy from 100% in PCS with no depression to 83% for PCS individuals with moderate/severe depression. By adding a third (depression) feature adopted from previous independent studies (the post potential trough (PPT)-area) to the previous PCS-specific features, the calculated accuracy improved to 89% for classifying PCS with moderate/severe depression from healthy controls. Moreover, in the case of three groups classification (PCS with no depression
versus PCS with mild depression versus PCS with moderate/severe depression), the accuracy improved from 64.6% when using two PCS previously applied features (AP area and IH33) to 81.5% when the depression feature was included. The overall results of this study (Chapter 4) indicate when depression and PCS are comorbid in a PCS group, the EVestG features could be used to separately detect both conditions with two different and relatively independent neurophysiological mechanisms that can be applied simultaneously. This result is important because not only it has been the first study to address identifying co-morbid depression in PCS individuals, but also because it is advantageous for clinical decision-making.

Correlation analysis indicated a significant correlation between the depression feature and the MADRS depression score (R=0.67, p<0.01) and between the PCS-specific feature and RPQ (R=-0.48, p<0.01) and RPQ13 (R=-0.45, P<0.01). No significant correlation was found between the PCS-specific feature and RPQ3 score (R=-0.22, P=0.20) or with the MADRS score (R=0.20, p=0.12). No significant correlation was found between the depression feature and the RPQ scores (RPQ (R=0.07, p=0.67), RPQ3 (R=0.08, p=0.63) and RPQ13 (R=0.12, p=0.53)).

To know whether EVestG features could be separately and relatively independently be applied as a diagnostic assist tool for PCS and or its comorbid depression was the goal of the first stage of the study (Chapters 3–4). Then in the next stage, we investigated whether the EVestG features applied can separately monitor both PCS and depression recovery following rTMS treatment when PCS and depression are comorbid (Chapter 5). The results indicate that the SPCS and LPCS subgroups who received active rTMS, showed a significant improvement in both the EVestG-depression and EVestG-PCS feature two months after treatment. This observation was consistent with the neuropsychological assessment outcomes. The EVestG-depression improvement was correlated with the MADRS score change (R=0.63, p=0.01) and the EVestG-PCS improvement
was correlated with RPQ (R=-0.78, p=0.001), RPQ3 (R=-0.78, P<0.01), and RPQ13 (R=-0.76, P<0.01) score change. The EVestG depression and PCS features are relatively independent measures each assistive in monitoring depression, PCS and comorbid PCS/depression changes. These results support rTMS treatment for both depression and PCS symptoms and also that the EVestG and in particular the AP area extracted feature has the potential to monitor the primarily PCS recovery. EVestG also has shown the potential to detect the depression comorbidity of PCS as well as monitor each pathologies recovery.

In the first part of this thesis, it was shown that the vestibular system is affected in PCS. However, there is evidence that other sensory modalities are also affected by PCS. The visual system is commonly impacted by PCS; and studies have shown that this might be reflected in vergence eye movements, one presentation of which is CI. As the link between the visual and vestibular systems is well established, this study aimed to explore the intimate relationship between these two systems in PCS. Given that the epidemiological prevalence of CI significantly increases in PCS, the correlation between CI and the extracted PCS feature FP-area was investigated and compared both of these measures to the RPQ scores that is a measure of the severity of PCS. Results indicate the average FP-area and the CI value were significantly correlated in patients with PCS (R=-0.68, P<0.01). Also, there was a significant correlation between RPQ3 (Headache, Dizziness, and Nausea symptoms) and CI (R=0.70, p<0.01) and between RPQ3 and FP-area (R=-0.56, P<0.02). When the PCS population was subgrouped based on their MADRS score, CI was: highest in the PCS with no-depression (CI=7.5 prism diopter (PD) ± 1.2(SE)); moderate CI (CI=5.2 PD ± 1.0) for PCS with mild depression and; lowest for PCS with moderate/severe depression (CI=1.6 PD ± 0.7). This finding is considered advantageous for clinical decision-making as well as for PCS-rehabilitation.
Besides the sample size limitation, the recording site and electrode type were also limitations. Some of the healthy control recordings were conducted in Australia where the power line and the hydraulic artefacts are different from Canada. These differences were considered within the NEER algorithm when removing these artifacts. As well, there were two electrode types used across the control population, one a BiopacTM and the second an in-house made one almost identically but with a cotton wool tip which had the advantages of being 1/10th of the price, potentially safer as they were softer, as well as producing a better electrode impedance match between reference and active electrodes which in turn produced a better signal to noise ratio (SNR). Future studies should not have these limitations.

This study was the very first pilot study on the application of EVestG for diagnosis of PCS and monitoring its recovery. The results of this study are very encouraging and warrants further investigation to establish EVestG as a promising tool in for PCS diagnosis and monitoring its recovery.

7.2 Recommendations for Future Work

While the sample size of the studies in this thesis was reasonable for exploratory studies, a larger dataset is needed to prove the findings with statistical rigor for clinical application.

However, our results are promising enough to merit further investigation. This pilot study may pave the way for further EVestG investigations in the PCS field. Below is the list of topics which worth further investigation.

7.2.1 PCS within the recovery time

In the third and fourth chapters, we have shown that using a quantitative physiological measure, the vestibulo-acoustic response (EVestG signals), is promising in identify SPCS and LPCS both
from each other and from healthy control populations. However, for both groups, the sample size was small particularly the SPCS group. Therefore, more data should be collected to validate the obtained classification between the PCS groups.

A substantial amount of literature agrees that most of the individuals who sustain a head injury are completely recovered in the first three months i.e.; the recovery time might lie within the first days, weeks, or up to 3 months after the injury. In chapters 3 and 4, we argued that some of the SPCS group were not in the persistent symptoms but they were still in the recovery time stage. Therefore, the SPCS sample contained individuals within the recovery time and beyond the recovery time with persistent symptoms. This statement is supported by our results wherein some of the SPCS individuals were classified in the LPCS cluster but not in the SPCS group. However, to make sure, we recommend conducting more examination to find out whether these SPCS individuals have been within their recovery time or they have been showing persistent symptoms.

The motivation of this study was to identify whether someone is still spending recovery time or not. This is very valuable for clinical decision-making. Usually, physicians advise their patients to take rest (from work, physical workout or playing) after receiving an impact to the head. The resting time duration is considered critical and can be a double-edged sword. On one hand, by taking rest, mTBI individuals will avoid sustaining another impact while they are still in the recovery time and wait until a complete recovery. On the other hand, an extended rest period has economic burden as well as the potential to cause negative consequences on the patients such as depression and fatigue.

The investigations during this proposed study suggest follow up assessments right after the impact, 1 month, 6 months and 1 year after the impact. The assessments should include EVestG recordings, clinical assessments by a neurologist and neuropsychological assessments such as RPQ
and MADRS. Moreover, since DTI has shown promising results in identifying DAI, it is well recommended to be used herein in order to validate the results.

7.2.2 The Neurophysiology of the EVestG FP

Throughout this research, all the hypothesis regarding the physiological meanings of the observed changes in the EVestG FP’s waveform were based on experiments conducted on the compound action potential (CAP) of the auditory nerve. In addition, we assumed that the building blocks of the EVestG FP’s and the auditory CAP waveforms were similar. However, some differences between the vestibular and the auditory systems exist. Some of these differences include the number of fibers, spikes rate, and the divergence of the efferent fibers (for review see Lithgow (2018) supplementary material). Therefore, the differences between both waveforms are expected.

While our physiological hypothesis about the FP changes observed in the PCS population is supported by other recent studies, it is recommended to conduct a series of animal experiments to better understand the neurophysiology and then the neuropathology behind the generation of different FPs. The experiments should include sectioning of the vestibular afferent nerve, sectioning the vestibular efferent, and applying physical changes to the vestibular nerve such as stretching, compression or twisting. Simultaneously, the changes in the EVestG FP’s waveform should be observed. This would help us first to understand the neuropathology behind the generation of each building block of the FP. Secondly, it will help us understand the changes in the FP waveform resulted from any physical change applied to the vestibular nerve.

The understanding of the FP waveform can be very helpful for future studies. It could be the key to tracing the changes in the extracted features obtained from different neurological pathologies. Consequently, this could become a game changer in the treatment world helping to
better understand the pathologies behind different neurodegenerative disorders, and particularly, it will be beneficial to medical research and testing.

7.2.3 Oculomotor stimulation

In our study, we have focused only on the analysis of the EVestG signals while being in a stationary position. Despite obtaining encouraging results by using stationary signals, it is recommended to have a tool that can trigger the vestibular system while participants are still in stationary position-maintaining minimal muscle artifacts.

By measuring the EvestG signals while stationary we basically measure the spontaneous activity of the vestibular hair cells. As we have explained in chapter 1, when a group of nerve fibers fires relatively synchronously, they can produce a FP. In mammalian species, the peripheral afferent response is influenced by the activity of the EVS. And there is a positive feedback loop formed by the vestibular afferents, the VN and the EVS (see chapter 2).

The vestibular nuclei are anatomically identifiable collections of neurons located in the dorsolateral regions of the pons and medulla. In humans and other mammals, there are four different nuclei: the superior, lateral, medial, and the inferior nucleus. Each of these nuclei projects to various targets in the brain and spinal cord. One of these projections is the one to the oculomotor, trochlear, and abducens nuclei, which mediate eye movements. Having such neural links between the vestibular nuclei and the motoneurons mediating eye movements, we may be able to evoke the vestibular nuclei via eye movement generation which in turn can evoke the EVS due to the positive feedback between the vestibular periphery and the VN mentioned above.

In this case, changes in the efferent response are supposed to be detectable using the IH33 feature as we believe that this feature is a measure of the efferent response.
We have already built a prototype for this study. The prototype includes a clock shaped design with LEDs located around the clock face. These LEDs are lit in a sequential format around the clock circle. Participants have to track the LED which turns up. This eye tracking will result in a circular eye movement which engages the oculomotor, trochlear, and abducens nuclei. Another prototype can be designed for saccade and pursuit eye movements.

This project can include a farther investigation to evaluate the oculomotor dysfunction which is common following a head injury. Particularly, this suggested study could be used to investigate whether the oculomotor dysfunction is the direct consequence of the vestibular abnormality or vestibular and oculomotor dysfunction are both have the same etiology after the head injury.

7.2.4 PCS treatment

Our results from the rTMS study are also encouraging for using EVestG as an assistive tool to measure recovery. Our finding from this pilot study has opened a window to look at the PCS treatment from a different aspect. When considering a treatment for PCS, it is recommended to take into account the time since the injury. Based on our findings, individuals with short-term symptomology (< 1 year since injury) were more likely to respond to the rTMS treatment. However, the reason why only these individuals responded positively to the rTMS treatment is still unknown. We may assume that this was possible as these individuals were having short-term symptoms or because these individuals were within their recovery time.

Therefore, it is worth conducting a future study on a larger rTMS group to confirm our findings. The recruited sample should include two subsets of data: (1) individuals with head injury less than three months, and (2) individuals with head injury more than three months but less than one year. Then, after applying the same treatment protocol as in our previous study, a comparison between these two groups should be conducted.
Additionally, it is recommended to investigate the possibility of using EVestG to determine/investigate whether from the baseline FP measurement individual PCS participants are likely to respond positively to the rTMS treatment or not.
RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: "Investigating the Feasibility of EVestG Assessment for Screening Concussion"


Principal Investigator: “Dr. Zahra Moussavi, Electrical & Computer Engineering, University of Manitoba, 204-474-7023 “

Co-Investigator: “Brian Lithgow, Riverview Health Center, Research Affiliate”

Sponsor: “Neural Diagnostics, Ltd. AND NSERC-CRC, AND Riverview Health Center”

You are being asked to participate in a Clinical Trial (a human research study). Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Purpose of Study

The main goal of this study is to investigate a rapid and objective screening for concussion. Signs and symptoms of a concussion may include, but are not limited to the following: having any blackout (or knocked out) (even for a few seconds) as a result of a hit to the head, any sustained headache after injury for more than 5 minutes and/or sustained headache within 24 hours after the injury, any sign of numbness, decreased coordination, weakness or vomiting within 24 hours after injury, any sign of confusion or amnesia or slurred speech immediately after or within 24 hours after the injury.

This Clinical Trial is being conducted to study the use of Electrovestibulography (EVestG) technology as a non-invasive screening tool for concussion. EVestG™ signals are recorded
painless and non-invasively from the external ear in response to a vestibular stimulus. They are in fact the brain signals modulated by the vestibular response. The EVestG system records signals during both spontaneous (no stimulus) and whole body tilt (stimulus) activity. The EVestG test is fundamentally electrocochleography (ECOG) with the acoustic input replaced by a series of tilts to evoke a vestibular response. Previous research has shown that following vestibular stimulation (for example being seated in a chair that tilts) there are clear differences in the neural responses measured from the ear canals of individuals with neurological pathology. We will be using EVestG to measure neural responses from ear canals to determine whether this recording and data analysis technique can help provide diagnostic information about head injuries. The use of EVestG is a research diagnostic tool and has been approved jointly by the research ethics boards of Monash University (Melbourne, Australia) and the University of Manitoba.

You are being asked to take part in this study because either you are in control group or you are suspected to have suffered a concussion. A total of 40 players from local junior sports teams, and 60 individuals involved in car accidents will participate in this study. The participants in this study may or may not have suffered a concussion.

**Eligibility**

To enrol in this study, you must be over age of 15 years old and have no other neurological condition other than a concussion.

**Study Procedures**

If you are participating in this study as a Control or as a person with plausible concussion, your participation involves one session testing of:

1. Completing a neuropsychology assessment done by an expert in neuropsychology as well as ImPCAT assessment.
2. Completing the standard Montgomery Asberg Depression Rating Scale (MADRS) questionnaire.
3. Hearing assessment by a simple computer program at different frequency range.
4. EVestG test: having measurements taken of brain activity in response to movement and at rest. This involves being seated in a chair and having electrodes placed on specific spots on your forehead, as well as one electrode placed in your ear. The electrodes measure brain activity while you relax in a chair and sit in a chair that is being slowly tilted forward and back as well as side to side.
Except the task 1 that is done at the Health Science Centre and may take up to 2 hours, the tasks 2-4 will be conducted at The Riverview Health Centre and take approximately up to 1.5 hour.

If you are participating in the study as a Control, you will be assessed by the above procedure only once. If you are participating in the study as a person with plausible concussion, you will be asked to repeat the above assessments 5 more times in the intervals of 2 weeks, 4 weeks, 3 months, 6 months and 12 months after the injury with both EVestG. In order to verify the results of the EVestG assessment, we will require access to your medical records and any imaging that may have been performed such as a CT, MRI or x-ray after your concussion incidence.

**Benefits**

If you participate in this study, you will visit a specialist who will give you a comprehensive neuropsychological assessment; usually having a neuropsychology assessment by a specialist requires referral and can take a couple of months but through this study will be assessed by them immediately. You will also be reimbursed for your transportation costs and/or time to participate in this study.

Other Possible benefit is gaining to a better understanding of brain activity associated with the vestibular system (brain regions involved in the control of balance) after sustaining a concussion. This would allow concussions to be diagnosed more easily, and gives us a better understanding of when individuals with concussion may be ready to return to their normal routine.

**Costs**

All clinic and professional fees, diagnostic and laboratory tests which will be performed as part of this study are provided at no cost to you. There will be no cost for the study treatment that you will receive.

**Risks and Discomforts**

Possible risks, side effects and discomforts are minimal; it may include the insertion of an electrode into your ear canal. However, to minimise potential discomfort only trained researchers will insert this electrode. Participants will be seated comfortably in a chair while the trained researcher inserts an electrode into the participant's ear canal to rest against the eardrum. The cotton wool electrodes soaked in a mixture of saline and conductive gel with a flexible stem (to minimise insertion risks) will be used. A potential side effect is a residue of electrode gel in the ear after electrode removal. You can remove it with a cotton wool or simply by washing your ear with warm water.

Some of the questions in the questionnaire might be uncomfortable (e.g. embarrassment, feeling upset) for you to answer. In that case, let us know and we can skip the question.

**Confidentiality**

Information gathered in this research study may be published or presented in public forums; however, your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal
Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law.

The University of Manitoba Biomedical Research Ethics Board may review research-related records for quality assurance purposes. All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the Riverview Health Centre. In case of any publication of results of this study, information will be provided in such a way that you cannot be identified.

With your permission your Family Physician (GP) will be notified about your participation in this study.

**Voluntary Participation/Withdrawal From the Study**

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time, and that will not affect your other medical care at this site. If your study doctor feels that it is in your best interest to withdraw you from the study, your study doctor will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

**Results of the Project**

All participants have the option of receiving a lay summary of group findings. If you wish to be informed of the results of this project, please inform the research staff at the time of your participation or you may contact the principle investigator of this study, Dr. Zahra Moussavi by email (moussavi@ee.umanitoba.ca).

**Questions**

If you require further information or if you have any problems concerning this project (for example, any side effects), you can contact the principal researcher Dr. Zahra Moussavi (474-7023). For questions about your rights as a research participant, you may contact the University of Manitoba Biomedical Research Ethics Board at 204-789-3389.

**Statement of Consent**

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Moussavi and/or their study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. Any relationship (such as employee, student or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my
participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by the University of Manitoba Biomedical Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

I agree to being contacted in relation to this study. Yes ☐ No ☐

I agree to my family physician being notified of my participation in this study. Yes ☐ No ☐

Participant signature ___________________________ Date ___________________

(day/month/year)

Participant printed name: ___________________________ Date of Birth: __________

Contact Number: _______________________________ Email: _______________________________

For participants below the age of majority, consent should be obtained from the parent or legal guardian and assent should be obtained from the participant as follows:

Parent/legal guardian’s signature ___________________________ Date _______________

(day/month/year)

Parent/legal guardian’s printed name: ___________________________ 

Player’s signature ___________________________ Date ___________________

(day/month/year)

Players’ printed name: ___________________________ 

The study coordinator Part

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: ___________________________ Date ___________________

(day/month/year)
Signature: ____________________________

Role in the study: ____________________________ [This must be done by an authorized/qualified member of the research team i.e. investigator, study nurse, etc.]

Relationship to study team members: ______________________ [eg. supervisor, teacher/professor or family member.]

Assigned Code: ______________________
### Appendix B- Demographics table of PCS participants

**Supplementary Table S0-1: A list of Post-concussion syndrome participants' demographics**

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<th>Patient Code</th>
<th>Age</th>
<th>Gender</th>
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<th>GCS</th>
<th>Impact site</th>
<th>MADRS</th>
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<td>1-2-3-4-5-6-</td>
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<td>KyPa</td>
<td>28</td>
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<td>R</td>
<td>1yr,9m</td>
<td>15</td>
<td>Back-head</td>
<td>6/60 Normal</td>
<td>1-2-3-4-5-6-</td>
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<td>R</td>
<td>7m</td>
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<td>Forehead</td>
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<td>1-2-3-4-5-6-</td>
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<td>Gender</td>
<td>Side</td>
<td>Years</td>
<td>Site</td>
<td>VOA</td>
<td>Severity</td>
<td>Symptoms</td>
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<td>31</td>
<td>ChSt</td>
<td>46</td>
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<td>R</td>
<td>6m</td>
<td>Top-head</td>
<td>23/60</td>
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<td>1- Headaches 2- Balance problems 3- Blurry vision</td>
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<tr>
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<td>Mild Depression</td>
<td>1- Headaches 2- Balance problems 3- Blurry vision</td>
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<td>NiLe</td>
<td>19</td>
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<td>R</td>
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<td>3yrs</td>
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<td>8/60</td>
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<td>1- Headaches 2- Fatigue 3- Blurry vision</td>
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<td>10/60</td>
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<td>Normal</td>
<td>1- Headaches 2- Dizziness 3- Concentration</td>
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<td>5/60</td>
<td>Normal</td>
<td>1- Dizziness 2- Fatigue 3- Blurry vision 4- Double vision</td>
</tr>
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</table>

*site of the impact is unknown.

** Participant experienced three Impacts.
Appendix C- Convergence Insufficiency measure and EVestG recording

**Convergence Insufficiency (CI) measure**

The CI was measured at near vision with a cross-cover test using a prism bar by a neuro-ophthalmologist for all participants. Cross-cover test is an objective measure and the gold standard in measuring the vertical and horizontal ocular misalignment. During the eye examination, the examiner covers one eye with the occluder at all time, which prevents fusion. A near reading chart is located at 33 Cm from the participants’ eyes and the participant is asked to read the smallest line they can comfortably read wearing their refractive corrective glasses for near. When the participant is concentrating on one letter the occluder is alternatively switched between the two eyes and the examiner observes any horizontal eye movement when one eye is uncovered. Movement of the eye from outside to inside (i.e. abduction to adduction) indicates exophoria. The presence of exophoria only at near vision suggests convergence insufficiency. At the next step of examination, horizontal prisms are used to measure the angle of convergence insufficiency. Starting at 2 prism diopter and base-in prism the examiner repeats the cross cover test and gradually increases the strength of the prism until the horizontal movement of the eye is neutralized. The strength of the prism at that point equals the angle of convergence insufficiency.

**Electrovestibulography (EVestG)**

Electrovestibulography (EVestG) that measures vestibulo-acoustic predominantly vestibular response changes. The recorded signal is a combination of acoustic and vestibular generated field potentials (FPs). EVestG measures the predominantly vestibular response either statically or in response to passive whole body tilts from the external ear. Electrovestibulography (EVestG)
has shown promise as a diagnostic assist tool for neurological and neuropsychiatric conditions such as Meniere’s\textsuperscript{29}, Depression\textsuperscript{30}, Parkinson’s Disease\textsuperscript{31} and PCS\textsuperscript{5–7}.

**E VestG Recording Procedure**

7. Placing the electrodes: the ear canal wick electrode was placed in each ear canal close to the ear drum (TM-EchoGtrode, Bio-logic, France). Identical reference electrodes were placed on each ipsilateral ear lobe close to the ear canal. One common ground (Biopac EL258S) electrode was placed on the forehead.

8. After placing the electrodes, the participant was positioned in an acoustically attenuated (>30dB) and electromagnetically shielded chamber, and seated in a stationary hydraulic chair, with their head supported by a headrest. Participants were instructed to close their eyes closed during the recordings.

9. The signals of both ears were recorded using Spike2\textsuperscript{TM} with a sampling rate of 41,666 Hz for compatibility with previous studies.

A high-pass filtering with 300Hz corner frequency is applied to the recorded signal to remove muscle artifacts. To detect the spontaneous and driven FPs and their firing patterns from the recorded signals buried in the noise, a wavelet-based signal processing technique to analyze phase changes across multiple scales called the Neural Event Extraction Routine (NEER)\textsuperscript{1} was used.

**References**


3. Ashiri, M., Lithgow, B., Suleiman, A., Moussavi, Z. & Mansouri, B. Visio-Vestibular


